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Teacher Notes

The Data Dilemma© — Modeling Scientific Practice

Introduction

Modeling is an important and integral part of scientific practice. The Data Dilemma© Activity is designed to model the process of incorporating evidence into a working external representation of the data. This activity may be used to facilitate a discussion on how scientific models evolve.

Materials

Five piece tangram set (all of the same color)
One extra data piece (a different color)

Procedure

1. Hand out the first piece of the tangram (the large triangle labeled “A”). Create a scenerio for the students explaining that while investigating a topic in the lab you have developed a “triangle model”.

2. Hand out the second piece of the tangram (the second large triangle labeled “B”). Have the students build upon the first triangle model with this second piece. You will notice that some groups will continue to form a triangle while others may form a square or a parallelogram. Stacking pieces is not allowed.

Class Discussion Questions

A. How many different shapes were created with this second piece of information collected from the lab?

B. How do you suppose scientists decide which model to continue building on as their investigation progresses?

3. Tell the students that because the initial evidence acquired suggested the simple triangle model, you decide to continue developing the triangle model and not the parallelogram or square models. Hand out the next three pieces (C, D and E) and have the students continue to build a more robust triangle model using these pieces. There are at least three solutions to this puzzle. If a group quickly discovers a solution, encourage them to find other solutions.

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Modeling Scientific Practice (continued)

Class Discussion Questions

C. Why do you think your teacher directed you to continue with the triangle model?

D. Why do you think researchers would choose to pursue the simplest model that would explain the data?

4. Introduce the "rogue" data piece (F). This part of the tangram is in a different color. Instruct the students that they now have to incorporate this latest piece of data into a geometric shape with the fewest sides possible. There are at least four solutions. If a group discovers a solution quickly, encourage them to find other solutions.

Class Discussion Questions

E. What shape did your model take once the extra piece was incorporated?

F. How did your model evolve when the new piece of data was uncovered?

G. How does this tangram activity model scientific practice?

H. Why might a scientist find it difficult to let an old model go?

I. Considering that there were multiple solutions to the data dilemma, how might a scientist determine if their explanation is the right one?

Teacher Tips

A. We recommend the students work in groups of two or three for this activity. Collaboration is an important skill to develop in the field of science.

B. Many teachers may opt to use this as an opening day activity to introduce the process of science to their students.

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Extension

The Missing Piece

Teachers may want to pose the question
Can you build another model that would require an additional piece of data while still maintaining the rectangle shape?

It is possible to construct a four sided rectangle model with a missing piece of data in the middle of the model. Other models may be built with a piece of data missing from an outside edge. Teachers may want to discuss the significance of the missing piece. Once a model has been developed, the researcher may find holes in the model that may direct the path of continued research on a given project in an effort to lend credibility to the work that has already been established. This is the crux of scientific practice. As basic researching progresses and engineering provides more sophisticated tools to collect and analyze data, the basic research that follows may provide models which evolve into more robust explanations of observed phenomena.

National Framework

Connections to A Framework for K-12 Science Education
Practices, Crosscutting Concepts, and Core Ideas

Dimension 1: Scientific and Engineering Practices
1. Asking questions and defining problems
2. Developing and using models
4. Analyzing and interpreting data
6. Constructing explanations and designing solutions
7. Engaging in argument from evidence

Dimension 2: Crosscutting Concepts
1. Patterns
4. Systems and system models

Dimension 3: Disciplinary Core Ideas

Life Sciences

LS 1: From molecules to organisms: Structure and processes

Engineering,Technology and the Application of Science

ETS 1: Engineering design

ETS 2: Links among engineering, technology, science, and society

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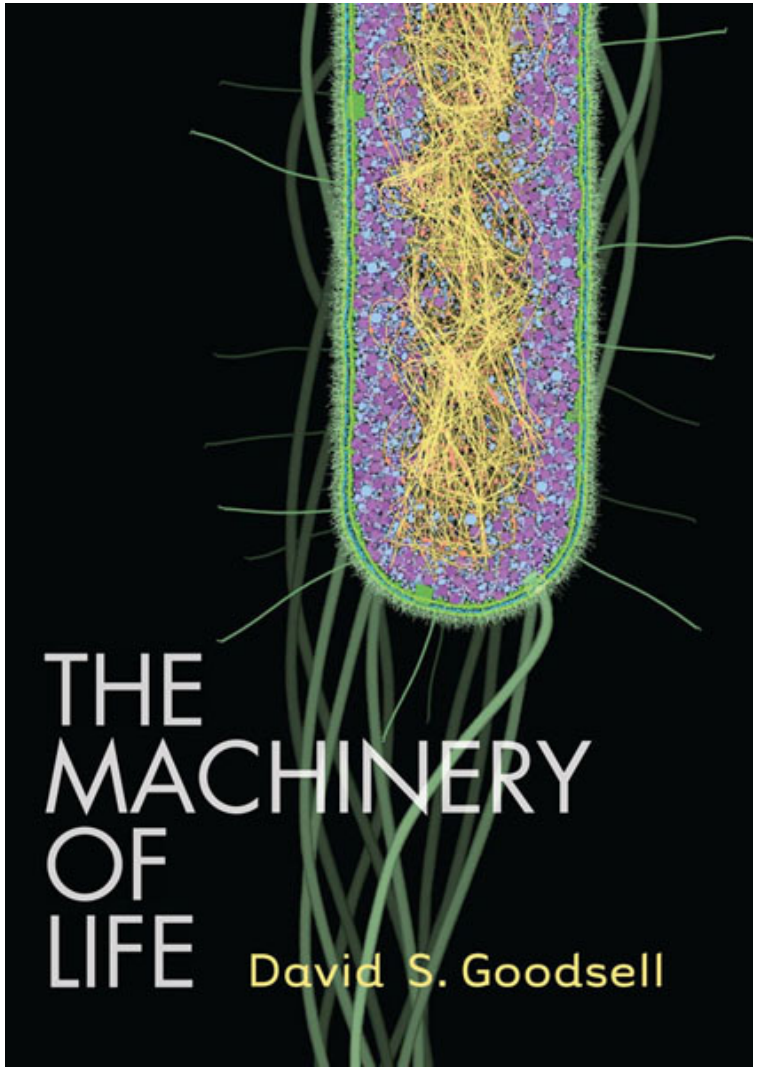
Center for BioMolecular Modeling

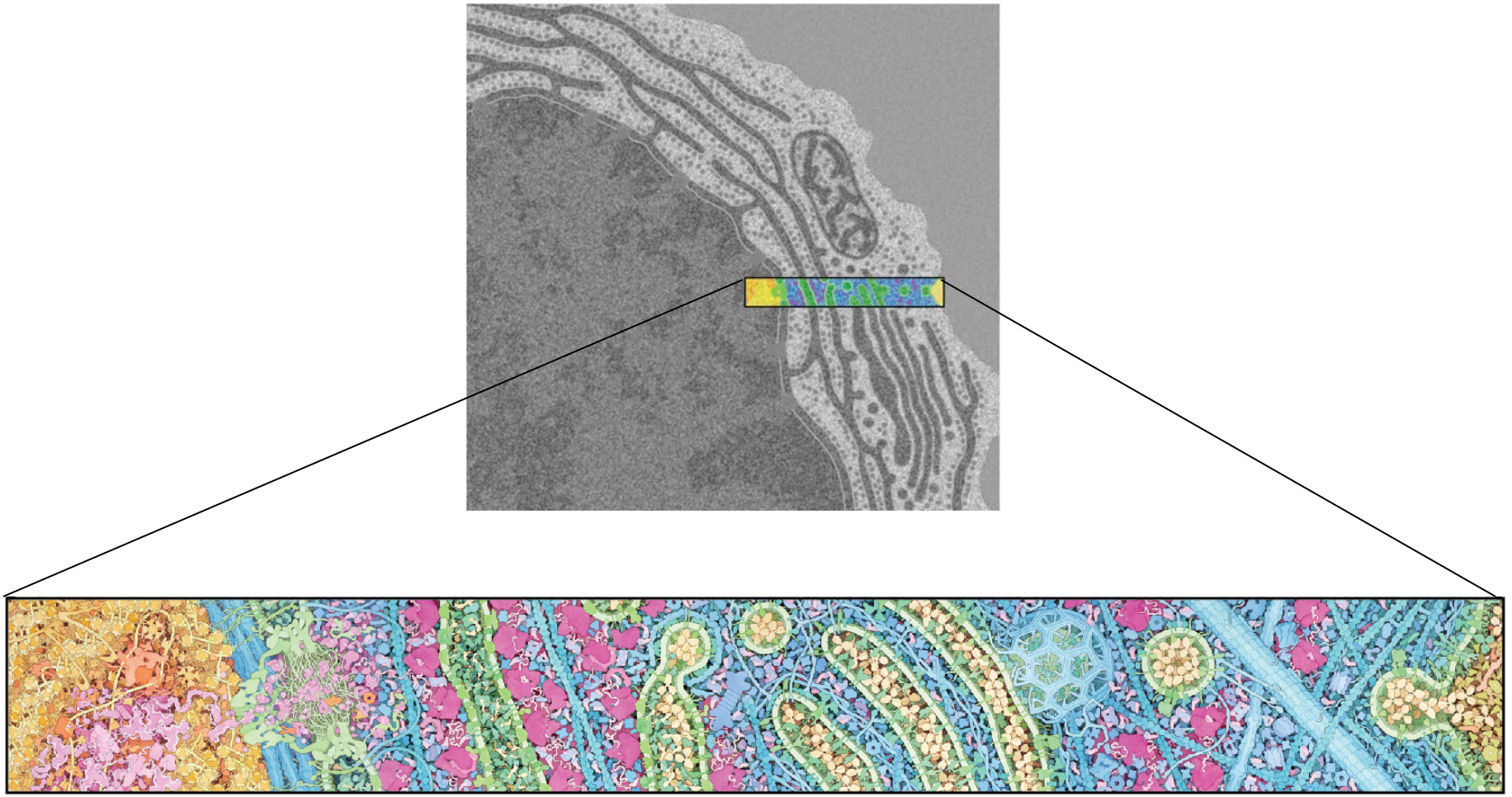
The Cellular Landscapes of David Goodsell



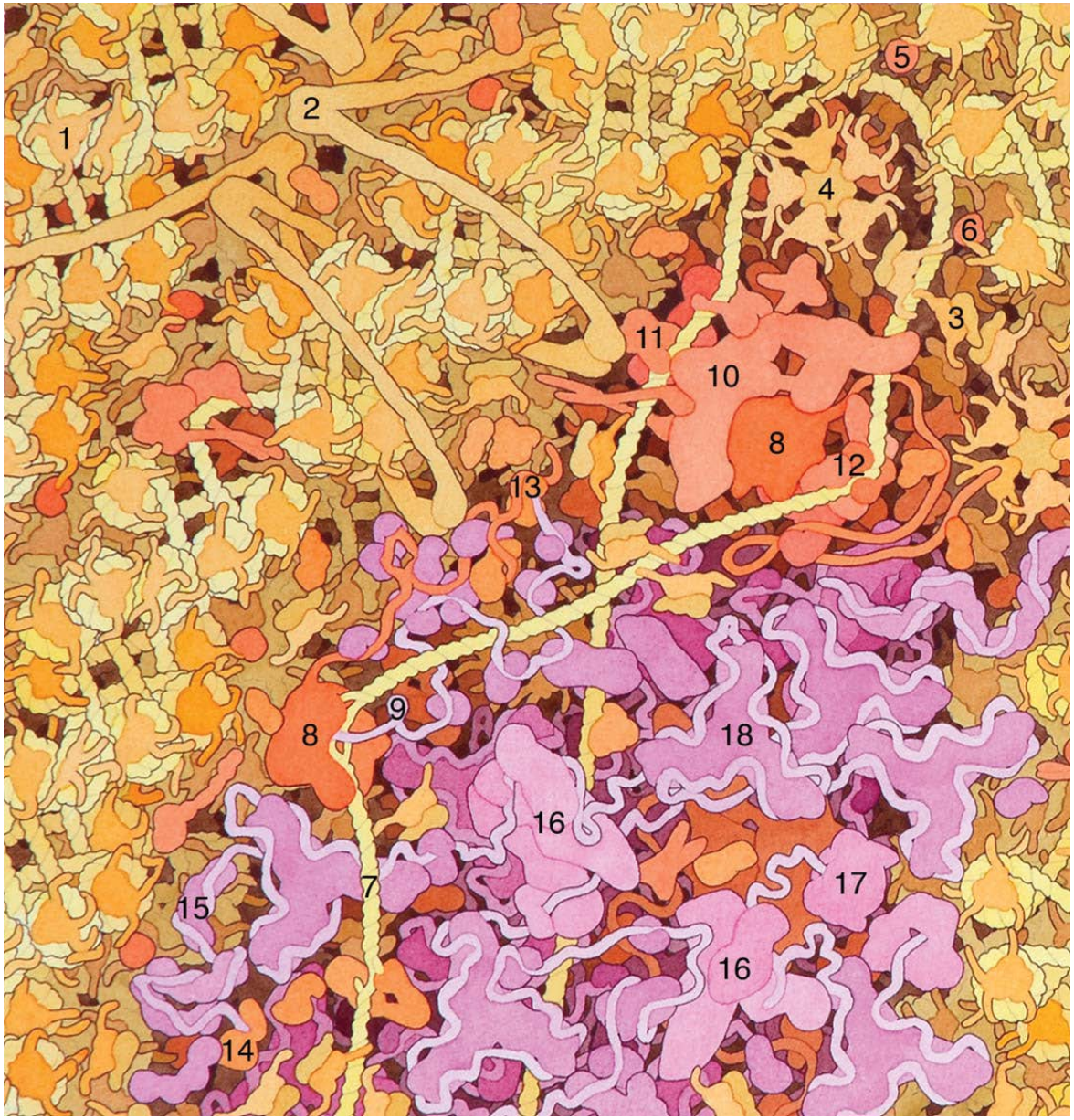
David Goodsell
Scripps Research institute

Scientist…, Author,… and Artist of all things small.

An electron micrograph of an antibody-producing B-cell



Your recent flu shot,….in action.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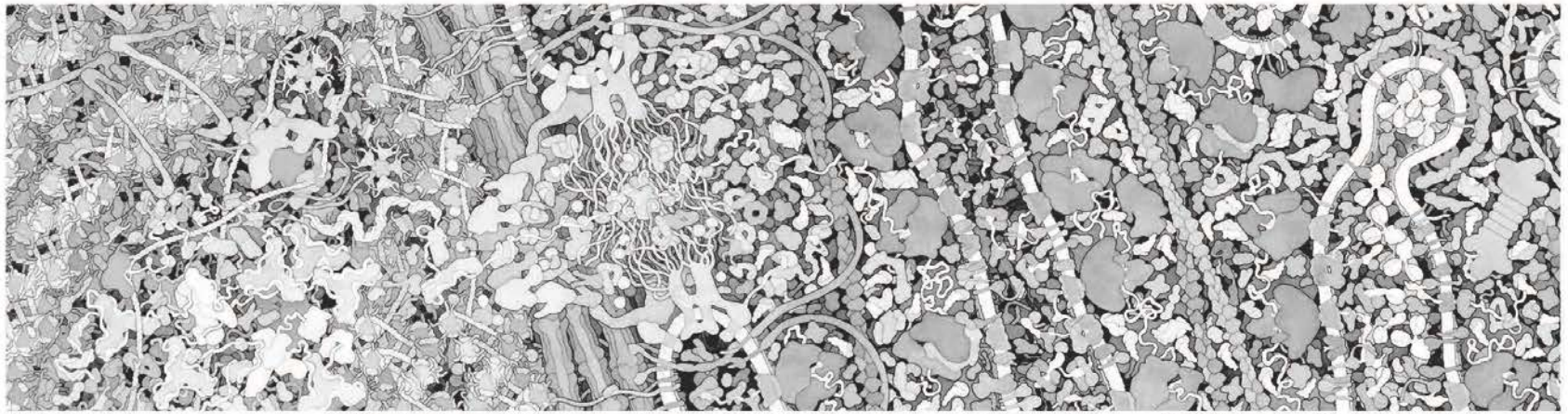
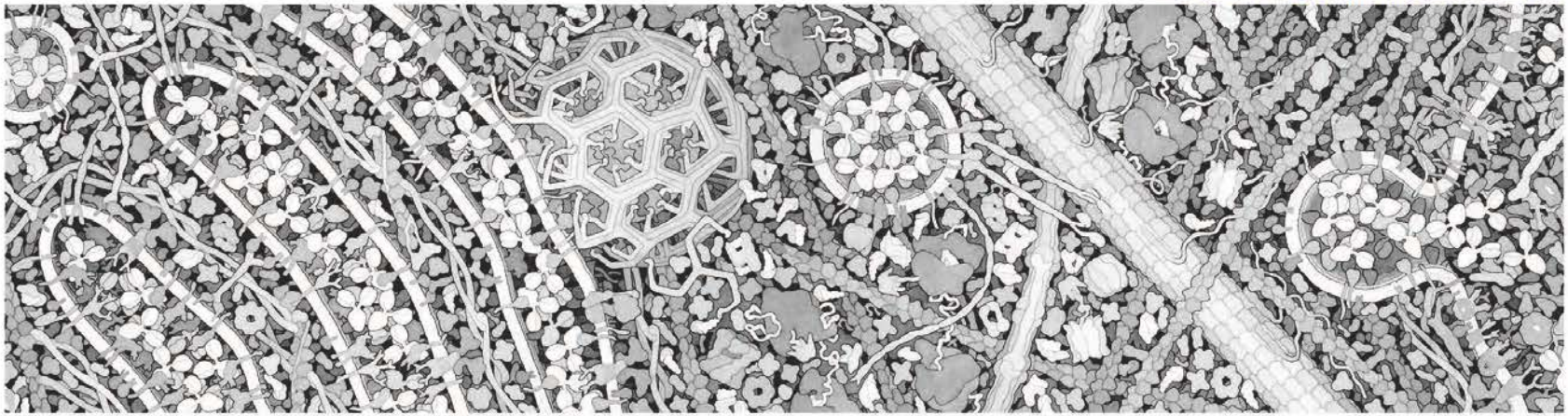
Tour of a Human Cell©



Find/Color/Label – a nucleosome
Find/Color/Label – the antibody gene
Find/Color/Label – an mRNA leaving the nucleus
Find/Color/Label – a ribosome, docked onto the E.R.
Find/Color/Label – a folded antibody protein

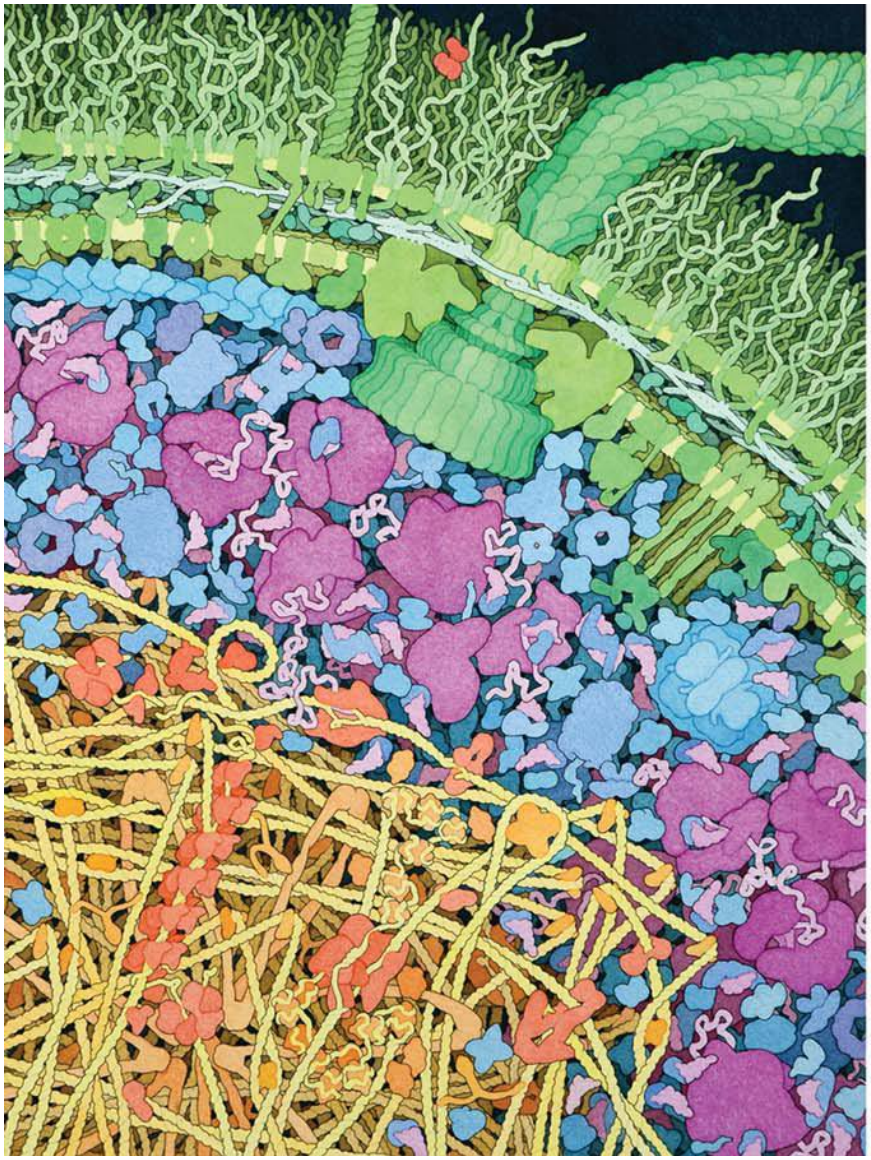
A Molecular Landscape by David Goodsell

3dmoleculardesigns.com/Education-Products/Tour-of-Human-Cell.htm

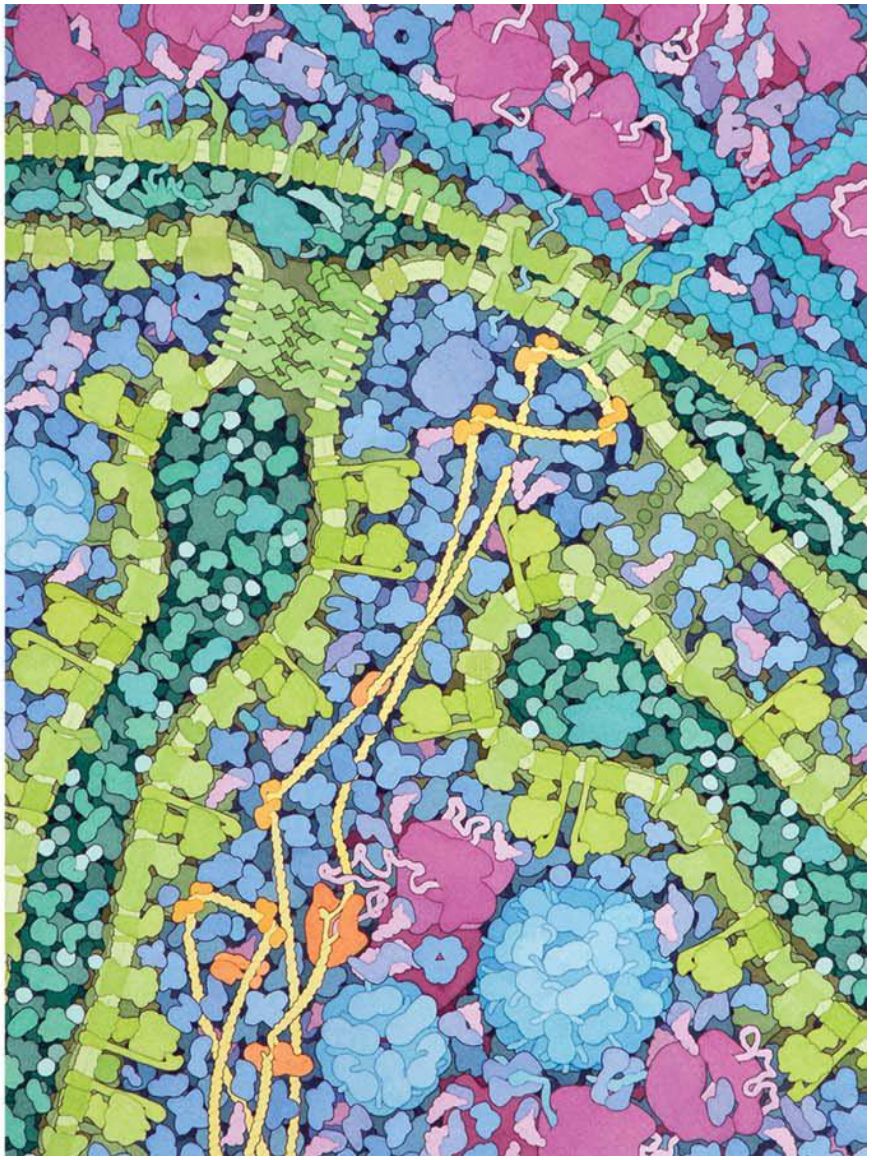


Find/Color/Label – the Golgi Apparatus
Find/Color/Label – a clathrin cage, forming an endosome
Find/Color/Label – kinesin motor proteins
Find/Color/Label – antibodies released into the circulation

E. coli



Mitochondrion



BIOCHEMISTRY AND MOLECULAR BIOLOGY EDUCATION
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Article

Miniseries: Illustrating the Machinery of Life

Eukaryotic Cell Panorama

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Diverse biological data may be used to create illustrations of molecules in their cellular context. This report describes the scientific results that support an illustration of a eukaryotic cell, enlarged by one million times to show the distribution and arrangement of macromolecules. The panoramic cross section includes eight panels that extend from the nucleus to the cell surface, showing the process of protein synthesis and export. Results from biochemistry, electron microscopy, NMR spectroscopy and x-ray crystallography were used to create the image.

Keywords: Eukaryotic cell structure, B-cell, cellular biology, molecular biology, molecular visualization, textbook diagrams, protein synthesis, nucleus, endoplasmic reticulum, Golgi.

INTRODUCTION

As part of the book, "The Machinery of Life," [1], I wanted to include a panoramic image that captures the major features of eukaryotic cells, including their many internal compartments and their extensive infrastructure for managing these compartments. Eukaryotic cells are typically too large to show in their entirety and still show individual molecules: at 1,000,000 X magnification, the entire cell would be several meters wide. So instead, I extracted a long rectangular portion that extends from the nucleus to the cell surface, and presented it on eight successive pages.

I had several goals when designing the image. The first was to include the major compartments of the cell: the nucleus, the endoplasmic reticulum, the Golgi, and the cytoplasm. I presented the mitochondria in a separate image, as they are too large to include in this compressed panoramic view. The mitochondrion image was presented in a previous article [2]. The second goal was to capture the entire process of protein synthesis and export, from DNA to final protein. Finally, I wanted to highlight a few of the unstructured proteins in the cell, and their unusual functions.

I chose a B-cell for the panorama, for several reasons. I wanted a free-living cell, so that there would not be complications with cell junctions. I also wanted a cell that synthesized and exported a familiar and recognizable protein, so that the reader could easily follow it along the process of synthesis.

This article describes the science supporting the illustration. The four letter codes presented here (1i6h, etc.) correspond to structures in the Protein Data Bank and UniProt codes (Q# or P#) are given for amino acid sequences. Full references are not given for these files, as they are available at the RCSB PDB and UniProtKB WWW sites, http://www.pdb.org and http://www.uniprot.org.

Ultrastructure

The overall layout of the panorama was based on electron micrographs of plasma cells, obtained from the electron microscopic atlas of cells, tissues and organs, on the WWW at: http://www.uni-mainz.de/FB/Medizin/Anatomie/workshop/EM/EMPlasmaZ.html.

The amount of cytoplasm separating the nucleus and cell surface is typically larger than what is depicted in the panorama, with many layers of endoplasmic reticulum, mitochondria, and Golgi. Many micrographs, however, include sections where the nucleus approaches fairly closely to the cell membrane, with only a few layers of endoplasmic reticulum, as shown in the panorama. So, the painting presents one extreme.

The panorama is broken into four sections, each with two halves to fit on two facing pages. The first section (Figs. 1 and 2) is the nucleus, with the nuclear interior on the left and the nuclear membrane on the right. The next section shows the endoplasmic reticulum, with one long compartment on the left (Fig. 3), and a budding vesicle on the right (Fig. 4). The third section shows the edge of the Golgi, with a vesicle docking on the left side (Fig. 5)

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 DOI 10.1002/bmb.20494

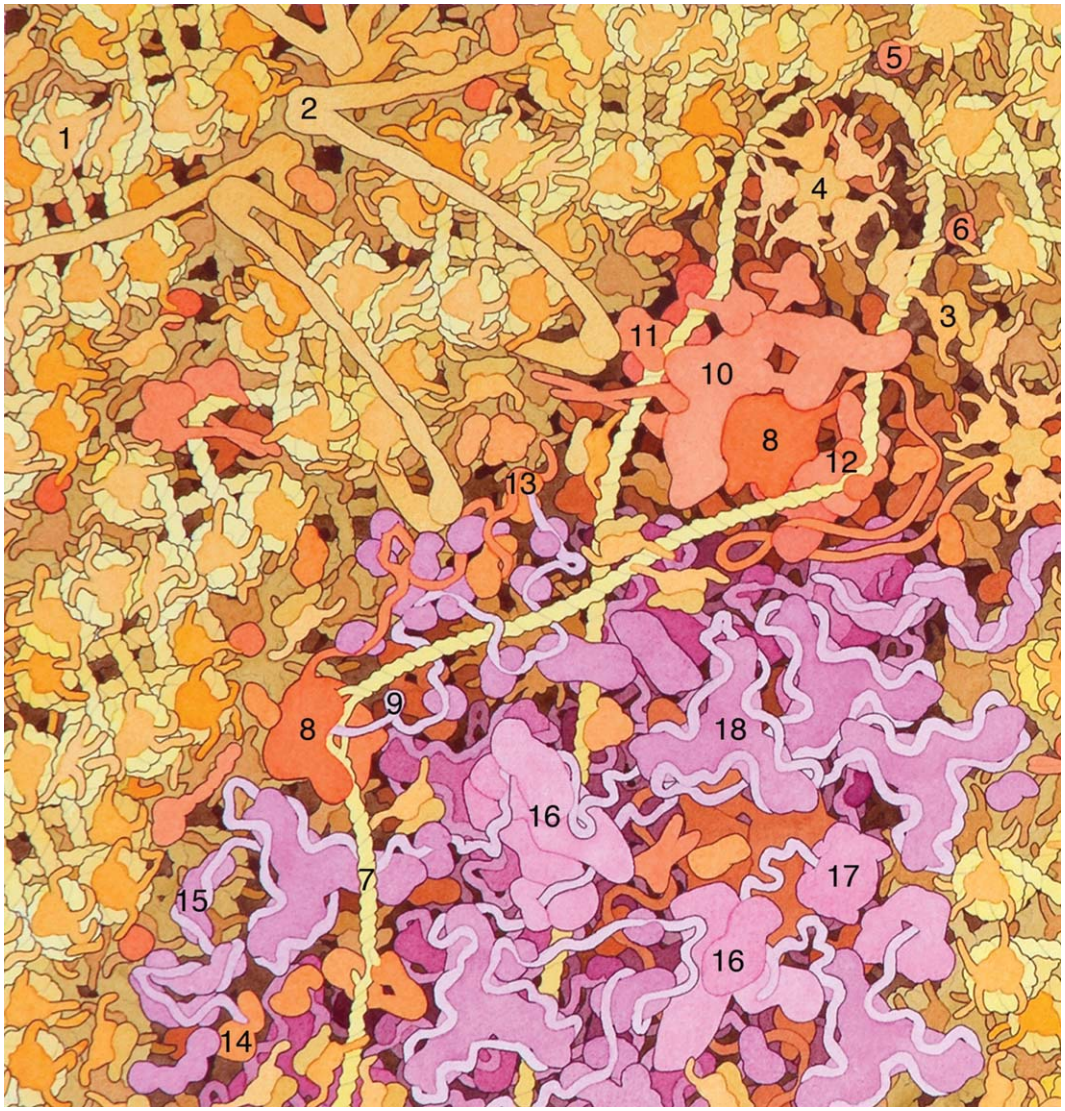

FIG. 1. **Transcription and RNA processing in the nucleus.** (1) nucleosome; (2) SMC; (3) CIA and histone; (4). nucleoplasmin and histones; (5) histone acetyltransferase; (6) histone deacetylase;. (7) DNA; (8) RNA polymerase; (9) messenger RNA; (10) Mediator; (11) enhanceosome; (12) transcription factors; (13) capping enzyme; (14). poly-A polymerase; (15) poly-A-binding protein; (16) spliceosome; (17) exosome; (18) hnRNP-C.

and another vesicle leaving at the right (Fig. 6). The final section shows a vesicle being transported along a microtubule (Fig. 7), and second vesicle fusing with the cell surface at the far right (Fig. 8).

Concentrations

The concentration of macromolecules was by far the most difficult parameter to define for this illustration. A wide range of values are reported, for instance: 200–300 mg/mL for "cytoplasmic protein" [3], 17–26% protein by weight for "actively growing cells" [4], and 15–25% for the "average protein content of animal cells" [5]. I chose a value at the higher end of this scale, roughly 25% protein.

Nucleus

The interior of the nucleus includes DNA in chromatin as well as DNA that is being transcribed. The chromatin is shown at upper left in Fig. 1 and adjacent to the nuclear membrane in Fig. 2. The nucleosomes are based on the crystal structure (1aoi), with the unstructured tails of the histones extended to their actual length. An alternating solenoidal model is used for the chromatin fiber [6]. I have also included a structural maintenance of chromosomes (SMC) protein [7], drawn as a large star-shaped complex as observed in bacterial cells [8]. At the nuclear membrane, several proteins interact with the nucleosomes, as described below.

Nucleosomes and histones are dynamic structures, and there is a substantial infrastructure for coordinating their action. I have included several histone chaperones [9], including the protein CIA (CCG1-interacting factor A) interacting with free histone dimers and the protein nucleoplasmin associating with five histone octamers. In addition, a variety of histone acetyltransferases and deacetylases are shown modifying the tails of histones [10].

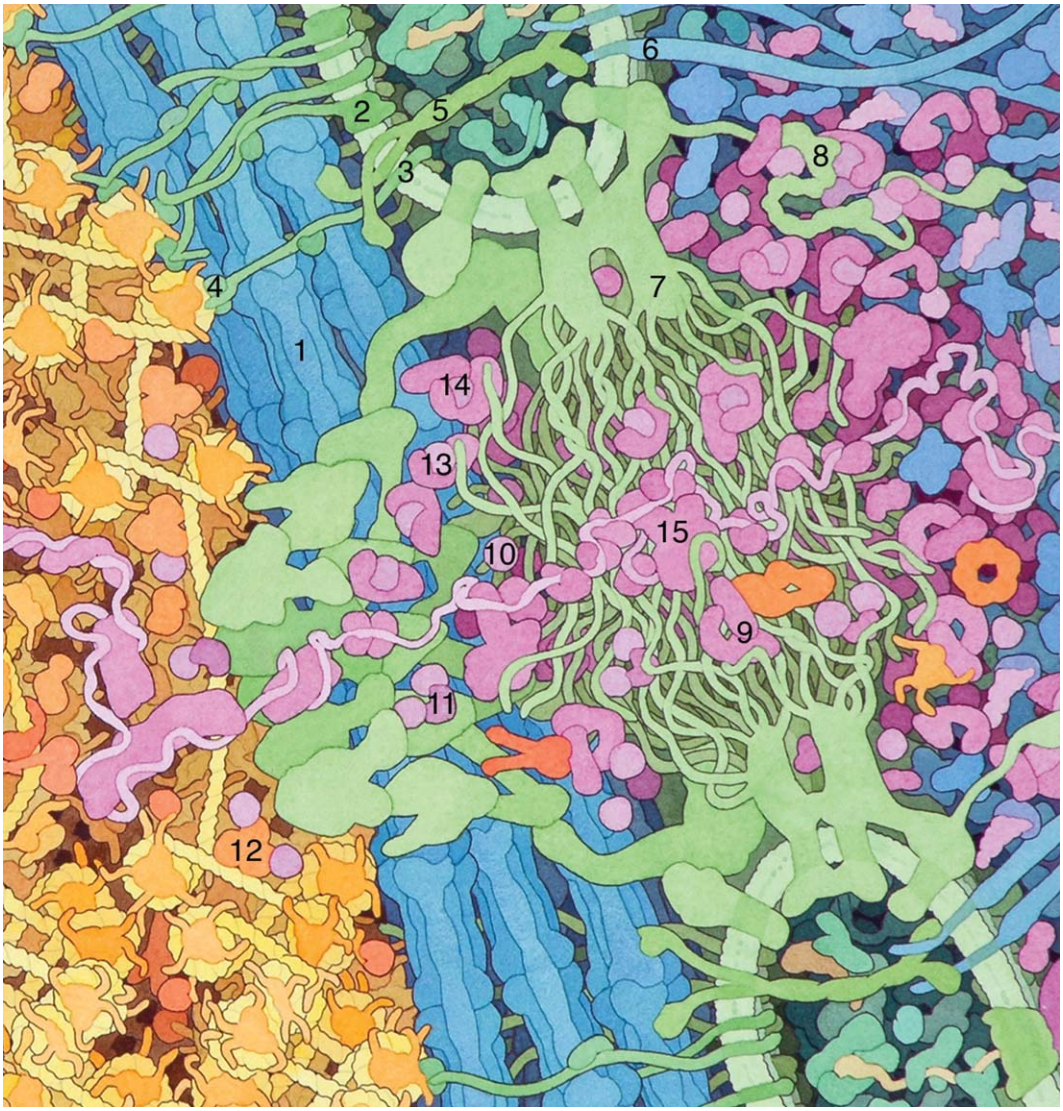

FIG. 2. **Transport through the nuclear pore.** (1) lamin; (2) LBR; (3) emerin; (4) BAF; (5) SUN; (6) nesprin; (7) nuclear pore complex; (8) Nup358 and associated proteins; (9) alpha/beta importin; (10) Ran; (11) NTF2; (12) RCC1; (13) Ran and beta importin; (14) alpha importin and CSE; (15) TAP/p15.

Two RNA polymerases are shown, one at initiation and one actively elongating an RNA transcript. The initiation complex includes a large Mediator complex based on an electron micrograph reconstruction [11], a complex of transcription factors based on crystallographic and electron microscope structures [12], and an enhanceosome taken from a structure-based model [13]. RNA polymerase is based on the crystal structure of the yeast enzyme (1i6h), with a long unstructured C-terminal tail based on the amino acid sequence (P24928). The second RNA polymerase is shown during elongation, with the transcribed RNA looping back and being processed by several capping enzymes [14]. The capping enzyme, along with other enzymes involved in modification, is shown bound to the unstructured tail of RNA polymerase [15].

RNA processing is shown at the bottom of the nuclear region in the image. At the 3′ end of the RNA chain, poly-A polymerase (1f5a) is adding the poly-A tail, which then associates with poly-A-binding protein (1cvj). Two spliceosome complexes are shown, one before splicing and one after splicing, based on structures from electron microscopy [16]. The introns are then degraded by exosomes (2nn6). The RNA is shown associating with hnRNP-C (heterogeneous nuclear ribonucleoprotein C), with the three-armed structure taken from electron micrograph structures [17]. These are shown dissociating as an RNA strand is transported through the nuclear pore.

Nuclear Membrane

The nuclear membrane, shown in Fig. 2, is composed of two lipid bilayers [18]. On the nuclear side, the membrane associates with the nuclear lamina, on the cytoplasmic side, it is continuous with the endoplasmic reticulum. The entire membrane is pierced by nuclear pores, which mediate transport into and out of the nucleus.

The nuclear lamina is composed of layers of lamin, a protein similar to cytoplasmic intermediate filaments. I have based these on electron micrograph structures of isolated intermediate filaments (described in more detail

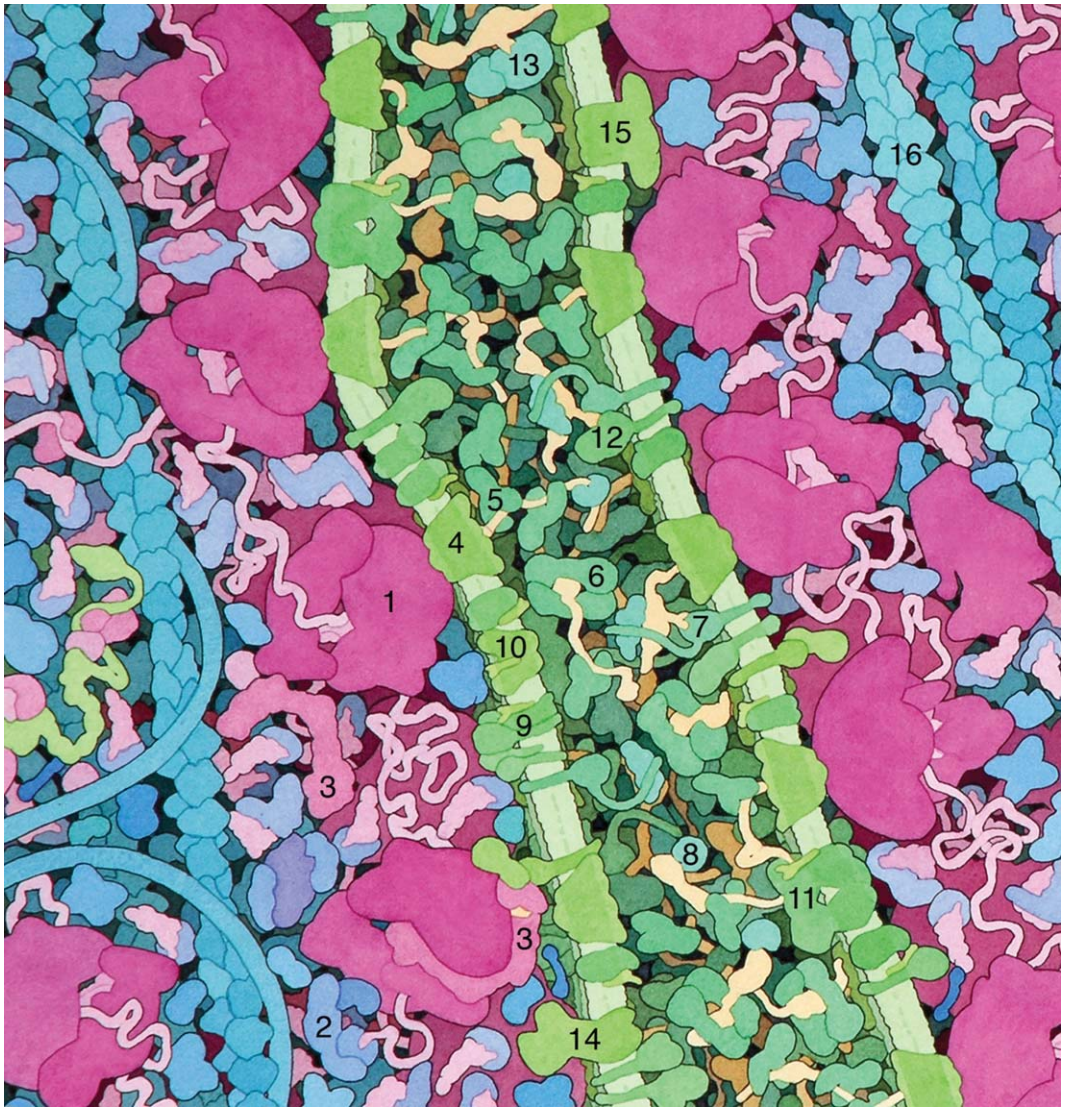

FIG. 3. **Endoplasmic reticulum.** (1) ribosome; (2) initiation factors; (3) signal recognition particle; (4) Sec61/SecY channel; (5) BiP; (6) GRP94; (7) calnexin and Erp57; (8) cyclophilin; (9) glycotransferases; (10) flippase RFT1; (11) oligosaccharide transferase; (12) GlsI; (13) GlsII; (14) calcium pump; (15) IP3R; (16) actin.

below) and electron micrographs of the lamina [19]. The lamina is connected to the membrane through a variety of membrane-bound proteins, including lamin-B receptor (LBR) and emerin. I based these structures on the amino acid sequences (Q14739 and P50402) and schematic diagrams [20]. They are drawn as unstructured proteins, with small proteins such as BAF (barrier-to-autointegration factor, 2odg) associating with the lamin and with nucleosomes.

A SUN protein (Sad1 and UNC84 domain containing protein) is shown bridging the two membranes and interacting with nesprin outside [21]. Nesprin is a long structural protein that interacts with cytoskeletal proteins [22]. I have included the chaperones and other proteins of the endoplasmic reticulum between the two membranes—these are described in more detail below.

The nuclear pore was the most exciting subject that I researched and rendered for this image. Current models of the pore include a large collection of unstructured nucleoporin proteins extending into the lumen of the pore, mediating the flow of proteins in and out [23–25]. The overall shape of the pore is based on electron micrograph reconstructions [26]. The Nup358 nucleoporin extending on the cytoplasmic side, and its interaction with transport proteins ras-related nuclear protein (Ran), Ran GTPase-activating protein (RanGAP), small ubiquitin-related modifier (SUMO), and ubiquitin-conjugating enzyme (UBE2I), is based on the annotation in UniProtKB entry P49792.

The entire transport cycle for nuclear proteins is shown [27]. This includes alpha and beta importin [28] transporting a cargo of a topoisomerase, a DNA clamp, and a repressor. Also, two copies of the Ran protein are being transported inwards by NTF2 (nuclear transport factor). Once inside, the GDP in Ran is exchanged for GTP by RCC1 (regulator of chromosome condensation, 1a12), which is shown bound to DNA [29]. Ran then associates with beta importin, and with a complex of alpha importin and CSE (importin alpha re-exporter, or chromosome segregation protein), carrying them back out through the nuclear pore. The export of RNA is also shown, with the

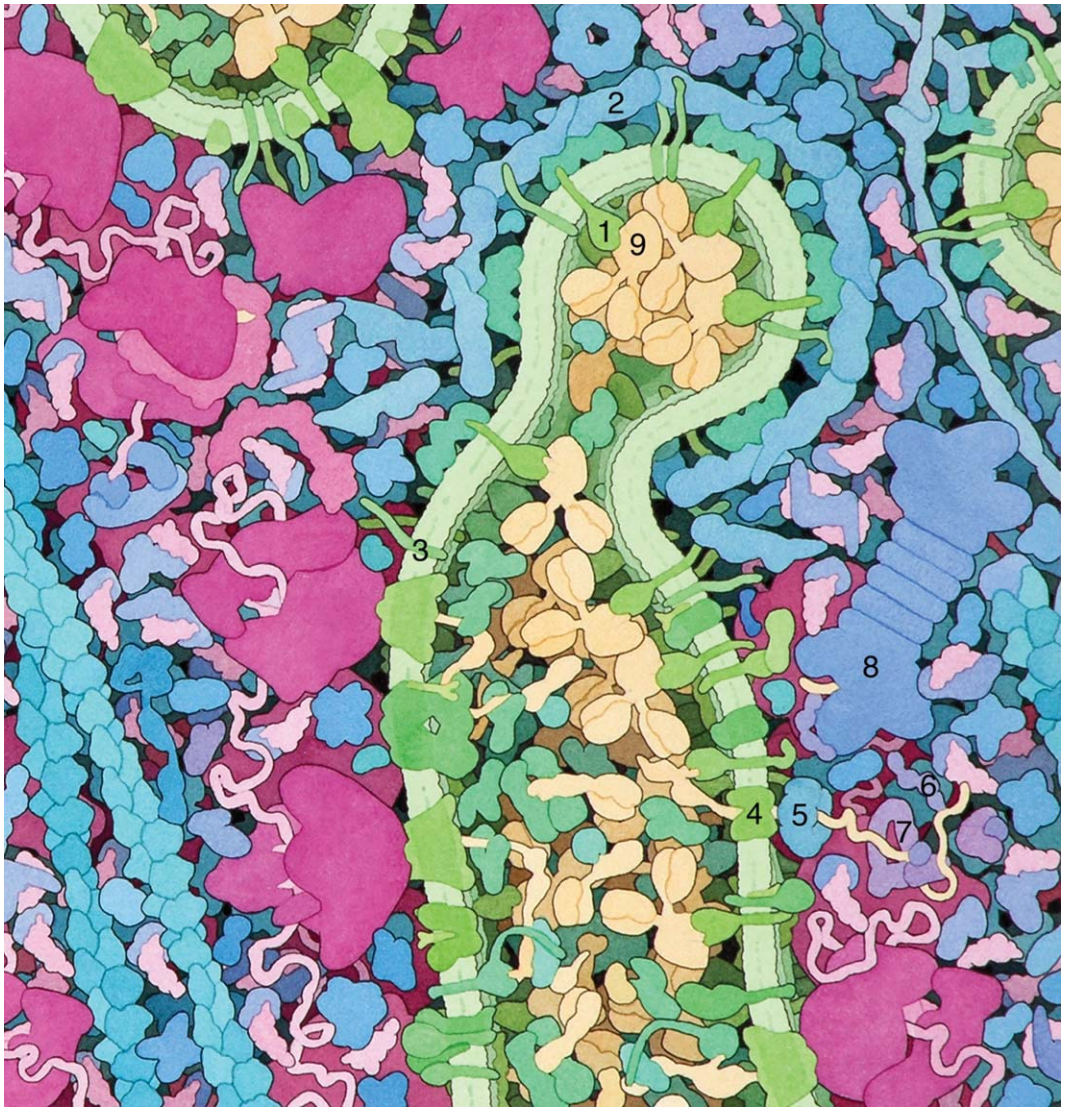

FIG. 4. **Transport from the endoplasmic reticulum.** (1) cargo receptor protein; (2) COPII; (3) SNAP; (4) EDEM; (5) AAA+ protein; (6) ubiquitin; (7) ubiquitin-conjugating proteins; (8) proteasome; (9) antibody.

complex of TAP (nuclear RNA export factor, or Tip-associated protein) and p15 proteins guiding the RNA outwards [27].

Endoplasmic Reticulum (ER)

Once the RNA leaves the nucleus, it is picked up by ribosomes and used to direct the synthesis of proteins, as shown in Fig. 3. At lower left, a ribosome has started synthesis, initiated by the complex of initiation factors [30]. A signal recognition particle (1ry1) has bound to the complex, recognizing the short signal sequence that is translated first. Interaction of the signal recognition particle with the ribosome was taken from a review [31], and eukaryotic ribosome structures were taken from electron micrograph reconstructions [32]. Binding of the signal recognition particle to its receptor delivers the ribosome to Sec61/SecY, the channel through the ER membrane, and the protein BiP (ER luminal binding protein) acts as a ratchet binding to the nascent protein inside the ER and ensuring that it stays inside [33, 34].

A variety of chaperonin proteins inside the ER assist with folding, including GRP 94 (glucose-regulated protein 94, modeled after HSP 90, 2cg9), calnexin (1jhn) [35] and protein disulfide isomerase ERp57 (2h8l), and cyclophilin (2cpl). Studies have shown that immunoglobulins are largely folded in the ER, not proceeding to the Golgi until they are in their proper oligomeric state [36, 37].

The ER also glycosylates proteins [38]. Ig-G molecules contain one N-linked site for glycosylation in the constant domain, and this glycosylation is important for structural stability, for interaction of antibodies with other serum proteins, and for transport and secretion of antibodies from the plasma cell [39]. The oligosaccharides are built on a lipid anchor by a collection of glycotransferases [40], shown here based on amino acid sequences of the yeast enzymes, taken from UniProtKB. Strangely, this process starts with the growing oligosaccharide chain facing outwards, and then the flippase RFT1 (based on the primary sequence from the yeast protein, UniProtKB P38206, which shows 13 transmembrane helices) flips the lipid toward the inside. Once the oligosaccharide is

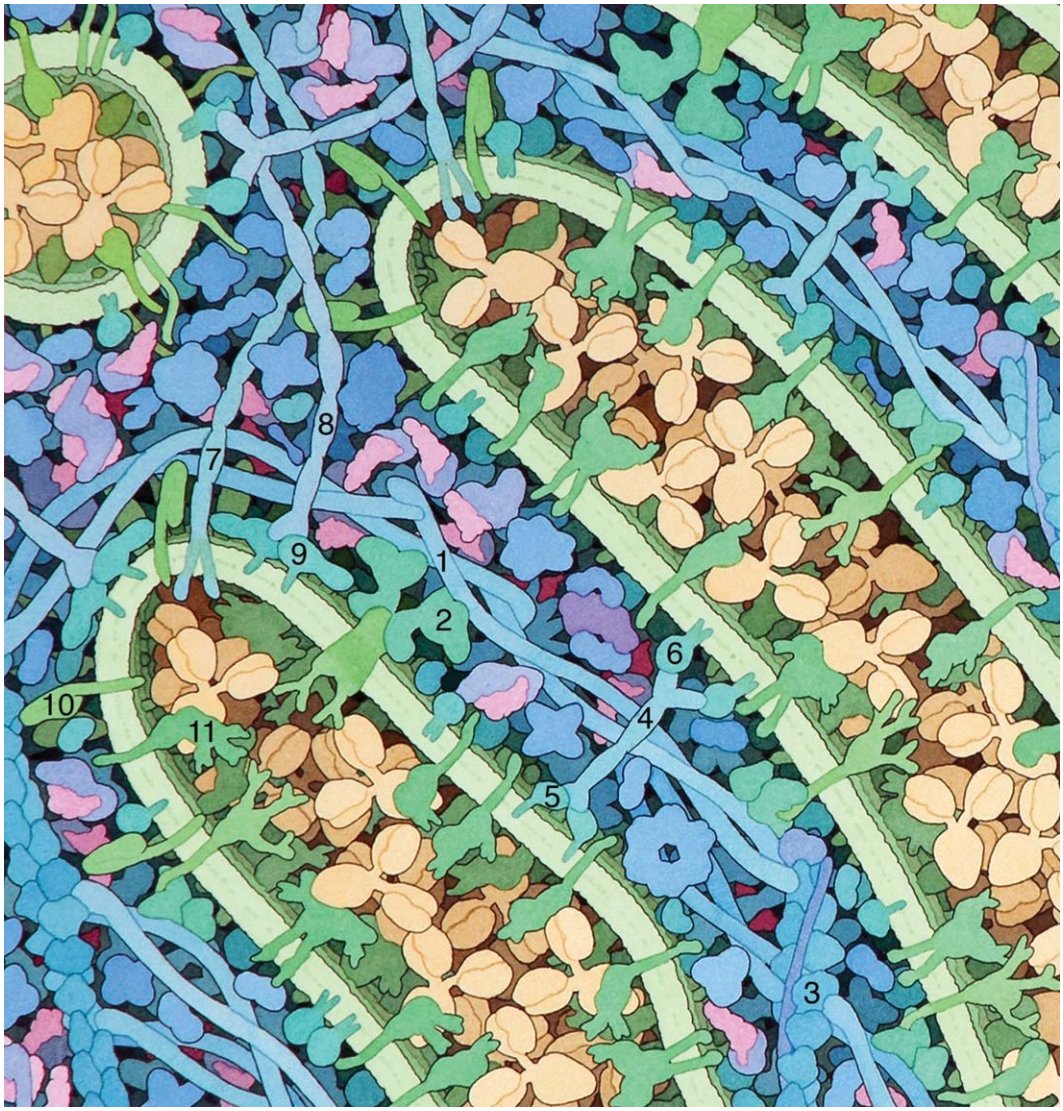

FIG. 5. **Protein sorting in the golgi.** (1) spectrin; (2) ankyrin; (3) actin; (4) golgin45; (5) GRASP55; (6) RAB2; (7) gigantin; (8) GM130; (9) GRASP65; (10) SNARE; (11) glycosyltransferase.

built, it is transferred from the lipid to the protein by oligosaccharide transferase, a large protein complex based on electron micrograph reconstructions [41]. Finally, several glucosidases, such as GlsI and GlsII [42] trim the oligosaccharides.

The ER is the major storage space for calcium ions in most cells, which is used for signaling [43]. I have included two proteins in ER membrane that are important in this process, a calcium pump (1su4), which transports calcium into the ER, and IP_3R (inositol 1,4,5-triphosphate receptor/calcium channel) [44], which releases calcium for signaling.

I found very little information on the infrastructure that is used to maintain the overall ultrastructure of the ER. There is evidence that microtubules are involved in the generation of the structure of the ER in human cells [45, 46], although actin plays this role in plant and yeast cells. However, there is also evidence that microtubules are not needed for maintaining the structure of the ER [46]. I chose to gloss over this point by simply including a few generic actin filaments in the image, without showing any specific interaction between the cytoskeleton and the ER membranes.

Figure 4 shows a vesicle being removed from the ER. Cargo receptor proteins, modeled here after ERGIC-53 (ER-Golgi intermediate compartment protein, 1r1z) [47], have captured antibodies and are interacting with COPII proteins (vesicle coat proteins) that are forming the vesicle, based on atomic structures (1m2v, 2pm7) and electron micrograph reconstructions [48]. Several SNAP proteins (synaptosomal-associated protein) are also included, which will be important for the fusion of the vesicle at its next stop, the Golgi. Finally, at lower right, I have included EDEM (ER degradation-enhancing alpha-mannosidase-like protein), a protein that recognizes faulty proteins from the ER [38]. I have shown a hypothetical transport protein powered by an AAA+ protein ejecting this protein from the ER, where it is ubiquinated and ultimately destroyed by a proteasome. Ubiquitin and ubiquitin-conjugating enzymes were based on crystal structures (1r4n, 1fxt, 1ldk, 1fqv), and the proteasome is based on an electron micrograph reconstruction [49].

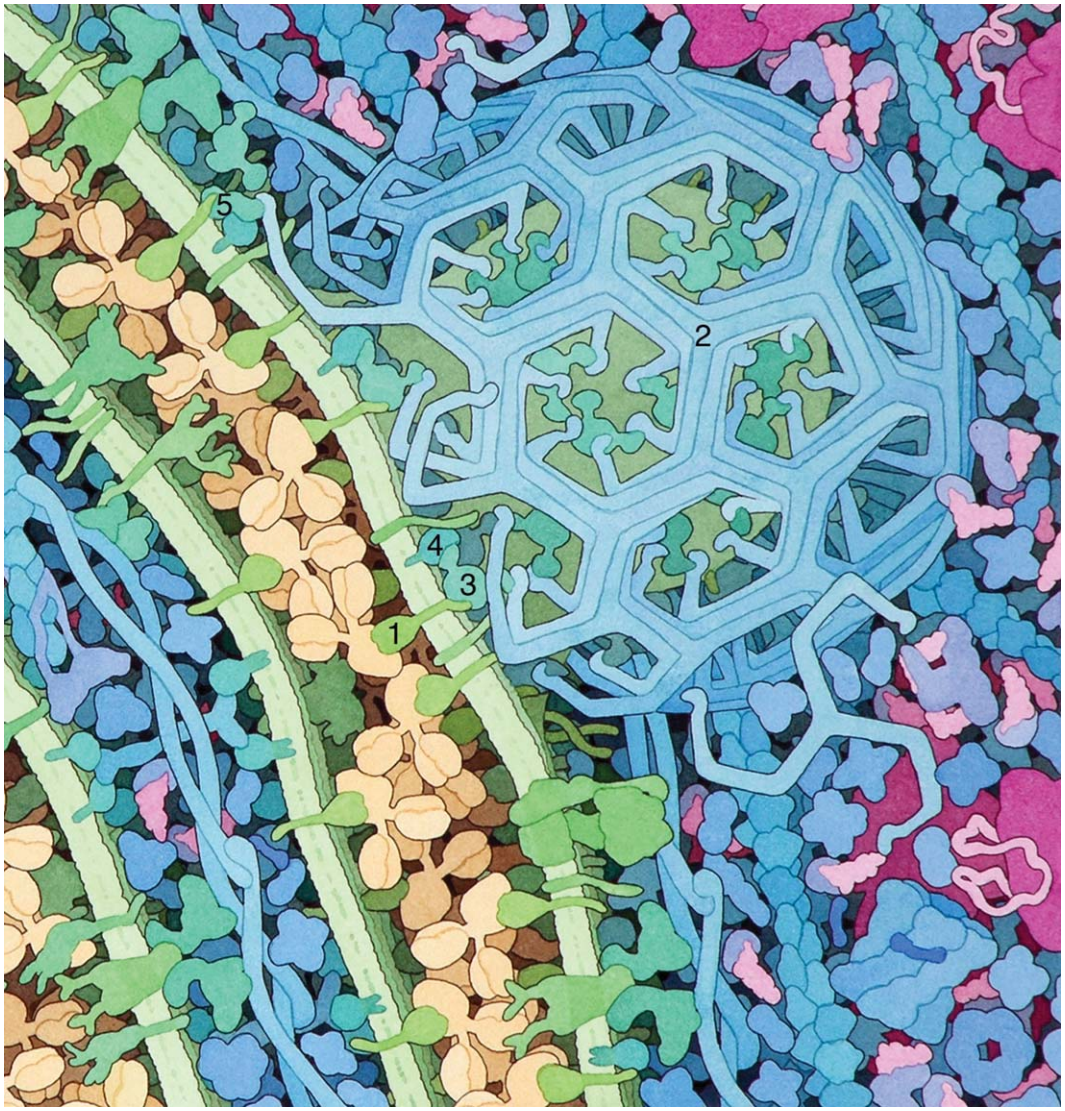

FIG. 6. **Transport from the golgi.** (1) cargo receptor protein; (2) clathrin; (3) AP2; (4) ARF; (5) GGA.

Golgi

The Golgi, shown in Figs 5 and 6, is the site of fine-tuning and sorting of proteins. I have included three layers of the Golgi, ranging from cis to trans as you move from left to right through the panorama. I have included many infrastructure proteins, based on biochemical studies and schematic diagrams. These include spectrin, bound to the Golgi membrane through ankyrin and linked through short filaments of actin [50]. The spectrin and ankyrin structures are based on a combination of primary sequence, electron microscopy and crystal structures of domains [51]. I have included a speculative complex of golgin45 protein linking between the Golgi stack, associating with GRASP55 (Golgi reassembly-stacking protein) and RAB2 (Ras-related protein) in the membrane surface [52]. Huge proteins like gigantin and GM130 (cis-Golgi matrix protein, bound to GRASP65) extend from the Golgi, acting as tethers to trap vesicles [53–56], bringing them close enough for membrane fusion by SNARE proteins [57]. Many of these proteins are characterized by long helical bundles, forming a flexible ropelike structure.

Inside the Golgi, a variety of glycosyltransferases modify the oligosaccharides on the antibodies [58]. I have drawn them as bound to the Golgi membrane by a transmembrane segment [59]. In the trans compartment, cargo receptor proteins capture antibodies and prepare them for transport to the surface. I have shown a clathrin-based mechanism for creation of vesicles, based on schematic diagrams from a review article [60], although other mechanisms may be important [61]. The clathrin coat is composed of three-armed triskelions based on the crystal structure (1xi4). The adaptor protein AP2 (1gq5, 1ky7, 2g30) links the clathrin coat to the cytoplasmic portion of the receptor, with the help of the regulatory protein ARF (ADP-ribosylation factor, 1rrf). GGA (Golgi-localized gamma-ear-containing ARF-binding protein) is also shown in the process of recruiting the receptor, AP2 (adapter protein) and ARF to the clathrin coat. The clathrin coat then disassembles after formation of

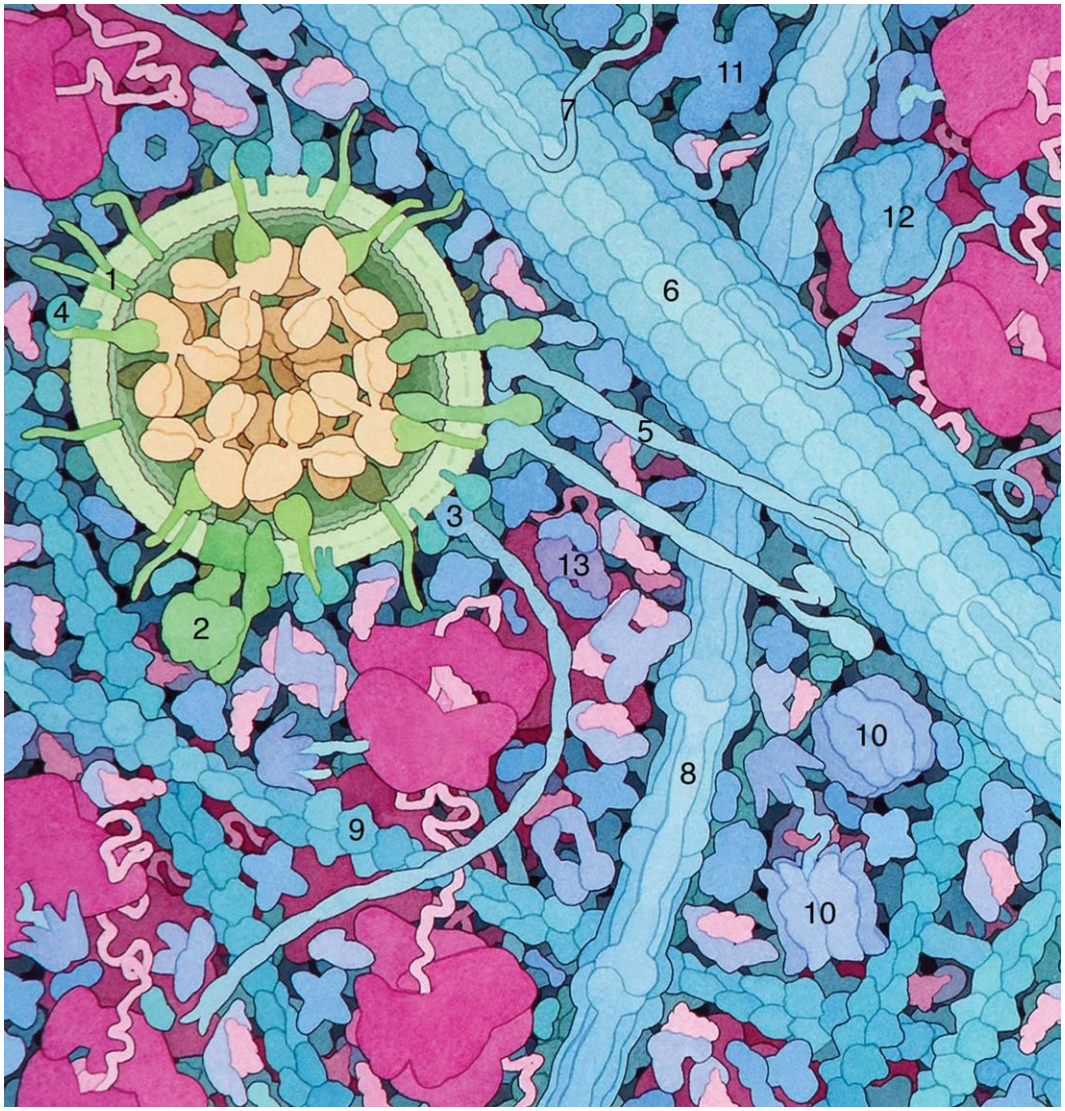

FIG. 7. **Transport of a vesicle through the cytoplasm.** (1) SNARE; (2) vacuolar ATPase; (3) golgin; (4) Rab; (5) kinesin; (6) microtubule; (7) MAP; (8) intermediate filament; (9) actin; (10) TRiC/CCT chaperonin; (11) fatty acid synthase; (12) calcium/calmodulin-dependent protein kinase II; (13) caspase 7 and XIAP.

the vesicle, but there was not room in the panorama to show this process.

Cytoplasm and Cell Surface

Figure 7 shows the journey of a vesicle to the cell surface. The vesicle includes SNARE proteins [62], a vacuolar ATPase (based on ATP synthase, 1c17 and 1e79), and several long golgin tether proteins connected to the vesicle through ARL (ADP-ribosylation factor-like protein) [54]. Small regulatory Rab proteins are bound to the vesicle surface with lipid anchors (3rab). Two kinesin proteins are transporting the vesicle along a microtubule (3kin) [63].

Three types of filaments from the cytoskeleton are shown. The microtubule is based on the crystal structure of a tubulin dimer fit to a cryo-EM map of the intact filament [64], shown here associated with unstructured MAP proteins, also based on electron microscopy [65]. The intermediate filament is based on electron micrographs and structural models of the coiled-coil regions [66], and the actin filaments are based on the crystal structure of the subunit and electron micrographs of the filament [67]. Several Arp2/3 (actin-related protein) junctions are shown in the actin network [68], which connect actin filaments with a characteristic 70 degree angle. The overall arrangement of filaments is based roughly on results from electron microscopy [69].

Many enzymes are shown in the cytoplasm. These include familiar enzymes of protein synthesis, glycolysis and other metabolic tasks, described in more detail in the previous article on the *Escherichia coli* illustration [70]. A few of the most showy examples include the TRiC/CCT chaperonin (TCP1-ring complex or chaperonin containing TCP1) in its open and closed forms (3iyg) [71], fatty acid synthetase (2cf2), and calcium/calmodulin-dependent protein kinase II [72] with calmodulin (3cln). Also included are caspase 7 bound to XIAP (X-linked inhibitor

FIG. 8. **Export of proteins across the cell membrane.** (1) Arp2/3; (2) c-Abl; (3) gelsolin; (4) spectrin; (5) ankyrin; (6) Fas receptor; (7) magnesium transporter; (8) immunoglobulin M; (9) interleukin receptor; (10) Src tyrosine kinase; (11) exocyst; (12) Rab; (13) Rho; (14) SNARE complex; (15) NSF.

of apoptosis, 1i4o), oncogene c-Abl tyrosine kinase (1opk), and gelsolin [73].

The cell membrane, shown in Fig. 8, is braced on the inside by a spectrin network, attached to the membrane through ankyrin. Cell surface proteins include Fas receptor (P25445), a magnesium transporter (based on bacterial MteE, 2yvy) and a membrane-bound form of immunoglobulin M (1igt). The large signaling complex is an interleukin receptor (3bpl) and Src tyrosine kinase (2src). Interleukins and similar signals are important for the survival of plasma cells [74].

A vesicle is shown midway through the process of fusion. The exocyst complex, bound to the membranes through Rab and Rho, acts as a tether to bring the vesicle to the membrane. The structure is based on electron microscopy [75]. The SNARE complexes shown on either side of the vesicle power the fusion of membranes [62]. The NSF (N-ethylmaleimide-sensitive factor) protein is shown separating a SNARE complex after membrane fusion (1d2n).

Aesthetics and Pedagogy

The design of this illustration was highly constrained, both by the need to be consistent with other illustrations in the book [1] and previous articles in BAMBED [2, 70, 76], and by the size of the book. The colors, magnification, and style are all similar to the other illustrations, so readers can compare the structure of the eukaryotic cell with the structure of a simpler bacterial cell. The consistent scheme, for instance, highlights the similar function of the bacterial nucleoid and the eukaryotic nucleus.

Each illustration is presented at 1,000,000× magnification in the book, and slightly reduced here. A simplified shape is shown for each macromolecule, since atoms are too small to be visible at this magnification. Molecules in the nucleus are colored yellow and orange and proteins in the cytoplasm are colored blue. Molecules with RNA, and many of the enzymes that process and transport RNA, are colored magenta. Membranes are colored green to highlight the different compartments in the cell, and mole-

cules inside the ER and Golgi are also colored green. The antibodies being produced are colored tan to set them apart from the cellular macromolecules.

The layout of the illustration is designed to tell the story of protein synthesis and export in a series of page-sized panels, while still connecting into a continuous panoramic cross-section. Each pair of panels presents a particular compartment on the left-hand page, and a mechanism of transport from the compartment on the right-hand page. In this way, readers are drawn through the panorama as they follow the transcription, translation, processing and export of antibodies.

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Miniseries: Illustrating the Machinery of Life

Mitochondrion*

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Diverse biological data may be used to create illustrations of molecules in their cellular context. I describe the scientific results that support a recent textbook illustration of a mitochondrion. The image magnifies a portion of the mitochondrion by one million times, showing the location and form of membranes and individual macromolecules, revealing the molecular basis of its role in energy metabolism and apoptosis. Results from biochemistry, electron microscopy, and X-ray crystallography were used to create the image.

Keywords: mitochondria, cellular biology, molecular biology, molecular visualization, textbook diagrams, energy metabolism, apoptosis, programmed cell death, endosymbiont hypothesis.

Mitochondria are organelles with many intriguing aspects [1]. They play a familiar role in energy metabolism, housing the machinery of ATP synthesis and harnessing chemical, electrical, chemiosmotic, and mechanical energy transformations in the process. They also play a major role in apoptosis (programmed cell death), amplifying the signal that ultimately leads to the death of the cell. In addition, mitochondria live their lives as more-or-less autonomous symbiotic units that live and reproduce inside our cells, which has been taken as evidence that mitochondria evolved from endosymbiotic cells that took up residence in eukaryotic cells early in the evolution of life [2, 3]. For the new edition of "The Machinery of Life" [4], I wanted to create an illustration that captured these many aspects of mitochondrial structure and function. Figures 1 and 2 show the illustrations used in the book, and Fig. 3 is a key to Fig. 2. I present here the scientific support for the molecular and ultrastructural details of the illustration, as well as some of the aesthetic and pedagogic choices that I made when designing the illustration.

MORPHOLOGY

The traditional model of mitochondria, inferred primarily from electron micrographs of thin sections, has a smooth outer membrane and a folded inner membrane, folded either into plate-like invaginations or tubules. The numerous folds of the inner membrane create a large surface area that is filled with the membrane-bound molecules of electron transport and ATP synthesis. However, more recent models based on electron tomography of mitochondria have shown that the traditional model may be too simplistic [5]. These studies suggest that the inner membrane folds to form two compartments that do not share soluble proteins. A portion of this membrane lies immediately inside the outer membrane, defining the intermembrane space between them. Another portion of the inner membrane surrounds the cristae, forming the intercristal space. The two compartments are connected by small (or no) openings that connect them, and the two compartments have a different complement of proteins.

I have drawn a speculative model that has the two spaces connected by narrow connections that are constricted by the protein OPA1 [6]. This protein is similar to dynamin, which forms helical assemblies that are important for pinching off membranes during the process of budding. I based the structure on a combined crystallographic/EM structure of dynamin [7]. There is also evidence for regions where the outer and inner membranes are closely opposed [8, 9], allowing transfer of small molecules and proteins between the cytoplasm and the matrix space. There are also connections between the infrastructure of the mitochondrion and the cytoskeleton. I have shown one such connection that links actin filaments in the cytoplasm with proteins in the mitochondrial membrane and inside to link to mitochondrial DNA, based on schematic diagrams from a review article [10]. To generate a model of this complex, the sizes and locations of intermembrane regions in the complex of MDM proteins (mdm10, mdm12, mmm1, mdm31, and mdm32) were taken from protein sequences at the ExPASy proteomic server (http://ca.expasy.org), and the Arp2/3 protein at the actin junction is taken from electron micrograph reconstruction [11].

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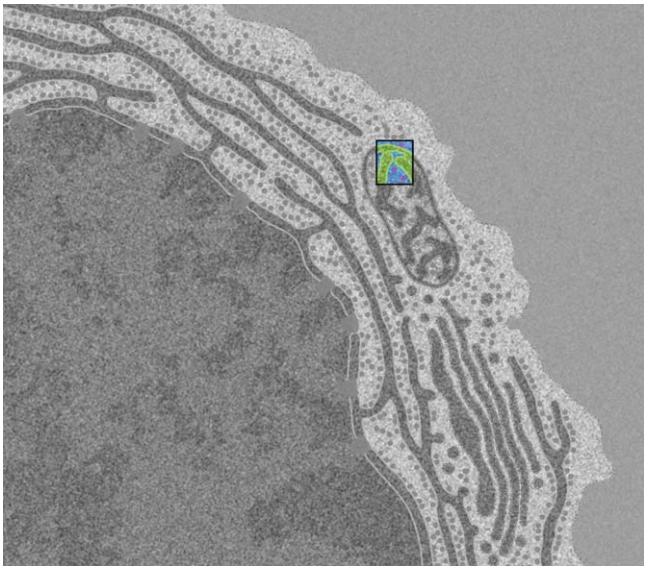

FIG. 1. **Simulated cross section through a eukaryotic cell, showing the location of the enlarged portion in Figure 2.**

The innermost compartment of the mitochondrion is termed the matrix. The matrix is one of the most densely packed portions of the cell; some estimates place it at greater than 50% protein [12]. It includes the enzymes of the tricarboxylic acid cycle and enzymes for fatty acid utilization, as well as an entire set of protein synthesis machinery, including DNA, polymerases, ribosomes, and transfer RNA, all described in more detail later.

ATP SYNTHESIS

The major task of the mitochondrion is the production of ATP. The enzymes of the tricarboxylic acid cycle are found in the matrix, along with a collection of enzymes for utilization of fat and other energy-rich molecules. These include two large multiprotein complexes, pyruvate dehydrogenase complex and alpha-ketoglutarate dehydrogenase complex, both drawn based on results from crystallography and electron microscopy [13, 14]. I have included little tails to represent the lipoic acid cofactors that are involved in substrate transfer between the subunits. Other tricarboxylic acid cycle enzymes are drawn based on crystallographic structures: citrate synthase (1cts), aconitase (1aco), isocitrate dehydrogenase (1lwd), succinyl-CoA synthetase (2fp4), succinate dehydrogenase (1zoy), fumarase (1yfm), and malate dehydrogenase (1mld). (4-letter codes included in this paper are accession codes for atomic structures at the Protein Data Bank, http://www.pdb.org.)

The electron transport chain is found in the membranes of the cristae. Decades ago, it was discovered that the large protein complexes of the electron transport chain are not arranged in a structured chain—rather, electrons are transferred by random diffusion of small carrier molecules between randomly-placed protein complexes in the membrane [15, 16]. More recently, however, there has been growing evidence that the proteins form a supercomplex in some organisms [17]. For this illustration, I have chosen to depict them as separate complexes in the membrane. NADH dehydrogenase complex is based on an electron micrograph reconstruction [18], and the other large complexes are based on atomic structures: cytochrome reductase (1bgy) and cytochrome oxidase (1oco). I have also included coenzyme q in the membrane and cytochrome c in the intercristal space (3cyt). Cytochrome c peroxidase (2pcc), also in the intercristal space, may play a role in detoxifying peroxide that leaks from the electron transport chain. ATP synthase (one of the wonders of the biomolecular world) is modeled after several crystal structures (1c17, 1e79, 1l2p, 2a7u).

I have also included a number of other enzymes involved in energy metabolism. I identified these using results from 2D gel electrophoresis [19] and by searching for "mitochondrial matrix human" in ExPASy. In the outer membrane, I included the membrane-linked enzyme monoamine oxidase (1gos). In the intermembrane and intercristal spaces, these include: creatine kinase (1qk1), adenylate kinase (1ak3), nucleotide diphosphate kinase (1ndl), and sulfate oxidase (based on molecular weight from ExPASy). In the matrix, I included manganese superoxide dismutase (1ja8) and copper-zinc superoxide dismutase (2sod), pyruvate carboxylase (2qf7), acyl-CoA dehydrogenase (3mdd), ornithine transcarbamoylase (1fb5), ornithine aminotransferase (2can), and glutamate dehydrogenase (1aup). I also included two forms of glycerol-3-phosphate dehydrogenase (1x0x), GPD1 in cytoplasm and GPD2 in the mitochondrion, which together form a shuttle.

APOPTOSIS

The mitochondria also play an important role in apoptosis (programmed cell death). Cytochrome c is a "moonlighting" protein that plays a secondary role entirely unrelated to its primary function as an electron transport protein. If it leaks into the cytoplasm, it initiates the cascade of apoptosis. A complex set of cellular machinery receives apoptotic signals and then ruptures the outer mitochondrial membrane, thus releasing cytochrome c and other apoptotic proteins.

In this illustration, I included many of the mitochondrial proteins of apoptosis, including several in the outer membrane and in the intermembrane space. In the outer membrane, BID (2bid) and Bcl-2 (1g5m) are ready to initiate an apoptotic cascade [20]. Fzo1 protein (based on a schematic diagram of its domain structure [21]) may play a role in fusion of mitochondria. In the intermembrane space, I included Smac (1few), which plays a role in activating caspases, and the apoptotic ribonuclease EndoG (3ism) and serine protease HtrA2/Omi (1lcy). I also included several cytoplasmic apoptotic proteins, including caspase-7 in an inactive complex with XIAP (1nw9) and Apaf-1 (1z6t), the protein that associates with cytochrome c when it is released from the mitochondrion, triggering the apoptotic cascade.

I created a separate illustration to show a rupturing mitochondrion in the process of losing its cytochrome c, and the consequences for the cell. A detail is included in

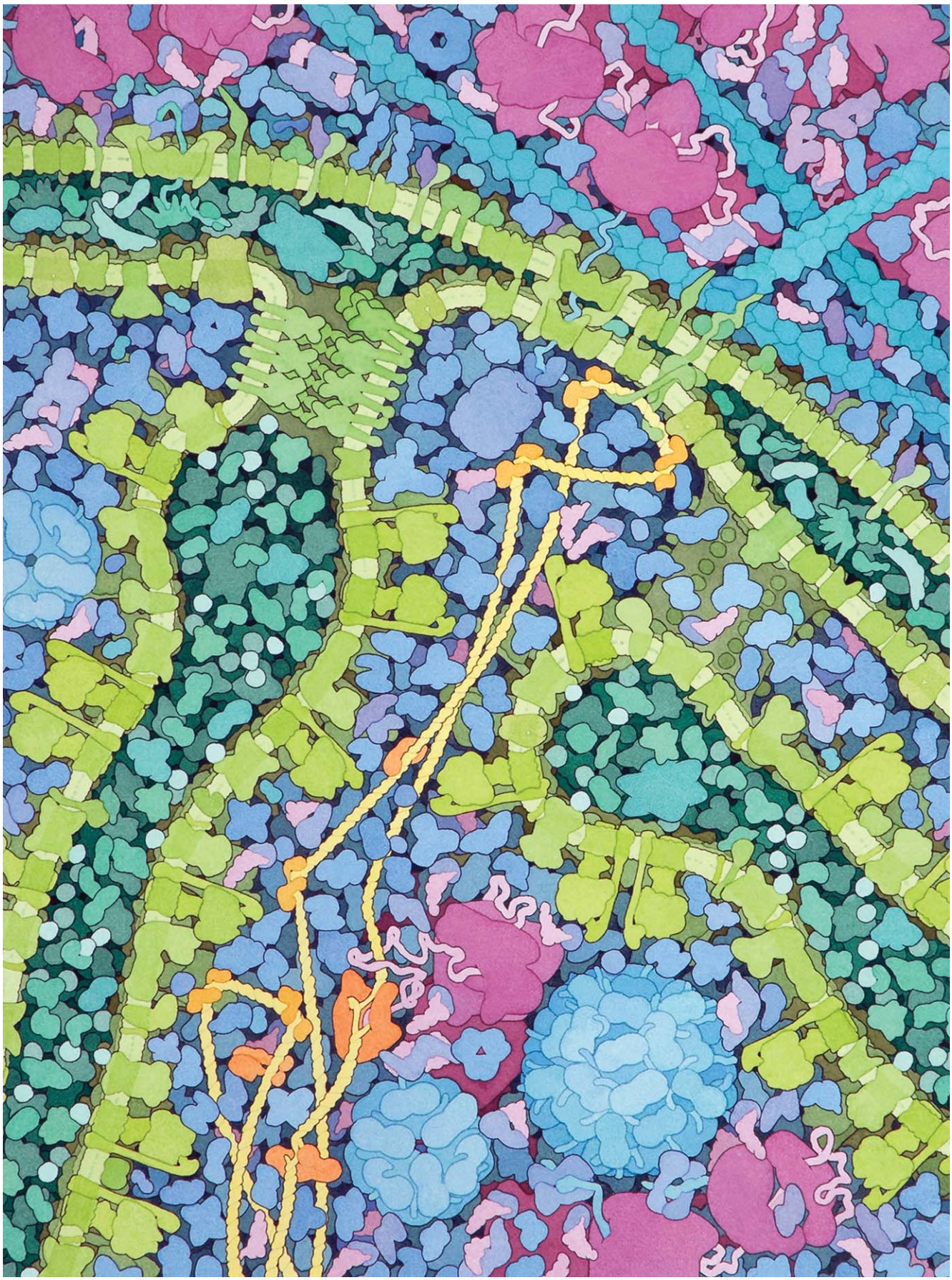

FIG. 2. **Cross section through a mitochondrion at 1,000,000× magnification.** All macromolecules and membranes are shown, but small molecules, ions and water are omitted for clarity. The cellular cytoplasm is at the top, and the mitochondrion fills most of the lower portion of the image. Soluble proteins are shown in shades of blue, and membranes and membrane-bound proteins are shown in green. RNA is shown in pink and ribosomes in magenta. DNA and DNA-associated proteins are shown in yellow and orange.

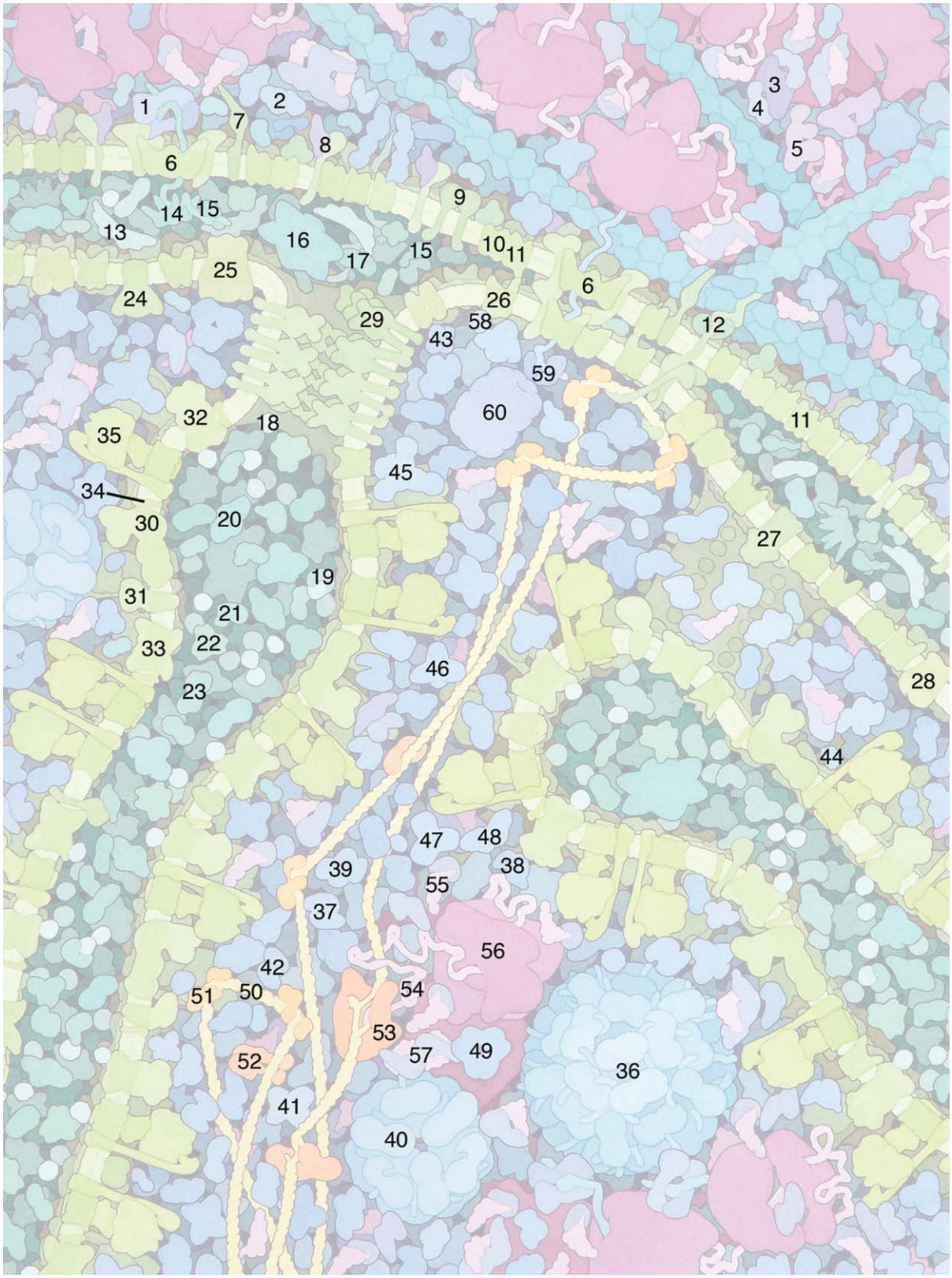

FIG. 3. **Key to Figure 2.** *Cytoplasm:* 1. Hsp90; 2. glycerol-3-phosphate dehydrogenase 1; 3. caspase-7; 4. XIAP; 5. Apaf-1. *Outer Membrane:* 6. protein transporter; 7. Fzo1; 8. BID/Bcl-2 complex; 9. monoamine oxidase; 10. PBR; 11. VDAC; 12. MDM complex bound to Arp2/3 and actin. *Intermembrane Space:* 13. Smac; 14. TIM9/10; 15. HtrA2/Omi; 16. creatine kinase; 17. EndoG. *Intercristal Space:* 18. cytochrome c; 19. cytochrome c peroxidase; 20. glycerol-3-phosphate dehydrogenase 2, 21. adenylate kinase; 22. nucleotide diphosphate kinase; 23. sulfate oxidase. *Inner Membrane:* 24. magnesium transporter; 25. RyR1; 26. ADP/ATP carrier; 27. potassium channel; 28. ABC-type transporter; 29. Opa1; 30. NADH dehydrogenase; 31. succinate dehydrogenase; 32. cytochrome bc1 reductase; 33. cytochrome oxidase; 34. coenzyme q; 35. ATP synthase. *Matrix: TCA enzymes:* 36. pyruvate dehydrogenase complex; 37. citrate synthase; 38; aconitase; 39. isocitrate dehydrogenase; 40. alpha-ketoglutarate dehydrogenase complex; (31. succinate dehydrogenase); 41. fumarase; 42. malate dehydrogenase; *Other enzymes:* 43. manganese superoxide dismutase; 44. copper-zinc superoxide dismutase; 45. pyruvate carboxytransferase; 46. acyl CoA dehydrogenase; 47. ornithine transcarbamoylase; 48. ornithine aminotransferase; 49. glutamate dehydrogenase; *Protein synthesis:* 50. DNA; 51. TFAM; 52. steroid receptor; 53. RNA polymerase; 54. messenger RNA; 55. transfer RNA; 56. ribosome; 57. aminoacyl-tRNA synthetase; 58. cyclophilin D; 59. MPP, 60. Hsp60.

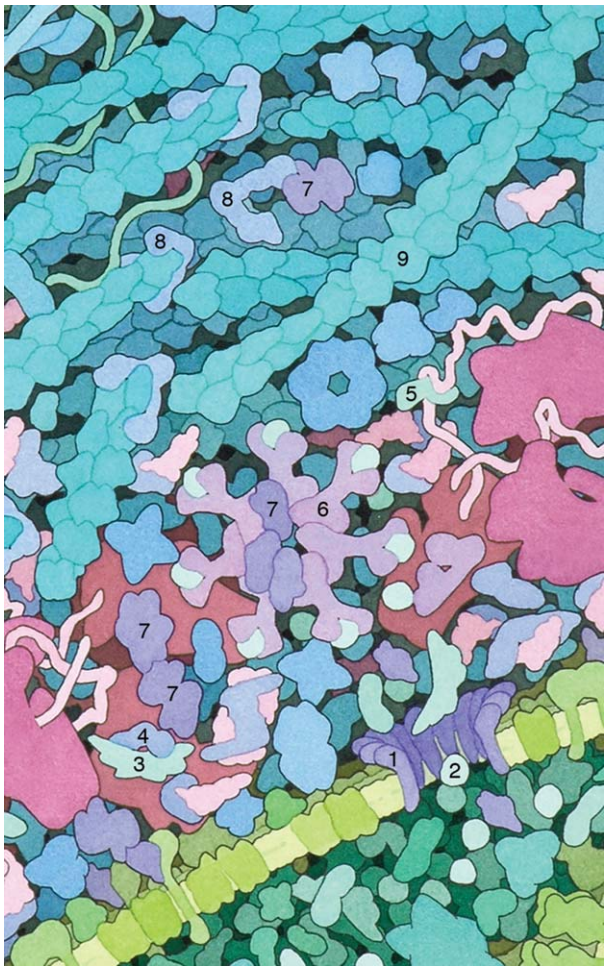

FIG. 4. **Apoptosis (detail)**. The outer membrane of the mitochrondrion has been ruptured, releasing mitochondrial proteins that trigger and assist with apoptosis. 1. BAX; 2 cytochrome c; 3. Smac; 4. XIAP; 5. EndoG; 6. Apaf-1; 7. caspase; 8. gelsolin; 9. actin.

Fig. 4. The BAX protein has formed a speculative pore through the membrane [22], and presumably the intercristal space is also breached, allowing cytochrome c, EndoG [23], HtrA2/Omi [24], and Smac to exit. Cytochrome c associates with Apaf-1 to form a beautiful seven-membered apoptosome [25], which then activates caspases and starts the destruction of proteins throughout the cell. Smac removes XIAP from caspases, further assisting the activation. EndoG attacks messenger RNA molecules, halting translation of new proteins, and caspases activate enzymes such as gelsolin [26], which disassembles actin filaments.

MITOCHONDRIAL DNA

The mitochondrial matrix includes an entire mechanism for protein synthesis, different and separate from the protein synthesis machinery in the cytoplasm. Moreover, the mitochondrial ribosomes, polymerases and other protein synthesis molecules are very similar to those found in bacteria. The presence of this bacteria-like protein synthesis machinery is one of the main clues that mitochondria evolved from endosymbiotic bacteria. This machinery is an evolutionary artifact, however, and is currently used to make only 13 proteins involved in the electron transfer chain and ATP synthase, as well as 22 mitochondrial transfer RNA and two ribosomal RNA [27]. The 700 or so other mitochondrial proteins [28], including the bacteria-like polymerases and translation factors, are made in the normal way by cytoplasmic ribosomes, and then imported into the proper compartment in the mitochondrion [29].

The mitochondrial ribosomes, transfer RNA, polymerases and other protein synthesis machinery are more similar to their bacterial counterparts than to the ribosomes and other machinery found in the cytoplasm of the cell. I have included many of these molecules in the illustration, including ribosomes modeled after the ones in *Thermus thermophilus* (1yl3, 1yl4), bacterial elongation factors EFTu (1ttt) and EFG (1dar), transfer RNA (1ttt), aminoacyl-tRNA synthetases (1asz, 1ffy, 1gax, 1euq, 1eiy, 1qf6), RNA polymerase (2e2i), and DNA. The chaperone Hsp60 was modeled after bacterial GroEL (1aon). The mitochondrial transcription factor TFAM is based on HMG-domain structures (1qrv, 2gzk), and the steroid receptor is based on the nuclear vitamin D receptor (1db1,1kb6).

TRANSPORT

Since mitochondria are surrounded by two membranes, there are potential challenges with transport. Like the outer membrane in *Escherichia coli*, the outer membrane of the mitochondrion is leaky. It is filled with voltage-dependent anion channel (VDAC), a protein similar to bacterial porins that forms a pore through the membrane. This pore is roughly 20–30 Å in size, large enough for small molecules like ATP and glucose to pass, but small enough to exclude larger molecules like proteins. VDAC is the most abundant protein in the outer membrane, and was found in large, densely packed clusters in a high-resolution atomic force microscopy study [30], where each pore was spaced by about 53 Å apart. I have modeled the structure after PDB entry 2k4t. I also included the PBR (peripheral-type benzodiazepine receptor), a protein involved in transport of cholesterol and other molecules [31]. I have shown it interacting with VDAC and the ADP/ATP carrier at the contact site shown at the center of membrane in Fig. 2 [9].

The inner membrane, however, must be sealed to allow generation of proton gradients to power ATP synthase. A large collection of transporters facilitate movement of molecules across this membrane. These include at least 49 different specific transport proteins of the mitochondrial carrier family [32]. The most abundant is the ADP/ATP carrier (1okc) that transports nucleotides in and out of the mitochondrion [33]. Other members of the mitochondrial carrier family, which have similar pore-like structures, transport pyruvate and other important metabolites, cofactors, and inorganic molecules. I have also included several other classes of transporters and channels [34], including the magnesium transporter (2bbj), RyR1 (ryanodine receptor, a possible calcium transporter), a potassium channel (1f6g) and several ABC-type transporters modeled after the vitamin B12 transporter system (2qi9).

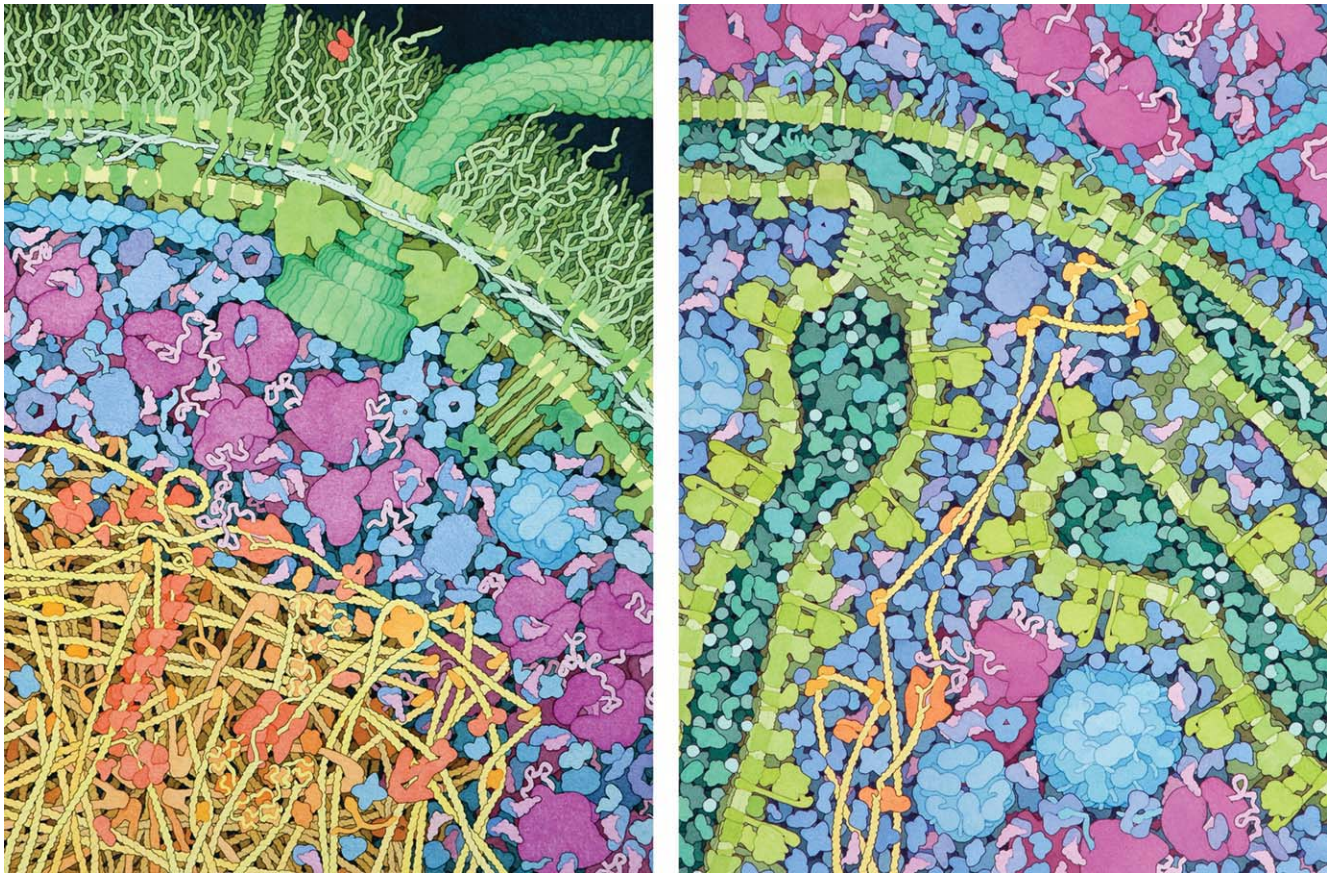

FIG. 5. **Illustrations of cross sections of *Escherichia coli* (left) and the mitochondrion (right) are designed to show structural and functional similarities.**

There are also several specific transporters for importing proteins into the intermembrane space or into the matrix [29]. I included two separate protein transporters. The one on the left in Fig. 2 is shown transporting an unfolded protein from Hsp90 (2cg9) in the cytoplasm and delivering it to the intermembrane space, where it is picked up by the chaperonin TIM9/10 (2bsk) and possibly HtrA2/Omi (1lcy). The one on the right is shown transporting the protein through both membranes into the matrix, where it is processed by the mitochondrial-processing peptidase MPP (1hr6).

AESTHETIC AND PEDAGOGIC CONSIDERATIONS

I designed the layout of this painting to match the layout of the *Escherichia coli* picture that was presented in an earlier chapter in the book (Fig. 5), to highlight the evolutionary relationship between mitochondria and bacteria [2, 35], and to show their many structural and functional similarities [3]. As with the other illustrations, a cross-section at 1,000,000× magnification is depicted, with the section chosen to place the membranes roughly perpendicular to the plane of the cut. The level of magnification is a compromise between two pedagogic goals: to show the entire subject in one comprehensive illustration, but still be able to see the shape and form of each macromolecule. This magnification allows display of a section of the mitochondrion that is large enough to see the major ultrastructural features as well as the molecular details of its major functions.

The colors are chosen to match the colors used for illustrations throughout the book. This scheme is designed to highlight the functional compartments of the cell/organelle, as described in my earlier article [36]. In this scheme, soluble proteins are shown in shades of blue, and membranes and membrane-bound proteins are shown in green. RNA is shown in pink and ribosomes in magenta. DNA and DNA-associated proteins are shown in yellow and orange. This consistent scheme integrates the illustrations throughout the book, allowing readers to compare the features presented in the different scenes, but it also poses a few problems. For instance, the mitochondrial matrix is rendered with similar colors as the cytoplasm. This has the advantage of showing the similarities between the two compartments (e.g., that both compartments are performing protein synthesis with similar molecular machinery), but it introduces the disadvantage of making it difficult to distinguish the mitochondrion from the cytoplasm. The consistent coloring scheme also required one unfortunate choice: after much vacillation, I chose to color cytochrome c in turquoise to match the other molecules in the intermembrane space, rather than coloring it its actual red color.

Acknowledgments— This manuscript 20555 is from the Scripps Research Institute.

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Basic Lesson Plans

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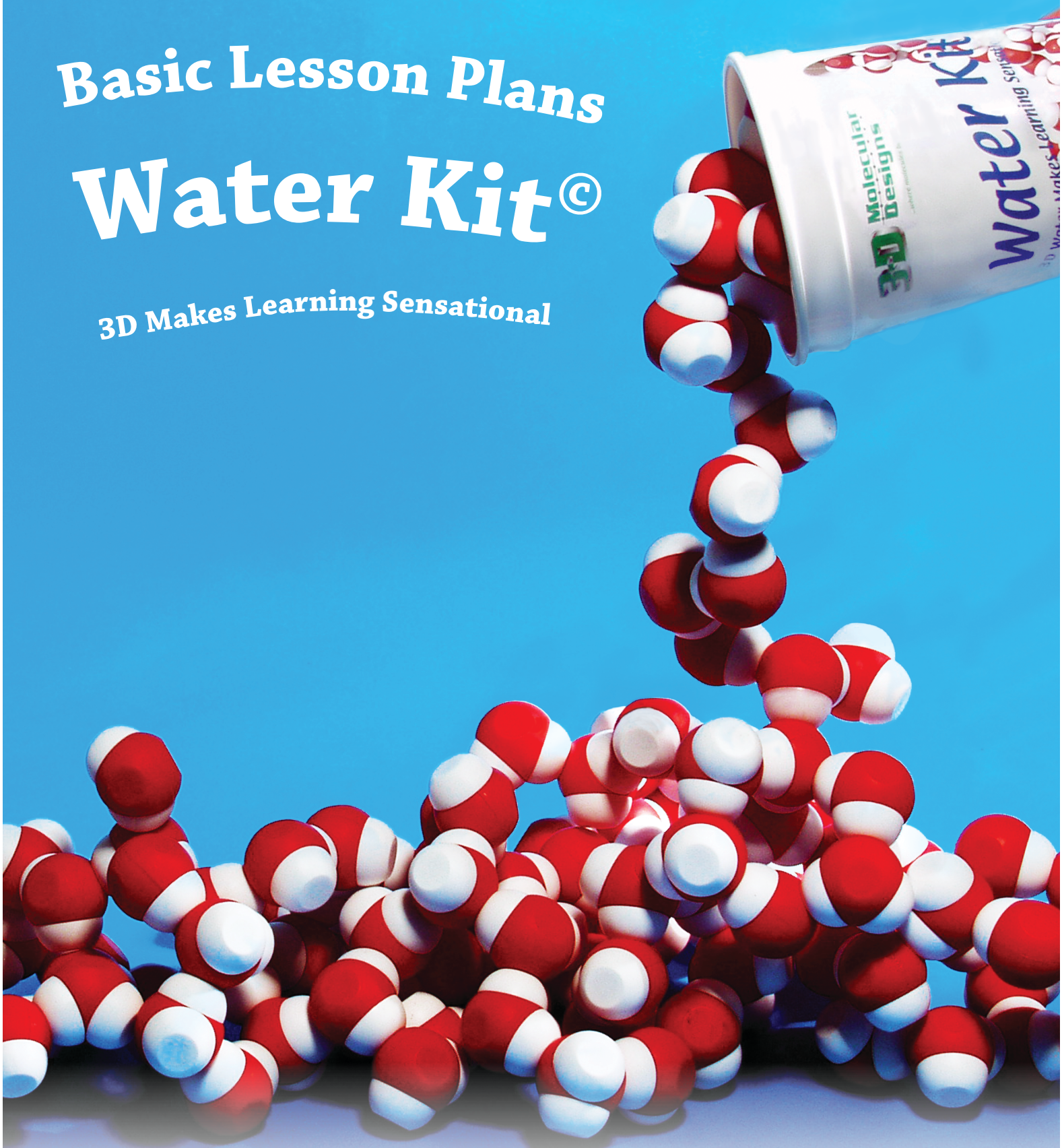

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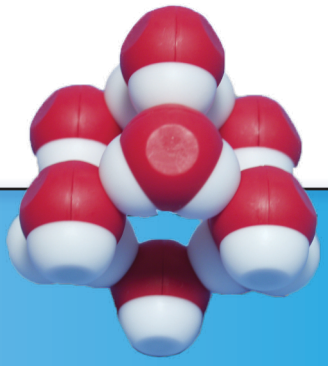

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Project WET (Water Education for Teachers)

3D Molecular Design's Water Kit© can be used to teach many of the concepts in Project WET's K-12 Curriculum Guide. Project WET is a nonprofit water education program that facilitates and promotes awareness, appreciation, knowledge and stewardship of water resources through the dissemination of classroom-ready teaching aids. Teachers can use the Water Kit© as part of many Project WET lessons or as an extension to the lessons. Please see the document Project WET and the Water Kit©. The chart shows which Project WET lessons the Water Kit© can be used with to enhance understanding.

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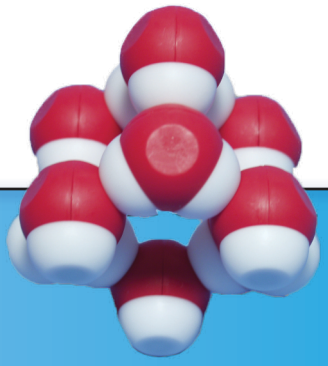
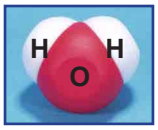
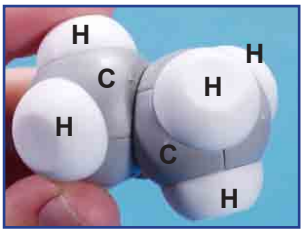


Contents of Water Kit©

Water Kit© Contents Per Cup

24 Oxygen* Pieces (Used to Assemble 12 Water Molecule Models)
24 Hydrogen* Pieces (Used to Assemble 12 Water Molecule Models)
1 Chloride* (Chlorine) Model
1 Sodium* Model
2 Carbon Pieces (Used to Assemble 1 Ethane Model)
6 Hydrogen** Pieces without Magnets (Used to Assemble 1 Ethane Model)
1 Post (Used to Assemble 1 Ethane Model)
1 Hydroxyl* Group Model (Preassembled)





Ethane molecule (CH_3CH_3)

Ethanol molecule (CH_3CH_2OH)

**North and south poles on embedded magnets simulate the partially positive and negative charges of oxygen and hydrogen atoms, and chloride and sodium ions.*

***The hydrogen pieces for the ethane model do not include embedded magnets, since ethane is nonpolar.*

Misconception Caution – Although the use of magnets to represent the partial positive and negative charges that exist on the hydrogen and oxygen atoms of water is a powerful teaching tool, it is important to explain to students that atoms are not magnets.

⚠WARNING:
SMALL MAGNETS — This product contains small magnets. Swallowed magnets can stick together across intestines causing serious infections and death. Seek immediate medical attention magnets are swallowed or inhaled.

⚠WARNING:
CHOKING HAZARD — This product contains small parts and should be kept out of the reach of children under the age of 3, because the parts or their pieces may present a choking hazard to small children.

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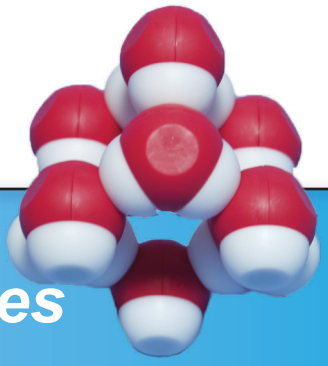
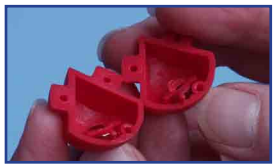


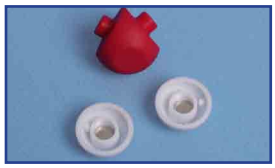
Assembling 3D Water Molecules

Start by selecting two red oxygen pieces and two white hydrogen pieces.



Take one red piece in each hand. You will see one post and one hole on the inside rim of each red piece.

Position the post of one piece into the hole of the other piece. Place your thumbs on the flat surface of each oxygen piece and push the two pieces together until they fit tightly and you no longer see a space between the pieces.



The red oxygen atom will now roughly resemble a sphere with two knobs sticking out of each half.

Take the red oxygen atom in one hand and one white hydrogen piece in the other and place the open side of the hydrogen piece onto the knob on the oxygen. Push the two together until the hydrogen fits tightly onto the oxygen, with no space between the two pieces.

Repeat with a second white hydrogen piece.

You now have one complete water molecule. Repeat steps 1 through 6, until 12 water molecules are assembled.

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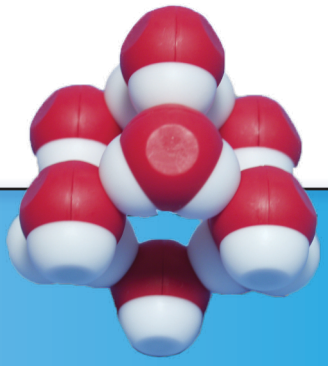


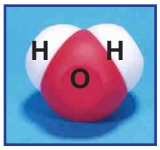
Basic Lesson Plans

Polarity

General Information

Understanding the unique characteristics of water begins with understanding polarity.

Polarity refers to the partial positive charge (+) and partial negative charge (-) that a molecule has when electrons are unequally shared between two or more atoms. Molecules that have partial charges are polar molecules. Water molecules are **polar molecules**.



In a water molecule, each hydrogen atom has a partial positive charge and the oxygen atom has two partial negative charges.

Some molecules do not have unequal regions of charges and therefore do not interact with polar molecules. These are **nonpolar molecules**. Oil and ethane are examples of a liquid and a gas composed of nonpolar molecules.

Water Kit©

The water molecules in the Water Kit© have been embedded with magnets to help students experience what positive (+) and negative (-) charges feel like as they interact with other polar molecules. Through manipulation of the 3D water molecule models, it becomes easy for students to understand the physical and chemical properties of water.

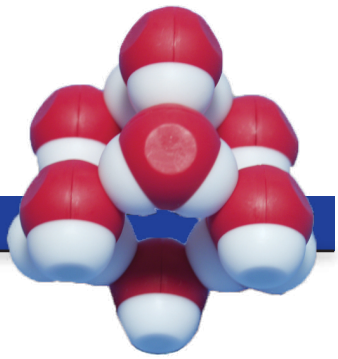
Embedded magnets are positioned at 105º from each other.

There are two magnets embedded in the oxygen at 105º degrees from each other. These represent the two partial negative (-) charges.

One magnet in each of the hydrogen atoms represents the partial positive (+) charges. The hydrogen molecules are also positioned 105º degrees apart.

The location of these four magnets (representing charges) makes it possible for your students to create a *tetrahedral* structure. A tetrahedral structure is formed when one water molecule interacts with four other water molecules.

Tetrahedral Structure



In the Classroom

Hand out one 3D water molecule model to each student.

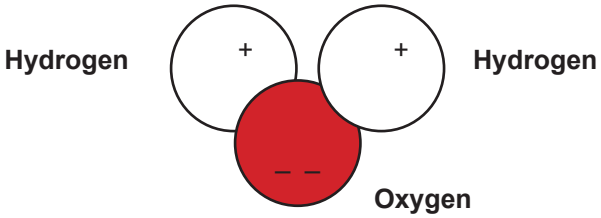
- What is the chemical formula of water?
  H_2O

Have your students discuss what the H and the O represent. Refer to the periodic table of elements.

- What does the 2 between the hydrogen and oxygen stand for?
  When a number # is placed after a symbol, it means there is more than one atom of that element and when there is no number after a symbol, it means there is only one atom of that element.

Have your students identify which parts of the model represent the hydrogen and which the oxygen. Clarify that the colors on the model are used by chemists to represent hydrogen (white) and oxygen (red), but water, as a real molecule, is not colored. Emphasize that it is impossible to see a single water molecule with the naked eye or even with the most powerful light microscope.

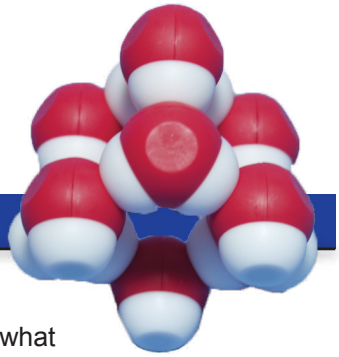
Have your students draw and label a water molecule. Remind your students that each hydrogen atom has a partial positive charge and the oxygen atom has two partial negative charges. Explain that they can show this on their drawing by placing a + symbol on each hydrogen and two – symbols on the oxygen.



Your students can now predict, what will happen when water molecules interact. Hand out one more 3D water molecule model to each student and let him or her investigate whether his or her prediction is correct.

- What will happen when two water molecules bump into each other?
  - When the oxygen atoms of 2 different water molecules come together, they repel.
  - When the hydrogen atoms of 2 different water molecules come together, they repel.
  - When an oxygen atom and a hydrogen atom from two different water molecules come together, they attract.

Have the students draw and label what they have discovered. Then, have your students write about what they have observed.



(In the Classroom Continue)

Difference Between Magnetism and Polarity

Display two large horseshoe-shaped, or other, magnets and ask your students what they know about magnets. List and diagram the student's answers on the board.

- What are the similarities and differences between magnets and water molecules?
  Atoms are not really magnetic. However, the north and south poles of magnets can be used to effectively model the partial positive and partial negative charges that exist on the polar water molecule.

Water Facts

- How many water molecules are in a single drop of water?
  There are 3 x 1021 of molecules in one drop of water (3,000,000,000,000,000,000,000). This number can be read, "three times ten to the twenty first power."

- At the scale of the water molecule models, how large would a water drop be?
  Clarify that it is impossible to see a single water molecule with the naked eye or even with the most powerful microscope. A water drop made of 3 x 1021 of 3D water molecule models would be ***larger*** than the earth.

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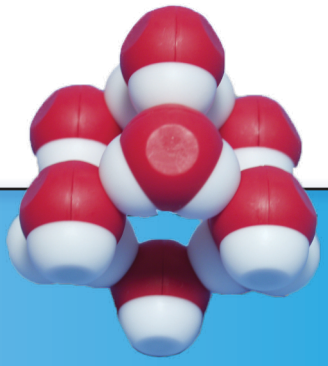


Hydrogen Bonding

General Information

A **covalent bond** is formed when two atoms share two electrons. A covalent bond is an ***intramolecular*** bond within one molecule. Covalent bonds can be either polar (which have partially charged atoms) or nonpolar (without charged atoms).

Hydrogen bonds are intermolecular forces between *two* molecules where a positively charged hydrogen atom interacts with a negatively charged fluorine, nitrogen or oxygen atom in a second molecule.

• ***Water fact:*** *A hydrogen bond is about 1/20 as strong as a covalent bond.*

An **ionic bond** is the complete transfer of an electron between two atoms resulting in one positively and one negatively charge atom. *Ionic bonds are intramolecular bonds within one molecule.*

Ions are charged atoms that have gained or lost electrons as a result of an ionic bond.

Water Kit©

The magnets in the 3D water molecule models simulate the intermolecular force of two polar water molecules forming a hydrogen bond. All of the bonds between multiple water molecules are hydrogen bonds. The bonds between water molecules and the OH group on the ethanol are hydrogen bonds.

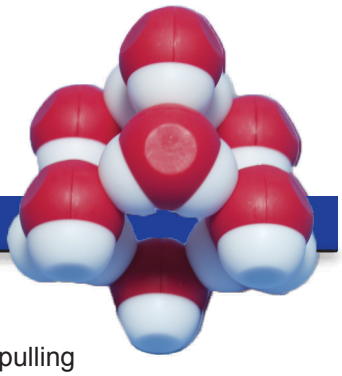
In each individual 3D water molecule models, the bonds between the hydrogen atoms and the oxygen atom are covalent bonds. Covalent bonds are also formed between all of the atoms that form ethane and ethanol. These are all intramolecular bonds. Water molecules and ethanol molecules are polar covalent molecules while the ethane is a nonpolar covalent molecule.

Ionic bonds are formed between the sodium and chloride ions. This is an ***intra***molecular bond.

The attraction between the negative charge of the oxygen and the positive charged of the sodium ion is an ***inter***molecular force.

Intramolecule force

Partially positively charged hydrogen atoms (see water and ethanol molecules) will interact with negatively charged chloride ions. ***This is not called a hydrogen bond because the chloride ion is not one of the three elements listed above (fluorine, nitrogen or oxygen)***. This is another type of intermolecular force that is covered in the Solubility Section.



Hydrogen Bond Activity and Questions

Give each student two 3D water molecule models.

Have your students *break* the hydrogen bond between the water molecules by pulling them apart. Next, have them compare this to the relative strength of a covalent bond by pulling a hydrogen atom off a water molecule. (Use a fingernail to pry the hydrogen off the oxygen if needed.)

- What is the intermolecular force that holds these two water molecules together?
  Hydrogen bond.

- What is the intramolecular bond that holds the hydrogen atoms and oxygen atom within a water molecule?
  Covalent bond.

- Is a water molecule a polar covalent molecule, or a nonpolar covalent molecule? Why?
  Water molecules are polar covalent molecules because electrons are shared within the molecule and the sharing of the electrons is unequal which results in partially positively charged hydrogen atoms and a partially negatively charged oxygen atom.

Ethane

Give each group of students an ethane molecule.


Ethane CH_3CH_3

- What is ethane?
  Ethane is a short hydrocarbon. Hydrocarbons are combinations of carbon and hydrogen atoms. Ethane is an odorless, colorless gas that can be used as a fuel, a freezing agent, and in making other chemicals.

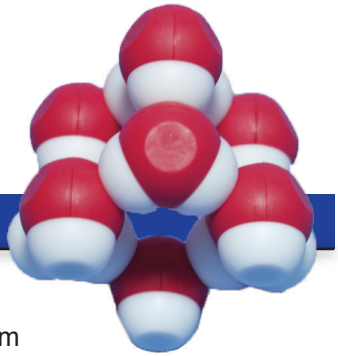
- What do the different colors on the ethane molecule represent? What is the chemical formula of ethane?
  White represents hydrogen atoms and the gray represents carbon. CH_3CH_3.

- What is the intramolecular bond that holds the hydrogen and carbon atoms within an ethane molecule?
  Covalent bonds.

- Is an ethane molecule a polar covalent molecule, or a nonpolar covalent molecule? Why?
  Ethane molecules are nonpolar covalent molecules because electrons are equally shared within the molecule. This equally charged molecule is nonpolar.

- Will ethane form a hydrogen bond with water? Why or why not?
  No, ethane will not form a hydrogen bond, or ionic bond with water or any other polar molecule because it is nonpolar. Ethane does not have any partial positive or negative charges.

- Will two ethane molecules form bonds with each other? Why or why not?
  No. Ethane molecules will not form covalent, ionic or hydrogen bonds with each other. Ethane molecules have small intermolecular forces and will interact under the certain temperature and pressure conditions to form liquids and solids.



Hydrogen Bond Activity and Questions (continued)

Give each student group an OH (hydroxyl) group* and have them replace the detachable hydrogen with the OH group. They have just changed the model from ethane to ethanol.

• What do the different colors represent? What is the chemical formula of ethanol?
White represents hydrogen atoms, the gray represents carbon atoms, and the red represents oxygen atoms. CH_3CH_2OH.

• Will ethanol and water interact? How?
The water molecules will form hydrogen bonds with the oxygen atom and the single polar hydrogen atom on the ethanol.

• Will ethanol molecules interact with each other? Why?
Yes. Ethanol will form hydrogen bonds with other ethanol molecules.

•What is sodium chloride?
Show a sodium chloride molecule to your class.

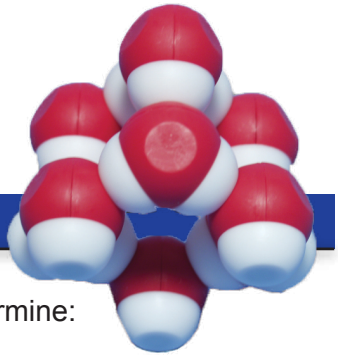
Sodium chloride is often called table salt or rock salt.

• What do the colors represent? What is the chemical formula of sodium chloride?
The blue atom represents sodium, the green chlorine. NaCl.

• The *intra*molecular bond that holds these ions together is ionic. What does this mean?
Ionic bonds are formed when an electron is completely transferred from one atom to another. This results in a positively charged ion (the atom that looses an electron) and a negatively charged ion (the atom that gains an electron).

• Will a water molecule interact with sodium chloride? Why?
Yes, water molecules and sodium chloride ions will interact. The partially charged water molecule will interact with the fully charged sodium chloride. These are intermolecular forces.

* The OH group, also called a hydroxyl group, is a functional group of the ethanol molecule. It is a way to refer to the part of the molecule that is polar – the oxygen atom and the hydrogen atom that is covalently bonded to the oxygen atom.



Hydrogen Bond Activity and Questions (continued)

Give each group of students the sodium chloride ions. Ask them to determine:
- which ion the hydrogen atoms interacts with.
- which ion the oxygen atom interacts with. Have your students record their findings.

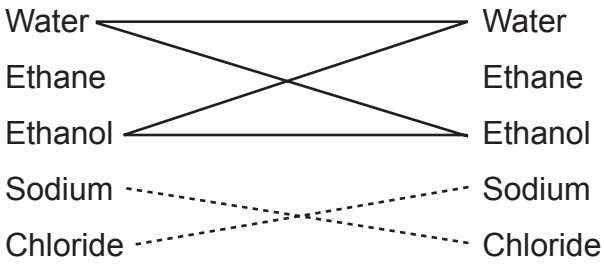
- Are chloride ions positively or negatively charged? Why?
  Chloride ions interact with hydrogen atoms in water molecules. Since hydrogen atoms are partially positively charged, the chloride ions are negatively charged.

- Do hydrogen atoms and chloride ions form hydrogen bonds? Why?
  No, the intermolecular force between the hydrogen atom and chloride ion is not a hydrogen bond because hydrogen bonds are only formed between hydrogen and fluorine, nitrogen and oxygen atoms.

- Are sodium ions positively or negatively charged? Why?
  Sodium ions interact with oxygen atoms in water molecules. Since oxygen atoms are partially negatively charged, the sodium ions are positively charged.

- Will sodium chloride interact with ethane? With ethanol? Why or why not?
  Sodium chloride will not interact with ethane because ethane is nonpolar. Sodium chloride will interact with the ethanol.

Have your students list water, ethane, ethanol, sodium and chloride in two columns. First, have them draw solid lines between the molecules that will form hydrogen bonds. Second, have them draw dotted lines between the ions that will form ionic bonds.



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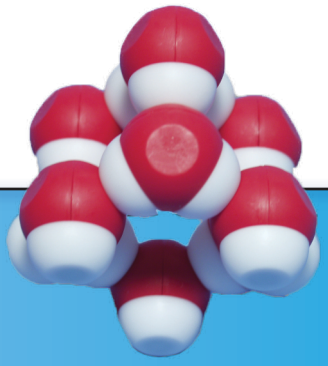


Adhesion, Cohesion & Capillary Action

General Information

The attraction between two like molecules is **cohesion**.

The attraction between two unlike molecules is **adhesion**.

Adhesion and cohesion are intermolecular forces between two molecules. These forces are only called hydrogen bonds when a hydrogen atom and a fluorine, nitrogen, or oxygen atom are attracted to each other. Capillary action is the spontaneous rising of a liquid in a capillary (small diameter) tube.

Capillary action in plants is a good example of adhesion and cohesion. The inner surface of the xylem, the cell wall of a plant, contains positive and negative charges. Water forms hydrogen bonds with the xylem. This is called adhesion. As water creeps up the sides of the xylem (adhesion) the water molecules in the middle connect to other water molecules because of cohesion. The water moves up as the water molecules at the top of the xylem enter the leaves and evaporate (move out of the stomata in the leaf). When a water molecule leaves the leaf, the molecule behind it moves up causing a general movement of the water up the tree.

Tip: *Adhesion* **adds** *a different molecule to the substance.*

Water Kit©

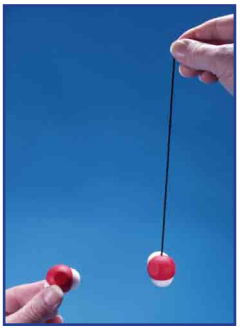
When 3D water molecule models are attracted to another substance, the force is adhesion.

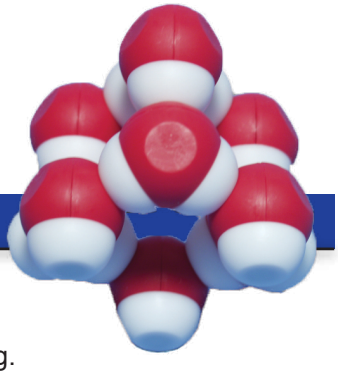
When 3D water molecule models are attracted to each other, the force is cohesion.

Activity

Demonstrate adhesion and cohesion by tying a string (dental floss or fishing line works well) around a 3D water molecule models. Hold the string in one hand and another 3D Water Molecule your other. Slowly bring the second 3D Water Molecule toward the one on the string. Students should see the hanging molecule move toward the molecule in your hand. Explain that this attraction is cohesion.

Next, bring an ethanol molecule toward the hanging 3D water molecule models.

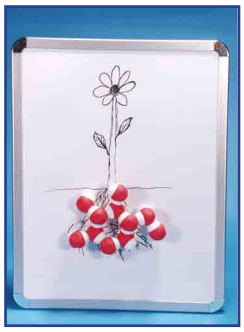




Adhesion, Cohesion & Capillary Action

- What is the attraction between these molecules?
  The force is called adhesion because two different molecules are attracting.
  Test your students' grasp of adhesion and cohesion by hanging an ethanol molecule. Bring a second ethanol molecule toward the first.

- What is the attraction between these molecules?
  The force is called cohesion because two like molecules are attracting.



Demonstrate capillary action by drawing a plant on a whiteboard. If your whiteboard is not magnetic, use a small magnet white message board. Place 11 of the 3D Water Molecules at the root of the outlined plant. Pull the molecules up the roots, through the stem and out the leaves of your plant with the molecule attached to the string. Note: Some of the water molecules will bond with the board and some to other water molecules.

Explain that this is capillary action. Water molecules form hydrogen bonds with cellulose in the xylem. The cellulose is composed of carbon atoms with hydroxyl groups (sugars). In addition, water molecules also form hydrogen bonds with each other.

- Does capillary action involve adhesion or cohesion?
  Capillary action involves both adhesion and cohesion. When water molecules are attracted to the xylem (as drawn on the board), adhesion is involved. When the water molecules are attracted to each other, cohesion is involved.

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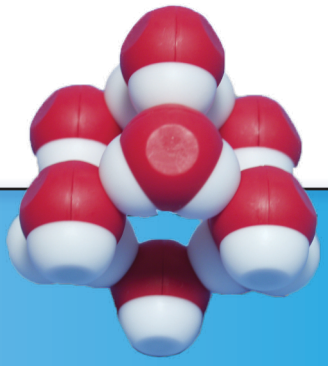


Surface Tension

General Information

Surface tension is due to the cohesion between molecules at the surface of a liquid. In a liquid, molecules are pulled in all directions by intermolecular forces. At the surface of a liquid, the molecules are only pulled downward and toward the sides. Surface tension is the amount of energy required to stretch or increase the surface of a liquid by a unit of measure.

Water Kit©

Water molecules are polar and have a relatively strong attraction to each other, called hydrogen bonds. This attraction (explained in the section labeled Hydrogen Bonding) is responsible for surface tension. Surface tension explains why water forms rounded drops on nonpolar surfaces like the waxy surface of an apple or a recently waxed car.

Activity

Have your students place drops of water on a variety of substances to test surface tension. Drops can be placed on paper, wax paper (or draw a solid rectangle with crayon on paper to simulate wax paper), paper towels, glass, plastic, fabric, plant leaves, etc. Have your students record the substance and draw the relative height of the drop.

Office Paper

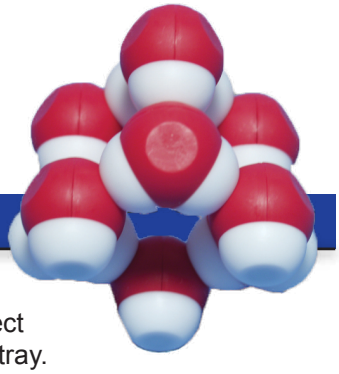
Wax Paper

Paper Towel

- Why does water bead up on some substances, like wax, and soak into others, like paper?
  Water does not interact with nonpolar substances. Wax is a nonpolar substance; the water molecules are only being pulled toward each other, forming a sphere. Office paper has a coating which is more polar than wax, but less polar than paper towels. This results in a dome-shaped drop. Paper towels are more polar than office paper. Paper towels actually break the surface tension of water to absorb it.

- Can anything walk on water?
  Yes, some insects, like the water strider, can walk on water.

- Can you explain how an insect does this?
  The hydrogen bonds are strong enough that certain insects can't break them and therefore can walk on the water molecules.



Surface Tension

Have your students pool 3D water molecule models together on a tray. Place a small toy insect on top and have your students gently shake the tray. The insect will stay on the molecules. Explain that this is surface tension.

- Why can't all animals walk on water?
  Their total mass is sufficient to break the intermolecular force of the hydrogen bonds between water molecules and they sink.

- What would happen if an insect tried to walk on a nonpolar liquid (molecules that do not have charges)? Record your hypothesis.

Pool several ethane molecules on a tray. Place a plastic insect on top and gently shake the tray. The insect will fall off.

- Why did the insect fall through the surface of the nonpolar molecules?
  There are no hydrogen bonds (cohesion) between the nonpolar molecules, therefore the insect falls between the molecules. While there are weak intermolecular forces between the ethane molecules, they are not strong enough to support the insect.

- Can you predict what would happen if an insect tried to walk on a slightly polar liquid?
  Have your students change the ethane to ethanol and repeat the activity. Results will vary with the weight of the insect and the number of hydrogen bonds formed by the ethanol. Now, have your students predict what will happen with actual liquids. Give each group a glass of water and one of vegetable oil representing 3D water molecule models and ethane, respectively. In addition, give each group a fork and several small metal paperclips.

- Predict what will happen when you lay a paperclip on top of each surface. Why? Record your hypothesis and reasons.
  Have students carefully place a small metal paper clip on the surface of a glass of water. Note: Students may have to practice this several times before they are successful. Using a fork to place the paperclip on the water can help. Paperclips should be dried thoroughly before trying to place them on a liquid again.

- What is keeping the paperclip on top of the water? Is it floating?
  No, the paperclip is not floating. The paperclip is resting on top of the water because the surface tension is stronger than the weight of the paperclip.

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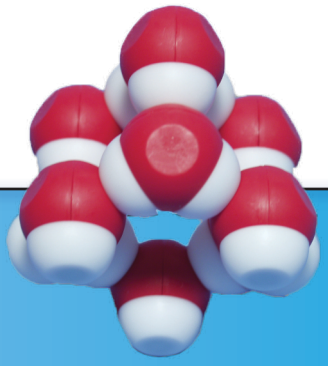


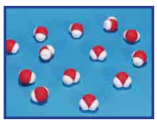
States of Water

General Information

Like other compounds, water can convert between solid, liquid, and gas phases. The remarkable property of water is that it is a liquid over a very large temperature range.

Water Kit©

3D water molecule models can be used to **demonstrate** the different phases of water as shown below.



Gas – 12 molecules separated. In the gas state individual water molecules are moving too fast and are too far apart to form hydrogen bonds.

Liquid – 12 molecules clumped together. In liquid water, the hydrogen bonds between water molecules are very short-lived. They are constantly forming, breaking, and reforming between other molecules.

Solid – 12 molecules connected in a repeating pattern or formation that form ice. In ice, the hydrogen bonds between water molecules are more stable and longer-lived.

Scientists have described 12 different structures of ice, many of which can be constructed with the Water Kit©. See pages 23 and 24 for directions on making hexagonal ice.

Activity

Demonstrate the three states of water as shown above.

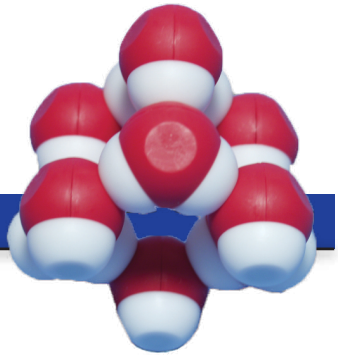
Give each group of students twelve 3D water molecule models. Challenge them to form an ice cube, an enclosed, stable ice lattice. After a few minutes, give students the hint that snowflakes, or ice crystals have six sides – they may want to try rings of six.

If your students are have trouble forming ice, print pages 23 and 24 for each group.

Display and discuss twelve 3D water molecule models in the gas, liquid, and solid states.

- What are the differences between these groups of molecules? How does their volume differ?
  Water vapor, the gaseous state, has the greatest volume. The water vapor condenses to form liquid water. Then the water molecules expand to form the solid form of water, ice.

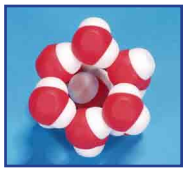
- Are these volume changes unique to water?
  All gases have greater volumes than liquids. Water, H_2O, is the only compound that expands as it freezes.



States of Water

Have your students discover how water expands as it freezes. They should form a lattice with 12 molecules and place it in a clear container, such as a bird feeder tube. Have your students measure and record the volume of water in this solid state. Next, they should gently push down on the molecules to break the hydrogen bonds until all the molecules are at the bottom of the container. Have you students measure and record the volume of the molecules in this liquid state.

Demonstrate that a 13th molecule can fit in the middle of an ice cube. Explain that when water freezes, the empty space, or void between the molecules increases.



Pool the ethane and ethanol molecules in separate containers and show them to your students.

- At room temperature, which substance is a gas and which substance is a liquid? Why?
  Ethane is a gas at room temperature because it is a nonpolar molecule which has a weak intermolecular force. It does not have a partial charge that would attract the molecules to each other. Ethanol is a liquid at room temperature because it is a polar molecule. The OH has a stronger intermolecular force, called hydrogen bonding, which attracts the molecules to each other.

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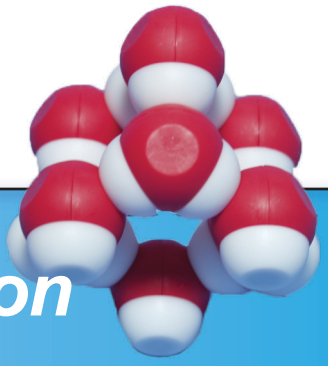


Evaporation & Condensation

General Information

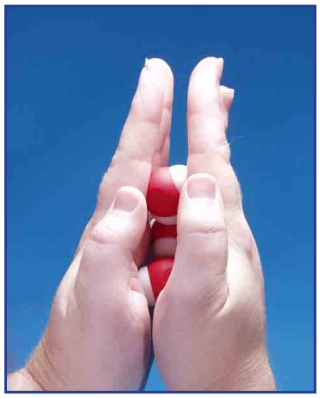
Evaporation is the transformation from a liquid to a gas. This occurs when a liquid absorbs enough heat, increasing the movement of the molecules, to cause the intermolecular forces between molecules to break the surface tension. This allows individual molecules to escape into the air. As water evaporates, the heat it absorbs breaks the hydrogen bonds and the liquid water becomes water vapor.

Condensation is the transformation from a gas to a liquid. This occurs when a gas is cooled enough that the molecules slow down to form stronger intermolecular forces with each other. As water condenses, the molecules slow down and form hydrogen bonds with each other as the water vapor cools.

Changes in pressure can also influence evaporation and condensation. As pressure increases, gases condense into liquids. As pressure decreases, liquids evaporate as gasses

Activity

- Have you ever tried to walk barefoot on hot sand, or black pavement in the summer? What was it like? What did you do?
  Tried to walk faster, keep my feet off the ground, find a cooler place to walk, put on shoes…



- When water molecules heat up they move faster as well. What happens when you heat, or boil, water?
  It evaporates.

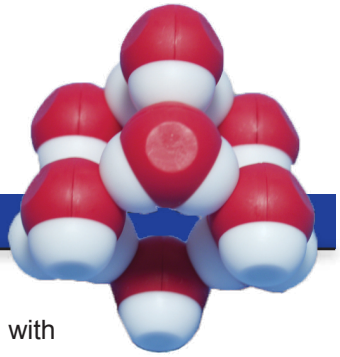
Have your students rub their hands together to create friction.

- What are you producing with your hands?
  Heat and/or energy.

Give each student three 3D water molecule models to hold between their hands. Have your students slowly move their hands together.

- What is happening to the water molecules (and the hydrogen bonds)?
  The molecules are moving around and slowly forming and breaking hydrogen bonds with each other.

This represents water in its liquid form. Have your students slowly increase the speed (heat) of their hands.



- What is happening to the water molecules now?
  The water molecules are moving fast and are not forming hydrogen bonds with each other.

This represents evaporation and water in its gas state. To simulate condensation, have your students slow down their hands, drawing heat away from the molecules.

To help your students understand evaporation, have them place one drop of water and one drop of rubbing alcohol 4 cm apart on their forearms. Start a timer. Your students should observe and record findings.

- What did you observe?
  The water forms a rounded drop due to its strong surface tension, while the rubbing alcohol flattens.

The rubbing alcohol evaporates within 2 minutes. Water takes much longer.

Explain to your students that body heat provides enough energy for the rubbing alcohol molecules to move fast enough to leave the surface of your skin. It takes about 290 cal per gram of rubbing alcohol for the molecules to evaporate, whereas, it takes 580 cal per gram of water to break hydrogen bonds between water molecules (calories are measurements of energy). Your body temperature at rest is not high enough to evaporate water rapidly.

Water fact: *580 cal of heat are needed to evaporate one gram of water at room temperature.*

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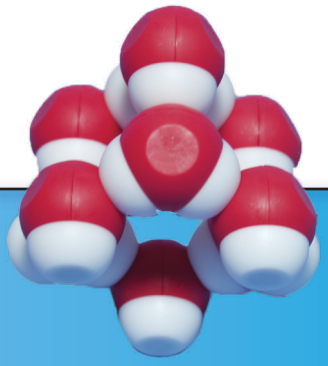


Solubility

General Information

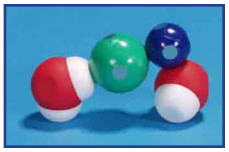
In the presence of water, salt dissolves. Water molecules always hydrate the sodium chloride.

Ions are charged atoms that have gained or lost electrons as a result of an ionic bond.

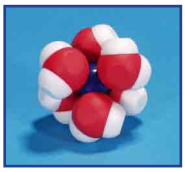
Water Kit©

The Water Kit© comes with a blue sodium (Na) ion model and a green chloride (Cl) ion model. These two ions form an ionic bond to make sodium chloride (NaCl), often called table salt.

When water dissociates sodium chloride, the hydrogen atom interacts with the negatively charged chloride ion and the oxygen atom interacts with the positively charged sodium ion.

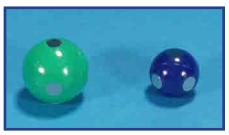


Note: The Water Kit© can demonstrate the principle behind hydration, but it cannot simulate the true chemical reaction. Many more water molecules would be needed.



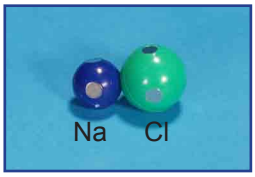
Activity

Hand out a sodium ion and a chloride ion to each group.
Have them explore what happens when they are brought together.

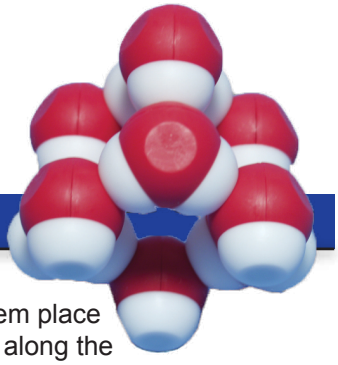


- Why did the sodium and chloride ions come together?
  Sodium has a positive (+) charge and chloride has a (-) negative charge. They form an ionic bond.

- What is the chemical formula for this? What is the compound?
  NaCl. NaCl is often called table salt.



- What happens when you put salt in a cup of water?
  It dissolves.



Solubility

Give each student group twelve 3D water molecule models and a tray. Have them place the sodium chloride in the center of the tray and the 3D water molecule models along the edges. Your students should gently shake the tray.

- Using your twelve 3D water molecule models and salt ions, can you explain what happens when you put salt in water?
  The sodium, a positively charged ion, is attracted to the oxygen of a water molecule and chloride, a negatively charged ion, is attracted to the hydrogen of the water molecule. Water molecules surround the entire sodium and chloride, breaking them apart.

- What happens when you keep adding salt to a glass of water? Why does this happen?
  Eventually, salt begins to form at the bottom of the glass when there are not enough water molecules to surround the salt.

- What is this called?
  This is a supersaturated solution.

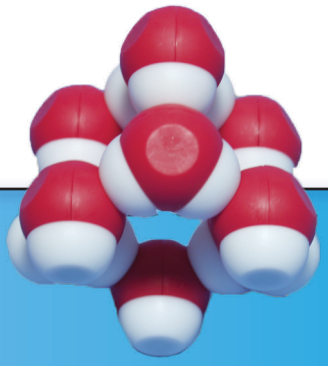
- What happens to a glass of salty water when it is heated to boiling?
  The water evaporates leaving the sodium and chloride to bond to form salt again.

Give each group of students an ethane molecule. Have them put it in the center of the tray and the 3D water molecule models along the edge. Have your students gently shake the tray.

- Will water dissolve ethane? Why or why not?
  No. Water cannot dissolve ethane because ethane is nonpolar. Water cannot form hydrogen bonds with ethane.

3-D Molecular Designs

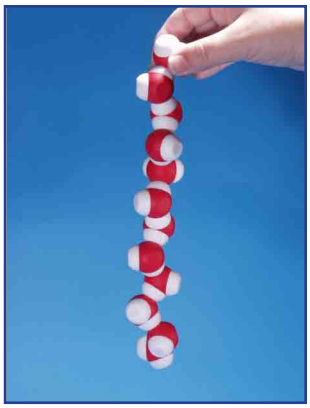
. . .where molecules become real™



Appendix

Just for Fun

The Water Kit© can be fun as well as educational! Here are some fun activities to do with your students.



Chain of Life

Have students guess how many 3D water molecule models can be strung on a chain before the chain breaks. Test their predictions.

Keep Score with Water!

Hang one 3D water molecule model from the ceiling. As each student correctly answers a water-related question, have them add a molecule to the chain. After enough students have correctly answered questions – and the chain breaks, declare No Homework!

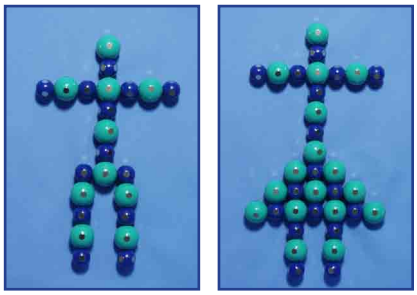
Building with Molecules

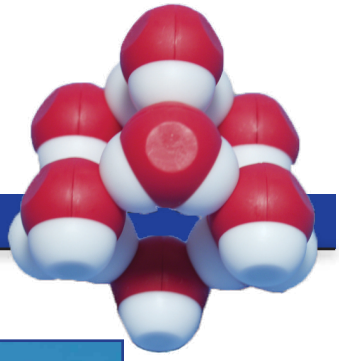
Try to build shapes with 3D water molecule models!

Snowflakes

Pyramid

NaCl

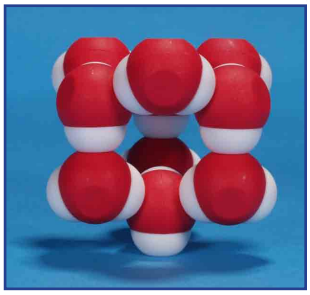




Just For Fun Activities

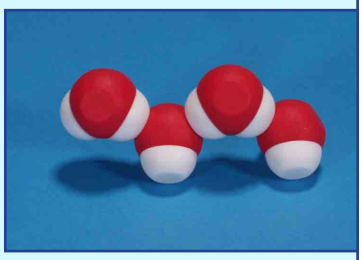
Cubic Ice

Cubic ice, (Ice 1c) is one of the twelve structures of ice identified by scientists. Cubic ice cubes can be created with as few as ten molecules, while hexagonal ice requires twelve. Check the 3D Molecular Designs website for more information on ice and how to construct some of the twelve different structures of ice. To create a Cubic Ice cube, follow the directions for either the *Step Method* or the *Pattern Method* (on the next page).

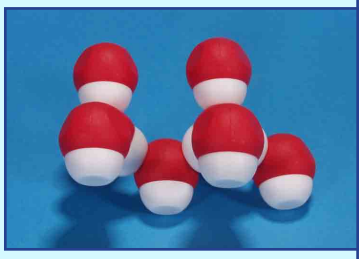


Step Method

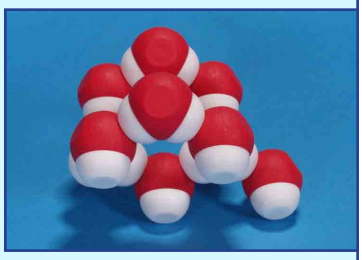
1. Create the body of a dragon as shown in the first picture.



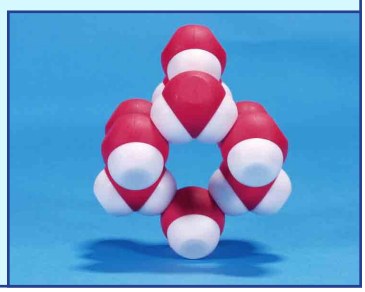
2. Add ears and wings to the dragon as shown in the second picture.
   Note: *the hydrogen atoms should point down on both the ears and wings.*

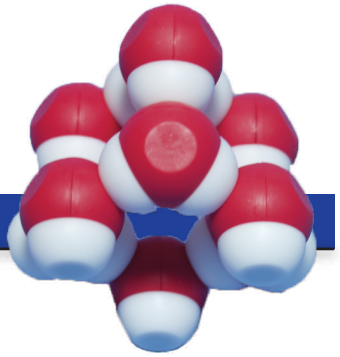


3. Connect each ear to a wing as shown in the third picture.



4. Remove the tail and place the final piece to create the cube as shown in the fourth picture.

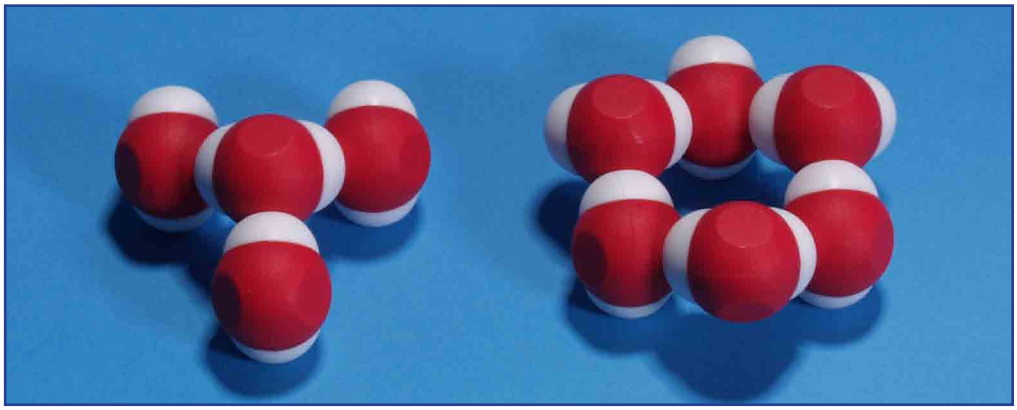


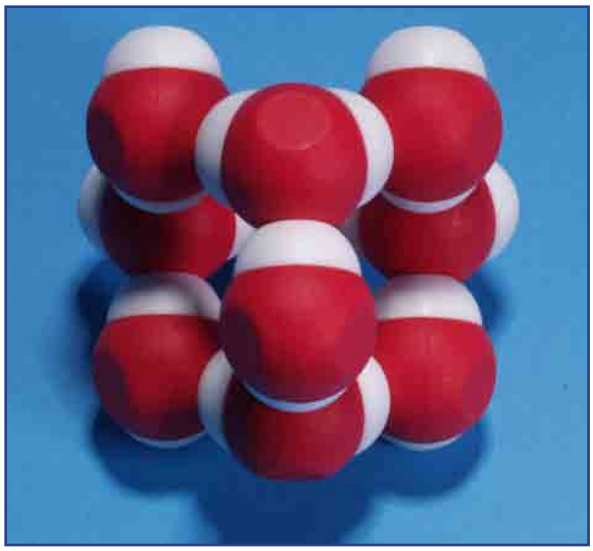


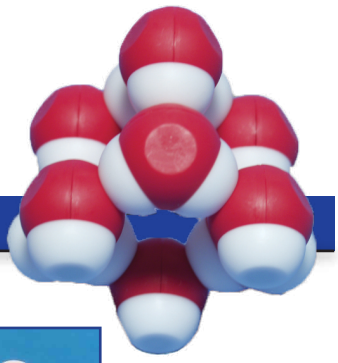
Just For Fun Activities (continued)

Pattern Method

Create a Y and a ring using the pattern below. Without rotating the Y, place it on top of the ring.



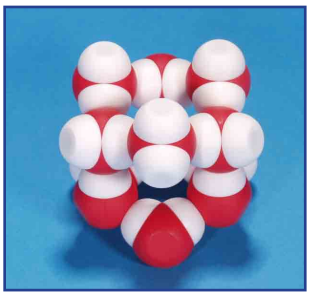




Just For Fun Activities (continued)

Hexagonal Ice

Scientists have described twelve structures of ice, many of which can be constructed with the Water Kit©. Check the 3D Molecular Designs website for more information on ice and how to construct some of the twelve different structures of ice. To construct Ice 1h, hexagonal ice, follow the directions for the *Step Method* or the *Pattern Method* (on the next page).

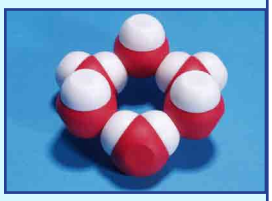


Step Method

1. Hold one molecule horizontally in front of you with the hydrogen atoms to the sides.

2. Add two vertical (hydrogen atoms pointing up and down) molecules to the two hydrogen atoms from the first step. *See the first picture on the right.*

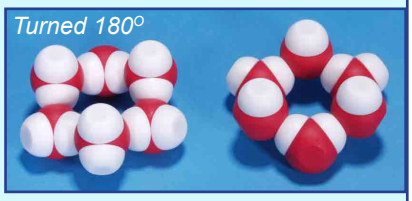
3. Add a horizontal molecule to the lower hydrogen on each molecule added in step 2. *See the 2nd picture to the right.*



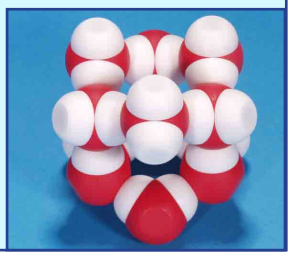
4. Add one vertical molecule to connect the hydrogen atoms from the molecules added in step 3. *See the 3rd picture to the right.*

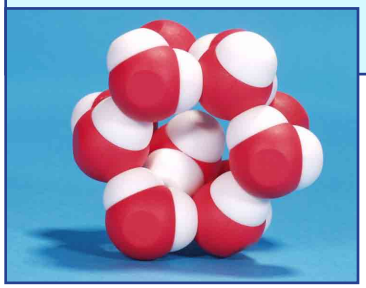
5. Create a second hexagonal ring following steps 1-4.

6. Orient the two hexagonal rings the same way – then rotate one ring 180 degrees.

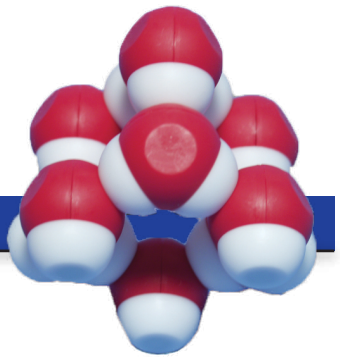


7. Place one hexagonal ring on top of the other. Do not flip one ring over – the hydrogen atoms on both rings should point the same way.





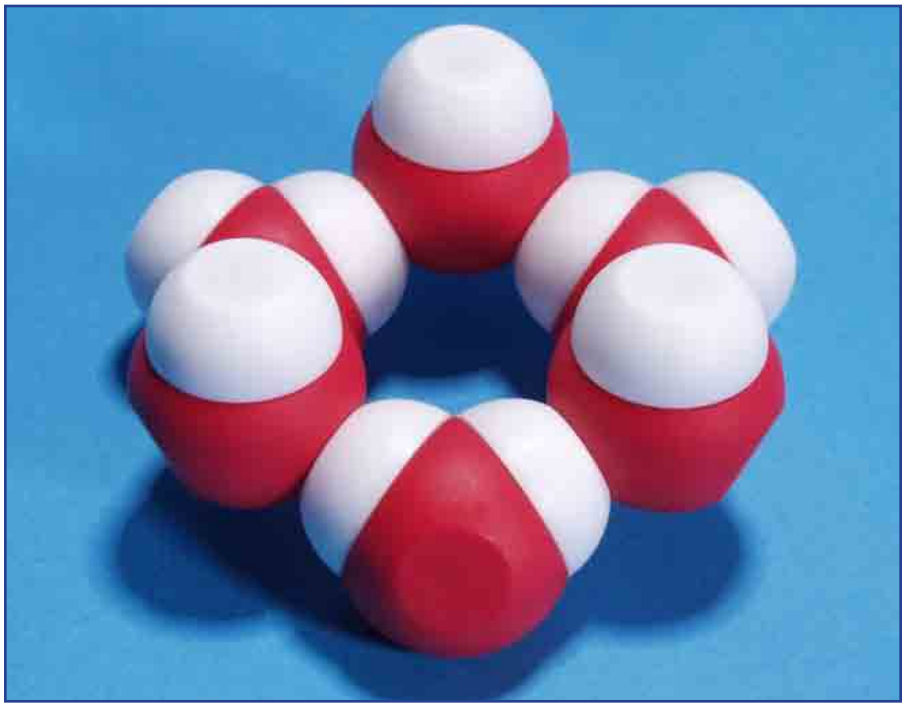
You have now formed a model of an ice cube. Note that it is possible to place a 13th water molecule into the hole formed by this lattice. Now you can see why ice floats. Ice has empty spaces in it. The same volume occupied by 12 water molecules in a solid ice lattice, can contain more water molecules in its liquid form.

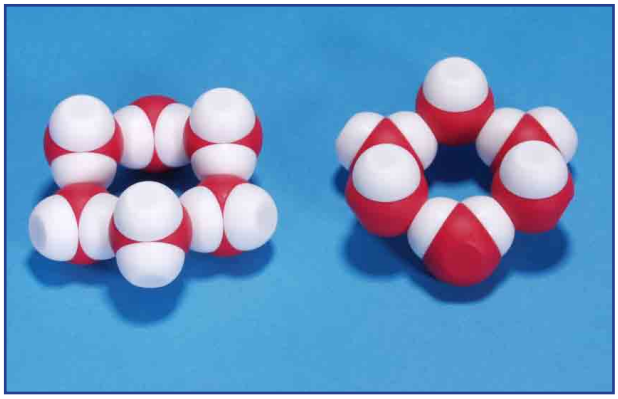


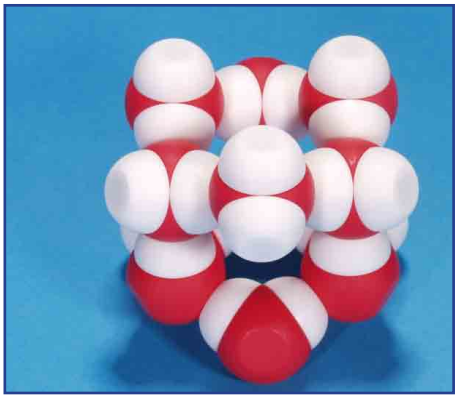
Just For Fun Activities (continued)

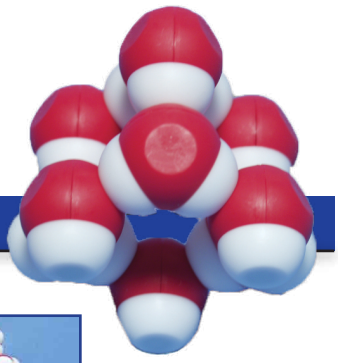
Pattern Method

Use this pattern to construct hexagonal Ice (Ice Ih). Create two rings, turn one 180 degrees, and stack one ring on top of the other.





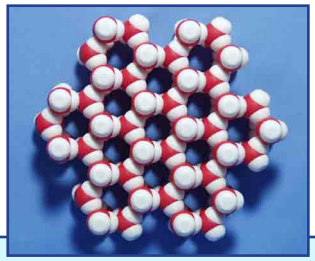




Just For Fun Activities (continued)

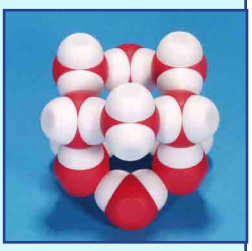
Snowflakes

Scientists have described twelve structures of ice, many of which can be constructed with the Water Kit©. To form a six-sided snowflake, you will need 6 or 7, 1-Cup Water Kits©.



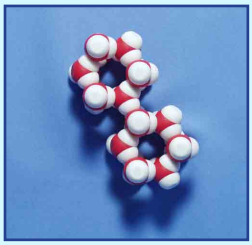
Hexagonal Snowflakes

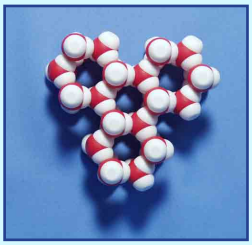
Make 6 or 7 ice cubes following the hexagonal ice instructions. Your cubes should look like the picture on the right.

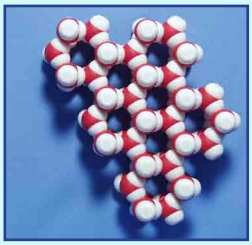


Connect your Hexagonal Ice cubes to form a hexagon. Each cube will be oriented the same way.

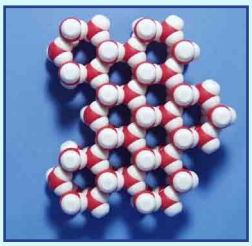
If you have problems creating a hexagon, then try to connect six cubes around a center cube.







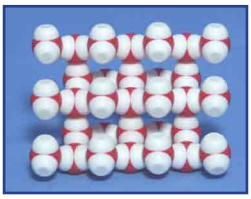
If you only have six cups, then remove the center cube and rotate it to complete the outer hexagon.

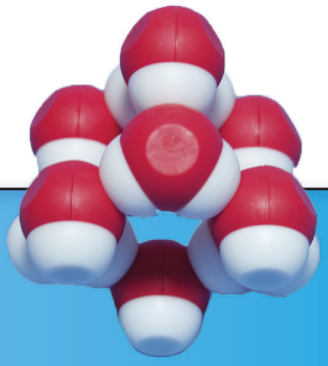


Without a center cube, which requires the seventh cup of the Water Kit©, this structure is not very strong.

Other Ice Crystals

If you do not create uniform cubes, it may be difficult to connect several cubes, and you will be unable to produce a six-sided snowflake. It is possible to create larger ice crystals by connecting several of the cubic ice cubes and some of the other twelve forms of ice described by scientists. Information on constructing other forms of ice will be posted on the 3D Molecular Designs website as they become available.





Glossary

Adhesion is the attraction between two unlike molecules.

Capillary action is the spontaneous rising of a liquid in a capillary (small diameter) tube.

Cohesion is the attraction between two like molecules.

Condensation is the transformation from a gas to a liquid. This occurs when a gas is cooled enough that the molecules slow down to form stronger intermolecular forces with each other. As water condenses, the molecules slow down and form hydrogen bonds with each other as the water vapor cools.

Covalent bonds are formed when two atoms share two electrons. A covalent bond is an intramolecular bond within one molecule. Covalent bonds can be either polar (which have partially charged atoms) or nonpolar (without charged atoms).

Evaporation is the transformation from a liquid to a gas. This occurs when a liquid absorbs enough heat, increasing the movement of the molecules, to cause the intermolecular forces between molecules to break the surface tension. This allows individual molecules to escape into the air. As water evaporates, the heat it absorbs breaks the hydrogen bonds and the liquid water becomes water vapor.

Hydrogen bonds are an intermolecular force between the two molecules where a positively charged hydrogen atom interacts with a negatively charged fluorine, nitrogen or oxygen atom in a second molecule.

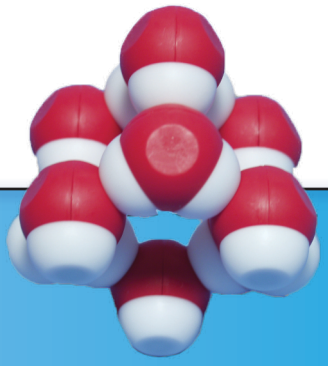
Ionic bonds are the complete transfer of an electron between two atoms resulting in one positively and one negatively charge atom. Ionic bonds are intramolecular bonds within one molecule.

Ions are charged atoms that have gained or lost electrons as a result of an ionic bond.

Nonpolar molecules do not have unequal regions of charges and therefore do not interact with polar molecules. Oil and ethane are examples of a liquid and a gas composed of nonpolar molecules.

Polar molecules have partial charges. Polarity refers to the partial positive charge (+) and partial negative charge (-) that a molecule has when electrons are unequally shared between two or more atoms. Water molecules are polar molecules.

Surface tension is the amount of energy required to stretch or increase the surface of a liquid by a unit of measure. Surface tension is due to the cohesion between molecules at the surface of a liquid. In a liquid, molecules are pulled in all directions by intermolecular forces. At the surface of a liquid, the molecules are only pulled downward and toward the sides.



National Standards

Connections to: A Framework for K-12 Science Education

Practices, Crosscutting Concepts, and Core Ideas*

Dimension 1: Scientific and Engineering Practices

1. Asking questions (for science) and defining problems (for engineering)
2. Developing and using models
5. Using mathematics and computational thinking

Dimension 2: Cross Cutting Concepts

1. Patterns
2. Cause and effect: Mechanism and explanation
3. Scale, proportion, and quantity
4. Systems and system models
5. Energy and matter: Flows, cycles, and conservation
6. Structure and function
7. Stability and change

Dimension 3: Disciplinary Core Ideas:

Physical Sciences

PS1: Matter and Its Interactions
PS1.A: Structure and Properties of Matter
PS1.B: Chemical Reactions
PS2: Motion and Stability: Forces and Interactions
PS2.A: Forces and Motion
PS2.B: Types of Interactions
PS2.C: Stability and Instability in Physical Systems

Life Sciences

LS1: From Molecules to Organisms: Structures and Processes
LS1.A: Structure and Function
LS2: Ecosystems: Interactions, Energy, and Dynamics
LS2.A: Interdependent Relationships in Ecosystems
LS2.B: Cycles of Matter and Energy Transfer in Ecosystems
LS2.C: Ecosystem Dynamics, Functioning, and Resilience

Earth and Space Sciences

ESS2: Earth's Systems
ESS2.A: Earth Materials and Systems
ESS2.C: The Roles of Water in Earth's Surface Processes
ESS2.D: Weather and Climate
ESS3: Earth and Human Activity
ESS3.A: Natural Resources
ESS3.D: Global Climate Change

**The NSTA Reader's Guide to A Framework for K-12 Science Education, National Research Council (NRC), 2011. A Framework for K-12 Science Education: Practices, Crosscutting Concepts, and Core Ideas. Washington, D.C.: National Academies Press.*

The Magic Disk Trick

--- or ---

A Macroscopic Demonstration of Hydrophobic vs. Hydrophilic Interactions.

We are always looking for ways to connect the macroscopic world in which kids live with the invisible world of molecules. For example, how can we help them construct an understanding of how hydrophobic and hydrophilic forces play a major role in determining the folded structure of a protein? Here is an activity that does just this.

Materials:

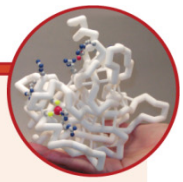
1. Hexane (~10 ml) (Lighter Fluid works as an alternative)
2. Water (~10 ml)
3. Paper….the cheap stuff, not shiny /glossy paper.
4. A number 2 graphite pencil
5. A capped test tube or vial (plastic or glass) that will hold ~20 ml of liquid.

Procedure:

1. Take the raw paper --- which is made of cellulose, and is therefore hydrophilic --- and scribble on one side with the # 2 pencil. This will coat one side of the paper with carbon --- which is hydrophobic.
2. Using a single hole punch, punch out 5 disks. One side of each disk is black --- and hydrophobic (graphite is pure carbon), while the other side is white --- and hydrophilic (paper is made from cellulose, which has a lot of OH groups).
3. Add both the water and the hexane to the test tube or vial. Cap and shake vigorously. These two solvents will quickly separate into two distinct phases, with the less dense hexane on top.
4. Add the bi-colored disks to the vial, cap and shake.
5. Observe how the disks orient themselves at the interface between the hydrophobic hexane and the hydrophilic water.

What can go wrong? Almost nothing, except:

1. You can use glossy paper….that has been treated with clay and other materials that decrease the hydrophilic nature of the cellulose surface.
2. You can scribble only very lightly, and therefore not completely cover the hydrophilic cellulose with hydrophobic carbon.
3. You can use a dirty or soapy test tube/vial….that will confound the disks.
4. You can handle the paper with oily/greasy fingers --- in which case the hydrophilic cellulose will become somewhat hydrophobic, confounding the disks.
5. You can fail to test all your reagents in advance, in which case this experiment is guaranteed …. not to work.



Membrane Starter Kit

Student Copy

A Message to Teachers

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Objectives: You will use the model pieces in the kit to:

- Examine the general amphipathic structure of a phospholipid.
- Compare and contrast various models of phospholipids.
- Explore the interaction between phospholipids and water.
- Construct a phospholipid monolayer, micelle and bilayer and relate it to plasma membrane structure.
- Identify and demonstrate the function of the various types of channel proteins involved in membrane transport.

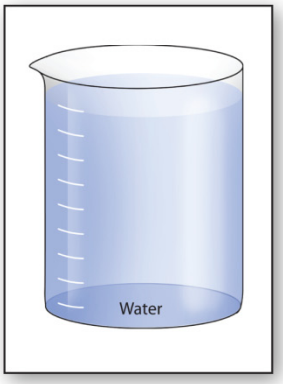
Introduction:

The **plasma membrane** is the structural boundary that separates the cell from its surroundings and controls what substances move into and out of the cell it surrounds. As only some substances are allowed to cross the membrane, the plasma membrane demonstrates the property of **selective permeability**. In particular, the plasma membrane of mammalian red blood cells (erythrocytes)

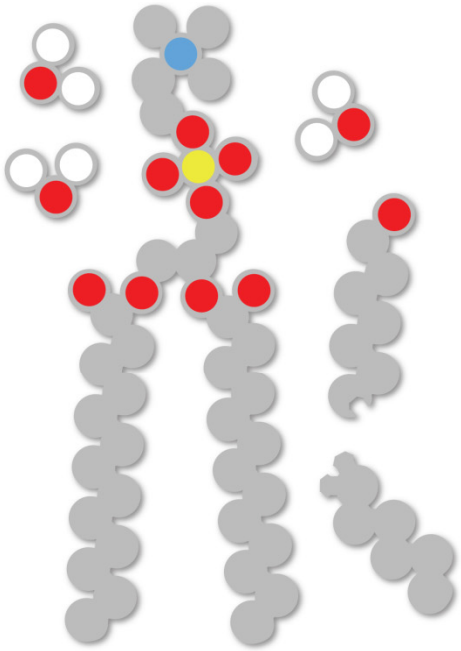
has been the focus of cell membrane study because these cells do not contain nuclei or internal membranes. As such, they represent a source from which a pure plasma membrane may be easily isolated for analysis. In 1925, the two Dutch scientists Evert Gorter and his research assistant F. Grendel extracted lipids from the membranes of a known number of red blood cells which corresponded to a known surface area of plasma membrane. The surface area occupied by a monolayer of the extracted lipid spread out at an air/water interface was determined. The results of their experiment showed that the surface area of the lipid monolayer was twice that occupied by the erythrocyte plasma membrane, leading to the conclusion that the plasma membrane consists of **lipid bilayers**. The most abundant lipids in most membranes are **phospholipids**. The ability of phospholipids to spontaneously form membranes is inherent to their **amphipathic** (meaning they have both a hydrophilic region and a hydrophobic region) nature.

Materials Needed:

Bag of Mini-Phospholipid Foam Models



Beaker Diagram



Large Foam Phospholipids

Large Molymod Phospholipids (optional)

Part 1: Focus on Phospholipids

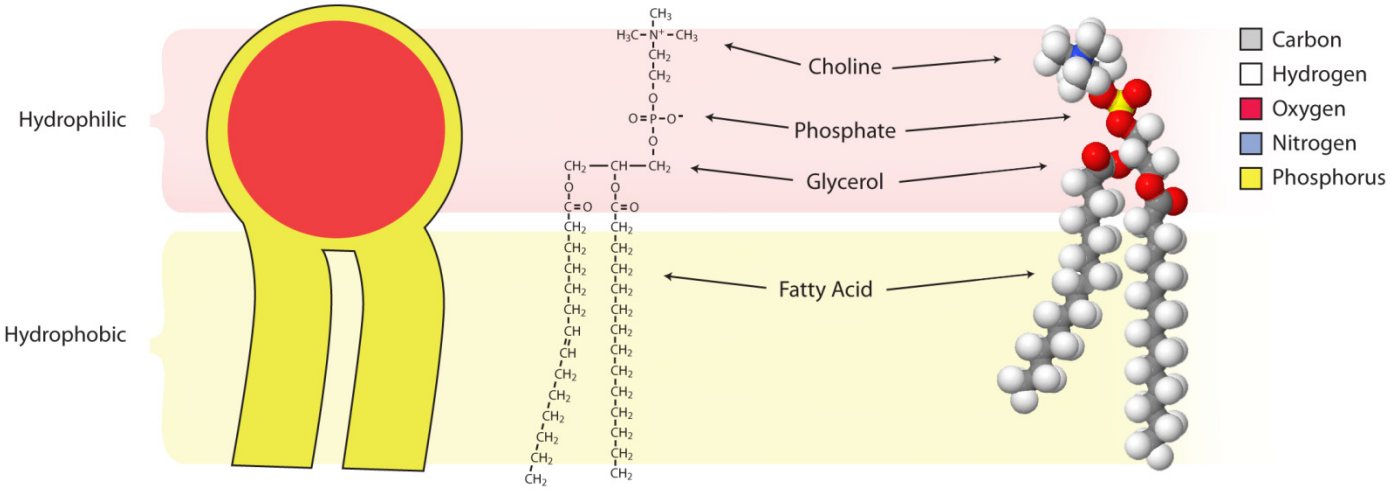
1a. Sketch the specific structural formal of the model you have been asked to examine in the space provide below. Label the hydrophilic and hydrophobic regions of your structure.

1b. Compare your structure to that of the other groups in the room. Record any similarities you observe in these phospholipid structures.

1c. Based on these similarities a simplified representation may also be used to indicate phospholipid structure. Sketch this simplified model in the space below. Label the hydrophobic and hydrophilic portions of this simplified model.

1d. Record any differences in the specific structures you have observed between these phospholipids.

The general structure of a phospholipid is most often represented by the phosphatidylcholine structure:



Part 2: The Kessler Membrane Activity

2a. Obtain ten of the simplified representations of a phospholipid. Using the diagram of the beaker of water, arrange the ten phospholipids demonstrating correct hydrophobic/hydrophilic interactions. Sketch your result below.

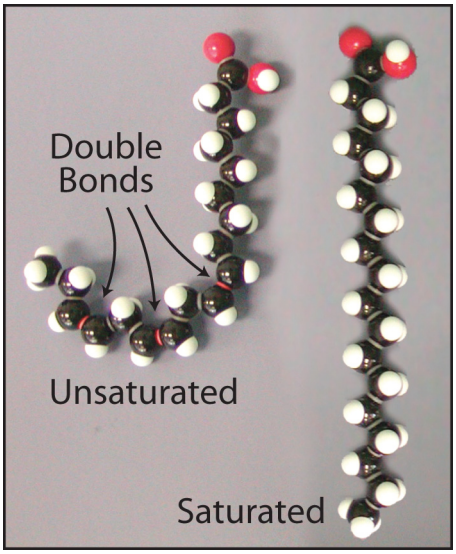
2b. Using the same ten phospholipids, rearrange them in the beaker so that they are submerged in the water while still maintaining the correct hydrophobic/hydrophilic interactions. Sketch your result below.

The resulting structure is referred to as a **micelle.** Micelles can act as emulsifiers that will allow a compound that is normally insoluble (in the solvent being used) to dissolve.

2c. Your next challenge is to construct a structure that is BOTH submerged in the water AND contains water on the inside. You may use as many of the phospholipids in your kit as you wish to complete the task. Sketch the resulting structure below.

2d. Based on the structure of a phospholipid, explain the reasoning for your arrangement.

The resulting structure is referred to as a **liposome**. Liposomes are artificially prepared vesicles that may be used as a vehicle for administration of nutrients or pharmaceutical drugs.

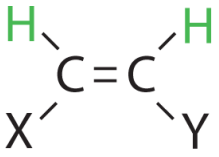


Upon closer examination of the hydrophobic tails, you may notice that in one of the tails, all of the carbons are connected with single bonds. These hydrocarbon tails are referred to as **saturated**. In the second tail there is a red connector, indicating that a double bond connects these two adjacent carbons. The presence of a double bond creates a "kink" in the structure of this hydrocarbon chain. The hydrocarbon tails containing a double bond or bonds between adjacent carbons are referred to as **unsaturated**.

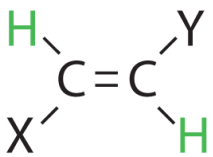
2e. Develop an explanation for the necessity of having "kinks" in the hydrophobic tail of the phospholipids that make up cell membranes.

Note to Teachers - Optional Activity:

Further comparison shows a difference in these double bonds. If the hydrogens associated with the double bonded carbons are on the same side, the configuration is said to be "cis". If the hydrogens associated with the double bonded carbons are on opposite sides, the configuration is referred to as "trans". (See illustrations below.) Teachers may also opt to include a discussion of "trans fats" using these models.



cis



trans

2f. Which configuration produces the bigger “kink” in the structure of the hydrophobic tail?

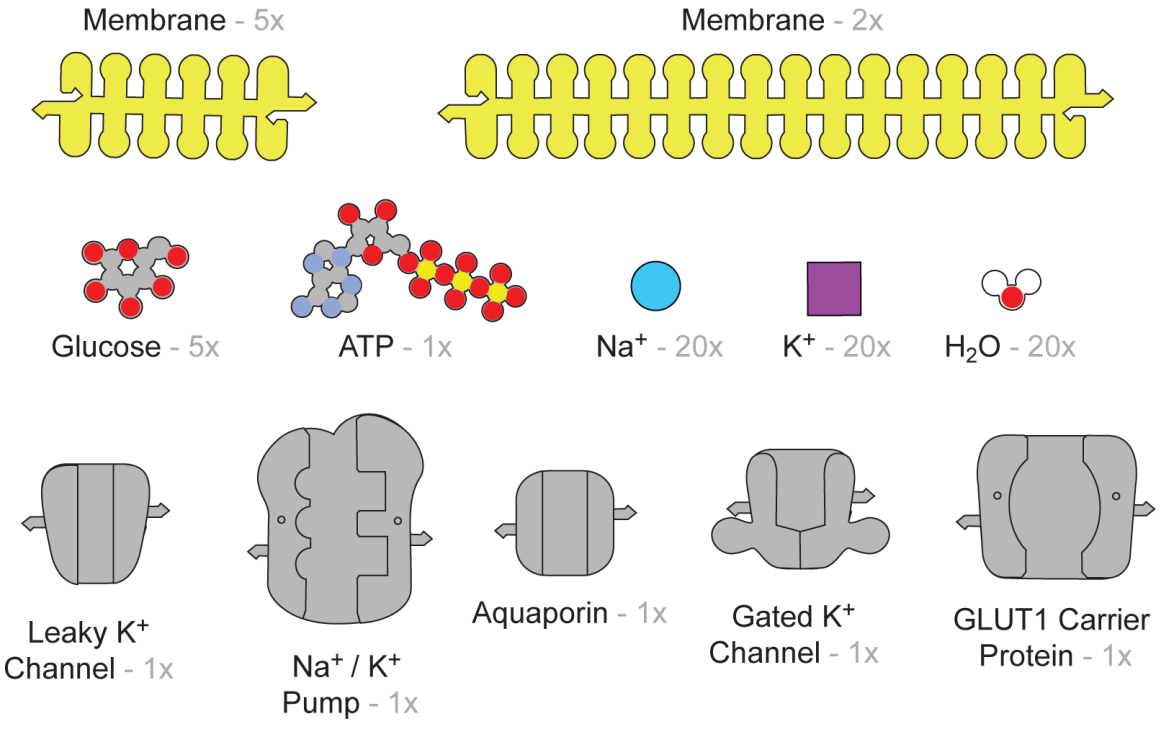
2g. How might this configuration contribute to the fluidity of the membrane?

Part 3: Exploring Membrane Permeability

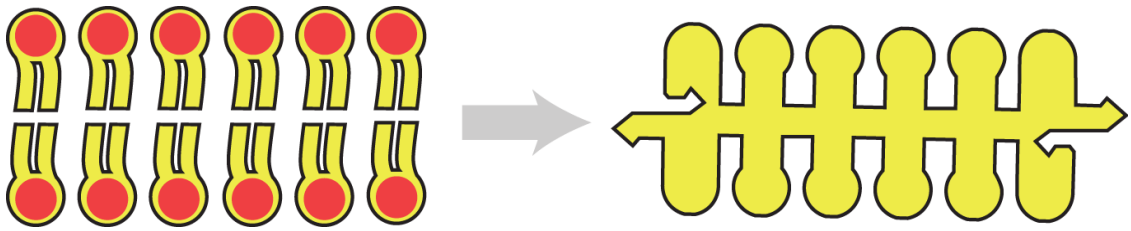
Introduction:

The phospholipid bilayer is only one aspect of the gatekeeper system responsible for the plasma membrane’s **selective permeability**. Membrane bound proteins play a key role in regulating the transport of ions and molecules through the plasma membrane.

Materials:



We will use a slightly different and simplified representation of the phospholipid bilayer in the next activity.



3a. Label the hydrophilic head and hydrophobic tail in the model pictured above.

3b. Nonpolar molecules, such as hydrocarbons, CO_2 and O_2 are hydrophobic. Explain why these molecules can easily cross the plasma membrane without the aid of proteins.

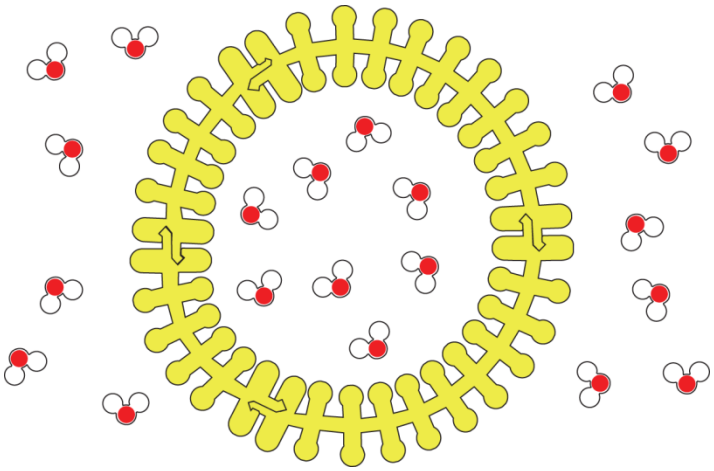
A variety of polar molecules and can't move through the plasma membrane on their own. Contact with hydrophobic lipid bilayer may be avoided by these hydrophilic substances as they cross the plasma membrane with the help of **transport proteins**.

Part 4: Channel Proteins

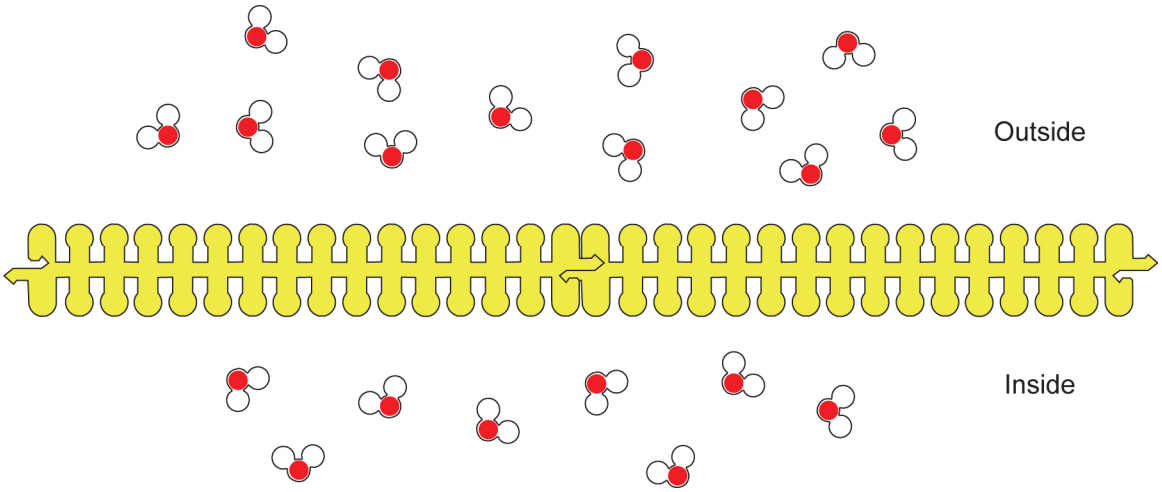
Some transport proteins, referred to as **channel proteins**, function by having a hydrophilic channel that certain molecules or ions use to cross the plasma membrane. There is a common misconception that a polar water molecule can easily cross the hydrophobic bilayer of the plasma membrane. Simple diffusion of water across the membrane does not occur at a rate fast enough in order to meet the survival needs of a cell. Channel proteins known as **aquaporins** allow entry of up to three billion (3×10^9) water molecules per cell per second!

4a. Construct a physical representation (a model) of the passage of water through a plasma membrane.

Use two short pieces and two long pieces of the simplified phospholipid membrane. Connect the pieces to form a "cell". We suggest placing 8 of the water molecules inside the cell (Intracellular) and 12 water molecules outside the cell (extracellular).

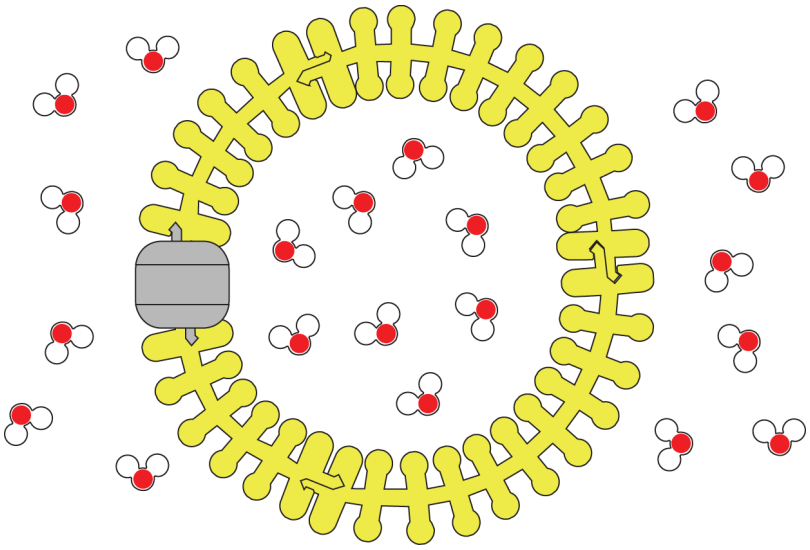


Note to Teachers - You may opt to set up the membrane in a linear fashion (shown below) if more conducive to your teaching environment.



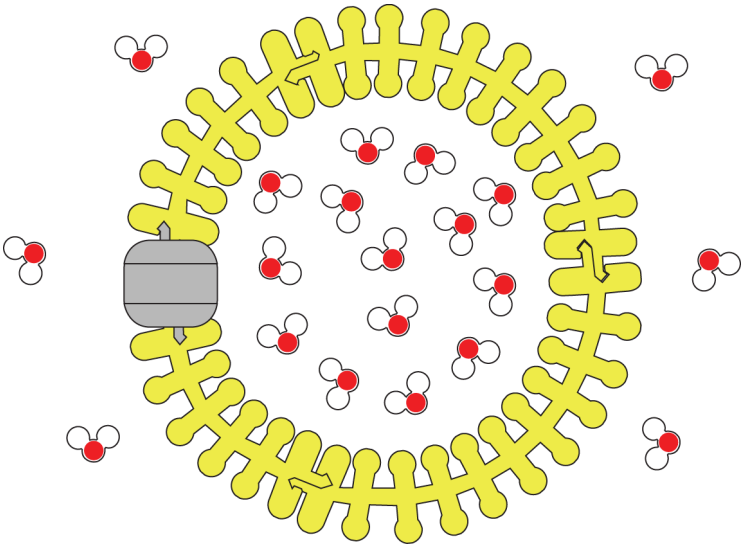
4b. Explain why water would have a difficult time diffusing across the cell membrane. Keep in mind the structure of water in your answer.

A substance will generally diffuse from where it is more concentrated to where it is less concentrated. In other words, the substance will diffuse down its **concentration gradient**. Insert a channel protein model, in this case representing **aquaporin**, into the cell membrane you have constructed. Aquaporin is an example of a channel protein found in the plasma membrane which transports water (see diagram at right).



4c. Move the water molecules to indicate the net flow of water in this system. What direction did the water molecules move?

4d. Construct a system where intracellular water molecule concentration is higher than extracellular water molecule concentration. Sketch your model in the space below and indicate the net flow of water.



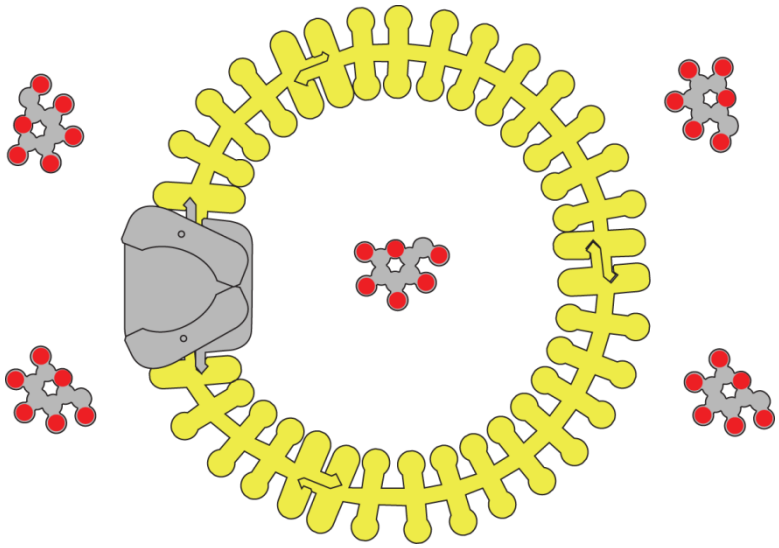
Note to Teachers -
You may choose to use the kit to introduce the students to the terms hypertonic, hypotonic and isotonic at this time.

4e. Predict what will happen to the cell due to the movement of the water.

In order for water move across the cell membrane in the above cases, the cell did **not** have to expend cellular energy (usually in the form of ATP) to move water across the plasma. Movement

of a substance across a membrane without the expenditure of energy is referred to as **passive transport**. Likewise, when the cell must expend energy to move a substance against its concentration gradient the process is referred to as **active transport**. Additionally, the channel protein aquaporin assisted in passively moving water across the membrane. This occurrence may be referred to as **facilitated diffusion**.

Part 5: Carrier Proteins



Remove the water molecules and aquaporin representation from the model cell you have constructed. Insert the carrier protein model into the plasma membrane model. Distribute the glucose molecules so that there are more extracellular glucose molecules than intracellular glucose molecules (see diagram).

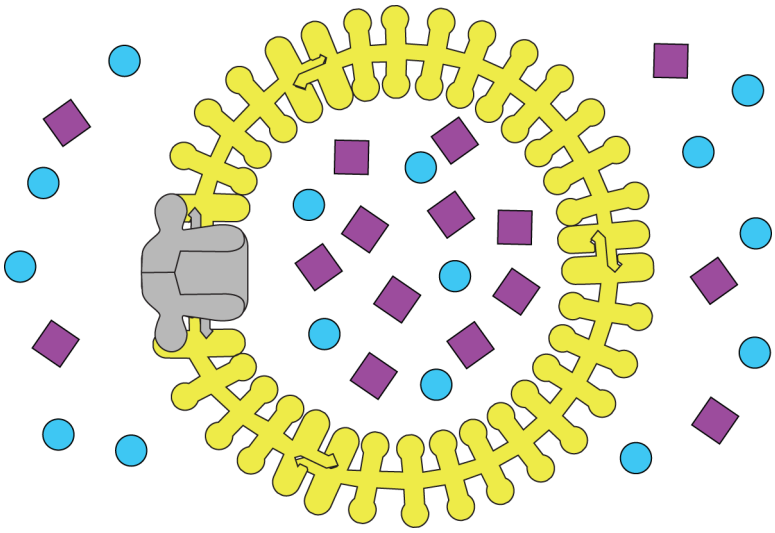
A **carrier protein** binds a solute molecule on one side of the membrane, undergoes a shape change (conformational change) and deposits the solute molecule on the other side of the membrane. GLUT 1 is an example of a protein channel frequently found in the plasma membrane of red blood cells that facilitates the movement of glucose across the cell's plasma membrane.

5a. Use the model to demonstrate the movement of glucose across the cell membrane. Sketch your model in the space below.

5b. Is this an example of passive or active transport? Explain your choice.

Part 6: Gated Channels

Remove the glucose molecules and carrier protein from the model cell you have constructed. Insert the gated channel protein model into the plasma membrane model (see diagram). Place 5 sodium ions (**round**) and 10 potassium ions (**square**) inside of the cell to simulate the intracellular environment ion concentrations. Place 10 sodium ions and 5 potassium ions outside of the cell to simulate the extracellular environment ion concentrations.



Gated channels are channel proteins that open or close in response to a stimulus. In nerve cells, a stimulus opens the gated sodium channel to allow a stream of sodium ions to enter the cell. Other gated channels open or close when a substance, different from the one to be transported, binds to the channel.

Begin the simulation with the sodium gated channel closed. After a stimulus the gates swing open.

6a. Based on what you know about concentration gradients, what direction will the sodium ions move through the channel?

6b. Does this gated channel demonstrate passive or active transport? Explain your answer.

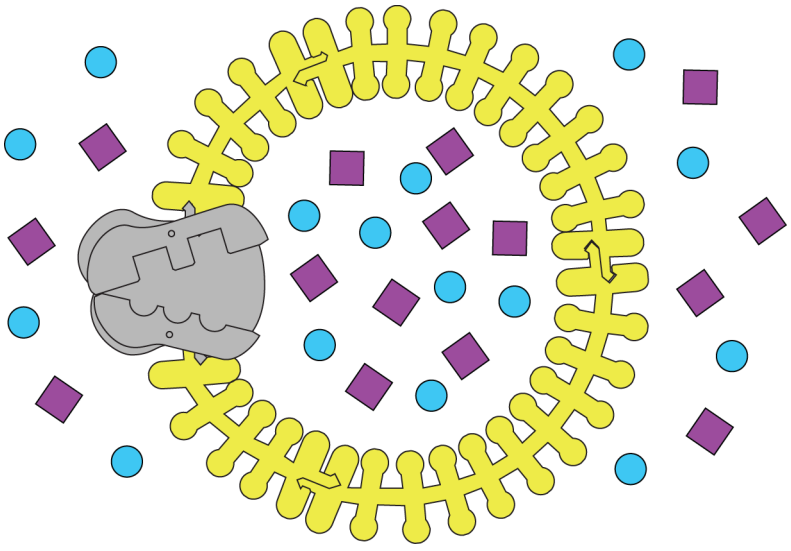
6c. Why may sodium ions move through the sodium channel while potassium ions typically do not?

6d. Speculate what other stimului may affect the operation of channel proteins.

6e. Devise a question you might have about the operation of this channel protein.

Part 7: Active Transport - The Sodium-Potassium Pump

Remove the gated channel from the model cell you have constructed. Instead, insert the sodium-potassium pump protein into the membrane of the model. (See diagram below.) Place 7 sodium ions (**round**) and 8 potassium ions (**square**) inside of the cell to simulate the intracellular environment ion concentrations. Place 8 sodium ions and 7 potassium ions outside of the cell to simulate the extracellular ion concentrations.



Transport proteins that move solutes against their concentration gradients are all carrier proteins. The sodium-potassium pump is a special carrier protein that moves sodium ions against their gradient OUT of the cell and potassium ions against their gradient IN to the cell.

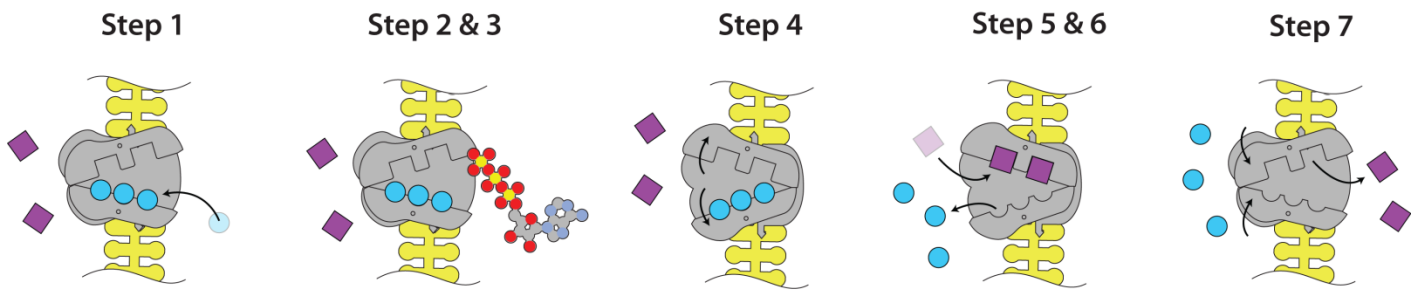
Because these ions are moving against their concentration gradients, the cell must expend energy to do the work resulting in **active transport** of these ions.

A typical animal cell has a much higher concentration of potassium ions (K^+) and a much lower concentration of sodium ions (Na^+) on the inside of the cell than the outside. The sodium-potassium pump uses energy in the form of ATP to move these ions against their concentration gradients and establish the "normal" intracellular ion concentrations. We will demonstrate the action of the sodium-potassium pump in the following simulation:

Set the sodium-potassium pump so that it is open to the inside of the cell as shown in the diagram on the previous page (page 14).

Record the initial ion concentrations in the table provided below:

Ion Types	Initial Amount	Amount After First Cycle	Amount After Second Cycle
Intracellular Na^+			
Intracellular K^+			
Extracellular Na^+			
Extracellular K^+			



Step 1: Bind three three intracellular sodium ions to the appropriate spots in the protein.

Step 2: Bring the ATP in close proximity to the pump.

Step 3: Sodium ion binding stimulates phosphorylation of the pump protein by ATP. In other words, a phosphate group is added to the sodium-potassium pump from the ATP molecule. (You will not be able to demonstrate this step with the model).

Step 4: Phosphorylation causes a change in the shape of the protein. You can demonstrate this by "swinging" the sides of the protein so that it opens to the outside of the cell.

Step 5: The shape change reduces the protein's binding affinity for sodium ions and increases the binding affinity for potassium ions. Remove the sodium ions from the protein and deposit them outside the cell and bind two potassium ions to the appropriate spots in the protein.

Step 6: Potassium ion binding triggers the release of the phosphate group from the protein. (Again, you will not be able to demonstrate this step with the model).

Step 7: Loss of the phosphate group results in the restoration of the protein's original shape which then releases the potassium ions. Swing the sides of the protein back so that they open to the inside of the cell and deposit the potassium ions.

Step 8: Repeat this process one more time.

Record the ion concentrations after completing the first cycle of the action of the sodium-potassium pump.

7a. What is the initial overall positive charge inside the cell compared to the outside the cell?

7b. Compare the total intracellular positive charge to the total extracellular positive charge after one cycle of the sodium-potassium pump.

Record the ion concentrations after completing the second cycle of the action of the sodium-potassium pump. Compare the total intracellular positive charge to the total extracellular positive charge after the second cycle of the action of the sodium-potassium pump.

7c. Where is the sodium ion concentration highest at the beginning of the sodium-potassium pump cycle?

7d. Where is the potassium ion concentration highest at the beginning of the sodium-potassium pump cycle?'

7e. What is the initial overall charge of the inside of the cell compared to the outside?

7f. Why is ATP required in this process?

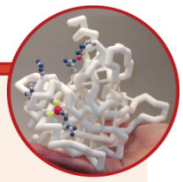
7g. After one cycle of the sodium-potassium pump, compare the overall charge of the inside of the cell to the outside? Explain how the distribution of ions changed.

7h. Is the sodium-potassium pump a channel protein or a carrier protein? Explain your answer.

7i. Devise a question you might have about the function of the sodium-potassium pump.

Fun Fact: In nerve cells, the sodium-potassium pump helps to reestablish the resting ionic concentrations after the nerve cell has fired.

Reference:
The Cell, 2nd edition
A Molecular Approach
Geoffrey M Cooper.
Boston University
Sunderland (MA): Sinauer Associates; 2000.
ISBN-10: 0-87893-106-6



Membrane Starter Kit

Teacher Copy

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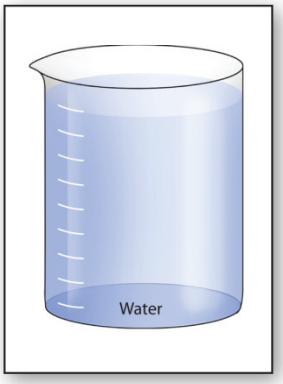

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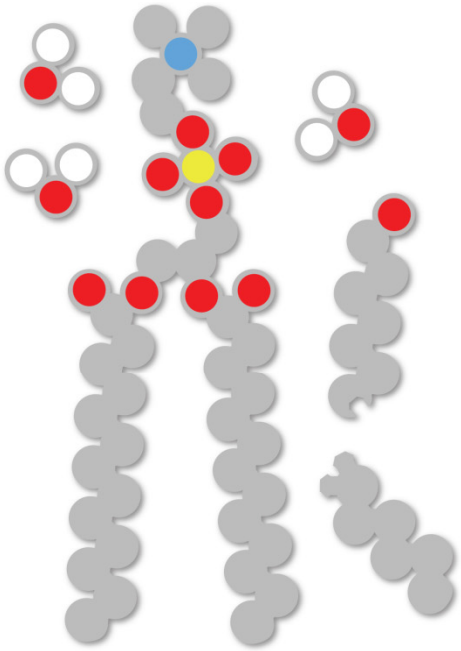

Large Foam Phospholipids

Large Molymod Phospholipids (optional)

Part 1: Focus on Phospholipids

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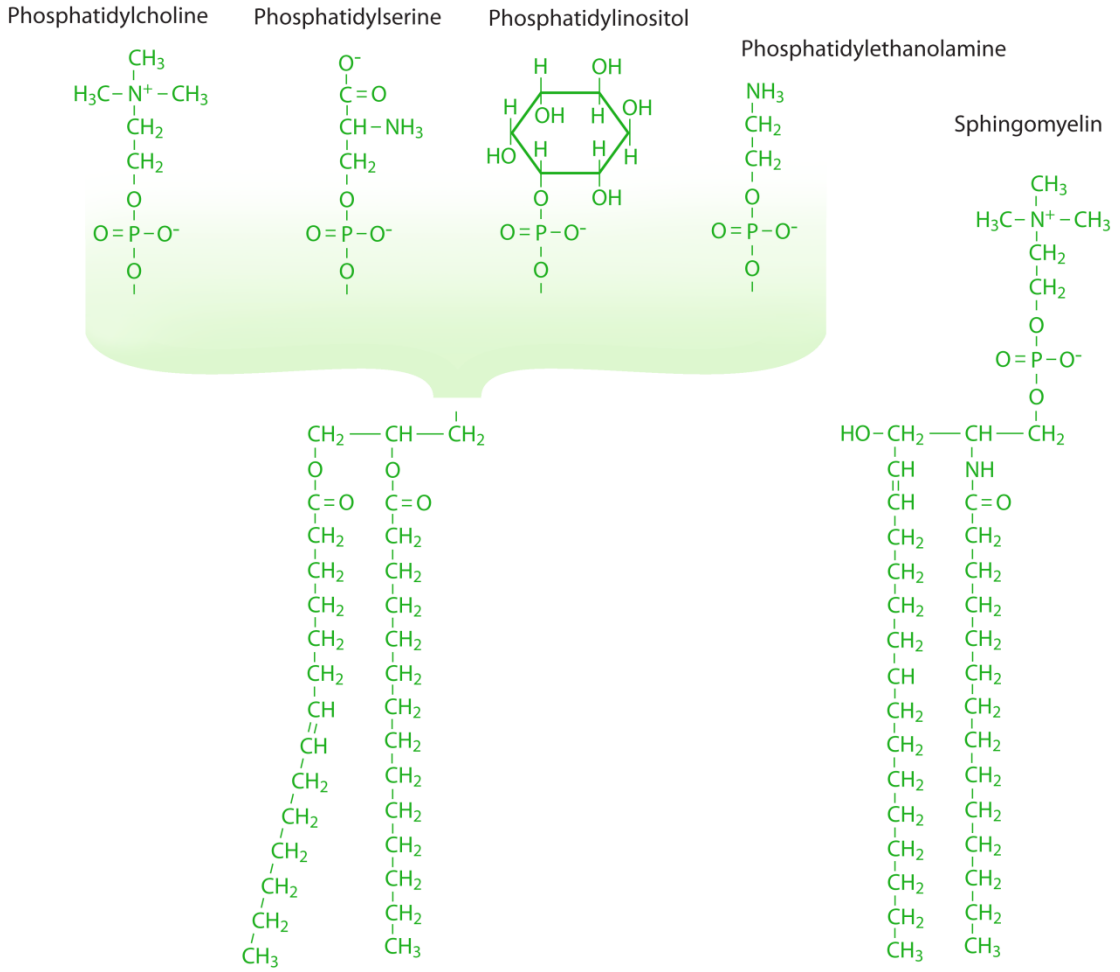
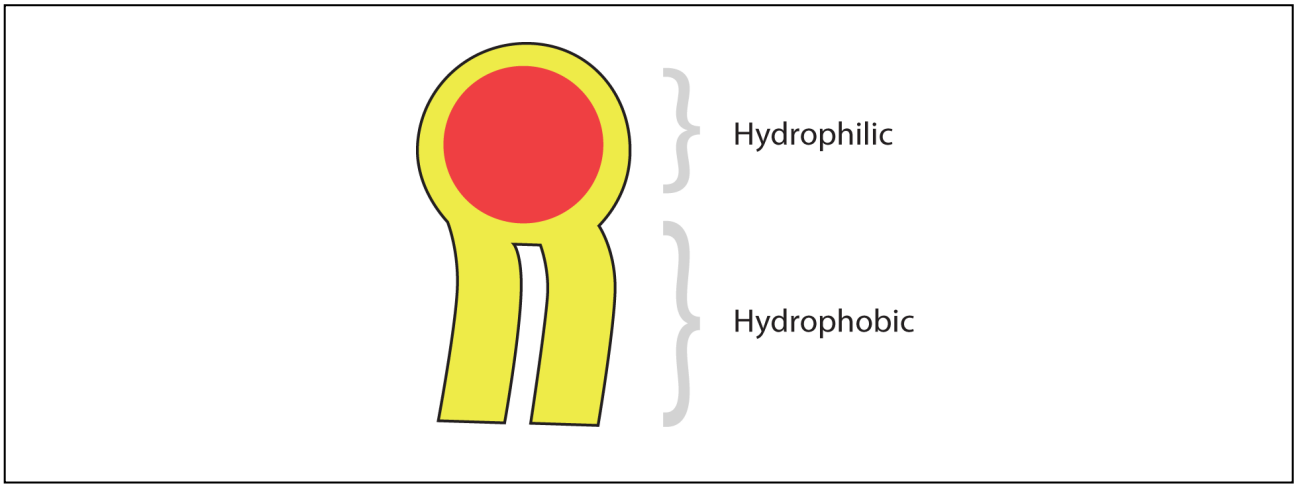
Answers will vary but should include one of the following:



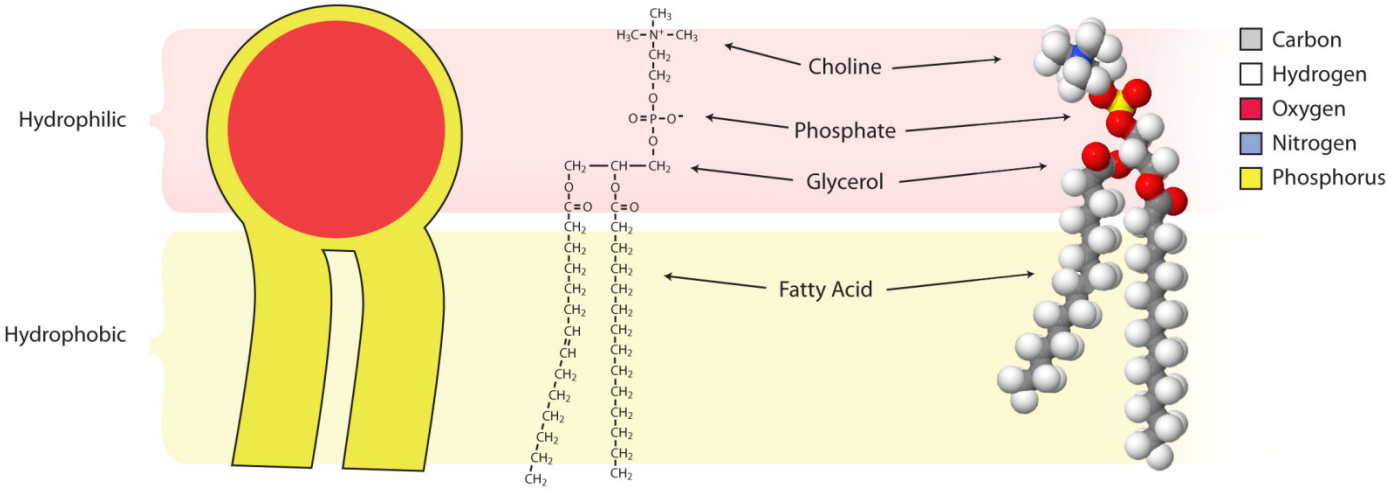
1b. Compare your structure to that of the other groups in the room. Record any similarities you observe in these phospholipid structures.
(All of the phospholipids have a hydrophilic head that contains a phosphate group and a glycerol. In addition, there are two hydrophobic tails in each phospholipid illustrated.)

1c. Based on these similarities a simplified representation may also be used to indicate phospholipid structure. Sketch this simplified model in the space below. Label the hydrophobic and hydrophilic portions of this simplified model.



1d. Record any differences in the specific structures you have observed between these phospholipids.
(The hydrophilic head contains some compositional differences. There are four major phospholipids that comprise the plasma membrane. Phosphatidylcholine and sphingomyelin make up the outer leaflet layer of the membrane while phosphatidylethanolamine and phosphatidylserine make up the inner leaflet layer of the membrane. A fifth phospholipid, phosphatidylinositol is also found in the inner leaflet layer of the plasma membrane. Although phosphatidylinositol is a minor membrane component, it plays a major role in cell signaling.)

The general structure of a phospholipid is most often represented by the phosphatidylcholine structure:



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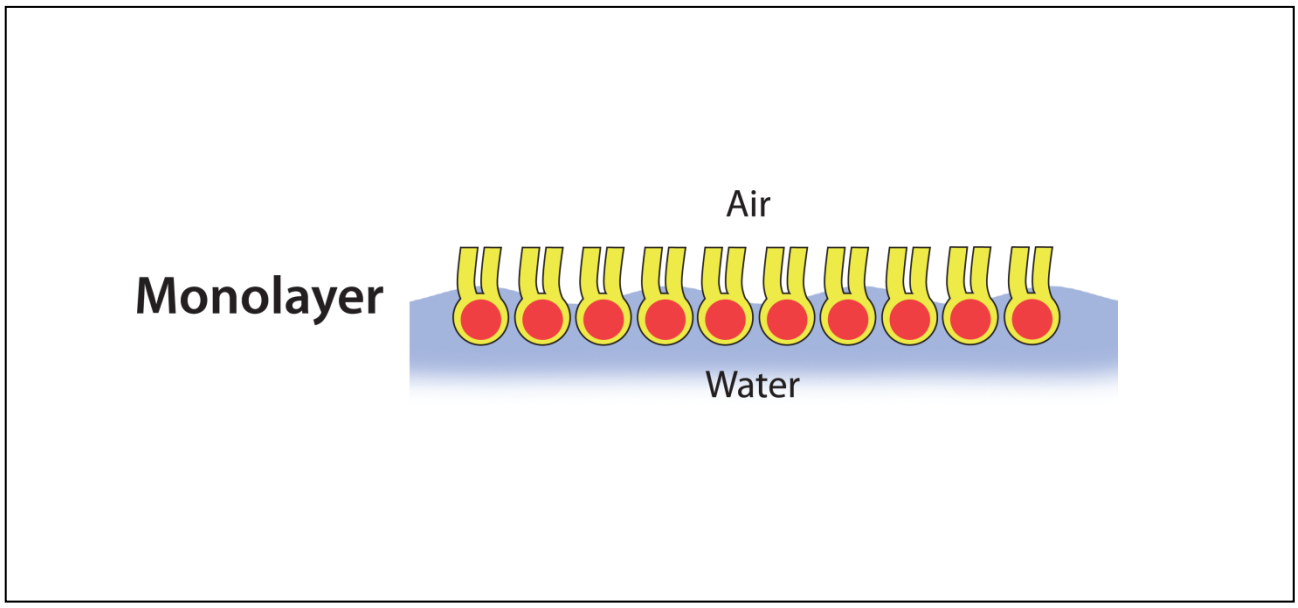
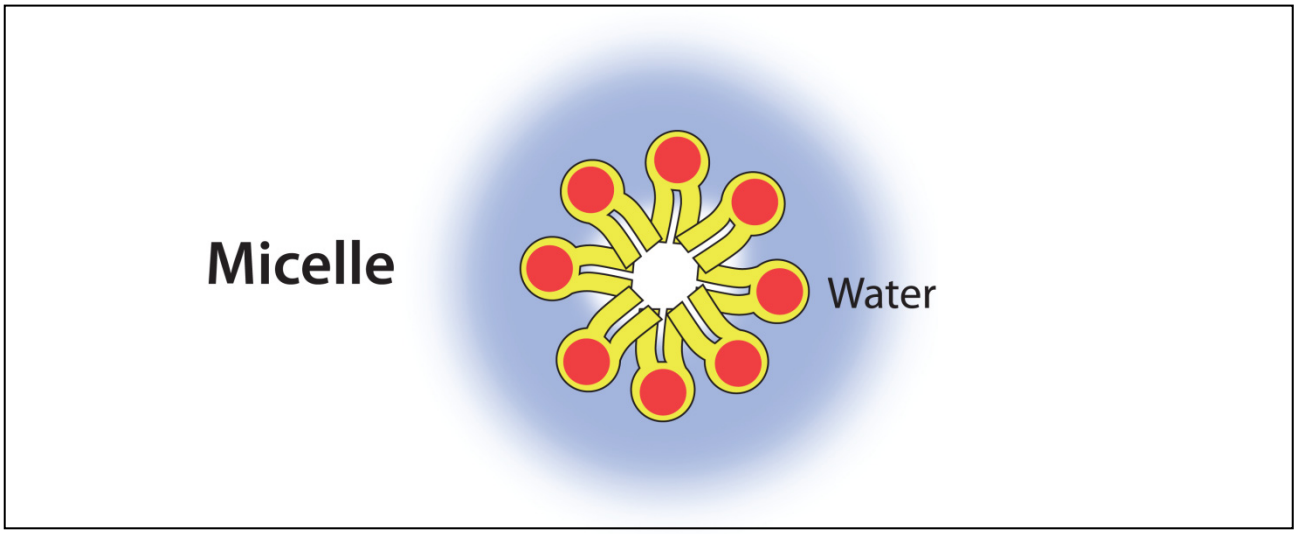
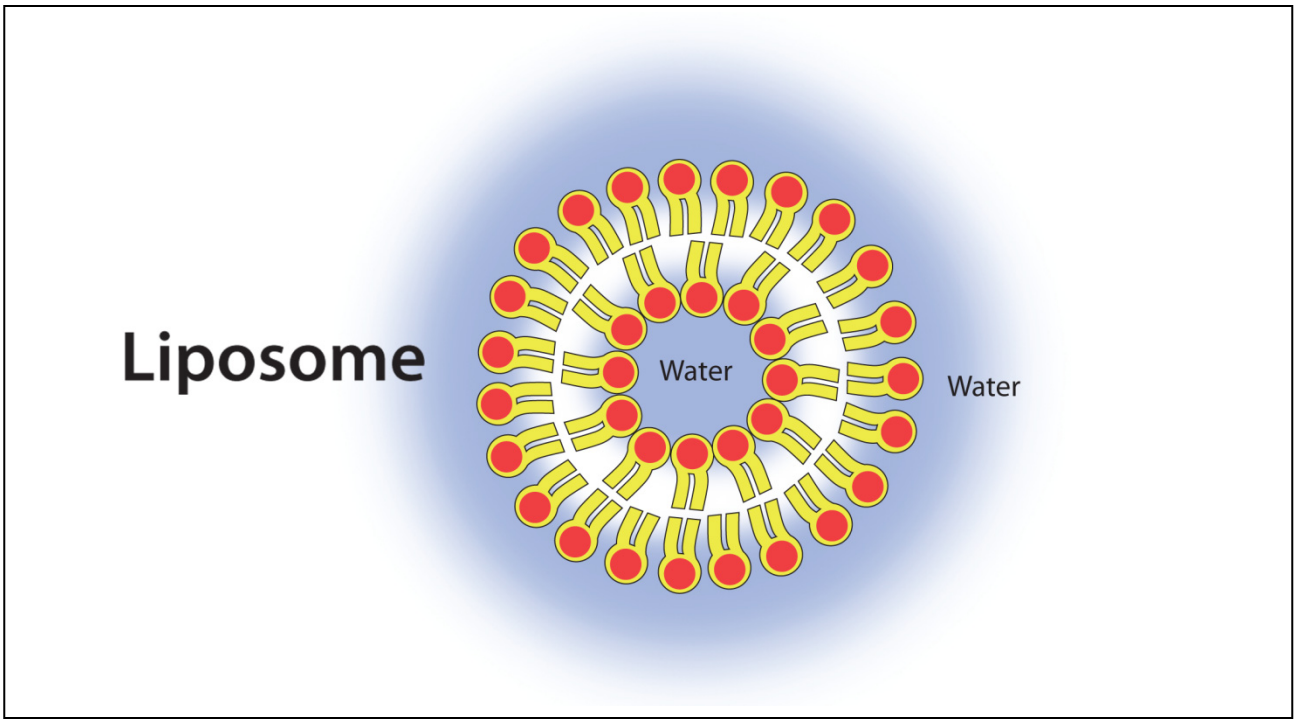


2b. Using the same ten phospholipids, rearrange them in the beaker so that they are submerged in the water while still maintaining the correct hydrophobic/hydrophilic interactions. Sketch your result below.



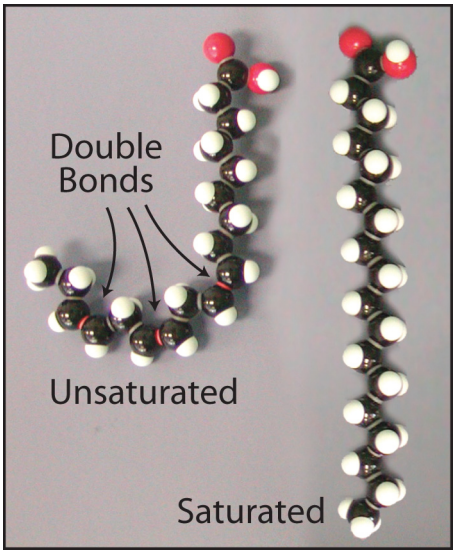
The resulting structure is referred to as a **micelle.** Micelles can act as emulsifiers that will allow a compound that is normally insoluble (in the solvent being used) to dissolve.

2c. Your next challenge is to construct a structure that is BOTH submerged in the water AND contains water on the inside. You may use as many of the phospholipids in your kit as you wish to complete the task. Sketch the resulting structure below.



2d. Based on the structure of a phospholipid, explain the reasoning for your arrangement.
(Because a phospholipid is amphipathic, this molecular arrangement shelters the hydrophobic tails of the phospholipids from water while exposing the hydrophilic heads to water.)

The resulting structure is referred to as a **liposome**. Liposomes are artificially prepared vesicles that may be used as a vehicle for administration of nutrients or pharmaceutical drugs.

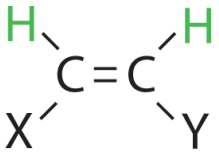


Upon closer examination of the hydrophobic tails, you may notice that in one of the tails, all of the carbons are connected with single bonds. These hydrocarbon tails are referred to as **saturated**. In the second tail there is a red connector, indicating that a double bond connects these two adjacent carbons. The presence of a double bond creates a "kink" in the structure of this hydrocarbon chain. The hydrocarbon tails containing a double bond or bonds between adjacent carbons are referred to as **unsaturated**.

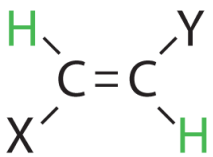
2e. Develop an explanation for the necessity of having "kinks" in the hydrophobic tail of the phospholipids that make up cell membranes.
(Because of the kinks in the tails where double bonds are located, unsaturated hydrocarbon tails cannot pack together as closely as saturated hydrocarbon tails contributing to the membrane fluidity.)

Note to Teachers - Optional Activity:

Further comparison shows a difference in these double bonds. If the hydrogens associated with the double bonded carbons are on the same side, the configuration is said to be "cis". If the hydrogens associated with the double bonded carbons are on opposite sides, the configuration is referred to as "trans". (See illustrations below.) Teachers may also opt to include a discussion of "trans fats" using these models.



cis



trans

2f. Which configuration produces the bigger “kink” in the structure of the hydrophobic tail?
(cis configuration)

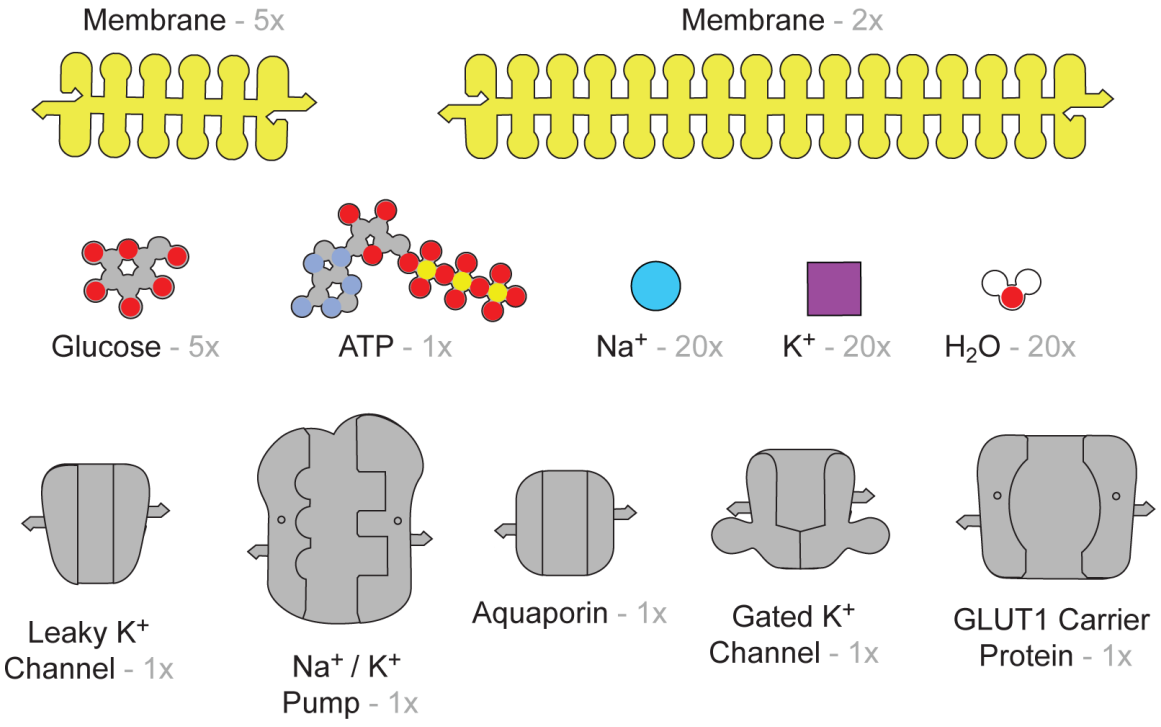
2g. How might this configuration contribute to the fluidity of the membrane?
(The “bend or kink” in the hydrophobic tail prevents these phospholipid tails from packing tightly together. The cis configuration would increase the fluidity of the membrane.)

Part 3: Exploring Membrane Permeability

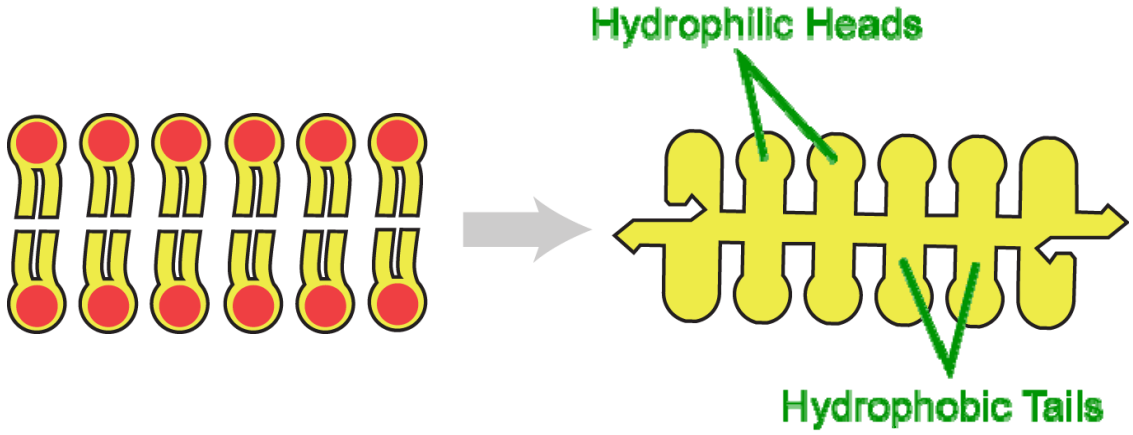
Introduction:

The phospholipid bilayer is only one aspect of the gatekeeper system responsible for the plasma membrane’s **selective permeability**. Membrane bound proteins play a key role in regulating the transport of ions and molecules through the plasma membrane.

Materials:



We will use a slightly different and simplified representation of the phospholipid bilayer in the next activity.



3a. Label the hydrophilic head and hydrophobic tail in the model pictured above.

3b. Nonpolar molecules, such as hydrocarbons, CO_2 and O_2 are hydrophobic. Explain why these molecules can easily cross the plasma membrane without the aid of proteins. (The hydrophobic nature of the interior of the membrane allows these hydrophobic molecules to cross the plasma membrane. Additionally, CO_2 and O_2 are small molecules that may diffuse across the membrane.)

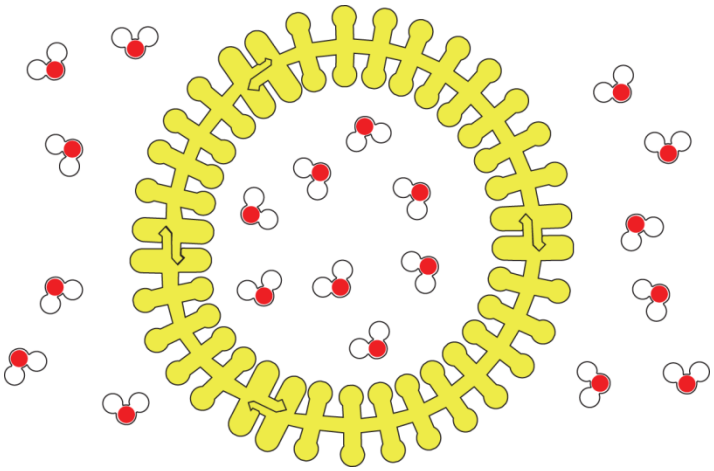
A variety of polar molecules and can't move through the plasma membrane on their own. Contact with hydrophobic lipid bilayer may be avoided by these hydrophilic substances as they cross the plasma membrane with the help of **transport proteins**.

Part 4: Channel Proteins

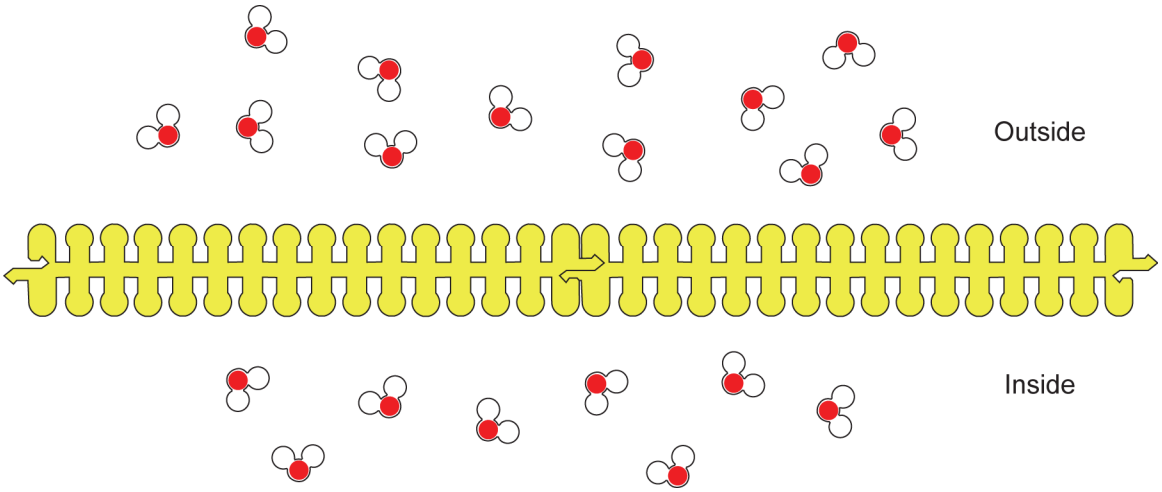
Some transport proteins, referred to as **channel proteins**, function by having a hydrophilic channel that certain molecules or ions use to cross the plasma membrane. There is a common misconception that a polar water molecule can easily cross the hydrophobic bilayer of the plasma membrane. Simple diffusion of water across the membrane does not occur at a rate fast enough in order to meet the survival needs of a cell. Channel proteins known as **aquaporins** allow entry of up to three billion (3×10^9) water molecules per cell per second!

4a. Construct a physical representation (a model) of the passage of water through a plasma membrane.

Use two short pieces and two long pieces of the simplified phospholipid membrane. Connect the pieces to form a "cell". We suggest placing 8 of the water molecules inside the cell (Intracellular) and 12 water molecules outside the cell (extracellular).

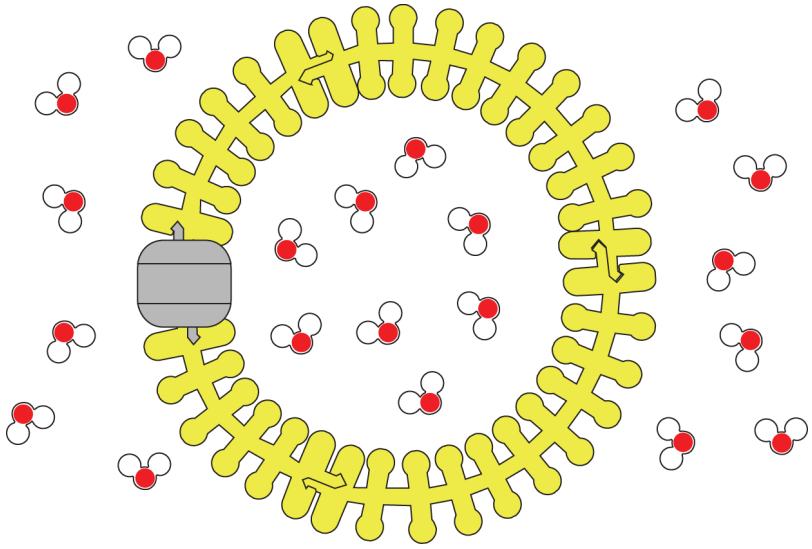


Note to Teachers - You may opt to set up the membrane in a linear fashion (shown below) if more conducive to your teaching environment.



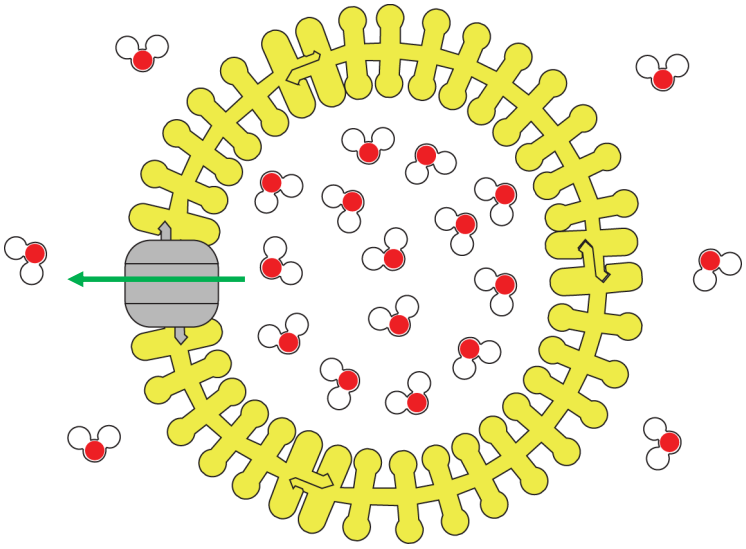
4b. Explain why water would have a difficult time diffusing across the cell membrane. Keep in mind the structure of water in your answer.
(The polar nature (slight negative charge on the oxygen end and slight positive charge on the hydrogen end) of water is not conducive to interaction with the hydrophobic phospholipid bilayer of a cellular membrane.)

A substance will generally diffuse from where it is more concentrated to where it is less concentrated. In other words, the substance will diffuse down its **concentration gradient**. Insert a channel protein model, in this case representing **aquaporin**, into the cell membrane you have constructed. Aquaporin is an example of a channel protein found in the plasma membrane which transports water (see diagram at right).



4c. Move the water molecules to indicate the net flow of water in this system. What direction did the water molecules move?
(In this configuration water molecules will move to the inside of the cell, moving from an area of high water molecule concentration to an area of low water molecule concentration.)

4d. Construct a system where intracellular water molecule concentration is higher than extracellular water molecule concentration. Sketch your model in the space below and indicate the net flow of water.

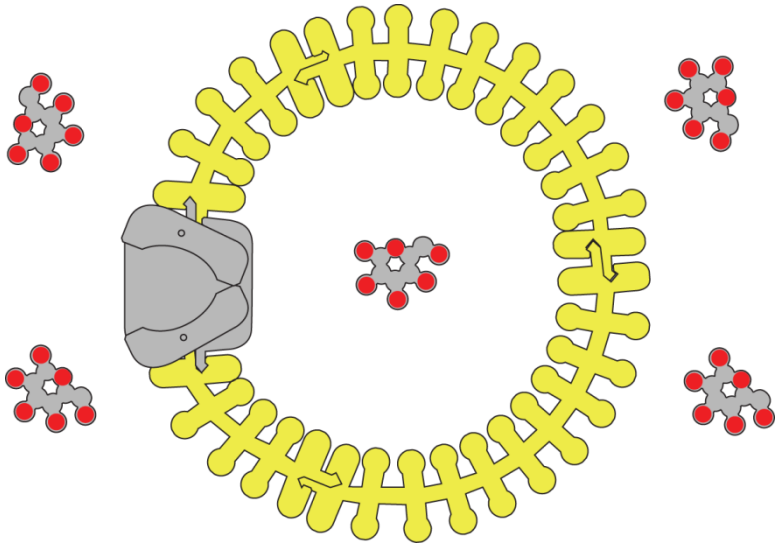


Note to Teachers -
You may choose to use the kit to introduce the students to the terms hypertonic, hypotonic and isotonic at this time.

4e. Predict what will happen to the cell due to the movement of the water.
(Since water is leaving the cell, the cell will shrink in size.)

In order for water move across the cell membrane in the above cases, the cell did **not** have to expend cellular energy (usually in the form of ATP) to move water across the plasma. Movement of a substance across a membrane without the expenditure of energy is referred to as **passive transport**. Likewise, when the cell must expend energy to move a substance against its concentration gradient the process is referred to as **active transport**. Additionally, the channel protein aquaporin assisted in passively moving water across the membrane. This occurrence may be referred to as **facilitated diffusion**.

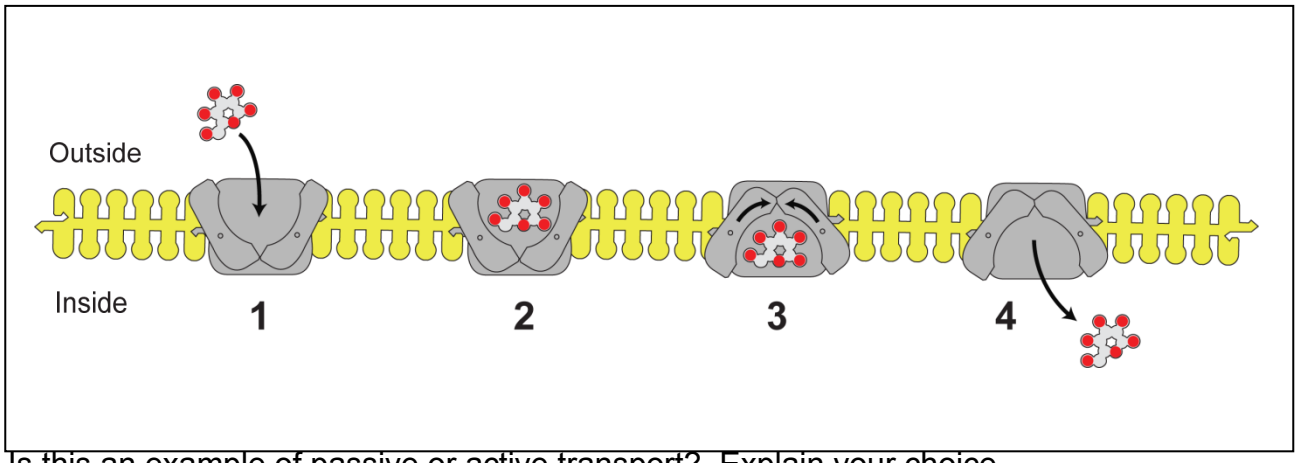
Part 5: Carrier Proteins



Remove the water molecules and aquaporin representation from the model cell you have constructed. Insert the carrier protein model into the plasma membrane model. Distribute the glucose molecules so that there are more extracellular glucose molecules than intracellular glucose molecules (see diagram).

A **carrier protein** binds a solute molecule on one side of the membrane, undergoes a shape change (conformational change) and deposits the solute molecule on the other side of the membrane. GLUT 1 is an example of a protein channel frequently found in the plasma membrane of red blood cells that facilitates the movement of glucose across the cell's plasma membrane.

5a. Use the model to demonstrate the movement of glucose across the cell membrane. Sketch your model in the space below.

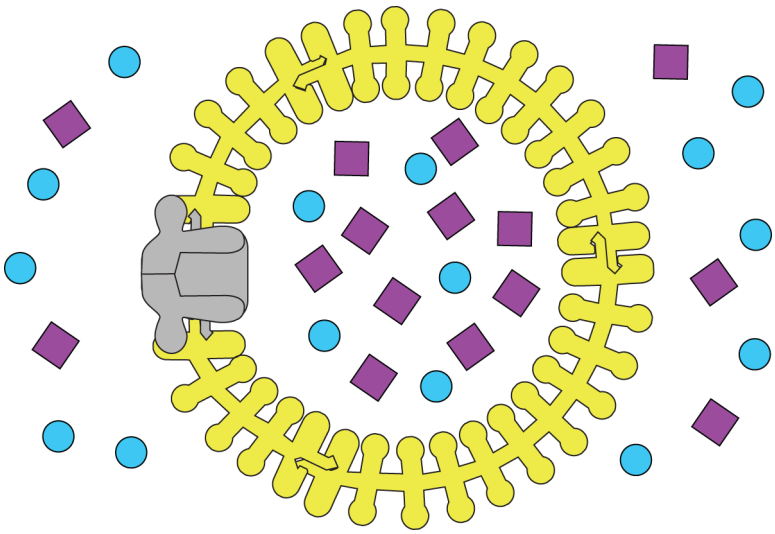


5b. Is this an example of passive or active transport? Explain your choice.

(Movement of glucose in this case is an example of passive transport because the cell does not need to expend energy to move the molecule against its concentration gradient.)

Part 6: Gated Channels

Remove the glucose molecules and carrier protein from the model cell you have constructed. Insert the gated channel protein model into the plasma membrane model (see diagram). Place 5 sodium ions (**round**) and 10 potassium ions (**square**) inside of the cell to simulate the intracellular environment ion concentrations. Place 10 sodium ions and 5 potassium ions outside of the cell to simulate the extracellular environment ion concentrations.



Gated channels are channel proteins that open or close in response to a stimulus. In nerve cells, a stimulus opens the gated sodium channel to allow a stream of sodium ions to enter the cell. Other gated channels open or close when a substance, different from the one to be transported, binds to the channel.

Begin the simulation with the sodium gated channel closed. After a stimulus the gates swing open.

6a. Based on what you know about concentration gradients, what direction will the sodium ions move through the channel?
(There are less sodium ions on the inside of the cell than the outside. Sodium ions will tend to move into the cell.)

6b. Does this gated channel demonstrate passive or active transport? Explain your answer.

(Sodium ions are moving from high concentration to low concentration. This is an example of passive transport because no energy is expended pushing them against their concentration gradient.)

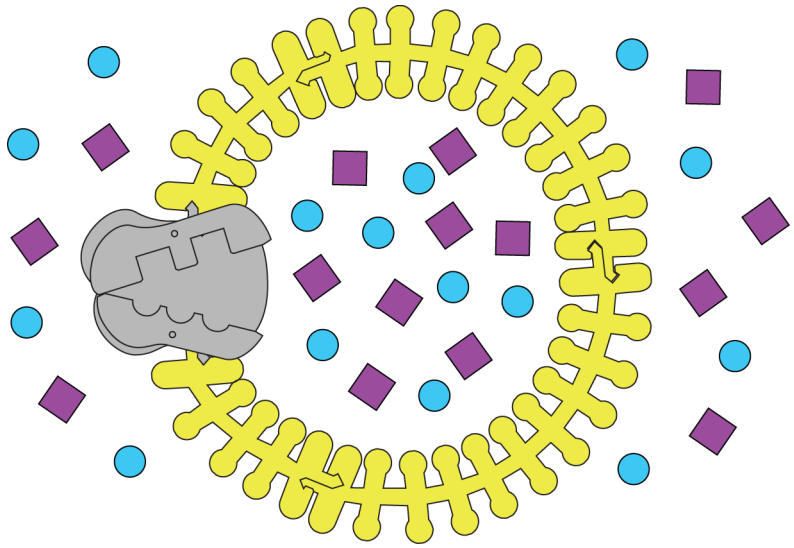
6c. Why may sodium ions move through the sodium channel while potassium ions typically do not?
(The sodium channel protein structure is such that sodium ions are allowed passage while potassium ions are not. Sodium ions are hydrated when they pass through the channel while potassium ions are not hydrated when passing through their channel.)

6d. Speculate what other stimului may affect the operation of channel proteins.
(Ligands may bind to a protein to trigger their opening or closing.)

6e. Devise a question you might have about the operation of this channel protein.
(Various answers)

Part 7: Active Transport - The Sodium-Potassium Pump

Remove the gated channel from the model cell you have constructed. Instead, insert the sodium-potassium pump protein into the membrane of the model. (See diagram below.) Place 7 sodium ions (**round**) and 8 potassium ions (**square**) inside of the cell to simulate the intracellular environment ion concentrations. Place 8 sodium ions and 7 potassium ions outside of the cell to simulate the extracellular ion concentrations.



Transport proteins that move solutes against their concentration gradients are all carrier proteins. The sodium-potassium pump is a special carrier protein that moves sodium ions against their gradient OUT of the cell and potassium ions against their gradient IN to the cell.

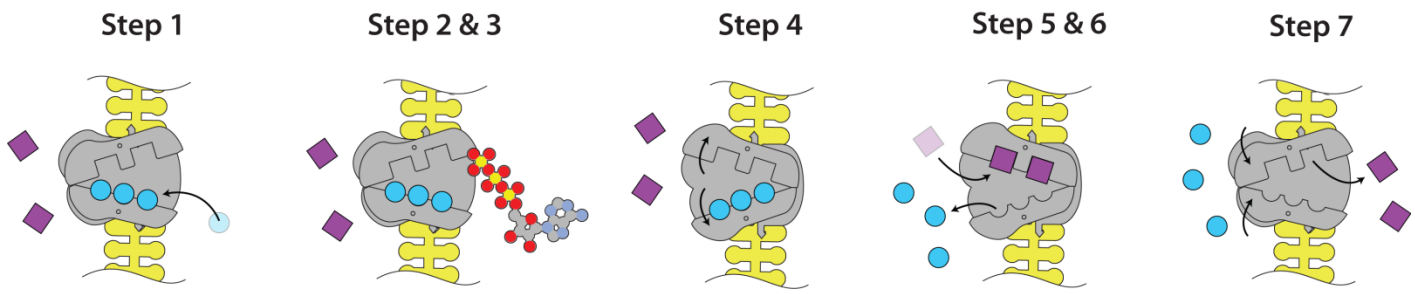
Because these ions are moving against their concentration gradients, the cell must expend energy to do the work resulting in **active transport** of these ions.

A typical animal cell has a much higher concentration of potassium ions (K^+) and a much lower concentration of sodium ions (Na^+) on the inside of the cell than the outside. The sodium-potassium pump uses energy in the form of ATP to move these ions against their concentration gradients and establish the "normal" intracellular ion concentrations. We will demonstrate the action of the sodium-potassium pump in the following simulation:

Set the sodium-potassium pump so that it is open to the inside of the cell as shown in the diagram on the previous page (page 14).

Record the initial ion concentrations in the table provided below:

Ion Types	Initial Amount	Amount After First Cycle	Amount After Second Cycle
Intracellular Na^+	**7**	**4**	**1**
Intracellular K^+	**8**	**10**	**12**
Extracellular Na^+	**8**	**11**	**14**
Extracellular K^+	**7**	**5**	**3**



Step 1: Bind three three intracellular sodium ions to the appropriate spots in the protein.

Step 2: Bring the ATP in close proximity to the pump.

Step 3: Sodium ion binding stimulates phosphorylation of the pump protein by ATP. In other words, a phosphate group is added to the sodium-potassium pump from the ATP molecule. (You will not be able to demonstrate this step with the model).

Step 4: Phosphorylation causes a change in the shape of the protein. You can demonstrate this by "swinging" the sides of the protein so that it opens to the outside of the cell.

Step 5: The shape change reduces the protein's binding affinity for sodium ions and increases the binding affinity for potassium ions. Remove the sodium ions from the protein and deposit them outside the cell and bind two potassium ions to the appropriate spots in the protein.

Step 6: Potassium ion binding triggers the release of the phosphate group from the protein. (Again, you will not be able to demonstrate this step with the model).

Step 7: Loss of the phosphate group results in the restoration of the protein's original shape which then releases the potassium ions. Swing the sides of the protein back so that they open to the inside of the cell and deposit the potassium ions.

Step 8: Repeat this process one more time.

Record the ion concentrations after completing the first cycle of the action of the sodium-potassium pump.

7a. What is the initial overall positive charge inside the cell compared to the outside the cell?
(There are 15 positive charges inside the cell as compared to 15 positive charges outside the cell.)

7b. Compare the total intracellular positive charge to the total extracellular positive charge after one cycle of the sodium-potassium pump.
(There are 14 positive charges inside the cell while there are 16 positive charges outside of the cell.)

Record the ion concentrations after completing the second cycle of the action of the sodium-potassium pump. Compare the total intracellular positive charge to the total extracellular positive charge after the second cycle of the action of the sodium-potassium pump.

7c. Where is the sodium ion concentration highest at the beginning of the sodium-potassium pump cycle?
(Sodium ion concentration is highest outside of the cell.)

7d. Where is the potassium ion concentration highest at the beginning of the sodium-potassium pump cycle?'
(Potassium ion concentration is highest inside the cell.)

7e. What is the initial overall charge of the inside of the cell compared to the outside?
(There are an equal amount of positive charges inside and outside the cell.)

7f. Why is ATP required in this process?
(In order for the cell to move sodium and potassium against their concentration gradients, energy must be expended by the cell.)

7g. After one cycle of the sodium-potassium pump, compare the overall charge of the inside of the cell to the outside? Explain how the distribution of ions changed.
(The inside of the cell has fewer positive ions than the outside of the cell. The sodium-potassium pump moves three sodium ions out of the cell for every two potassium ions in resulting in a redistribution of charge.)

7h. Is the sodium-potassium pump a channel protein or a carrier protein? Explain your answer.
(The sodium-potassium pump is a carrier protein because it is binds a substance, undergoes a shape change and deposits the substance on the other side of the plasma membrane.)

7i. Devise a question you might have about the function of the sodium-potassium pump.
(Various)

Fun Fact: In nerve cells, the sodium-potassium pump helps to reestablish the resting ionic concentrations after the nerve cell has fired.

Reference:
The Cell, 2nd edition
A Molecular Approach
Geoffrey M Cooper.
Boston University
Sunderland (MA): Sinauer Associates; 2000.
ISBN-10: 0-87893-106-6

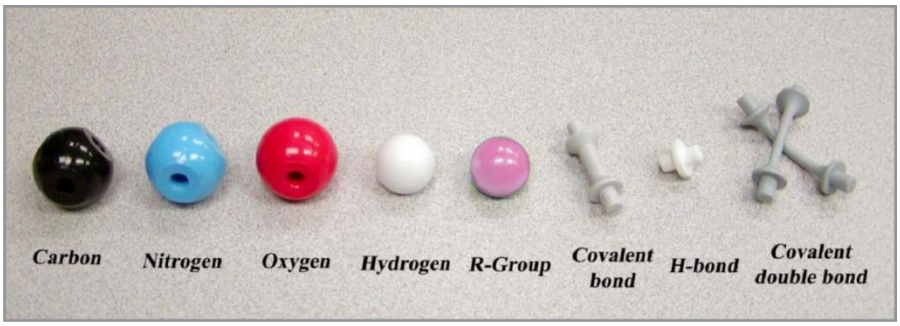
Center for BioMolecular Modeling

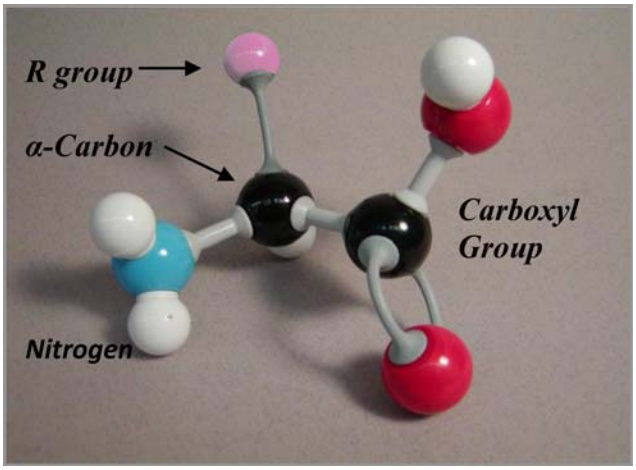
Amino Acids are the Building Blocks of Proteins

The purpose of this activity is to introduce students to the structure of an amino acid displayed in a ball and stick model format. Each atom is represented by a colored sphere; covalent bonds and hydrogen bonds are represented by "sticks". Students will build an amino acid and identify the atoms and parts of an amino acid. Students will build a dipeptide and identify components of the dipeptide. After this activity, students should be able to recognize an amino acid and identify the atoms. This knowledge is necessary to master specific commands in RasMol.

Molymod® Kit Contents:

4	Carbon (black)
4	Oxygen (red)
2	Nitrogen (blue)
2	R-groups (green), representing sidechains
10	Hydrogen (white)
6	Covalent bonds-single (thick, short, gray)
6	Covalent bonds-double (thin, long, gray)
10	Hydrogen bonds (short, white)
1	Molymod® link remover



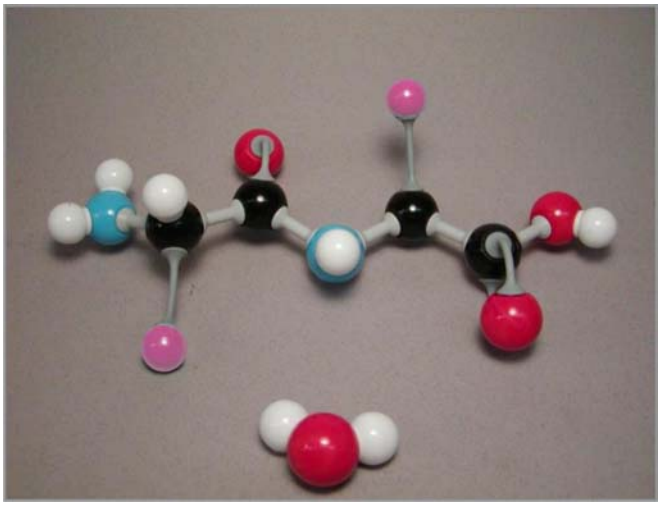


Activity:

1. Construct two separate amino acids using the Molymod® atoms, covalent bonds, and hydrogen bonds.
   a. Identify the following components: amino group, carboxyl group, the R-group or sidechain, α-carbon, carboxyl carbon, nitrogen. *(see labeled diagram above)*

b. Compare the two amino acids that have been built. Are they identical? How might two amino acids be different? *Amino acids are identical because they share the same "core" structure of NH_3-CHR-COOH. Amino acids are different because the composition of the "R-group" is different for each of the 20 amino acids. A second way that the amino acid structures may be different is their stereochemistry. The arrangement of atoms around the α-carbon may be "right-handed" or "left-handed" to form a D-amino acid or an L-amino acid. The L-amino acids are the naturally occurring form used to make proteins.*

2. Two amino acids can be chemically linked by a reaction called "condensation" to form a peptide bond linking two amino acids. A chain of amino acids linked by peptide bonds is called a polypeptide. Using the two amino acids built in step 1, create a dipeptide.



   a. What are the products of the condensation reaction? *The products are a dipeptide and a molecule of water.*
   b. Identify the following components of the dipeptide: amino groups, amino terminal end, carboxyl groups, carboxyl terminal end, carbonyl group, peptide bond, R-groups or sidechains, α-carbon, carbonyl carbon.

Teaching Points:

- Amino acids are the building blocks of protein
- Amino acid structure
  a. Identify atoms: nitrogen, oxygen, carboxyl carbon, α-carbon, oxygen, hydrogen
  b. Identify groups: amino terminal, carboxyl terminal, R-group or sidechain,
- There are twenty different amino acids
  a. Amino acids are identical because they share a core structure
  b. Amino acids are different because they have unique R-groups or sidechains
- Linear chain of amino acids is a polypeptide
- Primary sequence of protein is the linear sequence of amino acids

3-D Molecular Designs
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Amino Acid Starter Kit

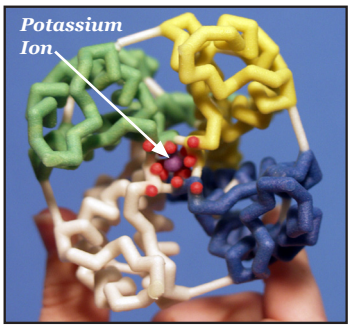
Key for Student Handout 1

Amino Acids - Building Blocks of Proteins

Introduction

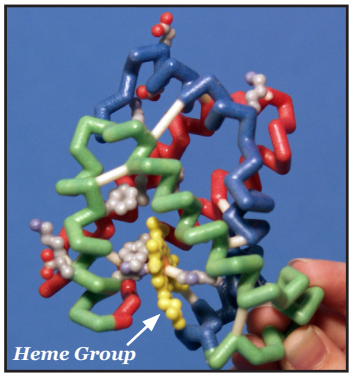
Proteins are more than an important part of your diet. Proteins are complex molecular machines that are involved in nearly all of your cellular functions. Each protein has a specific shape **(structure)** that enables it to carry out its specific job **(function)**.

A **core idea** in the life sciences is that ***there is a fundamental relationship between a biological structure and the function it must perform.*** At the macro level, Darwin recognized that the structure of a finch's beak was related to the food it ate. This fundamental structure-function relationship is also true at all levels below the macro level, including proteins and other structures at the molecular level. *For two examples of proteins and their functions, see the photos and cutlines at the right.*



The potassium channel (above) spans cell membranes and regulates the passage of potassium ions in and out of cells. It folds into a "pore" for the potassium ion to pass through.

The β-globin protein (below) transports oxygen in blood. It accomplishes this with the heme group (yellow structure in photo) in which an iron atom binds to O2. Other proteins perform other functions.



In this activity, you will explore the structure of proteins and the chemical interactions that drive each protein to fold into its specific structure, as noted below.

- Each protein is made of a specific sequence of **amino acids**. There are 20 amino acids found in proteins.
- Each amino acid consists of two parts — a **backbone** and a **sidechain**. The backbone is the same in all 20 amino acids and the sidechain is different in each one.
- Each sidechain consists of a unique combination of atoms which determines its 3D shape and its chemical properties.
- Based on the atoms in each amino acid sidechain, it could be ***hydrophobic, hydrophilic, acidic (negatively charged), or basic (positively charged)***.
- When different amino acids join together to make a protein, the unique properties of each amino acid determine how the protein folds into its final 3D shape. The shape of the protein makes it possible to perform a specific function in our cells.

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Amino Acid Starter Kit

Chemical Properties Circle & Amino Acid Chart

Hydrophobic and Hydrophilic Properties

What do you think hydrophobic means? Separate the word 'hydrophobic' into its two parts — hydro and phobic. Hydro means water and phobia means fear or dislike, so hydrophobic sidechains don't like water. Hydrophobic sidechains are also referred to as non-polar sidechains.

Now can you guess what hydrophilic means? Philic means likes or attracted to, so hydrophilic sidechains like water. Hydrophilic sidechains are also referred to as polar sidechains.

Acidic (Negatively Charged) and Basic (Positively Charged) Properties

Can you think of acids you have around your house? Lemon and fruit juices, vinegar and phosphoric acid (in dark sodas) are common household acids. Acids taste sour and are typically liquids.

Can you think of bases you have around your house? Tums®, baking soda, drain cleaner and soap are common bases. Bases taste bitter and can be a liquid or solid.

What happens when you mix lemon juice or vinegar with baking soda? They neutralize each other, in a bubbling chemical reaction.

Preparation

The activities described in this handout primarily focus on amino acid sidechains. They will help you understand how the unique properties of each sidechain contribute to the structure and function of a protein.

First look at the components in your Amino Acid Starter Kit. Make sure your 1-group set has:

1. 1 **Chemical Properties Circle**
2. 1 Laminated **Amino Acid Sidechain List**
3. 4' **Mini-Toober**
4. 1 Set of **Red and Blue Endcaps**
5. 22 Clear **Bumpers**
6. 22 **Amino Acid Sidechains**
   - -1 each of the 20 Amino Acids
   - -1 additional cysteine and
   - -1 additional histidine
7. 22 Plastic **Clips**
   - -8 yellow
   - -8 white
   - -2 blue
   - -2 red
   - -2 green
8. 6 **Hydrogen Bond Connectors**

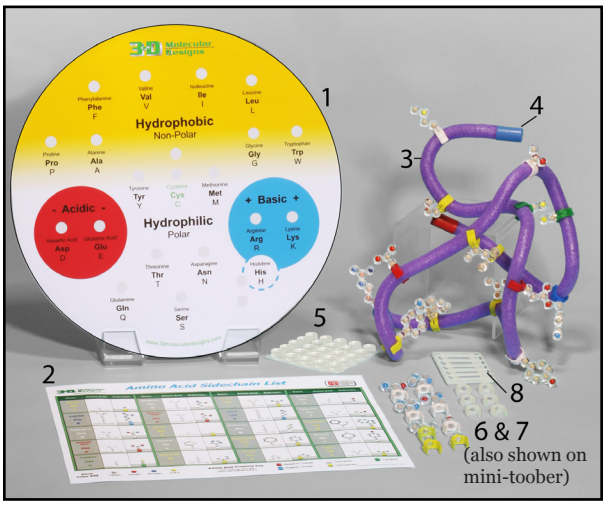

Photo shows a 1-Group Amino Acid Starter Kit.

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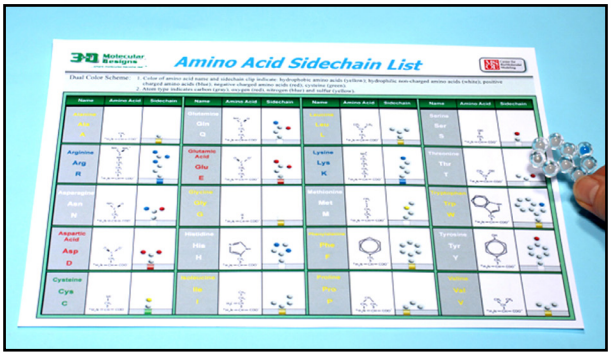
Chemical Properties Circle (continued)

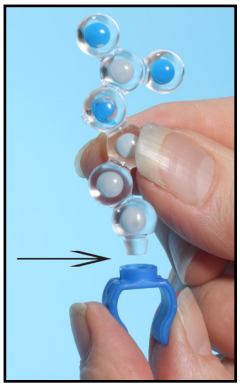
The colored areas on the Chemical Properties Circle, the color coding on the Amino Acid Sidechain List, the key below and the colored clips show the chemical properties of sidechains.

KEY

Hydrophobic Sidechains are **Yellow**
Hydrophilic Sidechains are **White**
Acidic Sidechains are **Red**
Basic Sidechains are **Blue**
Cysteine Sidechains are **Green**



Amino Acid Sidechain List.



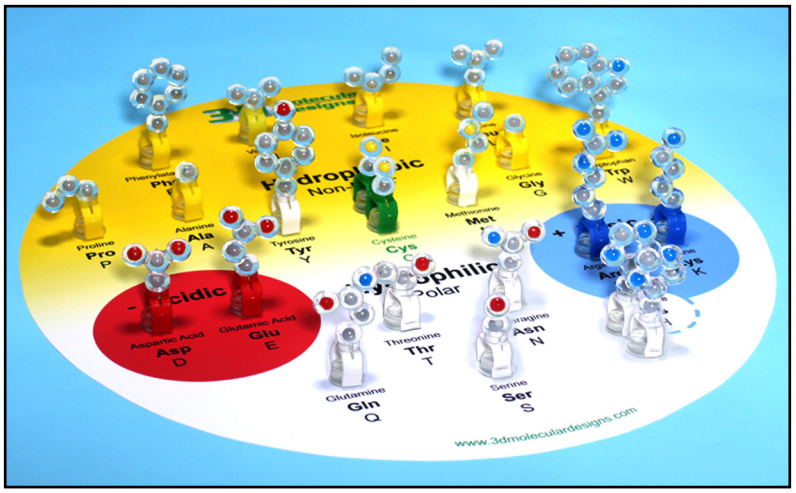
Insert sidechain into clip.

Directions

Select any sidechain and a colored clip that corresponds to the property of the sidechain. Insert the sidechain into the clip.

Place each amino acid sidechain attached to its clip on the bumper near its name and abbreviations. You will need to consult the Amino Acid Sidechain List in your kit to find the name of each sidechain, so you can position it correctly on the circle.

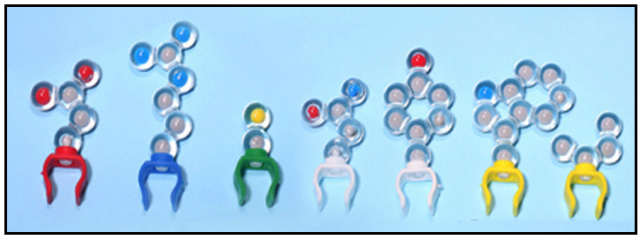
Place clip (with sidechain attached) onto the bumper.



Chemical Properties Circle with sidechains and clips.

Chemical Properties Circle (continued)

After each sidechain has been correctly positioned on the circle, look at the colored sheres in each sidechain. Scientists established a CPK coloring scheme (see chart below) to make it easier to identify specific atoms in models of molecular structures.



KEY

Carbon is **Gray**
Oxygen is **Red**
Nitrogen is **Blue**
Hydrogen is **White**
Sulfur is **Yellow**

Did you notice similarities of patterns in each group of sidechains? Describe Your Observations.

• Hydrophobic sidechains primarily contain _____carbon_____ atoms.

• Acidic sidechains contain two _____oxygen_____ atoms. This is called a carboxylic acid functional group.

• Basic sidechains contain ____one or two nitrogen____ atoms. This is called an amino functional group.

• Hydrophilic sidechains have various combinations of
oxygen, nitrogen and sulfur and carbon atoms.

• An exception to the above observation is:
Tryptophan - a hydrophobic amino acid that contains a nitrogen atom.

• **Optional Activity** - Amino Acids Jmol (see AASK Lessons on website)

Folding a 15-Amino Acid Protein

Once you have explored the chemical properties and atomic composition of each sidechain, think about how proteins spontaneously fold into their 3D shapes.

Predict what causes proteins to fold into their 3D shapes.

• Which sidechains might position themselves on the interior of a protein, where they are shielded from water?

The hydrophobic amino acids - tryptophan, leucine, isoleucine, valine, proline, alanine and glycine.

• From your experience with static electricity, which sidechains might be attracted to each other?

The basic amino acids (+ charge) and the acidic amino acids (- charge).

• Would the final shape of a protein be a high energy state or a low energy state for all of the atoms in the structure?

A low energy state.

Why?

A low energy state is more stable than a high energy state.

1. Unwind the 4-foot mini-toober (foam-covered wire) that is in your kit. Place a blue end cap on one end and the red end cap on the other end. The blue end cap represents the N-terminus (the beginning) of the protein and the red end cap represents the C-terminus (the end) of the protein (see photo on next page).

2. Choose 15 sidechains from the chemical properties circle as indicated in the chart below.

   Mix the Sidechains together and place them (in any order you choose) on your mini-toober.

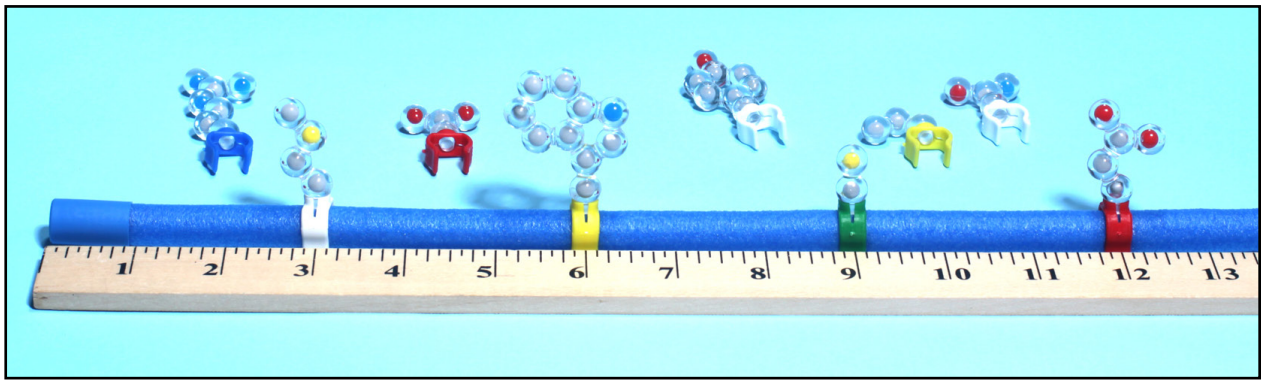
KEY

6 **Hydrophobic** sidechains
2 **Acidic** sidechains
2 **Basic** sidechains
2 **Cysteine** sidechains
3 **Hydrophilic** sidechains

Folding a 15-Amino Acid Protein (continued)

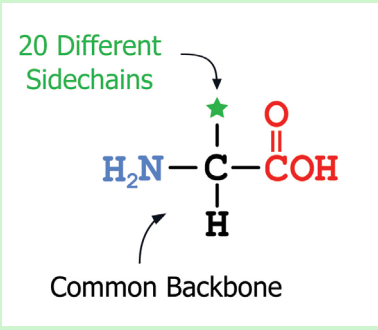
3. You may want to use a ruler to place your sidechains on you mini-toober.

Beginning at the N-terminus of your mini-toober, measure about three inches from the end of your mini-toober and slide the first colored clip with its sidechain onto the mini-toober. (See photo.) Place the rest of the clips three inches apart on your mini-toober until all are attached to the mini-toober.

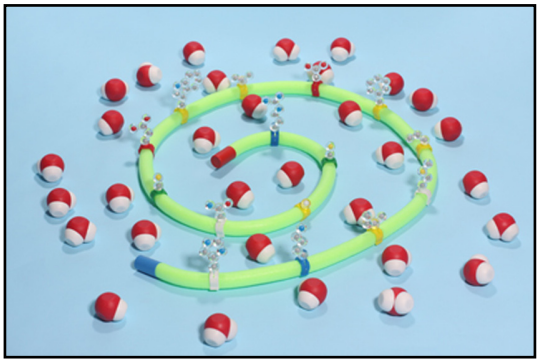


• This drawing represents the backbone section of an amino acid. What do you think the clips represent?

The alpha-carbon, because this is the atom that the sidechains are bonded to.



The *sequence* of amino acid sidechains that you determined when placing them on the mini-toober is called the **primary structure** of your protein. As a general rule the final shape of a protein is determined by its **primary structure**. Remember that protein folding happens in the watery environment of the cell.



Folding a 15-Amino Acid Protein (continued)

4. Now you can begin to fold your 15-amino acid protein according to the chemical properties of its sidechains. Remember all of these chemical properties affect the protein at the same time.

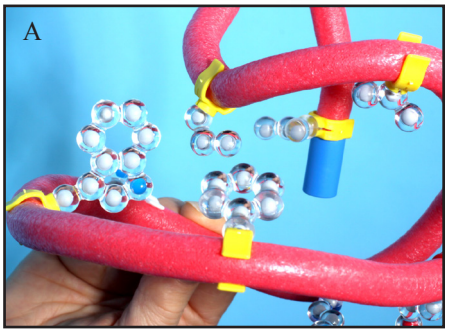


Photo A — Hydrophobic Sidechains
Start by folding your protein so that all of the hydrophobic (non-polar) sidechains are buried on the inside of your protein, where they will be hidden from polar water molecules.

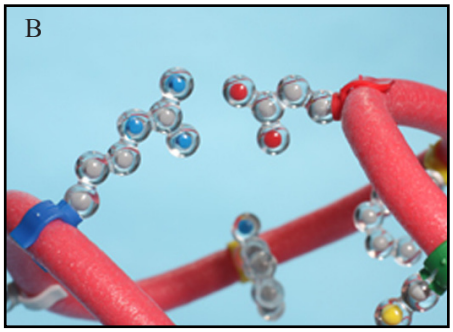


Photo B — Acidic & Basic Sidechains
Fold your protein so the acidic and basic **(charged)** sidechains are on the outside surface of the protein. Place one negative (acidic) sidechain with one positive (basic) sidechain so that they come within one inch of each other and neutralize each other. This positive-negative pairing helps stabilize your protein.

Note: As you continue to fold your protein and apply each new property listed below, you will probably find that some of the sidechains you previously positioned are no longer in place. For example, when you paired a negatively charged sidechain with a positively charged one, some of the hydrophobic sidechains probably moved to the outer surface of your protein. Continue to fold until the hydrophobic ones are buried on the inside again. Find a shape in which all the properties apply ***simultaneously***.

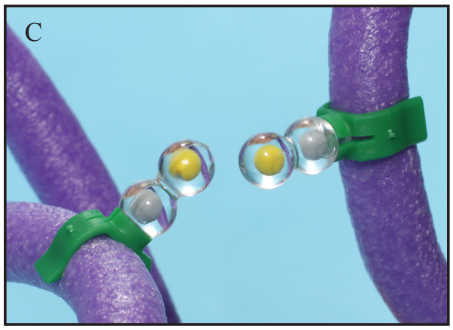


Photo C — Cysteine Sidechains
Fold your protein so that the two cysteine sidechains are positioned opposite each other on the inside of the protein where they can form a covalent-disulfide bond that helps stabilize your protein.

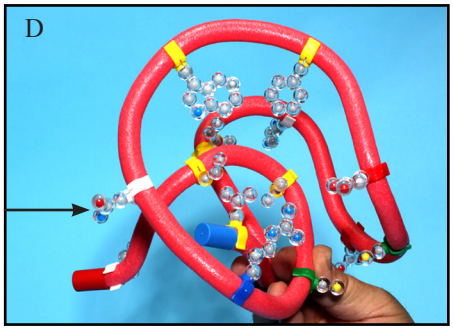


Photo D — Hydrophilic Sidechains
Continue to fold you protein making sure that your hydrophilic (polar) sidechains are also on the outside surface of your protein where they can hydrogen bond with water.

The final shape of your protein when it is folded is called the **tertiary structure.**

15-Amino Acid Protein Questions

• What happened as you continued to fold your protein and applied each new chemical property to your protein?

It became more compact and more complicated.

• Were you able to fold your protein, so that all of the chemical properties were in effect at the same time?

Yes. (Note to teachers: some students may answer "No".)

• If not, do you have any ideas why you weren't able to fold your protein in a way that allowed all of the chemical properties to be in effect simultaneously?

Some sequences simply do not allow for a single shape that simultaneously satisfies all the principles of chemistry that drive protein folding.

• Did your protein look like the proteins other students folded? No
Explain.

Because everyone had a different sequence of amino acids.

• How many different proteins, 15 amino acid long, could you make given an unlimited number of each of the 20 amino acids?

$20^{15} = 3.28 \times 10^{19}$

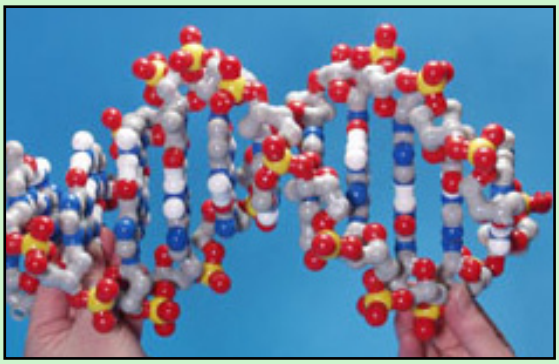
• Most real proteins are actually in the range of 300 amino acids long. How many different possible proteins, 300 amino acids in length, could exist?

$20^{300} = 2 \times 10^{390}$

15-Amino Acid Protein Questions (continued)

• Research how many different proteins are found in the human body. Hint: how many different genes are there in the human genome*?

25,000 or 2.5 x 10^4



• Assuming that all human proteins are 300 amino acids long, what fraction of the total number of possible different proteins is found in the human body?

1 x 10^{-386} = miniscule!

• Why do you think there are fewer actual proteins than possible ones?

Because only a relatively small number of amino acid sequences can adopt a stable shape that simultaneously satisfies all of the principles of chemistry.

* Completed in 2003, the Human Genome Project (HGP) was a 13-year project coordinated by the U.S. Department of Energy and the National Institutes of Health. During the early years of the HGP, the Wellcome Trust (U.K.) became a major partner; additional contributions came from Japan, France, Germany, China, and others.

Project goals were to:

- Identify all of the approximately 20,000-25,000 genes in human DNA,
- Determine the sequences of the 3 billion chemical base pairs that make up human DNA,
- Store this information in databases,
- Improve tools for data analysis,
- Transfer related technologies to the private sector, and
- Address the ethical, legal, and social issues (ELSI) that may arise from the project.**

*** U.S. Department of Energy Genome Programs website http://ornl.gov/sci/techresources/Human_Genome/home.shtml*

• **Optional Discussion:** Genes can code for multiple proteins through the process of alternative splicing.

15-Amino Acid Protein Questions (continued)

Record the sequence of amino acids in your protein, starting with the N-terminus (blue end cap). Use the single letter abbreviation for each amino acid (Methionine = M).

This is the **primary structure** of your protein.

In the space below, sketch the **tertiary structure** of your protein.

Discussion

Proteins perform critical functions in all our cells. Without proteins, life wouldn't exist. With your group or class, can you think of some of some specific proteins and describe what function they perform? *Proteins are involved in your metabolism, cell structure, immune system, DNA expression, protein folding, transport, movement, communication and storing energy.*

- **Optional Jmol Activity**
  - Basic Priciples of Chemistry that Drive Protein Folding Part 1 Jmol
  - Basic Priciples of Chemistry that Drive Protein Folding Part 2 Jmol

  (See AASK Lessons on website.)

The next student handout provides folding activities and information that will help you understand the **secondary structure** of proteins.

3-D Molecular Designs

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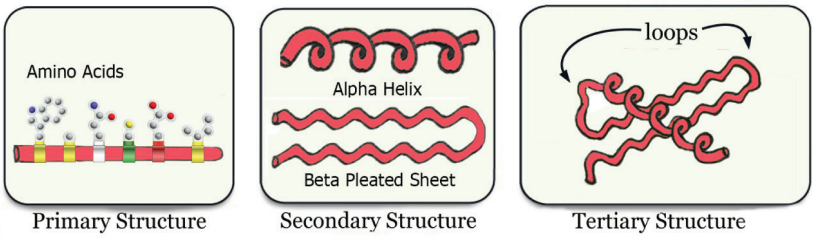
Amino Acid Starter Kit

Key for Student Handout 2

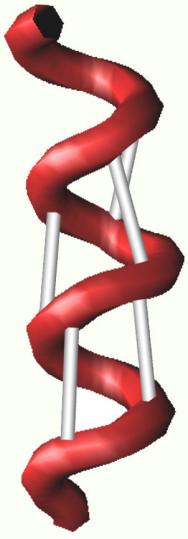
Secondary Structure

In the previous protein folding activity, you created a hypothetical 15-amino acid protein and learned that basic principles of chemistry determine how each protein spontaneously folds into its characteristic 3-dimensional shape. You learned that the sequence of amino acids in a protein (from N-terminus to C-terminus) is called its **primary structure**. The final folded, 3D shape of your protein is called its **tertiary structure**.

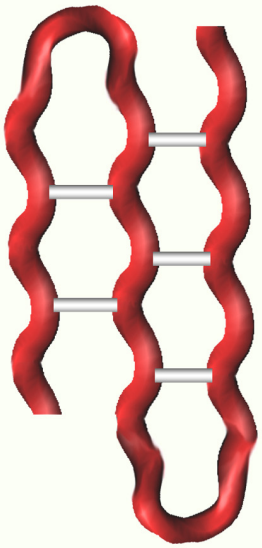
In this second protein-folding activity, you will learn about the **secondary structure** of proteins. This secondary structure consists of alpha helices and/or beta sheets. Proteins commonly contain a combination of alpha helices and beta sheets. Proteins can be described as a series of alpha helices and beta sheets, joined by **loops** of less regular protein structure.



Primary Structure | Secondary Structure | Tertiary Structure



An **alpha helix** is a compact right-handed helix, with 3.6 amino acids per turn of the helix. The amino acid sidechains are bonded to the alpha carbon of each amino acid and radiate outward from the helix. The alpha helix is stabilized by hydrogen bonds – weak bonds between the amino nitrogen of one amino acid (x), and the carbonyl oxygen of another amino acid (x+4) located four sidechains further along the chain.



A **beta sheet** is an extended, zig-zag structure in which individual strands are positioned parallel or anti-parallel to each other to form flat sheets in proteins. Since the amino acid sidechains are bonded to the alpha carbons of each amino acid, they are alternately orientated above and below the plane of the sheet. The beta sheet is stabilized by hydrogen bonds between the amino nitrogen of one amino acid and the carbonyl oxygen of another amino acid in an adjacent beta strand.

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Folding a Toober Model of the Zinc Finger

In this activity, you will fold a model of the first of three zinc fingers of the Zif268 protein. Zinc finger proteins regulate the transcription of DNA into mRNA – by binding to DNA and attracting RNA polymerase. A zinc finger protein contains two cysteine amino acids and two histidine amino acids which simultaneously bind to a single zinc atom. These four amino acids are contained within a 30 amino acid sequence that folds into a two-stranded beta sheet and short alpha helix. Many zinc finger proteins (like zif268) are composed of three consecutive fingers with similar features (motifs) which bind to a nine base pair sequence of double-stranded DNA.

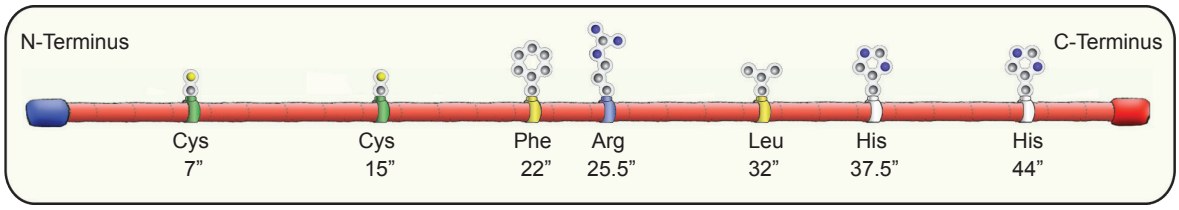
The primary structure of this zinc finger is below.

N-Terminus P Y A (C) P V E S (C) D R R (F) S (R) S D E (L) T R (H) I R I (H) T G C-Terminus

The sidechains of the seven circled amino acids in the above sequence will be included in the model you fold.

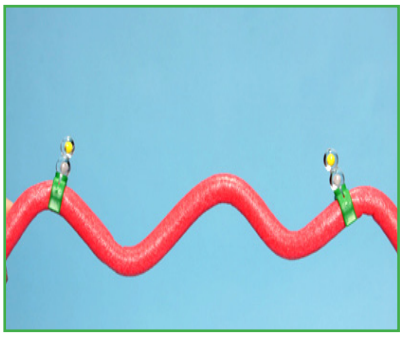
1. ***Primary Structure***

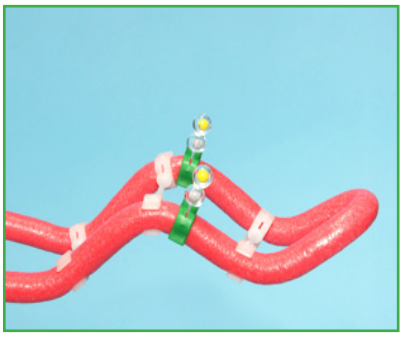
Map the positions of the seven amino acids on your mini-toober. Since the toober is 48 inches long and the zinc finger is 28 amino acids long, each amino acid occupies 1.7 inches of toober. Using a ruler, measure the distances shown below and add the appropriate sidechains to the mini-toober at each position.



2. ***Secondary Structure***

Fold the toober into its secondary structure. The first 13 amino acids (the first 22 inches from the N-terminus) should be folded into a 2-stranded beta sheet. This can be made by creating a zig-zag structure that is bent in the middle as shown in the photos below. Add the plastic hydrogen bonds connectors to your model as shown in the far right photo below.





Folding a Toober Model of the Zinc Finger (continued)

The last 15 amino acids of the zinc finger exist as a compact, right-handed alpha helix. This can be made by wrapping the mini-toober around your finger or an empty paper towel tube to create three full turns as shown in the photos below. Loosen the loops and add the hydrogen bond connectors as shown in the far right photo.

Your mini-toober should look similar to the one shown below.

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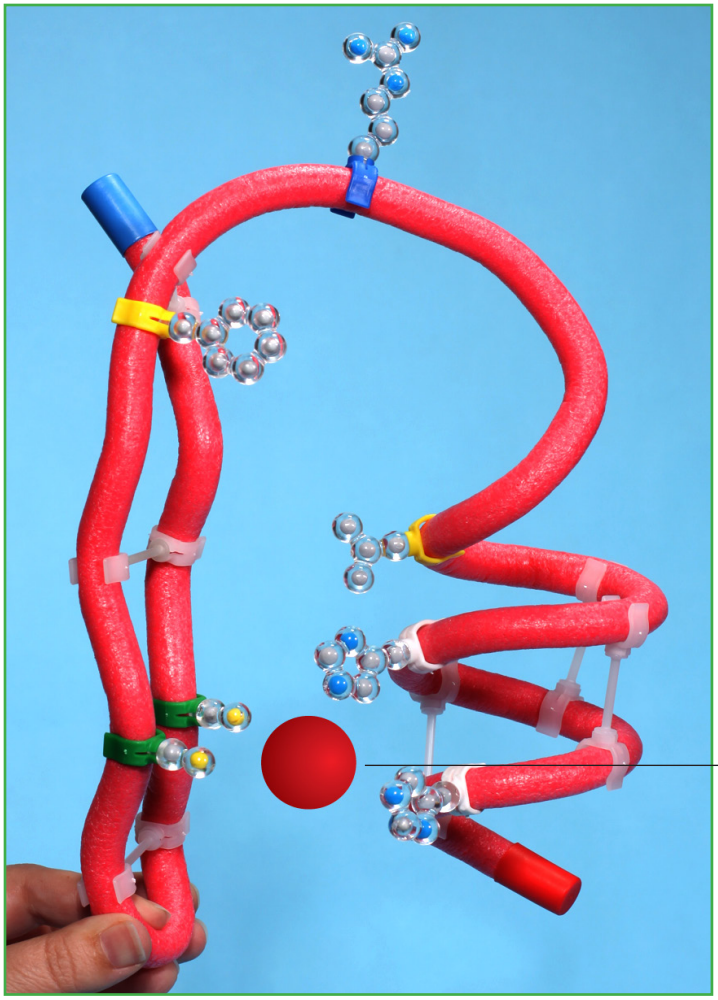
Folding a Toober Model of the Zinc Finger (continued)

3. *Tertiary Structure*

Fold the beta sheet and alpha helix into the final tertiary structure of the zinc finger.

In its final tertiary structure, the seven sidechains will be positioned such that:

- The two cysteine and two histidine sidechains will be oriented to simultaneously bind to a single zinc atom (not included) in the center of the structure (see photo).
- The two hydrophobic amino acid sidechains phenylalanine and leucine will be orientated toward the inside of the structure.
- The positively-charged arginine sidechain will be exposed at the top of the alpha helix, where it is available to bind to the negatively-charged phosphate backbone of DNA.



The zinc ion (not included with the kit) binds simultaneously to the two histidines and two cysteines.

As a folding guide, you can either use the photo shown below or the interactive Jmol image of a zinc finger on website (www.3dmoleculardesigns.com/resources.php).

Note: As you fold your mini-toober, you may need to rotate the sidechains around the mini-toober to make them adopt to the desired final shape.

Folding a Toober Model of the Zinc Finger (Questions)

1. Both alpha helices and beta sheets are stabilized by hydrogen bonds.
   - Which atoms share the hydrogen in these weak bonds?

   The nitrogen of an amino group and the oxygen of a carbonyl group.

   - Are these backbone atoms or sidechain atoms?

   Backbone atoms.

2. Describe the secondary structural elements that comprise a zinc finger:

   A 2-stranded beta sheet and a short alpha helix.

3. How is a zinc atom involved in the stabilization of the zinc finger motif?

   The zinc atom is simultaneously bound by the 2 cysteine and the 2 histidine sidechains.

4. Zinc fingers often bind to DNA. How might the arginine sidechain (positively-charged) shown on your model be involved in DNA binding?

   DNA has a negatively-charged phosphate backbone. Therefore, the positively-charged arginine of the zinc finger can bind to DNA via an electrostatic interaction.

- **Optional Activity** - Zinc Finger Jmol (see AASK Lessons on website)

Teaching Points on page 6.

Teaching Points

When proteins fold into their tertiary structures, there are often subdivisions within the protein, designated as domains, which are characterized by similar features or motifs. One such motif is the zinc finger in which a specific domain of the protein is arranged into a **finger-like** structure where two beta sheets and one alpha helix are positioned around a zinc ion. The zinc finger motif is commonly found in eukaryotic transcription factors, which are proteins that bind to specific sequences of DNA in order to regulate transcription.

One common class of zinc finger is the C_2H_2 class which is the one modeled in this collection. In this class of zinc fingers, the zinc ion is bound to two cysteine residues and two histidine residues.

3-D Molecular Designs

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Amino Acid Starter Kit

Key for Student Handout 3

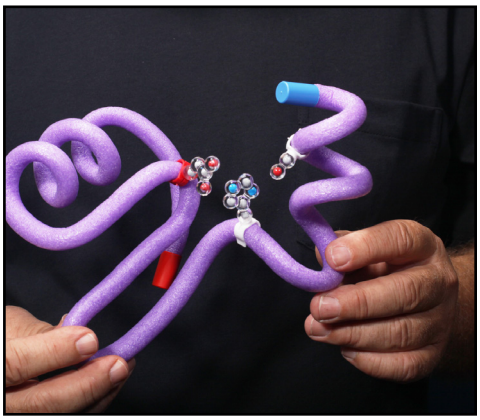
Understanding an Enzyme Active Site

In the first protein folding activity, you learned that a protein begins as a linear sequence (primary structure) of amino acids that spontaneously folds into a compact 3D shape (tertiary structure) following basic principles of chemistry.

In the second activity, you learned that the 3D shape of a protein consists of stretches of alpha helices and/or beta sheets (secondary structure) connected by short turns of less regular protein structure.

In the space below, draw and label examples of primary, secondary and tertiary structures.

Proteins perform many different functions in cells. Some proteins function as structural supports for the cell's architecture. Others transport small molecules — such as oxygen or neurotransmitters — between cells.



Enzyme Active Sites

In this third activity, you will explore enzymes — a major class of proteins. Enzymes bind a specific small molecule — a substrate — and then catalyze a chemical reaction that changes the substrate in some way. The active site of an enzyme is the **region** of the protein that is able to bind a specific substrate (usually a small molecule) and then catalyze the reaction.

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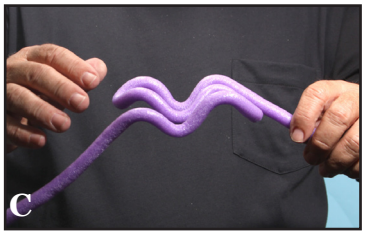
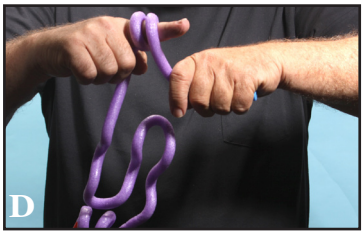
Modeling an Active Site

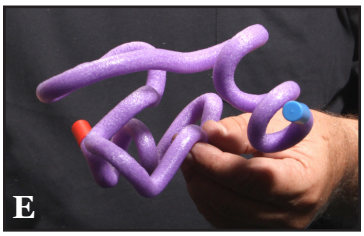
Imagine that your 4-foot mini-toober represents a protein consisting of 200 amino acids.

1. Begin folding your mini-toober into the shape of a protein by creating a three-stranded beta sheet and two short alpha helices. The beta sheet and alpha helices represent your protein's secondary structure. See photos A through D.



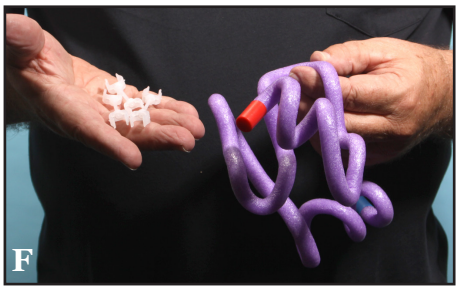


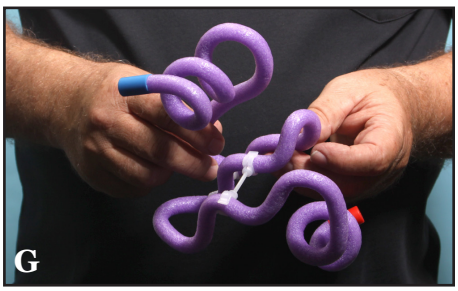
2. Fold the beta sheet and the alpha helices into a compact, globular shape. See photo E.



3. Use three connectors to stabilize the overall 3D shape of the folded protein. See photos F and G.

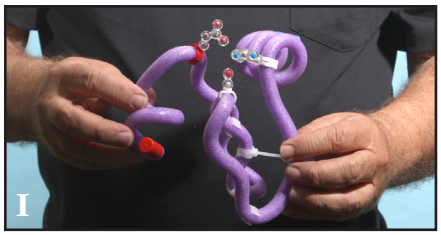
   These connectors stabilize your protein's structure in the same way that hydrogen bonds, which are present in alpha helices and beta sheets, stabilize the structure of a real protein. You now have a stable 3D structure – upon which you can precisely place three specific amino acid sidechains to create an enzyme active site.





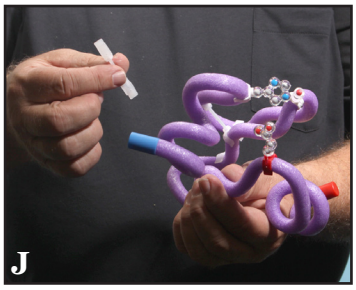
Modeling an Active Site (continued)

4. Create an active site in a shallow crevice on the surface of your protein by adding three amino acid sidechains – a serine, a histidine and a glutamic acid – to your mini-toober in such a way that all three sidechains are within 2 cm of each other. See photos H and I.

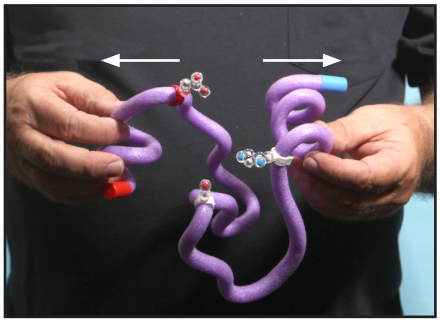


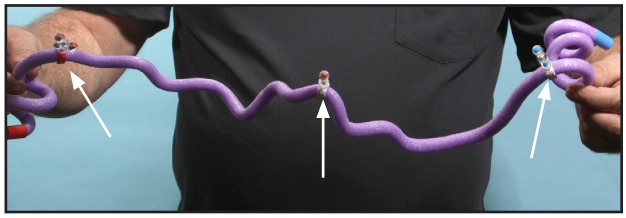
5. The three amino acid sidechains that make up your enzyme's active site interact with a substrate to catalyze a specific chemical reaction. This requires that the sidechains be precisely positioned in 3D space. Examine you protein, noting how its secondary and tertiary structure combines to provide a stable scaffolding, or framework, upon which the active site amino acids are precisely positioned relative to each other.

6. Now carefully remove the connectors that were stabilizing your folded protein. See photo J.



7. Holding your protein with one hand near the N-terminus end and the other near the C-terminus end, slowly move your hands away from each other – simulating the unfolding (denaturation) of your protein.

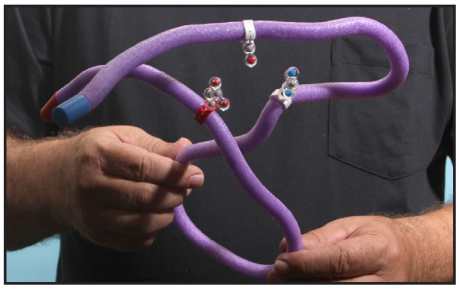


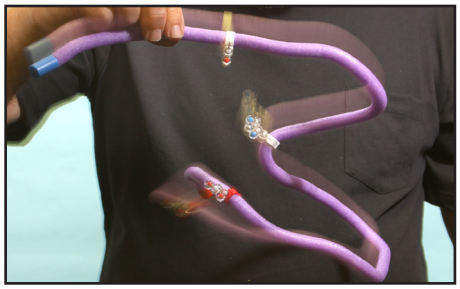


The 3 active site amino acids — that were close together in a folded enzyme — are now far apart in the linear sequence of the protein.

Modeling an Active Site (continued)

Notice that without the stabilizing effect of the hydrogen bonding in your protein's secondary structure, the normal thermal motion experienced by proteins would cause them to unfold (denature).





• Describe the kinds of interactions (bonds) that are present in your protein's secondary and tertiary structure that contribute to the stability of this scaffolding.

The protein's secondary structure (both alpha helices and beta sheets) are stabilized by **hydrogen bonds** — between the polar nitrogen and carbonyl oxygen atoms of the protein's backbone.

The protein's tertiary structure is stabilized by a variety of bonds and interactions between the amino acid sidechains that make up the protein. Bonds that stabilize the protein include: **hydrogen bonds** between polar sidechains and **electrostatic bonds** between oppositely-charged sidechains (acidic and basic sidechains). **Hydrophobic interaction** between hydrophobic sidechains — as they try to minimize their interaction with water — is another major force that stabilizes a protein's tertiary structure.

• Describe your observations of the distribution of the three active site amino acids in your enzyme?

The surprising thing about an enzyme active site is that the three amino acids — that were positioned very close together in the 3D shape of the protein — are actually very far apart in the linear sequence of the amino acids that make up the protein. The protein has to fold into its 3D shape for the sidechains that make the active site to come together, so they can perform their function.

• **Optional Jmol Activity** - Active Site Jmol (see AASK Lessons on website)

Teaching Points

Although most enzymes consist of 200 or more amino acids, the active site of an enzyme is made up of only 2 to 3 amino acids that are precisely positioned in 3D space. In this activity, your students will be asked to think about how all the other amino acids in the enzyme create a compact, stable scaffold upon which the 2-3 active site amino acids can be positioned. This activity will also demonstrate the role of protein secondary structure in achieving this stable scaffold. In addition your students may be surprised to discover that the three active site amino acids in this example are very far apart from each other in the linear sequence of amino acids that make up the protein.

Key Points

Enzyme active sites are composed of a small number (2-3) of amino acids that are precisely positioned in 3D space such that their sidechains create the chemistry needed to catalyze a reaction.

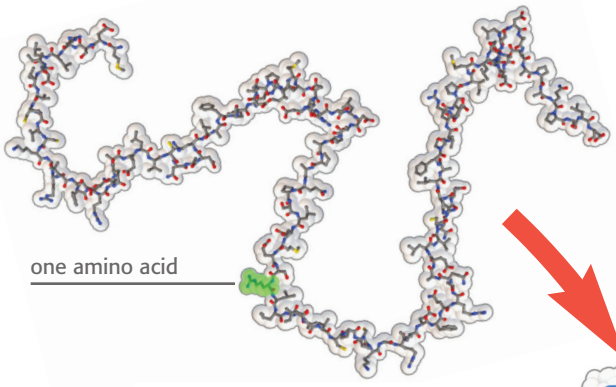
Protein secondary structure (alpha helices and beta sheets) provides that stable scaffolding upon which the critical active site amino acids can be precisely positioned in 3D space.

The 2-3 amino acids that come together in 3D space to create an enzyme active site are very far apart in the linear sequence of the amino acids that make up the protein.

What is a Protein?

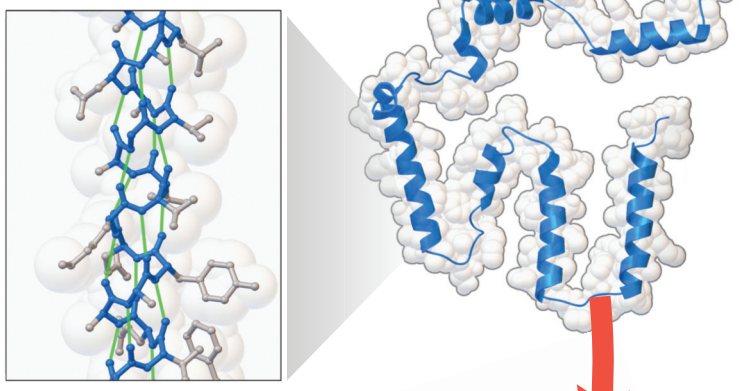
Proteins play countless roles throughout the biological world, from catalyzing chemical reactions to building the structures of all living things.

Despite this wide range of functions all proteins are made out of the same twenty amino acids, but combined in different ways. The way these twenty amino acids are arranged dictates the folding of the protein into its unique final shape. Since protein function is based on the ability to recognize and bind to specific molecules, having the correct shape is critical for proteins to do their jobs correctly.



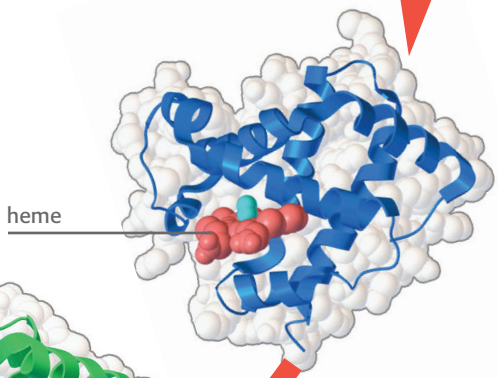
Primary Structure

Primary structure is the linear sequence of amino acids as encoded by the DNA. This sequence defines how the protein will fold and therefore also defines how it will function. A single change in the amino acid sequence of hemoglobin can cause the proteins to clump together, resulting in the disease sickle cell anemia.



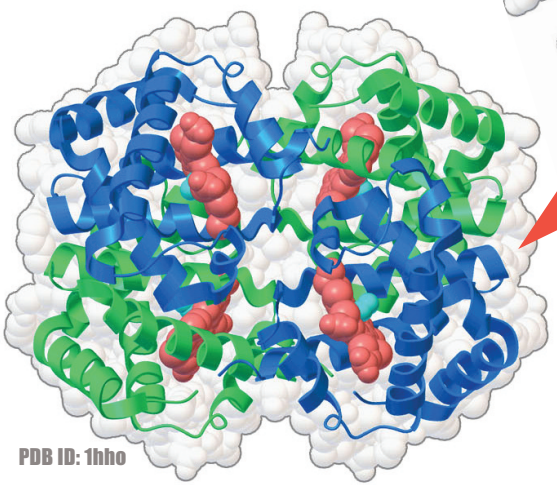
Secondary Structure

Hydrogen bonds between amino acids form two particularly stable structural elements in proteins: alpha helices and beta sheets. Alpha helices (shown in blue) are the basic structural elements found in hemoglobin, but many other proteins also include beta sheets. The inset highlights the pattern of hydrogen bonds (shown in green) that stabilizes alpha helices.



Tertiary Structure

Many functional proteins fold into a compact globular shape, with many carbon-rich amino acids sheltered inside away from the surrounding water. The folded structure of hemoglobin includes a pocket to hold heme, which is the molecule that carries oxygen as it is transported throughout the body.



Quaternary Structure

Two or more polypeptide chains can come together to form one functional molecule with several subunits. The four subunits of hemoglobin cooperate so that the complex picks up and delivers more oxygen than is possible with single subunits.

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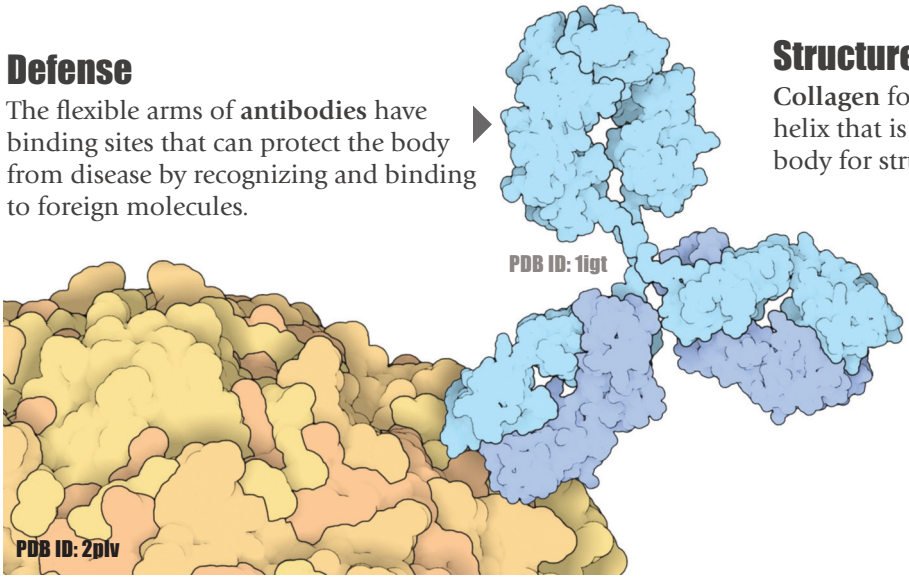
Protein
Shape & Function

Specific amino acid sequences give proteins their distinct shapes and chemical characteristics. Protein shape is important because many proteins rely on the recognition of specific 3D molecular shapes to function correctly.

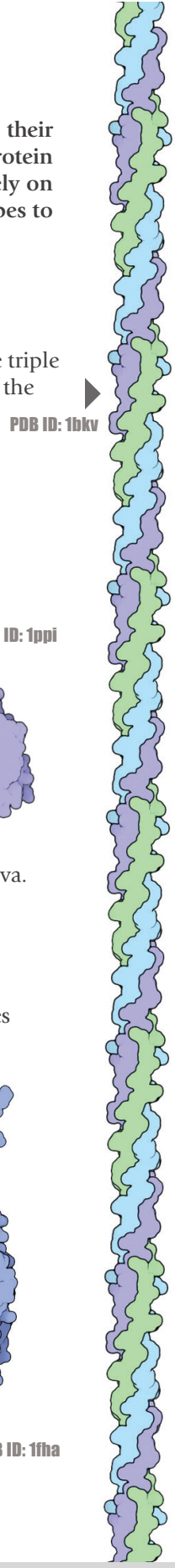
Defense

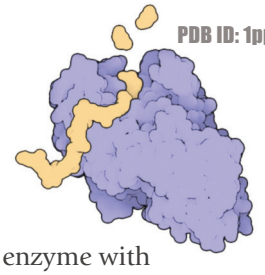
The flexible arms of **antibodies** have binding sites that can protect the body from disease by recognizing and binding to foreign molecules.



Structure

Collagen forms a strong and flexible triple helix that is widely used throughout the body for structural support.



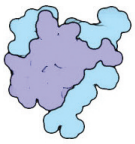


Enzymes

Alpha amylase is an enzyme with a specific catalytic site that begins the breakdown of carbohydrates in our saliva.

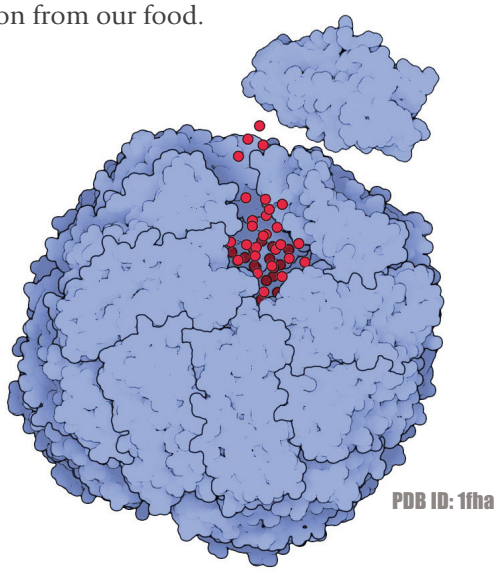
Communication

Insulin is a small, stable protein that can easily maintain its shape while traveling through the blood to regulate blood sugar levels.



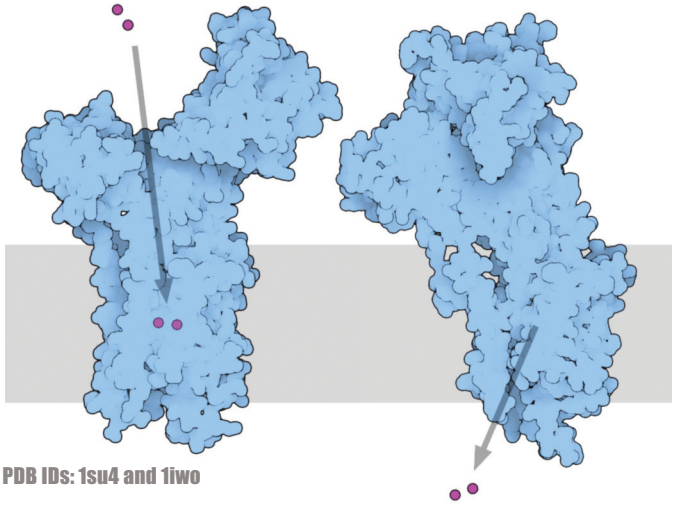
Storage

Ferritin forms a hollow shell that stores iron from our food.



Transport

The **calcium pump** moves ions across cell membranes allowing the synchronized contraction of muscle cells.



To learn more about these and other proteins please visit PDB-101 at **www.rcsb.org/pdb-101**

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Text and illustrations by Jessica May and David S. Goodsell

3-D Molecular Designs

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Teacher Notes

From Amino Acids to Proteins - in 4 Easy Steps

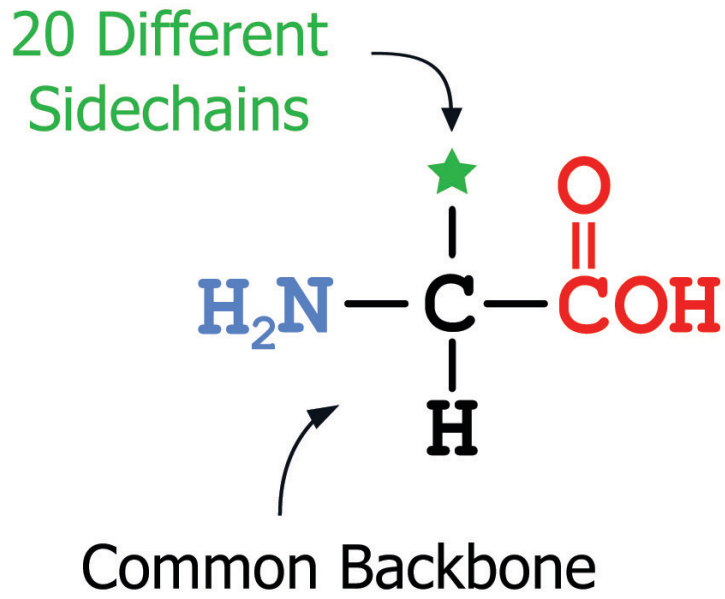
Although protein structure appears to be overwhelmingly complex, you can provide your students with a basic understanding of how proteins fold by focusing on the following four teaching points.

- The 20 amino acids are at the same time identical and different.
- In a single amino acid at neutral pH, the backbone amino group (NH_3^+) is positively charged, and the backbone carboxyl group (COO^-) is negatively charged.
- In a protein, the backbone amino group of the N-terminal amino acid is ***positively charged***, and the backbone carboxyl group of the C-terminal amino acid is ***negatively charged***. All other backbone charges have been ***neutralized*** by peptide bond formation.
- In a protein, the chemical properties of each sidechain are the major determinant of the final, folded 3D structure.

Four Easy Steps

1. The 20 amino acids are at the same time identical and different. How can that be?

The 20 amino acids all share a common backbone and have different sidechains, each with different chemical properties.



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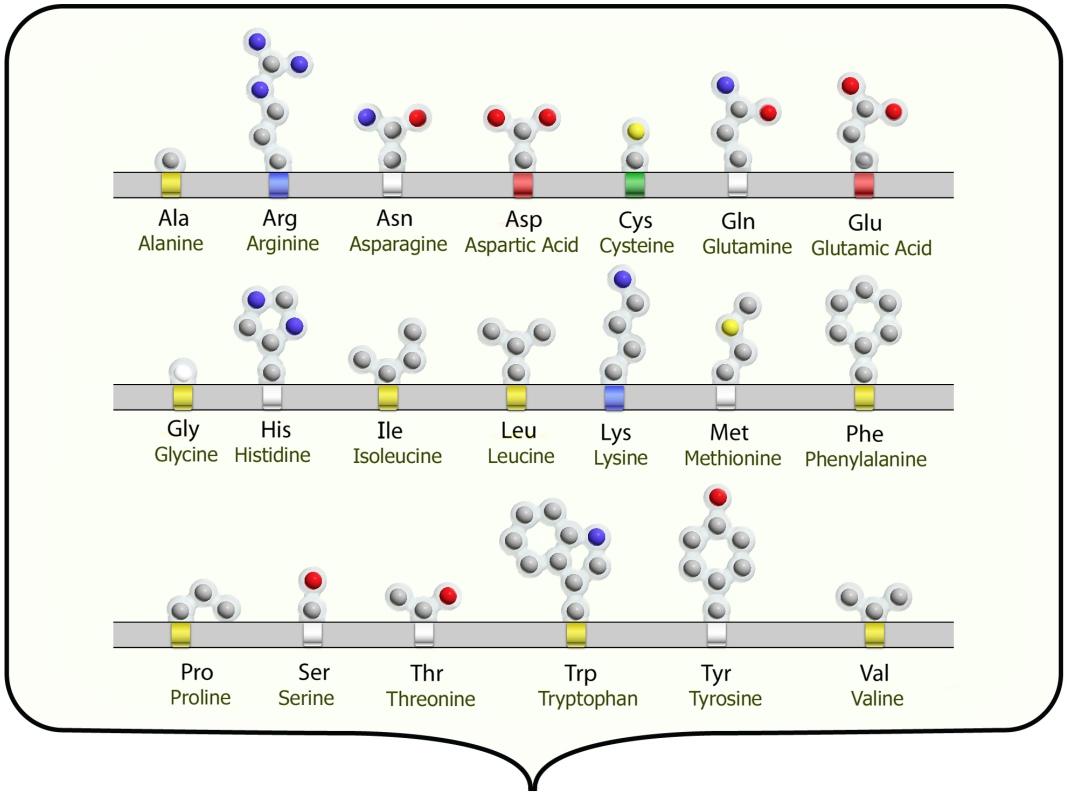
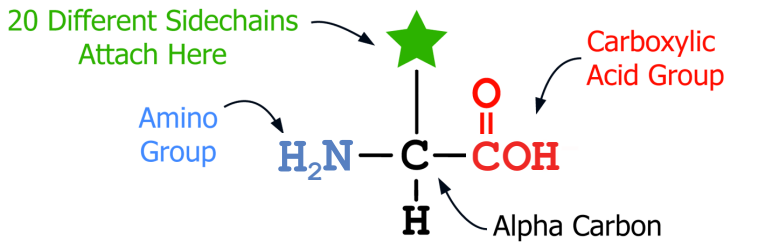
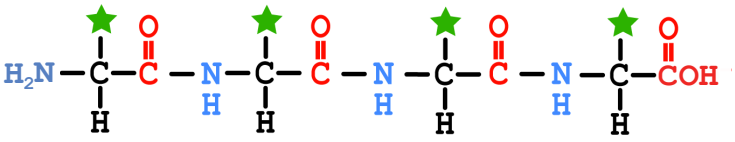
From Amino Acids to Proteins (continued)



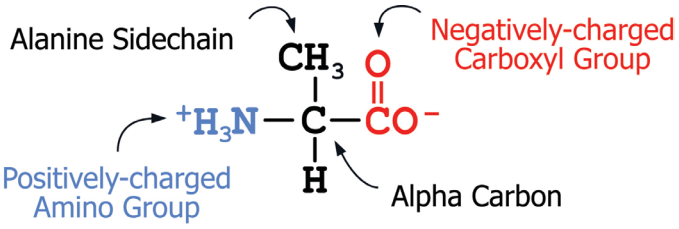


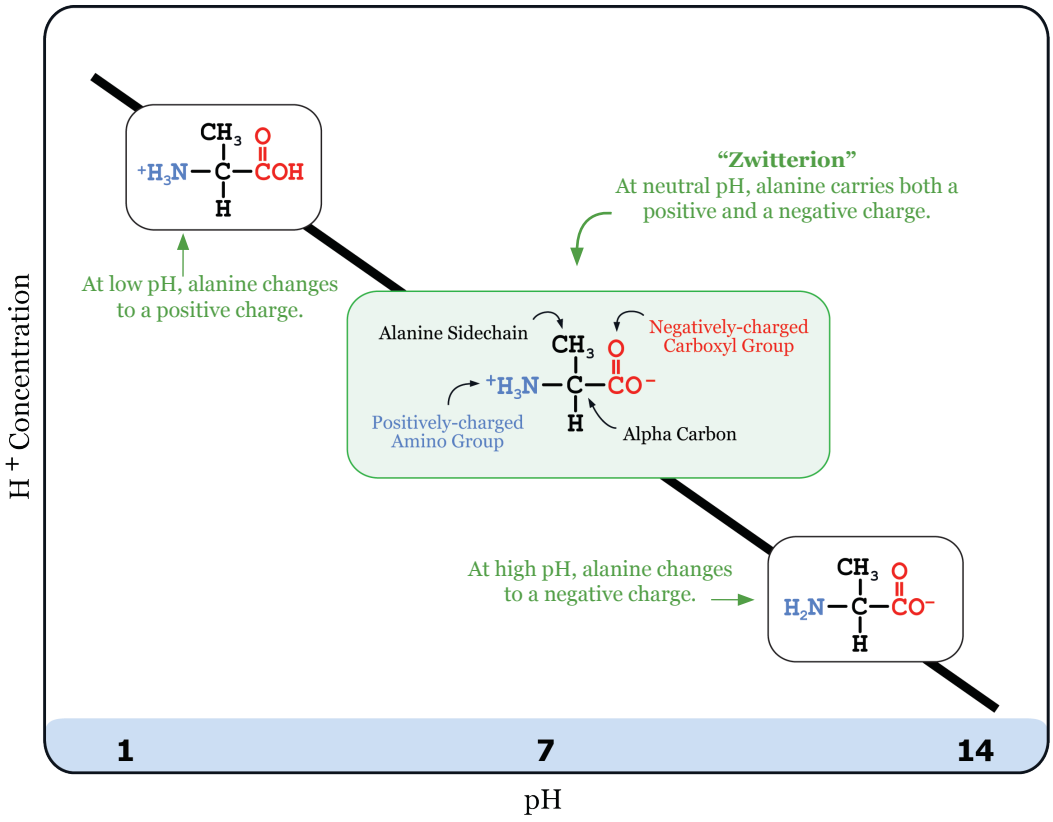


A polypeptide composed of 4 amino acids.

From Amino Acids to Proteins (continued)

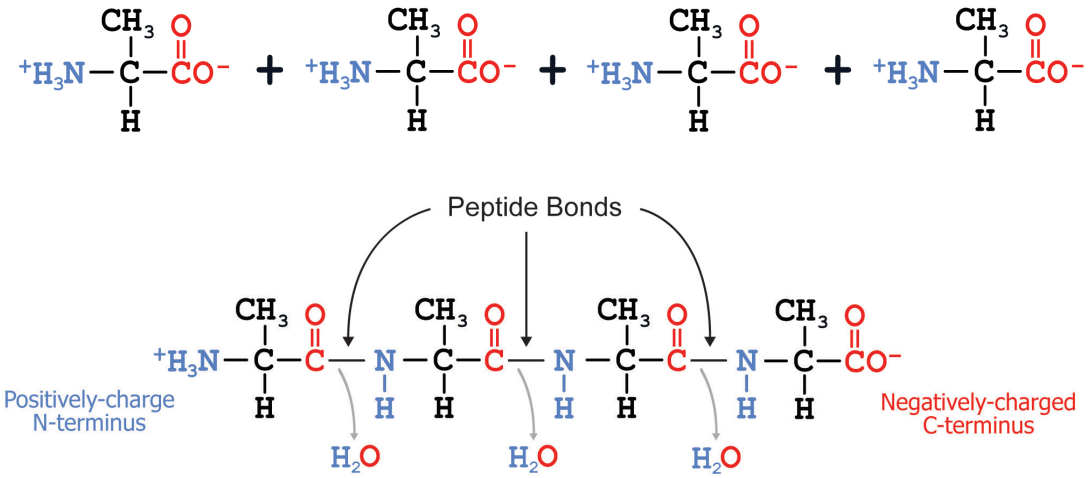
2. In a single amino acid at neutral pH, the backbone amino group (NH_3^+) is positively charged, and the backbone carboxyl group (COO^-) is negatively charged.



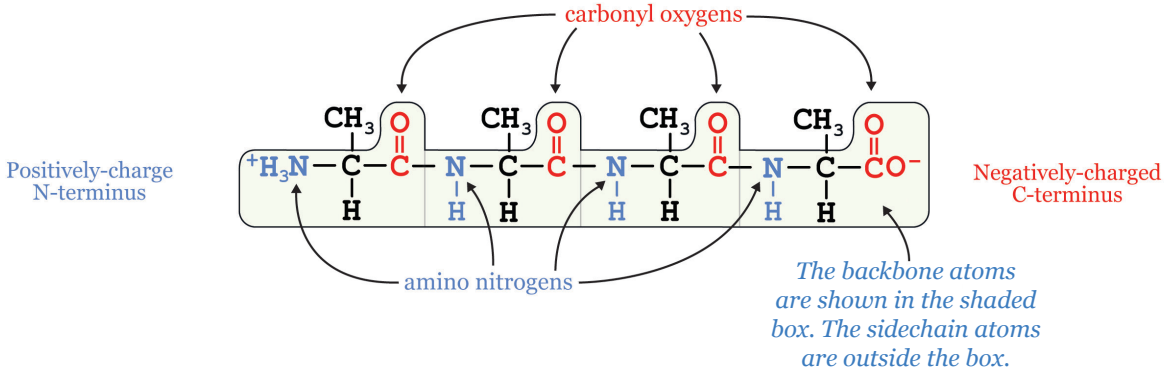


From Amino Acids to Proteins (continued)

3. In a protein, the backbone amino group of the N-terminal amino acid is ***positively-charged***, and the backbone carboxyl group of the C-terminal amino acid is ***negatively-charged***. All other backbone charges have been ***neutralized*** by peptide bond formation.



Note that the formation of each peptide bond results in the production of a water molecule. This is an example of a condensation reaction, also called dehydration synthesis.



From Amino Acids to Proteins (continued)

4. In a protein, the chemical properties of each sidechain are the major determinants of the final, folded 3D structure.

Basic Principles of Chemistry Drive Protein Folding

A. Hydrophobic amino acids are buried in the interior of a globular protein.

- Hydrophobic amino acids are composed primarily of carbon atoms, which cannot form hydrogen bonds with water. In order to form a hydrogen bond with water, a polar molecule, the amino acid sidechains must also be polar, or have an unequal distribution of electrons. Carbon atoms have a uniform distribution of electrons and create a non-polar sidechain. In a soluble, cytosolic protein, these amino acids can be found buried within the protein, where they will not interact with water.

B. Hydrophilic amino acids are usually exposed on the surface of globular proteins.

- Hydrophilic amino acids have oxygen and nitrogen atoms, which can form hydrogen bonds with water. These atoms have an unequal distribution of electrons, creating a polar molecule that can interact and form hydrogen bonds with water. These polar amino acids will be found on the surface of a soluble, cytosolic protein, where they can hydrogen bond with water.

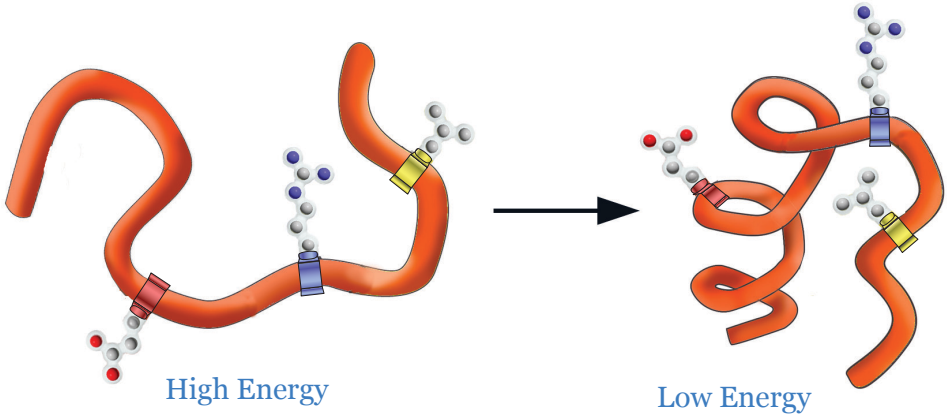
C. Acidic and basic amino acids can form salt bridges, or electrostatic interactions.

- Two of the polar amino acids (glutamic acid and aspartic acid) contain carboxylic acid functional groups and are therefore acidic (negatively charged).
- Two of the polar amino acids (lysine and arginine) contain amino functional groups and are therefore basic (positively charged).
- These two groups of amino acids (acidic and basic) are attracted to one another and can form electrostatic interactions.

D. Cysteine amino acids can form disulfide bonds.

- The cysteine sidechain contains a sulfur atom that can form a covalent disulfide bond with other cysteine sidechains. Disulfide bonds often stabilize the structure of secreted proteins.

When a protein is viewed as a system of interacting components, thermodynamic principles dictate the final shape should represent a low energy state for all of the atoms in the structure.

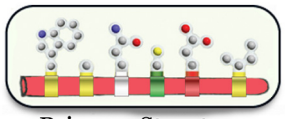


High Energy Low Energy

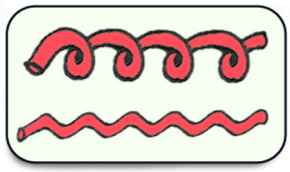
Protein Structure

The previous section focuses on the primary and the tertiary structures of proteins. However, it is useful to think about protein structure in a hierarchical manner, starting with the **primary structure**, and then proceeding to the **secondary**, **tertiary** and **quaternary structure**.

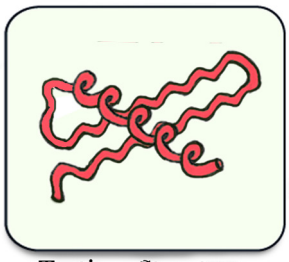
- **The primary structure of a protein** is simply the amino acid sequence of the protein. The final shape of a protein is encoded in its primary structure – the sequence of amino acids in a protein determines its final 3D structure.


Primary Structure

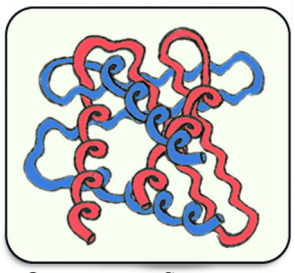
- **The secondary structure of a protein** refers to the alpha helices or beta sheets in the protein. These two common secondary structural elements are stabilized by hydrogen bonding between backbone atoms (the sidechains are not involved in protein secondary structure). A protein can be thought of as a collection of alpha helices and strands of beta sheet that are connected by loops.


Secondary Structure

- **The tertiary structure of a protein** refers to the overall 3D folded structure of a protein. This final folded structure represents a global low-energy state of all the atoms that make up the protein. The final tertiary structure of a protein is stabilized by a combination of many non-covalent interactions including hydrophobic forces, hydrogen bonds between polar atoms, ionic interactions between charged sidechains and Van der Waals forces. Covalant disulfide bonds can also provide stability in some proteins.


Tertiary Structure

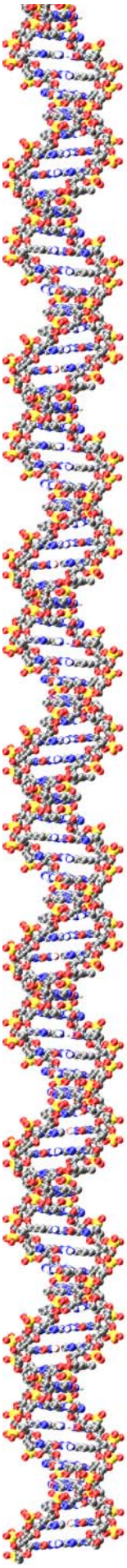
- **The quaternary structure of a protein** refers to protein complexes composed of more than one protein chain. Although some proteins exist as monomers (and therefore have no quaternary structure), many proteins interact to form multi-component protein complexes. Hemoglobin is a good example of a protein with quaternary structure. It is composed of two alpha chains and two beta chains.


Quaternary Structure

Summary

To construct a robust mental model of a protein, students will:

- **conclude** that the primary structure of a protein (its amino acid sequence) is a major determinant of its final 3D shape.
- **determine** that local regions of proteins first adopt a secondary structure (either alpha helices or beta sheets), which are stabilized by hydrogen bonding between backbone atoms.
- **establish** that the basic principles of chemistry act on the amino acid sidechains to determine the tertiary structure of the protein.
- **recognize** that many proteins assemble into quaternary structures, where they function as complex molecular machines.



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The Discovery of DNA

On April 25, 1953, a one-page paper entitled, *A Structure for Deoxyribonucleic Acid,* appeared in the British journal, *Nature*. The authors of this paper were James Watson, a young American post-doctoral candidate who had recently received a Ph.D. from the University of Illinois, and Francis Crick, a physicist who was completing his doctoral dissertation at Cambridge University, England. The paper began; *"We wish to suggest a structure for the salt of deoxyribose nucleic acid (D. N. A.). This structure has novel features which are of considerable biological interest."*

This initial description of the structure of DNA marked a major milestone in the development of molecular biology. In addition to reporting the correct structure of DNA, the paper also contained their classic understatement in scientific literature: *"It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material."* Their paper serves as an excellent example of what has become a recurring theme in the molecular biosciences — ***Forms Follows Function***. That is, the structure of a macromolecule often explains the macromolecule's function (how the macromolecule) works.

Watson and Crick's achievement is notable in several ways, including the fact that they determined the structure of DNA without performing a single experiment. They used the information from numerous other scientists who were investigating various properties of DNA. Modeling was the major approach Watson and Crick used. Using paper cut-outs of the shapes of the four nitrogenous bases (A,T, G and C), they were able to combine all of the different facts that had accumulated to that date into a plausible model for the structure of DNA.

> *...The structure has two helical chains coiled around the same axis (see diagram). We have made the usual chemical assumptions, namely, that each chain consists of phosphate diester groups joining B-D-deoxyribofuranose residues with 3',5' linkages. The two chains (but not their bases) are related by a dyad perpendicular to the fibre axis. Both chains follow right-handed helices, but owing to the dyad the sequences of the atoms in the two chains run in opposite direction.*
>
> — Watson, J.D. and Crick, F.H.C., *Nature*, 171, 737-738 (1953)

Student Handout

The DNA Student Challenge

Your challenge today is to see if you can discover the correct structure of double-stranded DNA, just as Watson and Crick did over 50 years ago.

Your model should satisfy all of the pieces of experimental information that was known in 1953, as noted in the blue box below. Rather than using paper cut-outs to represent the DNA bases, you will use plastic models of the four deoxyribonucleotides whose 3D structures are based on known atomic coordinates of the B-form DNA. In these nucleotide models, magnets are used to represent both:

- the phosphodiester bonds that link the nucleotide units together into a long, linear polymer
- the hydrogen bonds that bond one base to another.

Information Available to Watson and Crick in 1953

DNA is a Polymer: Previous studies identified DNA as the genetic material of cells, and that DNA was a polymer consisting of three components:

- A nitrogenous base
- A pentose (5-carbon) sugar called deoxyribose
- A phosphate group.

Moreover, experiments suggested that the DNA molecule was unbelievably large, with molecular weights ranging from 25 x 10^6 to 3 x 10^9 daltons. (Since each nucleotide has a mass of 330 daltons, DNA molecules were believed to be composed of between 76,000 and 9,000,000 nucleotides.)

DNA is more dense than protein. At a density of 1.6 gm/cm3, DNA was known to be more dense than protein (1.3gm/cm3). This suggested that DNA was a densely packed structure.

Chargaff's Rules: In 1947, Erwin Chargaff demonstrated that while the four nucleotides were not present in equal amounts in the DNA from different organisms, the amount of adenine was the same as thymine, and the amount of guanine was the same as cytosine. This became known as *Chargaff's Rules:*

- The proportion of A always equals that of T, and the proportion of G always equals that of C. Thus, A = T and G = C.

X-ray Crystallography Data: In the laboratory of Maurice Wilkins, Rosalind Franklin used X-ray diffraction to analyze fibers of DNA. The pattern of spots on the X-ray diffraction pattern suggested that:

- Phosphate was on the outside, nitrogenous bases were on the inside.
- DNA was a double helix, made up of two strands.
- The two strands of DNA run in opposite directions (anti-parallel).
- There are 10 base pairs per turn of the double helix.

Background information for students

Each group of students should have physical models of the four nucleotides, separated into their component parts. These include:

- Phosphate group – which is negatively charged
- Deoxyribose group — which is a cyclic ring structure
- Four nitrogenous bases (A, G, C and T)

Each component of the nucleotides is color coded according to atom type, following the standard CPK coloring scheme:

Oxygen is RED **Nitrogen is BLUE**

Carbon is GRAY Hydrogen is WHITE

equipment, and to Dr. G. E. R. Deacon and the captain and officers of R.R.S. *Discovery II* for their part in making the observations.

[1] Young, F. B., Gerrard, H., and Jevons, W., *Phil. Mag.*, **40**, 149 (1920).
[2] Longuet-Higgins, M. S., *Mon. Not. Roy. Astro. Soc., Geophys. Supp.*, **5**, 285 (1949).
[3] Von Arx, W. S., Woods Hole Papers in Phys. Oceanog. Meteor., **11** (3) (1950).
[4] Ekman, V. W., *Arkiv. Mat. Astron. Fysik.* (*Stockholm*), **2** (11) (1905).

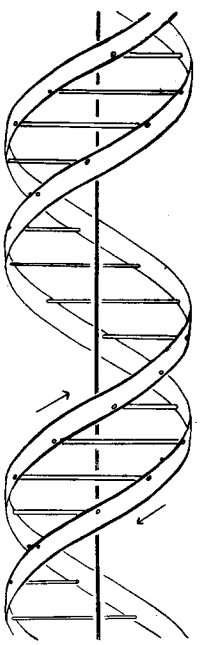
MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribose Nucleic Acid

WE wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

A structure for nucleic acid has already been proposed by Pauling and Corey[1]. They kindly made their manuscript available to us in advance of publication. Their model consists of three intertwined chains, with the phosphates near the fibre axis, and the bases on the outside. In our opinion, this structure is unsatisfactory for two reasons: (1) We believe that the material which gives the X-ray diagrams is the salt, not the free acid. Without the acidic hydrogen atoms it is not clear what forces would hold the structure together, especially as the negatively charged phosphates near the axis will repel each other. (2) Some of the van der Waals distances appear to be too small.

Another three-chain structure has also been suggested by Fraser (in the press). In his model the phosphates are on the outside and the bases on the inside, linked together by hydrogen bonds. This structure as described is rather ill-defined, and for this reason we shall not comment on it.



This figure is purely diagrammatic. The two ribbons symbolize the two phosphate—sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis

We wish to put forward a radically different structure for the salt of deoxyribose nucleic acid. This structure has two helical chains each coiled round the same axis (see diagram). We have made the usual chemical assumptions, namely, that each chain consists of phosphate diester groups joining β-D-deoxyribofuranose residues with 3′,5′ linkages. The two chains (but not their bases) are related by a dyad perpendicular to the fibre axis. Both chains follow right-handed helices, but owing to the dyad the sequences of the atoms in the two chains run in opposite directions. Each chain loosely resembles Furberg's[2] model No. 1; that is, the bases are on the inside of the helix and the phosphates on the outside. The configuration of the sugar and the atoms near it is close to Furberg's 'standard configuration', the sugar being roughly perpendicular to the attached base. There is a residue on each chain every 3·4 A. in the z-direction. We have assumed an angle of 36° between adjacent residues in the same chain, so that the structure repeats after 10 residues on each chain, that is, after 34 A. The distance of a phosphorus atom from the fibre axis is 10 A. As the phosphates are on the outside, cations have easy access to them.

The structure is an open one, and its water content is rather high. At lower water contents we would expect the bases to tilt so that the structure could become more compact.

The novel feature of the structure is the manner in which the two chains are held together by the purine and pyrimidine bases. The planes of the bases are perpendicular to the fibre axis. They are joined together in pairs, a single base from one chain being hydrogen-bonded to a single base from the other chain, so that the two lie side by side with identical z-co-ordinates. One of the pair must be a purine and the other a pyrimidine for bonding to occur. The hydrogen bonds are made as follows: purine position 1 to pyrimidine position 1; purine position 6 to pyrimidine position 6.

If it is assumed that the bases only occur in the structure in the most plausible tautomeric forms (that is, with the keto rather than the enol configurations) it is found that only specific pairs of bases can bond together. These pairs are: adenine (purine) with thymine (pyrimidine), and guanine (purine) with cytosine (pyrimidine).

In other words, if an adenine forms one member of a pair, on either chain, then on these assumptions the other member must be thymine; similarly for guanine and cytosine. The sequence of bases on a single chain does not appear to be restricted in any way. However, if only specific pairs of bases can be formed, it follows that if the sequence of bases on one chain is given, then the sequence on the other chain is automatically determined.

It has been found experimentally[3,4] that the ratio of the amounts of adenine to thymine, and the ratio of guanine to cytosine, are always very close to unity for deoxyribose nucleic acid.

It is probably impossible to build this structure with a ribose sugar in place of the deoxyribose, as the extra oxygen atom would make too close a van der Waals contact.

The previously published X-ray data[5,6] on deoxyribose nucleic acid are insufficient for a rigorous test of our structure. So far as we can tell, it is roughly compatible with the experimental data, but it must be regarded as unproved until it has been checked against more exact results. Some of these are given in the following communications. We were not aware of the details of the results presented there when we devised our structure, which rests mainly though not entirely on published experimental data and stereochemical arguments.

It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

Full details of the structure, including the conditions assumed in building it, together with a set of co-ordinates for the atoms, will be published elsewhere.

We are much indebted to Dr. Jerry Donohue for constant advice and criticism, especially on interatomic distances. We have also been stimulated by a knowledge of the general nature of the unpublished experimental results and ideas of Dr. M. H. F. Wilkins, Dr. R. E. Franklin and their co-workers at

King's College, London. One of us (J. D. W.) has been aided by a fellowship from the National Foundation for Infantile Paralysis.

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April 2.

[1] Pauling, L., and Corey, R. B., *Nature*, **171**, 346 (1953); *Proc. U.S. Nat. Acad. Sci.*, **39**, 84 (1953).
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Molecular Structure of Deoxypentose Nucleic Acids

While the biological properties of deoxypentose nucleic acid suggest a molecular structure containing great complexity, X-ray diffraction studies described here (cf. Astbury[1]) show the basic molecular configuration has great simplicity. The purpose of this communication is to describe, in a preliminary way, some of the experimental evidence for the polynucleotide chain configuration being helical, and existing in this form when in the natural state. A fuller account of the work will be published shortly.

The structure of deoxypentose nucleic acid is the same in all species (although the nitrogen base ratios alter considerably) in nucleoprotein, extracted or in cells, and in purified nucleate. The same linear group of polynucleotide chains may pack together parallel in different ways to give crystalline[1–3], semi-crystalline or paracrystalline material. In all cases the X-ray diffraction photograph consists of two regions, one determined largely by the regular spacing of nucleotides along the chain, and the other by the longer spacings of the chain configuration. The sequence of different nitrogen bases along the chain is not made visible.

Oriented paracrystalline dooxypentose nucleic acid ('structure *B*' in the following communication by Franklin and Gosling) gives a fibre diagram as shown in Fig. 1 (cf. ref. 4). Astbury suggested that the strong 3·4-A. reflexion corresponded to the internucleotide repeat along the fibre axis. The ~ 34 A. layer lines, however, are not due to a repeat of a polynucleotide composition, but to the chain configuration repeat, which causes strong diffraction as the nucleotide chains have higher density than the interstitial water. The absence of reflexions on or near the meridian immediately suggests a helical structure with axis parallel to fibre length.

Diffraction by Helices

It may be shown[5] (also Stokes, unpublished) that the intensity distribution in the diffraction pattern of a series of points equally spaced along a helix is given by the squares of Bessel functions. A uniform continuous helix gives a series of layer lines of spacing corresponding to the helix pitch, the intensity distribution along the nth layer line being proportional to the square of J_n, the nth order Bessel function. A straight line may be drawn approximately through

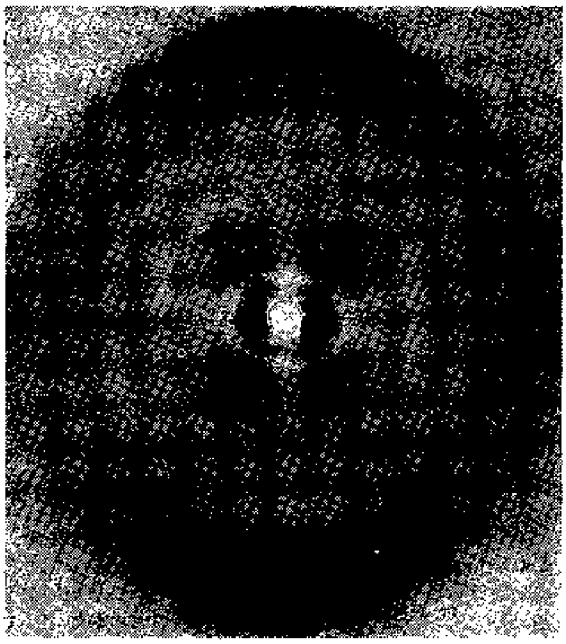

Fig. 1. Fibre diagram of deoxypentose nucleic acid from *B. coli*. Fibre axis vertical

the innermost maxima of each Bessel function and the origin. The angle this line makes with the equator is roughly equal to the angle between an element of the helix and the helix axis. If a unit repeats n times along the helix there will be a meridional reflexion (J_0^2) on the nth layer line. The helical configuration produces side-bands on this fundamental frequency, the effect[6] being to reproduce the intensity distribution about the origin around the new origin, on the nth layer line, corresponding to *C* in Fig. 2.

We will now briefly analyse in physical terms some of the effects of the shape and size of the repeat unit or nucleotide on the diffraction pattern. First, if the nucleotide consists of a unit having circular symmetry about an axis parallel to the helix axis, the whole diffraction pattern is modified by the form factor of the nucleotide. Second, if the nucleotide consists of a series of points on a radius at right-angles to the helix axis, the phases of radiation scattered by the helices of different diameter passing through each point are the same. Summation of the corresponding Bessel functions gives reinforcement for the inner-

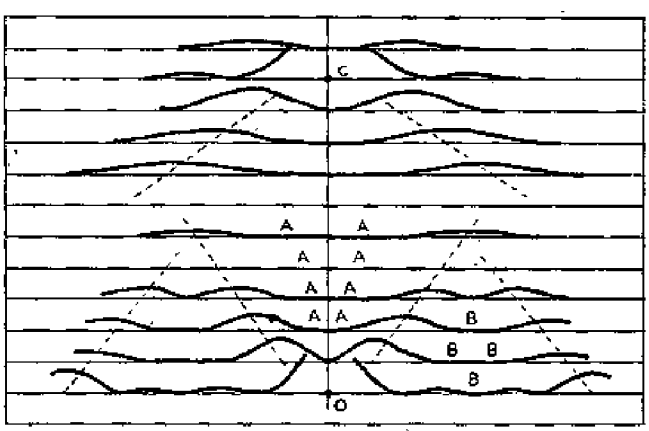

Fig. 2. Diffraction pattern of system of helices corresponding to structure of deoxypentose nucleic acid. The squares of Bessel functions are plotted about 0 on the equator and on the first, second, third and fifth layer lines for half of the nucleotide mass at 20 A. diameter and remainder distributed along a radius, the mass at a given radius being proportional to the radius. About *C* on the tenth layer line similar functions are plotted for an outer diameter of 12 A.

which has never been since surpassed. Dr. Schonland expressed disappointment that the membership in recent years has been but a little more than a thousand, for South Africa has expanded enormously since 1906 and with this expansion the need for, and potential value of, such a body as the Association. The general aims of the Association have not changed at all with the passing of years: "We exist," he said, "primarily to create and foster a scientific fraternity in South Africa, not to publish original work. We exist to provide a common meeting-ground for South African scientists and a forum for general discussion of the problems of this country from the scientific angle." He defended the use of Afrikaans by those who preferred it, for "we were intended by our founders to be parochial, and we should pride ourselves on being parochial. I would suggest that if we try to be anything else we will have mistaken our real aim".

Having thus firmly and, most people would agree, wisely placed the Association in its proper perspective, Dr. Schonland went on to make some concrete suggestions. The *South African Journal of Science* should have a series of semi-popular articles reviewing and surveying the new ideas of science and so bridge the gap between those who teach and do advanced research work and those who pay for it. This, he thought, is the proper function of the *Journal*, and it is but one aspect of the Association's duty, as representative of all sections of scientific opinion in South Africa, "to take a stronger, a more continuing and a more active interest in all scientific developments, national and university, in South Africa and to study carefully what is being done in other countries".

Besides his plea that the Association needs to form a standing committee to watch over scientific education in schools, Dr. Schonland suggested that the Association might consider taking a part in the formation of a body on the lines of the British Parliamentary and Scientific Committee and also help in the creation of better facilities for advanced research in South Africa. On this last-named point, he cited the instances of the National University in Canberra and the Institute for Advanced Studies in Dublin, but he made the interesting suggestion that a more acceptable solution might be the creation of a number of specialized institutes for advanced study, attached to and forming part of those universities which for one reason or another are best suited for them.

BASIS OF TECHNICAL EDUCATION

GENERAL education to-day should be planned so as to enable the ordinary citizen to adapt himself to the needs of technological society and to understand what is happening and what is required of him. This was the theme of an international conference convened by the United Nations Educational, Cultural and Scientific Organization at Unesco House in June 1950*.

Broadly, the Conference found that organized social foresight is essential to enable the educational system of a country to prepare children for the type of life and work they are likely to encounter, and that a substantial development of technical education is required at all levels: at present it is wholly inadequate for future needs, while the practical content of general education is also inadequate for the needs of future citizens of a technological society. The cultural content of technical education is also generally inadequate; technical education requires special consideration, and training for adaptability is an outstanding requirement in an age of ultra-rapid technological change. The education of women and girls also demands particular attention in view of their dual role as workers and home-makers, and improved administrative arrangements are essential if education is to fulfil its true function in such a society.

The report does not suggest that all these propositions apply equally to every country, though the Conference considered that, so far as its knowledge extended, they are generally valid for the world as a whole. The stress is laid on the need for adapting technology to man, not man to technology. The questions formulated in this report—and which merit attention in current discussions on the expansion of both technical and technological education in Great Britain—are raised in the belief that mastery of the machine by man is not an end in itself: it is a means to the development of man and of the whole society.

The distinction between technician and technologist is not always kept clear in this report, particularly in the chapter on the content of technical education. Nevertheless, the report directs attention to some fundamental issues which no sound policy for either type of education can disregard. In both fields it must be recognized that we are concerned not simply with the efficiency of production, but also with the fundamental attitude which the men and women of to-morrow will adopt in facing the problems of a technological society. Both, too, in seeking to foster flexibility, must recognize that flexibility is determined not only by education and training but also by social, economic and technical conditions; and the administrative measures required to ensure that education becomes more adapted to the needs of a changing technological society are themselves likely to be most effective when they are informal and varied rather than concentrated and uniform. The administrator, no less than the teacher and student, has need of frequent opportunities of contact with the industrial world, and requires experience of the difficulties and problems created by technological development in society; just as the teacher and student should keep abreast of developments in research and of practical applications in industry.

* Education in a Technological Society: a Preliminary International Survey of the Nature and Efficacy of Technical Education. (Tensions and Technology Series.) Pp. 76. (Paris: Unesco; London: H.M.S.O., 1952.) 200 francs; 4*s*.; 75 cents.

GENETICAL IMPLICATIONS OF THE STRUCTURE OF DEOXYRIBONUCLEIC ACID

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THE importance of deoxyribonucleic acid (DNA) within living cells is undisputed. It is found in all dividing cells, largely if not entirely in the nucleus, where it is an essential constituent of the chromosomes. Many lines of evidence indicate that it is the carrier of a part of (if not all) the genetic specificity of the chromosomes and thus of the gene itself.

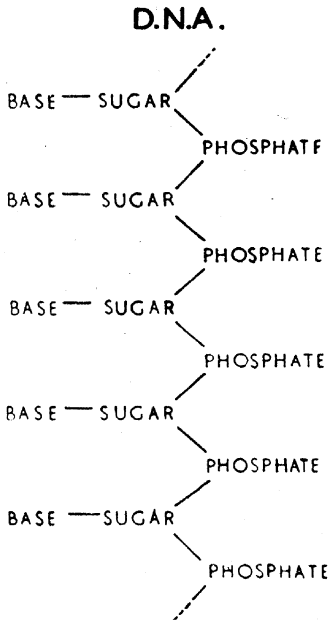

Fig. 1. Chemical formula of a single chain of deoxyribonucleic acid

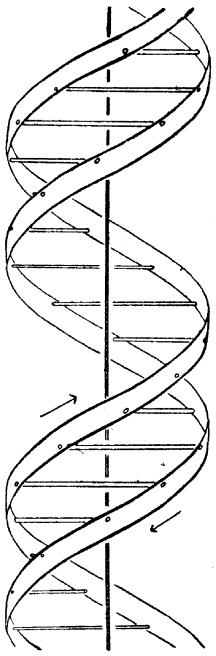

Fig. 2. This figure is purely diagrammatic. The two ribbons symbolize the two phosphate-sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis

Until now, however, no evidence has been presented to show how it might carry out the essential operation required of a genetic material, that of exact self-duplication.

We have recently proposed a structure[1] for the salt of deoxyribonucleic acid which, if correct, immediately suggests a mechanism for its self-duplication. X-ray evidence obtained by the workers at King's College, London[2], and presented at the same time, gives qualitative support to our structure and is incompatible with all previously proposed structures[3]. Though the structure will not be completely proved until a more extensive comparison has been made with the X-ray data, we now feel sufficient confidence in its general correctness to discuss its genetical implications. In doing so we are assuming that fibres of the salt of deoxyribonucleic acid are not artefacts arising in the method of preparation, since it has been shown by Wilkins and his co-workers that similar X-ray patterns are obtained from both the isolated fibres and certain intact biological materials such as sperm head and bacteriophage particles[2,4].

The chemical formula of deoxyribonucleic acid is now well established. The molecule is a very long chain, the backbone of which consists of a regular alternation of sugar and phosphate groups, as shown in Fig. 1. To each sugar is attached a nitrogenous base, which can be of four different types. (We have considered 5-methyl cytosine to be equivalent to cytosine, since either can fit equally well into our structure.) Two of the possible bases—adenine and guanine—are purines, and the other two—thymine and cytosine—are pyrimidines. So far as is known, the sequence of bases along the chain is irregular. The monomer unit, consisting of phosphate, sugar and base, is known as a nucleotide.

The first feature of our structure which is of biological interest is that it consists not of one chain, but of two. These two chains are both coiled around a common fibre axis, as is shown diagrammatically in Fig. 2. It has often been assumed that since there was only one chain in the chemical formula there would only be one in the structural unit. However, the density, taken with the X-ray evidence[2], suggests very strongly that there are two.

The other biologically important feature is the manner in which the two chains are held together. This is done by hydrogen bonds between the bases, as shown schematically in Fig. 3. The bases are joined together in pairs, a single base from one chain being hydrogen-bonded to a single base from the other. The important point is that only certain pairs of bases will fit into the structure. One member of a pair must be a purine and the other a pyrimidine in order to bridge between the two chains. If a pair consisted of two purines, for example, there would not be room for it.

We believe that the bases will be present almost entirely in their most probable tautomeric forms. If this is true, the conditions for forming hydrogen bonds are more restrictive, and the only pairs of bases possible are :

adenine with thymine ;
guanine with cytosine.

The way in which these are joined together is shown in Figs. 4 and 5. A given pair can be either way round. Adenine, for example, can occur on either chain ; but when it does, its partner on the other chain must always be thymine.

This pairing is strongly supported by the recent analytical results[5], which show that for all sources of deoxyribonucleic acid examined the amount of adenine is close to the amount of thymine, and the amount of guanine close to the amount of cytosine, although the cross-ratio (the ratio of adenine to guanine) can vary from one source to another. Indeed, if the sequence of bases on one chain is irregular, it is difficult to explain these analytical results except by the sort of pairing we have suggested.

The phosphate-sugar backbone of our model is completely regular, but any sequence of the pairs of bases can fit into the structure. It follows that in a long molecule many different permutations are possible, and it therefore seems likely that the precise sequence of the bases is the code which carries the genetical information. If the actual order of the

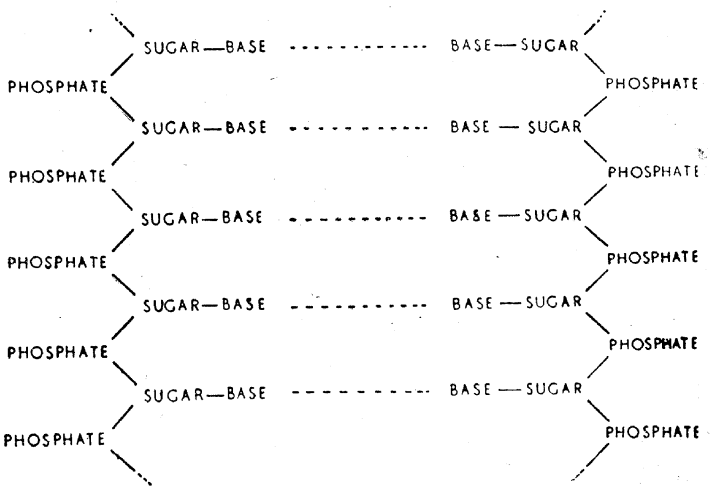

Fig. 3. Chemical formula of a pair of deoxyribonucleic acid chains. The hydrogen bonding is symbolized by dotted lines

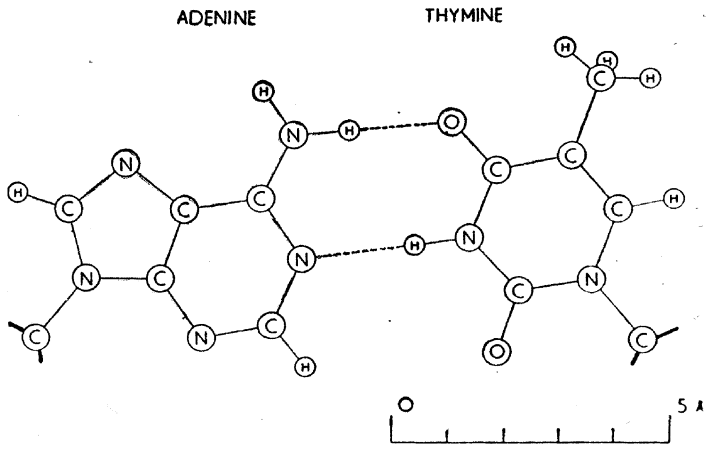

Fig. 4. Pairing of adenine and thymine. Hydrogen bonds are shown dotted. One carbon atom of each sugar is shown

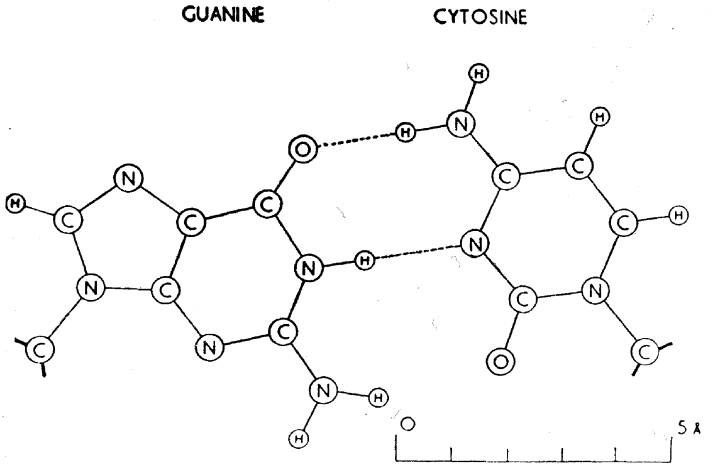

Fig. 5. Pairing of guanine and cytosine. Hydrogen bonds are shown dotted. One carbon atom of each sugar is shown

bases on one of the pair of chains were given, one could write down the exact order of the bases on the other one, because of the specific pairing. Thus one chain is, as it were, the complement of the other, and it is this feature which suggests how the deoxyribonucleic acid molecule might duplicate itself.

Previous discussions of self-duplication have usually involved the concept of a template, or mould. Either the template was supposed to copy itself directly or it was to produce a 'negative', which in its turn was to act as a template and produce the original 'positive' once again. In no case has it been explained in detail how it would do this in terms of atoms and molecules.

Now our model for deoxyribonucleic acid is, in effect, a *pair* of templates, each of which is complementary to the other. We imagine that prior to duplication the hydrogen bonds are broken, and the two chains unwind and separate. Each chain then acts as a template for the formation on to itself of a new companion chain, so that eventually we shall have *two* pairs of chains, where we only had one before. Moreover, the sequence of the pairs of bases will have been duplicated exactly.

A study of our model suggests that this duplication could be done most simply if the single chain (or the relevant portion of it) takes up the helical configuration. We imagine that at this stage in the life of the cell, free nucleotides, strictly polynucleotide precursors, are available in quantity. From time to time the base of a free nucleotide will join up by hydrogen bonds to one of the bases on the chain already formed. We now postulate that the polymerization of these monomers to form a new chain is only possible if the resulting chain can form the proposed structure. This is plausible, because steric reasons would not allow nucleotides 'crystallized' on to the first chain to approach one another in such a way that they could be joined together into a new chain, unless they were those nucleotides which were necessary to form our structure. Whether a special enzyme is required to carry out the polymerization, or whether the single helical chain already formed acts effectively as an enzyme, remains to be seen.

Since the two chains in our model are intertwined, it is essential for them to untwist if they are to separate. As they make one complete turn around each other in 34 A., there will be about 150 turns per million molecular weight, so that whatever the precise structure of the chromosome a considerable amount of uncoiling would be necessary. It is well known from microscopic observation that much coiling and uncoiling occurs during mitosis, and though this is on a much larger scale it probably reflects similar processes on a molecular level. Although it is difficult at the moment to see how these processes occur without everything getting tangled, we do not feel that this objection will be insuperable.

Our structure, as described[1], is an open one. There is room between the pair of polynucleotide chains (see Fig. 2) for a polypeptide chain to wind around the same helical axis. It may be significant that the distance between adjacent phosphorus atoms, 7·1 A., is close to the repeat of a fully extended polypeptide chain. We think it probable that in the sperm head, and in artificial nucleoproteins, the polypeptide chain occupies this position. The relative weakness of the second layer-line in the published X-ray pictures[3a,4] is crudely compatible with such an idea. The function of the protein might well be to control the coiling and uncoiling, to assist in holding a single polynucleotide chain in a helical configuration, or some other non-specific function.

Our model suggests possible explanations for a number of other phenomena. For example, spontaneous mutation may be due to a base occasionally occurring in one of its less likely tautomeric forms. Again, the pairing between homologous chromosomes at meiosis may depend on pairing between specific bases. We shall discuss these ideas in detail elsewhere.

For the moment, the general scheme we have proposed for the reproduction of deoxyribonucleic acid must be regarded as speculative. Even if it is correct, it is clear from what we have said that much remains to be discovered before the picture of genetic duplication can be described in detail. What are the polynucleotide precursors ? What makes the pair of chains unwind and separate ? What is the precise role of the protein ? Is the chromosome one long pair of deoxyribonucleic acid chains, or does it consist of patches of the acid joined together by protein ?

Despite these uncertainties we feel that our proposed structure for deoxyribonucleic acid may help to solve one of the fundamental biological problems—the molecular basis of the template needed for genetic replication. The hypothesis we are suggesting is that the template is the pattern of bases formed by one chain of the deoxyribonucleic acid and that the gene contains a complementary pair of such templates.

One of us (J. D. W.) has been aided by a fellowship from the National Foundation for Infantile Paralysis (U.S.A.).

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[2] Wilkins, M. H. F., Stokes, A. R., and Wilson, H. R., *Nature*, **171**, 738 (1953). Franklin, R. E., and Gosling, R. G., *Nature*, **171**, 740 (1953).
[3] (*a*) Astbury, W. T., Symp. No. 1 Soc. Exp. Biol., 66 (1947). (*b*) Furberg, S., *Acta Chem. Scand.*, **6**, 634 (1952). (*c*) Pauling, L., and Corey, R. B., *Nature*, **171**, 346 (1953); *Proc. U.S. Nat. Acad. Sci.*, **39**, 84 (1953). (*d*) Fraser, R. D. B. (in preparation).
[4] Wilkins, M. H. F., and Randall, J. T., *Biochim. et Biophys. Acta*, **10**, 192 (1953).
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GEOPHYSICAL AND METEOROLOGICAL CHANGES IN THE PERIOD JANUARY–APRIL 1949

IN a recent article[1] Lewis and McIntosh have considered the geophysical data for the period January–April 1949, which we presented in an earlier communication[2]. On the basis of certain probability criteria they appear to show that the apparent regular variations in ionospheric and meteorological phenomena which occurred in that period were not significant. We have studied their article and made a separate statistical analysis of the *unsmoothed* data, and conclude that in all respects our original suggestions seem to be valid.

In our original article we presented graphs showing five-day moving averages in four parameters: (*a*) ground pressure, p; (*b*) E-layer critical frequency, fE; (*c*) F-layer critical frequency, $fF2$; and (*d*) K-index of geomagnetic activity. The connexion between ionospheric and geomagnetic phenomena is well known. Thus, Appleton and Ingram[3] in 1935 established the correlation between geomagnetic activity and depressions in $fF2$. It is worthy of note that in the period under discussion the inverse correlation between K and $\Delta fF2$ is, as Lewis and McIntosh point out, considerably less striking than that between p and ΔfE (cf. Figs. 1 and 2 in our original article). It would seem, then, that if statistical analysis can be successfully applied to show that there is no significance between the variations in p and ΔfE, it is, *a fortiori*, evident that a similar analysis might, in the present instance, be used for discrediting the established relationship between K and $\Delta fF2$. Conversely, of course, the fact that a phenomenon appears to be statistically significant over a short period must likewise be treated with reserve. The need for the utmost care in the application and interpretation of statistical analyses to such a limited time series is thus clear.

From inspection of our graphs it seemed to us that, so far as p and ΔfE were concerned, the period was unusual in three respects: (i) there appeared to be four oscillations in ground pressure showing a progressive diminution of amplitude, with an average period of about 27 days; (ii) in like manner there appeared to be four marked oscillations of period about 27 days in ΔfE; (iii) oscillations (i) and (ii) appeared to be almost exactly out of phase. In addition, we noted that the period was characterized by an unusual 27-day recurrence of great sudden commencement (S.C.) magnetic storms.

In our original communication we merely directed attention to these matters, and suggested that there might be some connexion between them. We did not then suggest, nor do we now suggest, that from a period of length only four months any conclusions can be drawn regarding the general behaviour over a long period of any of the geophysical parameters considered. The severely limited number of observations available, together with the fact that there is considerable uncertainty about the correct statistical approach to time series analysis, seemed to us sufficient reason for not entering into an extended statistical analysis.

However, the contrary conclusions reached by Lewis and McIntosh (see below) have prompted us to re-examine the data. Briefly, their conclusions are: (i) the 27-day oscillation in ground pressure is of no significance, since the amplitude is no more than would be expected from mere chance considerations; (ii) the 27-day oscillation in ΔfE is probably significant; (iii) oscillations (i) and (ii) are exactly in anti-phase; (iv) there is no significant correlation coefficient between the p and ΔfE data; (v) our conclusions arise from smoothing of the data.

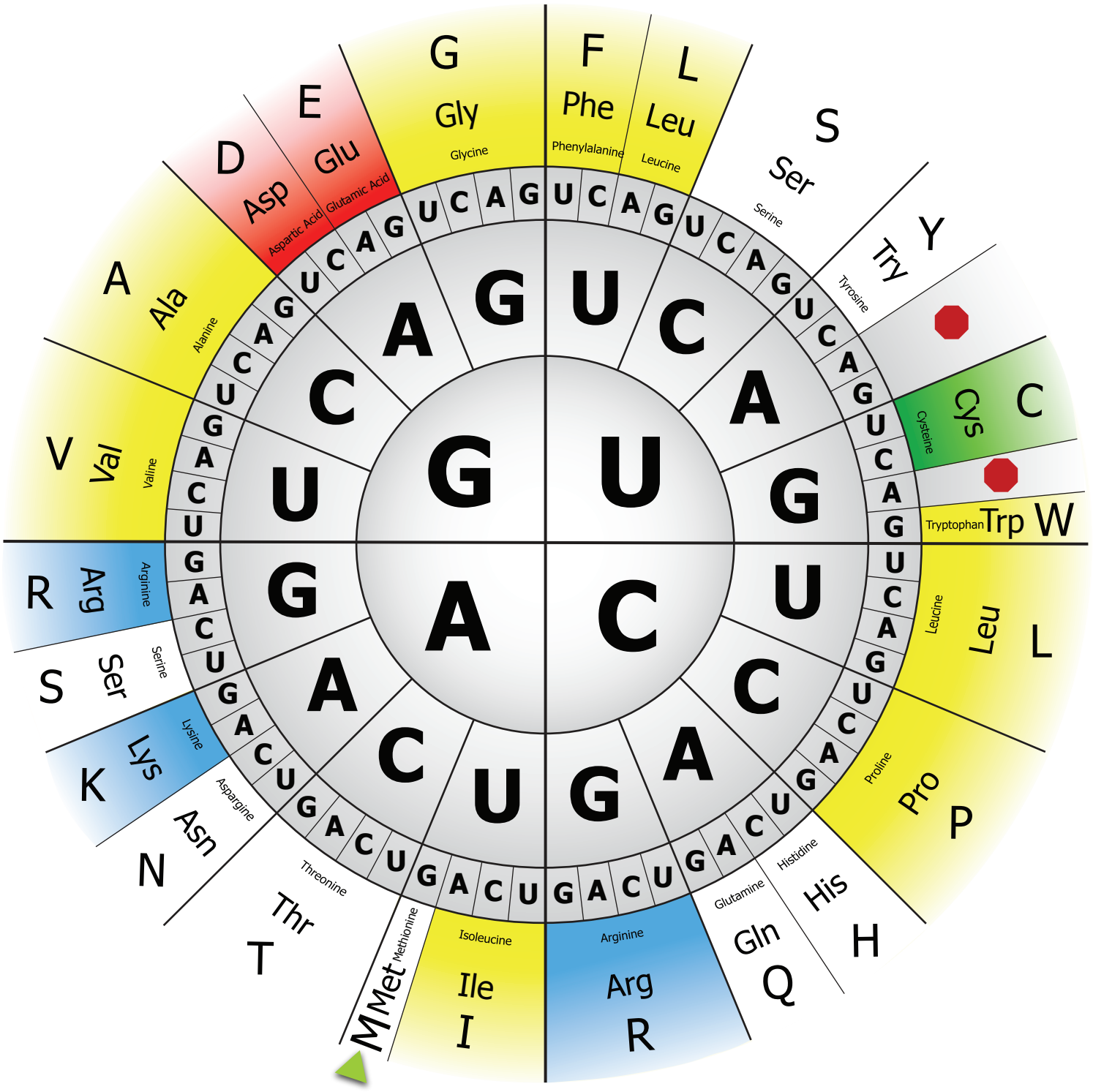
We shall now outline our own analysis. In various communications[4–6], Kendall has made it abundantly clear that most of the methods generally used for studying periodicities in time series (for example, periodograms, Fourier analysis, etc.) may yield very misleading results when applied to the kind of time series with which we are here concerned. He has also questioned the reliability of the usual significance tests for periodicities when applied in time series analysis. Kendall has shown that the most reliable approach is that of serial correlation coefficients as exhibited in the correlogram. He points out that although the correlogram may be insensitive, it does give a lower limit to the oscillatory effects, and that if it oscillates there is almost certainly some systematic oscillation in the primary series explored. Figs. 1 and 2 show the correlograms for Δp and ΔfE respectively for the period under consideration. In both of these the original *unsmoothed* data have been used.

It is important to note that there is a marked trend in the pressure data, and to eliminate this we have dealt with values of pressure departures, Δp (as with the fE data), rather than with the absolute magnitudes p. The oscillations in both correlograms are clear, with a maximum at 26–27 days in each case. These correlograms provide strong support for our original deductions (based, as they were, on simple inspection of graphs), and make it essential for us to repeat Lewis and McIntosh's calculations.

At the outset we must again stress that the pressure data exhibit a marked downward trend (approximately linear), and it is imperative initially to eliminate this before proceeding with any numerical analysis. It appears that Lewis and McIntosh have overlooked this point, and as a result have arrived at quite contrary conclusions. This will be clear from an examination of Table 1, in which we present the results of calculations made by us using (i) pressure, p, (ii) pressure departures, Δp, and (iii) fE departures, ΔfE. The nomenclature employed (c, φ, σ, etc.) is that used by Lewis and McIntosh.

Without going into details, it can be stated that there is little significant difference between the present results *using pressure*, p, and those given by Lewis and McIntosh. The slight differences in the values of amplitude c and first serial correlation coefficient r_1 are of no significance and can be ascribed to different ways of deducing the amplitude and phase

The Genetic Codon Chart©



Amino Acid Properties

- Translation Start Codon
- Hydrophilic / Polar
- Hydrophobic / Non-polar
- Cysteine
- Translation Stop Codon
- Negative Charge
- Positive Charge

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 Version 3.0

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The Standard Genetic Code

Center for BioMolecular Modeling

	U	C	A	G	
U	UUU → Phe	UCU → Ser	UAU → Tyr	UGU → Cys	U
	UUC → Phe	UCC → Ser	UAC → Tyr	UGC → Cys	C
	UUA → Leu	UCA → Ser	UAA → Stop	UGA → Stop	A
	UUG → Leu	UCG → Ser	UAG → Stop	UGG → Trp	G
C	CUU → Leu	CCU → Pro	CAU → His	CGU → Arg	U
	CUC → Leu	CCC → Pro	CAC → His	CGC → Arg	C
	CUA → Leu	CCA → Pro	CAA → Gln	CGA → Arg	A
	CUG → Leu	CCG → Pro	CAG → Gln	CGG → Arg	G
A	AUU → Ile	ACU → Thr	AAU → Asn	AGU → Ser	U
	AUC → Ile	ACC → Thr	AAC → Asn	AGC → Ser	C
	AUA → Ile	ACA → Thr	AAA → Lys	AGA → Arg	A
	AUG → Met	ACG → Thr	AAG → Lys	AGG → Arg	G
G	GUU → Val	GCU → Ala	GAU → Asp	GGU → Gly	U
	GUC → Val	GCC → Ala	GAC → Asp	GGC → Gly	C
	GUA → Val	GCA → Ala	GAA → Glu	GGA → Gly	A
	GUG → Val	GCG → Ala	GAG → Glu	GGG → Gly	G

translation start codon

translation stop codon

hydrophobic amino acids

hydrophilic non-charged amino acids

- charged amino acids

+ charged amino acids

cysteine

Pilot Study Version

DNA Starter Kit

1-Group Set

3-D Molecular Designs

...where molecules become real ™

DNA Replication and Transcription Activity

The purpose of this exercise is to explore the steps involved in DNA replication and transcription, simulating these activities using foam models of the nucleotides. At the end of this activity, you should be able to describe each process and identify the enzymes involved.

Open the bag labeled "DNA Starter Kit" and separate the parts. Each kit should contain:

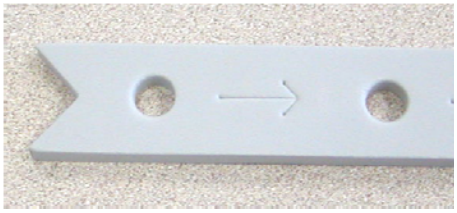
- 12 red adenine
- 12 blue cytosine
- 12 green guanine
- 12 yellow thymine
- 6 white uracil
- 4 long gray DNA backbone pieces – note the arrows on the backbone – point from 5' phosphate end to 3' OH end
- 24 gray sugar-phosphate backbone pieces (representing deoxyribose linked to a phosphate for DNA)
- 12 purple sugar phosphate backbone pieces (representing ribose linked to a phosphate for RNA)
- 56 orange phosphate pieces
- 48 orange phosphate linkers

If something is missing from your kit or you have too many pieces, notify your instructor.

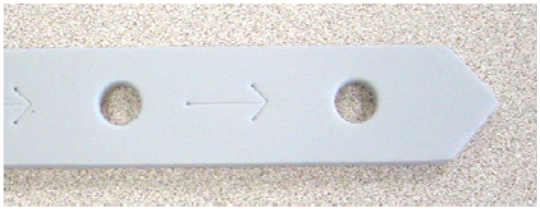
Construct Double-Stranded DNA

1. Look at the long gray backbone piece. Note the arrows on one side of these pieces:

5 ' phosphate end of the backbone



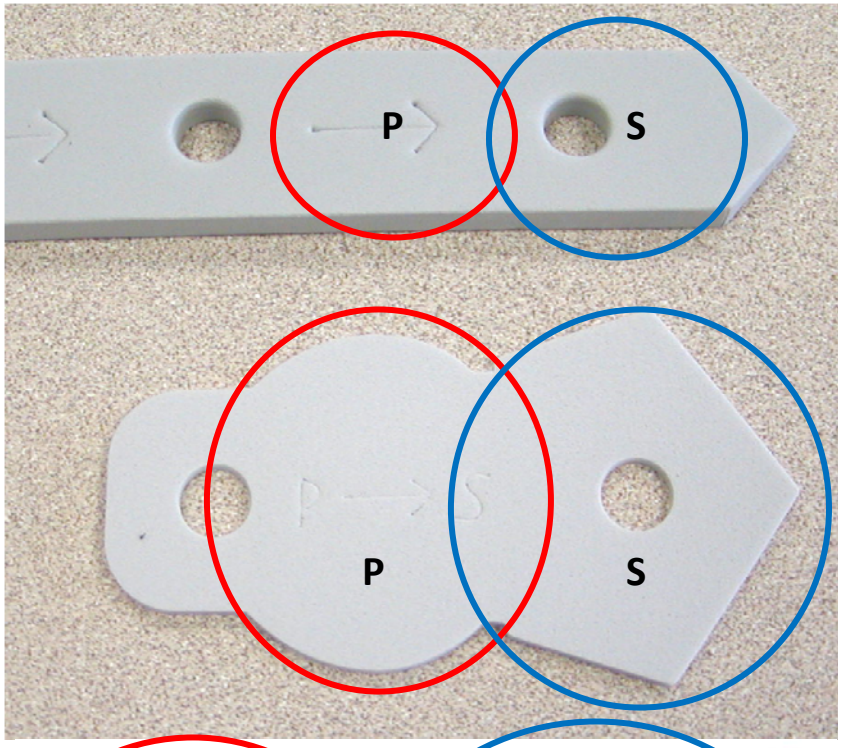
3' OH (sugar) end of the backbone



5' ⟶ 3'

This model is a schematic model – since the sugar and phosphate structures are not clearly evident. The holes in the backbone are the attachment sites for the nucleotides, so they represent the 1' carbon of the sugar molecules.

The arrows point from the 5' phosphate end of the backbone towards the 3' OH (sugar) end of the backbone. The smaller individual sugar-phosphate pieces also have an arrow that points from the 5' end to the 3' end of the piece:



In the smaller pieces, the shape of the sugar (pentagon) is more visible, and the phosphate is represented by the circle. You'll also see a stamp that shows P → S on this model as well.

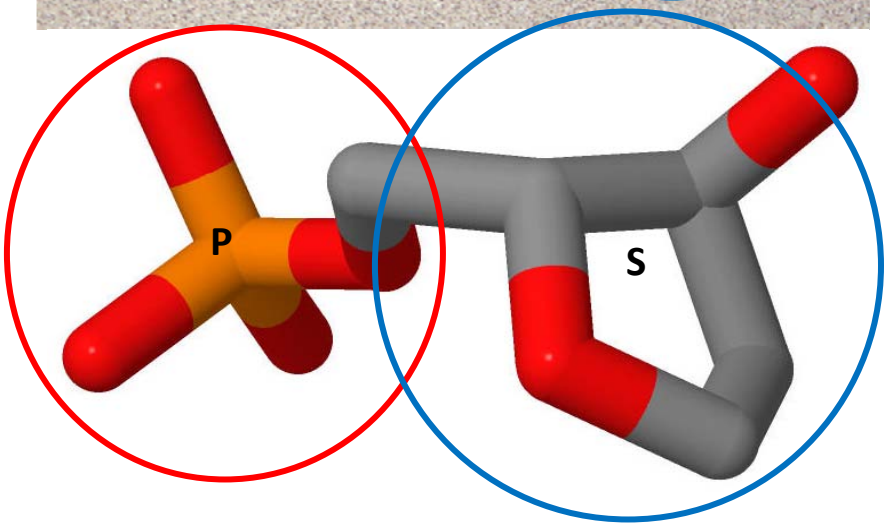
Below the images of the schematic phosphate-sugar backbone pieces is a more accurate representation of the backbone, based on the X-ray crystal structure, displayed in Jmol.

The Jmol image is color-coded, such that:

Gray is carbon
Red is oxygen
Orange is phosphorus

In each depiction, the phosphate group is circled in **red**, and the sugar group is circled in **blue**.

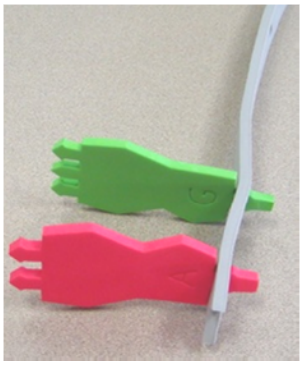
Each of these models, though different, represents the same structure.



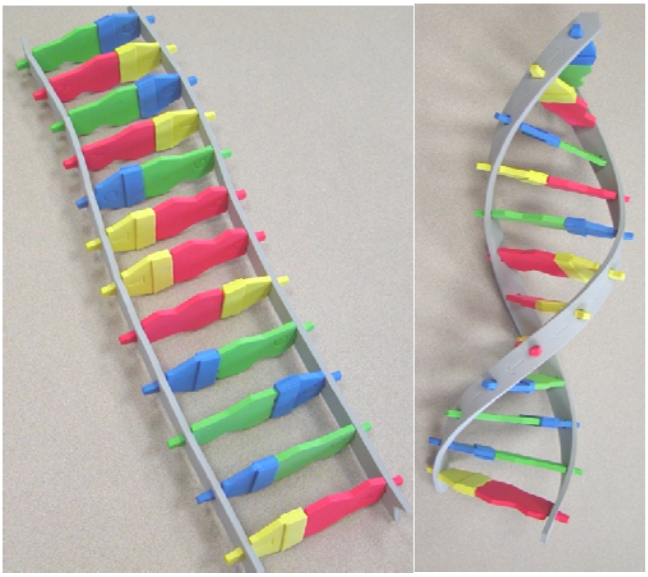
5' ⟶ **3'**

All nucleic acids (both DNA and RNA) are synthesized in the 5' to 3' direction – by polymerases. DNA polymerases make DNA, and RNA polymerases make RNA.

2. Using 3 each of adenine, cytosine, guanine and thymine and a long DNA backbone piece, construct a segment of single stranded DNA. Place the nucleotides in any order on the chain. (See figure at right.)



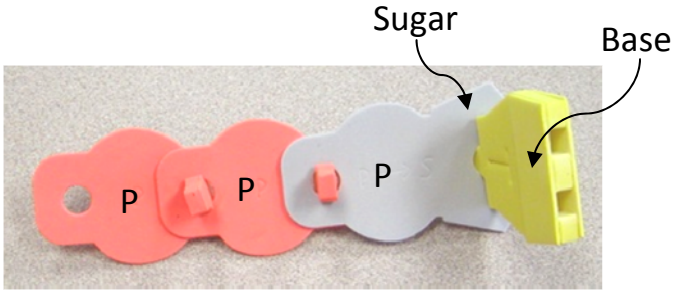
3. Next, build the second strand of DNA **complementary** to the first, using 3 of each of the nucleotides A, G, C and T, and a second long DNA backbone. Be sure the backbone is running **antiparallel** (in the opposite direction) to the first backbone. Add the diphosphate to the 5' end of this DNA strand, and join the base pairs.



4. Twist your DNA into a right handed helix by holding the two ends in your hands and twisting toward you with your right hand and away from you with your left hand. Verify that this is a right-handed helix. (Hint: Imagine walking up the DNA as a spiral staircase. Which hand is on the OUTSIDE railing?)

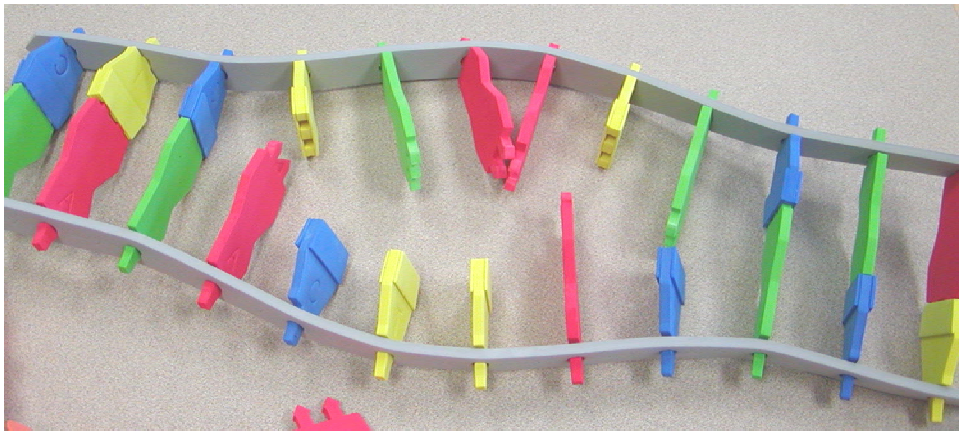
5. **Complete Part A of the DNA Replication and Transcription Worksheet before proceeding.**

DNA Replication



1. DNA is synthesized by DNA polymerase using nucleotide triphosphates as substrates. Build nucleotide triphosphates with the remaining A, G, C and T bases. Each of these will have a base (A, G, C or T) joined to a gray sugar (the pentagon side of the gray sugar phosphate). See illustration at right. The base is attached to the 1' carbon and the phosphate group is attached to the 5' carbon of the sugar molecule, but these individual atoms are not identifiable in this model of DNA. Two phosphate groups will be attached to the phosphate using the orange phosphate connectors.

2. Create replication bubble – pulling the middle 6 bases apart. The end bases will remain intact for the time being.



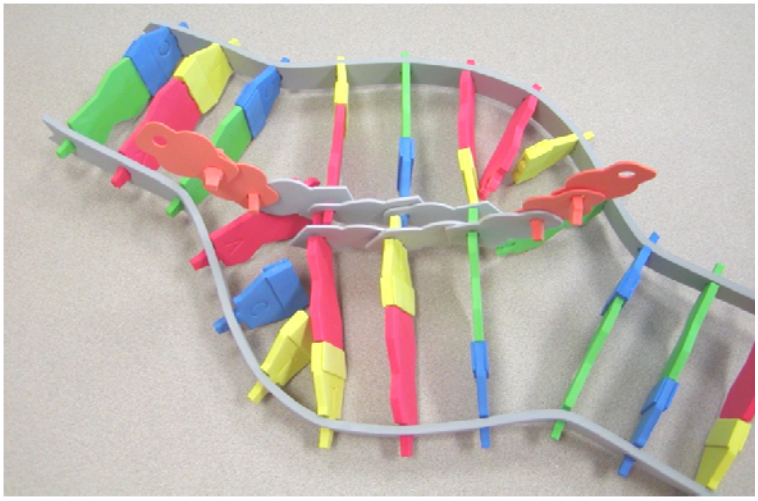
3. New DNA strands are synthesized in the 5’ to 3’ direction – so opposite directions on each strand.

4. Energy for each nucleotide addition comes from splitting off the two phosphates. Just as ATP is an energy carrier molecule, so are GTP, CTP and TTP.

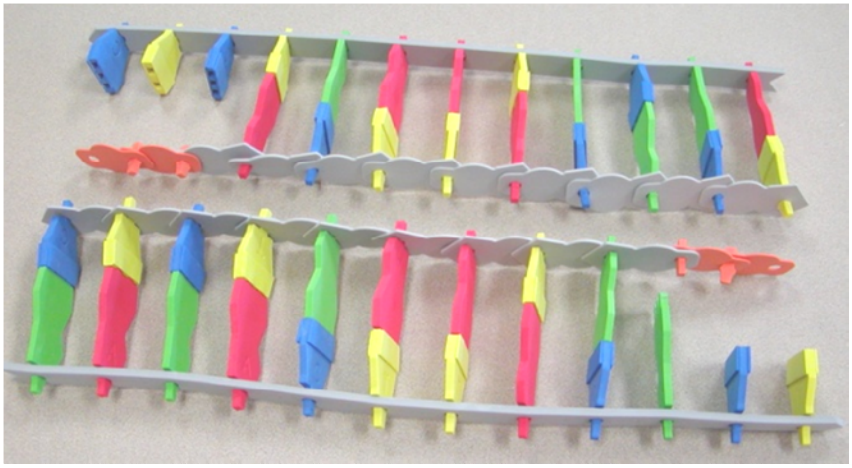
5. Initiation of DNA replication begins with a short RNA primer. Since you are working with such a short strand of DNA, we’re not going to simulate the RNA primer in this activity.

6. Add a nucleotide that is complementary to the nucleotide at the 3’ end of one of the template strands within the replication bubble. Be sure you orient the nucleotide so the arrows on the sugar-phosphate piece are running in the *opposite* direction from the arrows on the backbone of the template strand. Similarly, add a nucleotide complimentary to the nucleotide at the 3’ end of the *other* template strand. Make sure that the arrows on the backbone pieces on the two strands point in opposite directions. Note that replication on the two strands does not start in the same place.

7. Bring in the next nucleotide on each strand – being sure to ‘grow’ your new chain in the 5’ to 3’ direction. Remove 2 phosphate groups as you add each new nucleotide to the sugar of the preceding nucleotide. Note that the first nucleotide retains its phosphate groups. Recall that ATP is considered the “energy currency” of the cell, and that removing one or more phosphate groups from ATP releases energy to drive chemical reactions. Similarly, CTP, GTP and TTP also have stored energy in the phosphate bonds. Energy released from cleaving the phosphates from each of these molecules is used to form the phosphodiester bond in the DNA backbone.



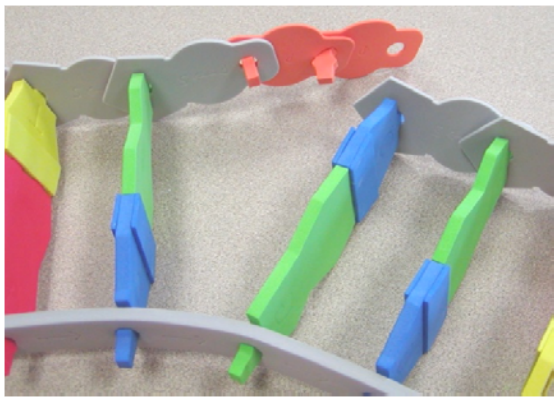
8. Build your two DNA strands, 5’ to 3’, opening the bubble and adding bases complimentary to the template strand.



9. When you are done, you should have two double stranded DNA sequences that lack two nucleotides on the 5’ end. Because the replication bubble proceeds in BOTH directions, and DNA synthesis can only occur from 5’ to 3’, for each replication fork, there is a leading strand (synthesized 5’ to 3’) and a lagging strand (running 3’ to 5’). In order to fill in the gaps on the lagging strand, synthesis is delayed, then build 5’ to 3’ in

segments of approximately 200 nucleotides. These short segments were discovered by Okazaki and coworkers, they are aptly named "Okazaki fragments".

10. Simulate the filling in of the Okazaki fragments by adding bases at the 5' end of your DNA chains. Be sure to add the bases 5' to 3'. Remember that DNA polymerase can add bases one at a time in the 5' to 3' direction. But DNA polymerase cannot join the backbone between two nucleotides that have already been added to the chain.



11. A separate enzyme, DNA ligase, joins the backbone between the Okazaki fragments. Simulate DNA ligase to create intact sugar-phosphate backbones on both of your DNA strands.

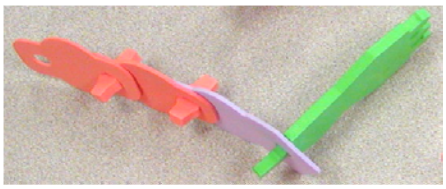
12. At this point you should have two nearly identical DNA double strands. What is the difference between the two strands? [Hint: Observe the solid vs. the pieced backbone pieces. Which strand is pieced on the two models?] The solid strand represents the ORIGINAL DNA, and the pieced strand represents the newly synthesized DNA. Back in the 1950's scientists didn't know exactly HOW DNA replication occurred – whether the old sequence remained intact and a completely new DNA was synthesized (termed "conservative replication") or whether each strand served as a template for a new strand, so that the daughter DNA contained one old strand and one new strand (termed "semi-conservative replication" since one half of the DNA is conserved in the new strand). Through a series of experiments using two isotopes of phosphorous, Meselson and Stahl were able to demonstrate that DNA replication is semi-conservative and that each strand serves as a template to build a new complementary strand of DNA. Your model demonstrated semi-conservative replication.

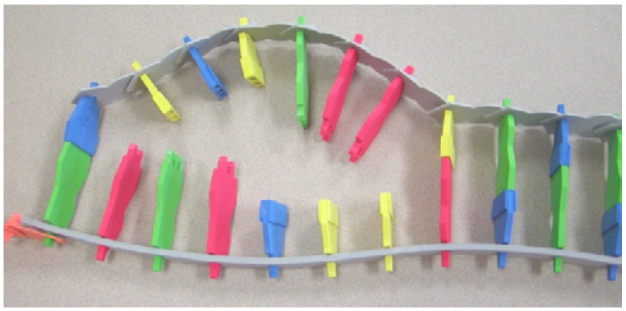
6. Complete Part B of the DNA Replication and Transcription Worksheet before proceeding.

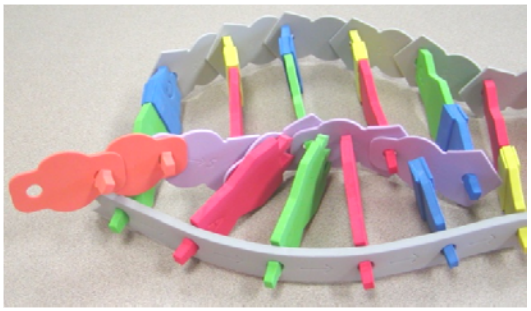
Transcription into mRNA

1. Human DNA contains sequences coding for genes, sometimes thousands of bases long, as well as regulatory sequences that are involved in turning genes on and off. Sometimes regulators function more like dimmer switches, fine tuning the expression of genes. Your segment of DNA is too short to model regulatory sequences. – Instead, you'll focus on the basics of transcription into RNA.

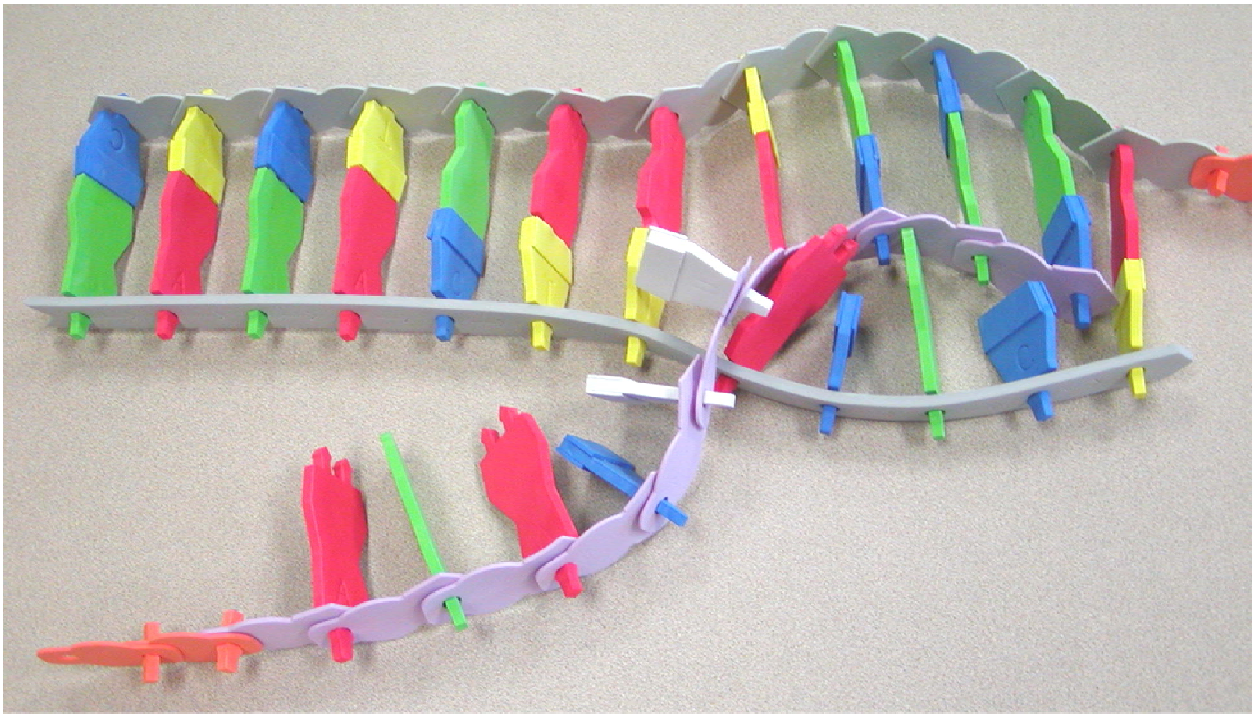
2. When DNA is transcribed into mRNA, only one strand of DNA is used. This is termed the template strand. Select one of your double-stranded DNA molecules to use. Take apart the other model of DNA. (You'll be using pieces of it for the next step.) Next, create ribonucleotide triphosphates by attaching the lavender ribose phosphate, then two additional orange phosphate groups, to each of three A (red), C (blue), G (green) and U (white) nucleotides.



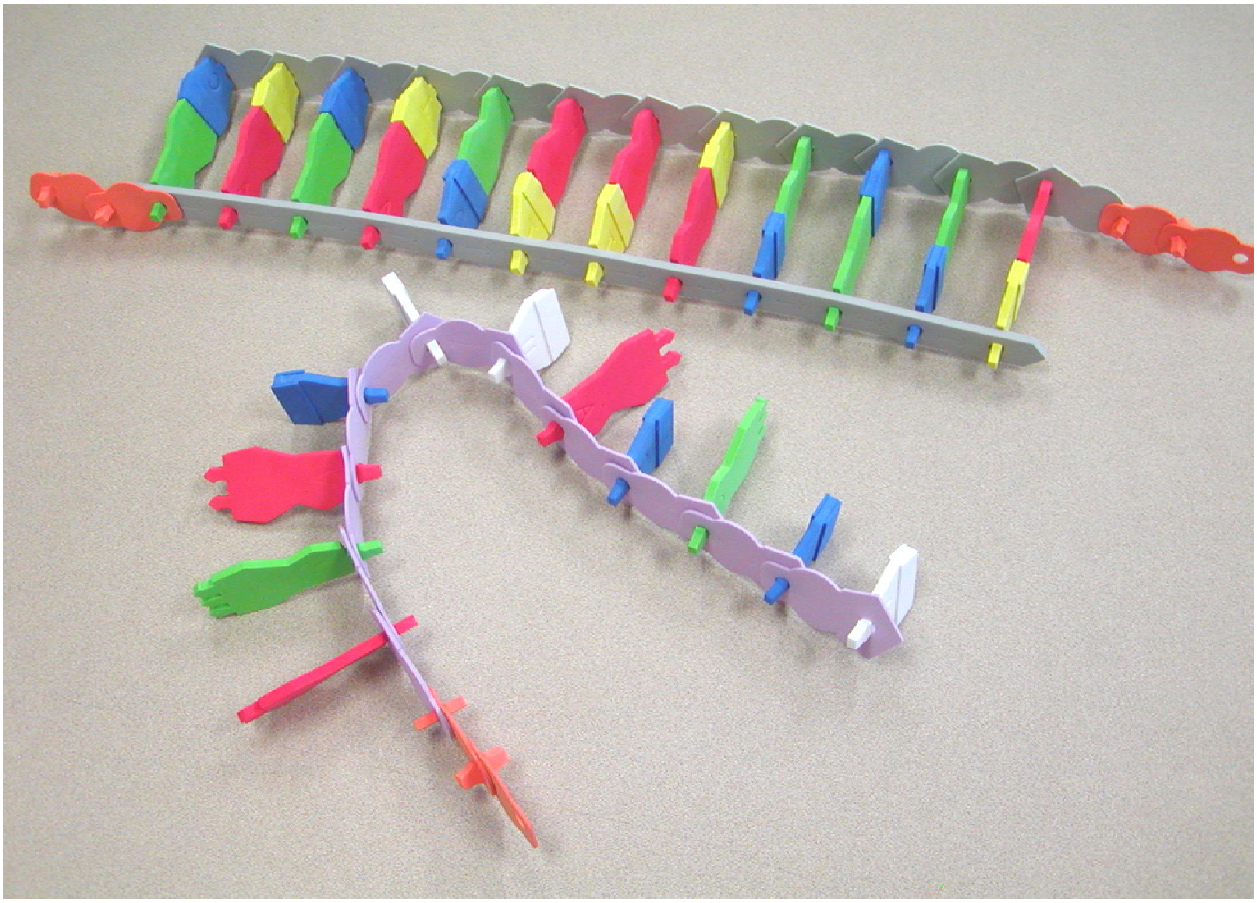




You will be using the newly synthesized strand of DNA (the one with the pieced backbone) as the template strand. The RNA polymerase binds to the DNA and unzips the DNA, forming a bubble similar to replication. In this simulation, you should form a bubble of 6 bases beginning two bases in from the 3' end of the template strand. Since RNA polymerase builds the RNA from 5' to 3', it must read the DNA from 3' to 5'. Using the template strand, build a complementary mRNA. As the transcription bubble moves along the DNA, each time you unzip one DNA base pair to add a ribonucleotide to the growing mRNA chain, you should remove one ribonucleotide from the start of the mRNA molecule and rezip the DNA. In this simulation, only 4 base pairs of DNA should be unzipped at once. (In reality, about 10 base pairs are unzipped and bound to mRNA at any given time.) You will have a double stranded DNA model with a few bases unzipped. One strand of the unzipped DNA will be bound to mRNA, which will have a single stranded "tail" that grows as RNA polymerase moves down the DNA.



3. Once you reach the end of the DNA, the mRNA will continue detaching from the DNA and the DNA will rezip. In the end, you should have a single-stranded mRNA molecule complementary to the DNA template strand, and a double-stranded DNA molecule.



4. **Complete Part C of the DNA Replication and Transcription Worksheet before proceeding.**

5. Disassemble your DNA and mRNA and place all the parts in the appropriate bags.

DNA Replication and Transcription Worksheet

Lending Library: DNA Starter Kit© (DS)

Part A: DNA Structure

1. Explain what is meant by each of the following terms as they relate to DNA structure:

   A. Complementary base pairing

   B. Antiparallel strands

   C. Sugar-phosphate backbone

2. Record the sequence of your DNA structure, and indicate 5’ phosphate and 3’ carbon ends of the strands. Draw lines between complementary base pairs:

Part B: DNA Replication

1. Explain what is meant by semi-conservative DNA replication.

2. Forming the phosphodiester linkage in the DNA backbone is an endergonic reaction. What is the source of the energy that drives this reaction?

3. In what direction is the newly synthesized DNA made?

4. Recall that the two DNA strands are antiparallel. In what direction is the DNA polymerase reading the *template* strand?

5. What is an Okazaki fragment?

6. What is the purpose of DNA ligase?

Part C: Transcription

1. What are two differences between DNA and RNA?

2. Complete the following table comparing and contrasting DNA replication with transcription:

DNA replication	Characteristic	Transcription
	How many strands of DNA are used as the template?	
	Direction of synthesis of the new strand?	
	Direction of movement of polymerase along the template strand?	
	What happens to the new strand as it is being made?	
	Enzyme(s) involved in the process	

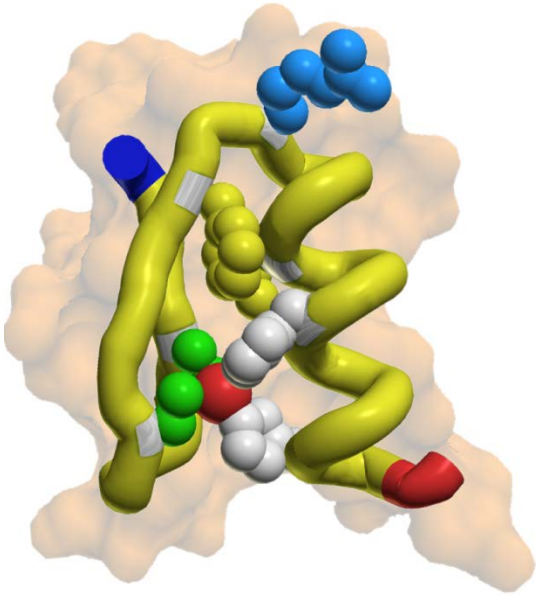
Center for BioMolecular Modeling

Zinc Finger Folding Activity

Based on amino acids 4-31 of 1zaa.pdb

Parts List

- Toober segment – 28 amino acids long (approx 72 cm)
- Sidechains
  - 2 Cys 2 His 1 Phe
  - 1 Arg 1 Leu
- 7 metal clips
- Zinc Atom
- Zinc Finger Folding Map
- 1 Blue end cap (for designating the amino terminus)
- 1 Red end cap (for designating the carboxy terminus)



Introduction

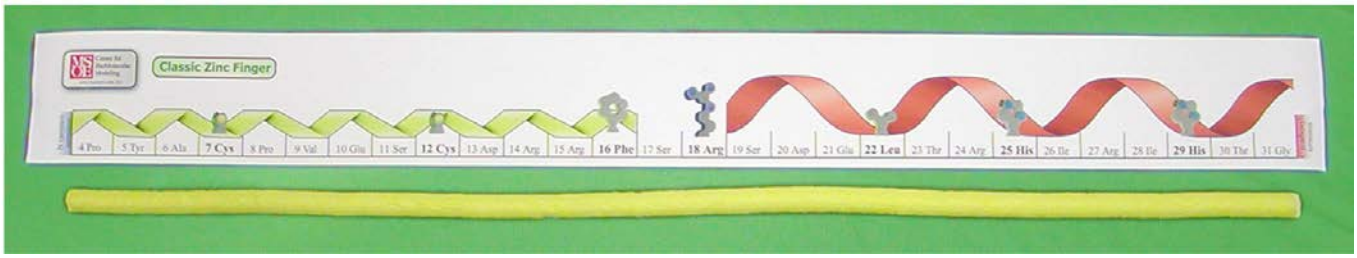
A C_2H_2 zinc finger is a 28 amino acid protein motif composed of a short alpha helix and a two-stranded beta sheet. The structure of the zinc finger is stabilized by a zinc atom that binds 2 cysteine and 2 histidine sidechains, and by hydrophobic amino acid sidechains that are buried on the inside of the folded motif. Zinc finger proteins function as regulators of gene expression. They bind to the negatively-charged backbone of DNA through a positively-charged arginine amino acid sidechain located at the beginning of the short alpha helix.

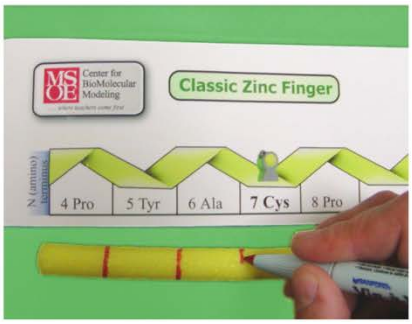
The construction of a physical model of the 3D structure of a zinc finger serves as a good example of how Toobers can be used to model protein structures.

This kit is based on 1ZAA.pdb and represents amino acids 4-31.

Getting Started: Laying out the primary sequence

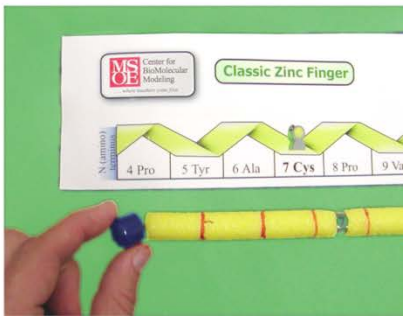
1. Lay out the protein folding map and position the toober below it.



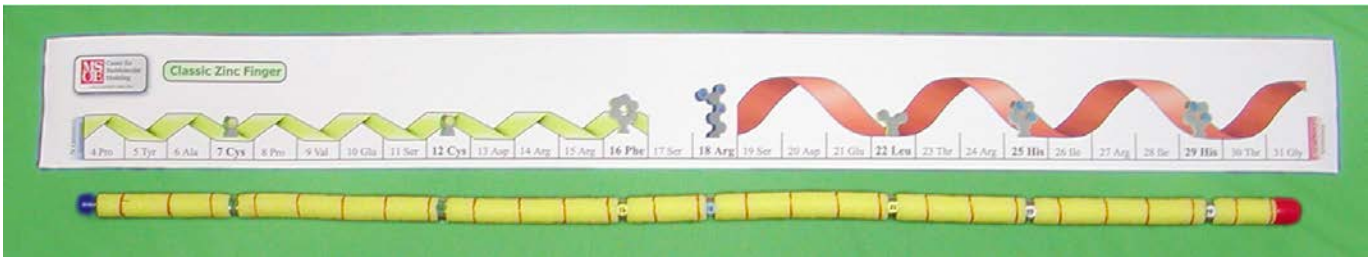


2. Align the toober with the protein folding map and draw a line for each amino acid.

3. Number and place the seven clips on the toober according to the protein folding map.

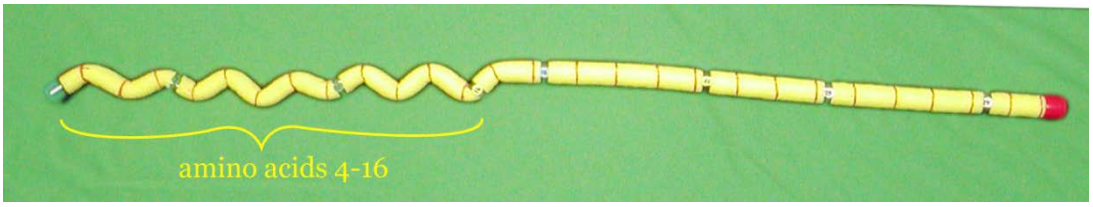


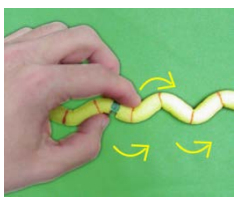
4. Add the blue and red end caps.



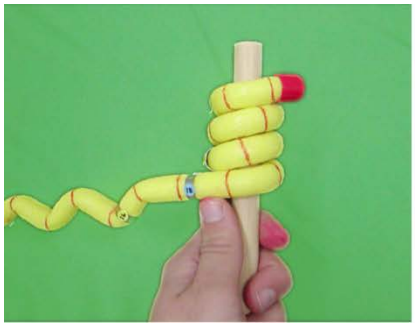
Folding the Protein

1. Bend the first 13 amino acids into a β-sheet (amino acids 4-16). You can create a β-sheet by bending the toober at every amino acid (approximately every 2 cm).

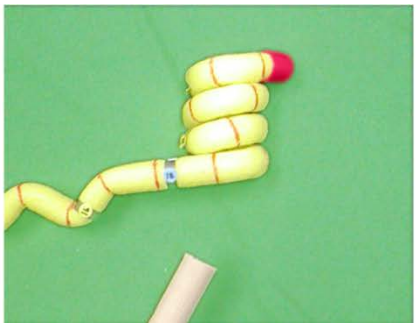




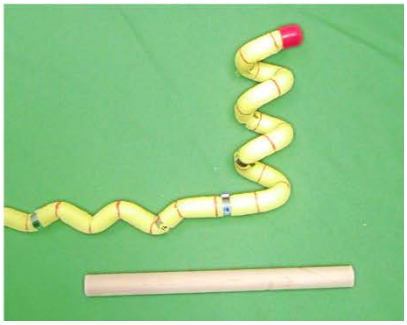
2. Next, create an α-helix with amino acids 19-31 (the last 13 amino acids of the protein). Do this by wrapping the toober segment around an alpha helix bending jig (wooden dowel or your finger) and then stretch it out so that so that there are approximately 3.6 amino acids per turn of the helix.



Wrap the toober around the jig.



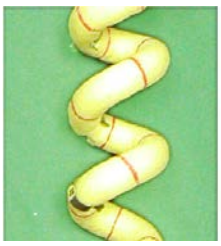
Slide the toober off the jig.

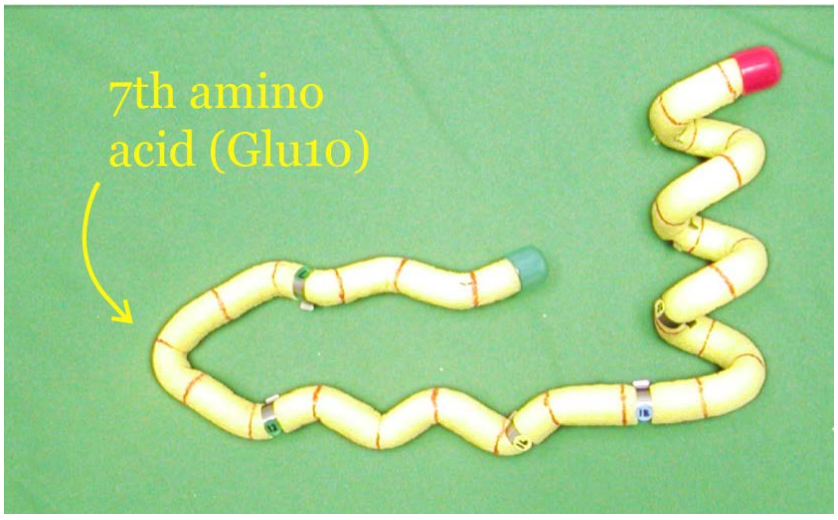


Stretch the toober.

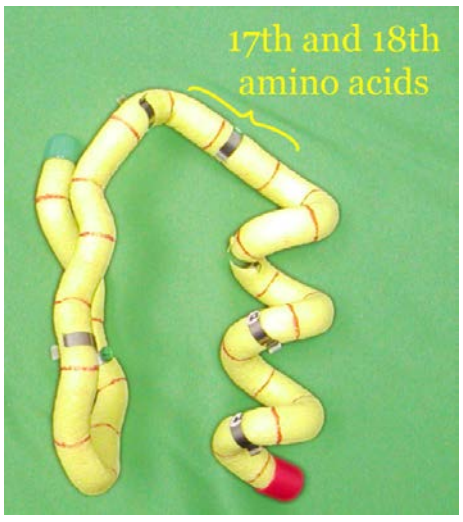
Is your helix right-handed or left-handed?

Alpha-helices are right-handed. Make sure that your model has a right-handed helix. To do this, imagine that your alpha helix is a spiral staircase. If you can climb that staircase with your right hand on the outside railing (the toober), then you have a right-handed helix.



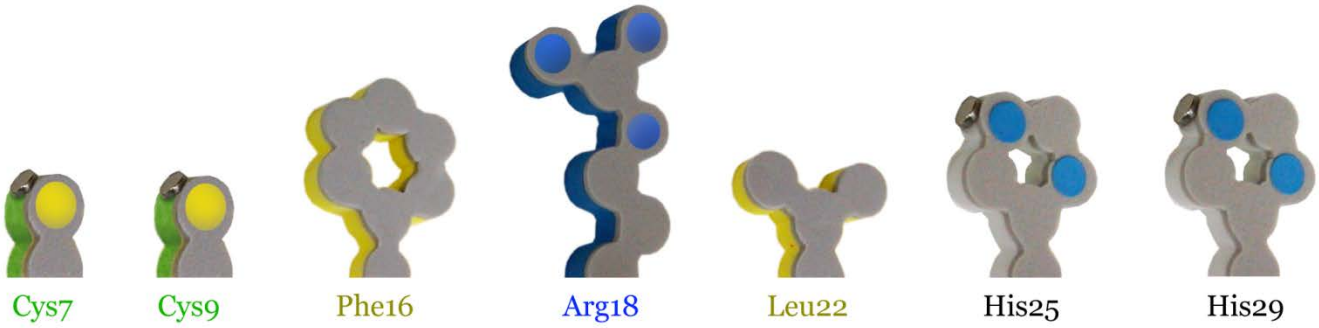


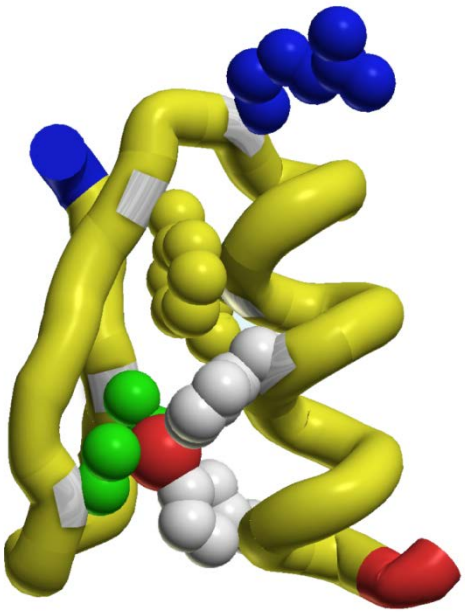
3. Now, bend the beta pleated strand in half at the 7th amino acid (Glu10).



4. Next bend the toober segment at the "turn" (between the 17th and 18th amino acids) so that it resembles the picture shown to the right.

5. Finally, using the zinc finger folding map included within this activity, decipher which amino acids have sidechains represented and attach the sidechains to the clips at their correct amino acid locations. There are two cysteines (positions 7 and 9), two histidines (positions 25 and 29), one phenylalanine (position 16), one arginine (position 18) and one leucine (position 22). Then position the zinc atom in the middle so that it is coordinated by the two histidines and two cysteines.





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Enzymes in Action Kit©

Creating Catalytic Connections with Models

Teacher Key

Objectives

You will use the model pieces in the kit to:

- **Simulate** enzymatic actions.
- **Explain** enzymatic specificity.
- **Investigate** two types of enzyme inhibitors used in regulating enzymatic activity.
- **Examine** how an enzyme may affect activation energy.

Introduction

Enzymes are specialized proteins that **catalyze** or speed up chemical reactions within cells. The substance upon which an enzyme acts is called a **substrate**. Substrates are small molecules.

Enzymes:

- Accomplish catalysis without being consumed in the reaction.
- Catalyzes a specific chemical reaction.

The Enzyme in Action Kit© allows you to explore how enzymatic reactions occur.

Catabolism

Model pieces needed

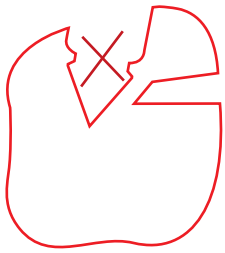
gray A foam piece without stickers

green B_1 and B_2 foam pieces

orange C_1 and C_2 foam pieces

1. The gray foam piece is a model of an **enzyme**. Place it with the **A** label facing up. Assemble the two green pieces (B_1 and B_2) into a single unit to model the **substrate** in this reaction.

2. Draw and label the **enzyme** and **substrate** before the enzymatic action.



enzyme

Substrate

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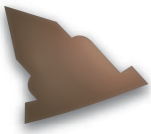
Enzyme Action Continues






3. In this first metabolic action, the enzyme will act on the substrate to break it apart. Experiment with the pieces to model how the enzyme and substrate might interact.

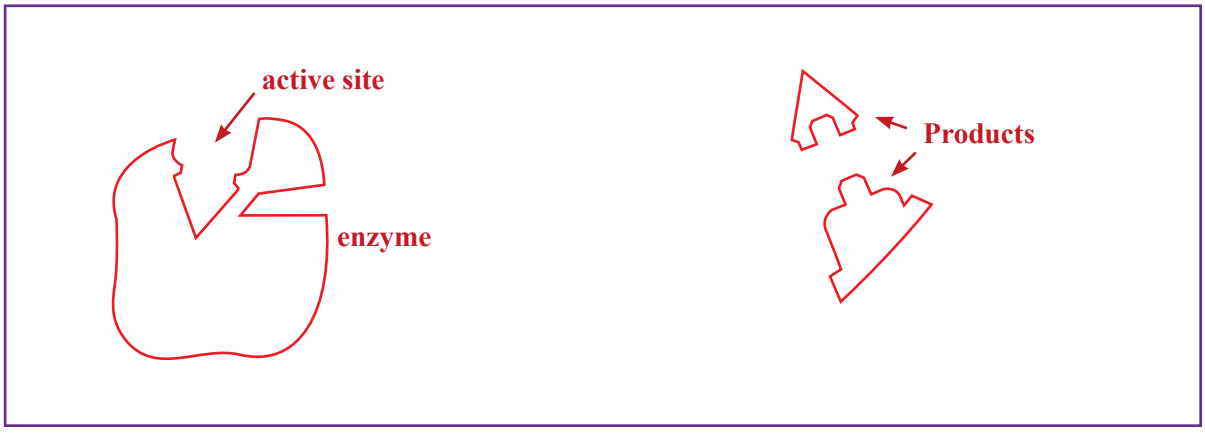
4. The substance an enzyme acts upon is referred to as the **substrate**.

5. Place an "**X**" on the drawing of the enzyme and substrate you created on page 1 to show where the substrate binds to the enzyme.

The part of the enzyme that binds the substrate to be acted on is referred to as the **active site**.

Once the substrate is locked into the enzyme, the two green substrate pieces may be easily pulled apart. This type of metabolic process is called **catabolism** (the breaking down of complex molecules into simpler molecules).

6. Draw and label the **enzyme**, **products** and **active site** after enzymatic action.



7. Although the substrate model changed in this reaction, what changes did you observe in the model of the enzyme during this reaction?

When the substrate was locked into place on the enzyme, a slight shape change took place in the enzyme to allow for the substrate to dock.

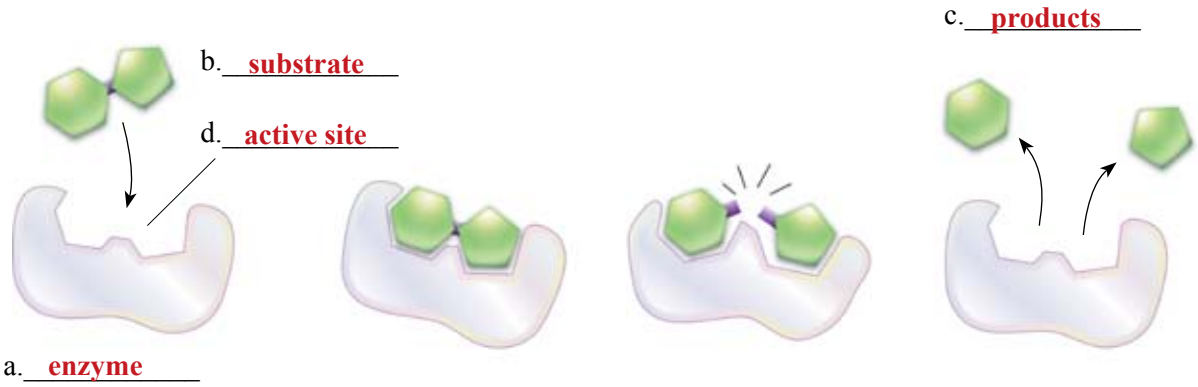
8. Why do you think it is an advantage for the enzyme to remain unchanged while catalyzing a chemical reaction?

Answers will vary but may include: The enzyme may be used over and over again reducing the amount of resources the cell would have to use to make more enzyme. The enzyme is not a direct reactant in the cellular chemical reaction and doesn't change in order to avoid interference with cellular reactions.

Enzyme Action Continues

Note: A real life example of catabolism occurs when the enzyme **sucrase** breaks down the substrate sucrose into glucose and fructose (monosaccharides).

9. Given what you now know about catabolism, identify the following components in the model illustrated below: enzyme, substrate, products and active site.



Induced Fit Model of Enzyme Action

In 1958 scientist Daniel Koshland, Jr., PhD., proposed the **induced fit model** to describe enzyme-substrate interaction. This model suggests that enzymes are flexible structures in which the binding of the substrate results in small changes to the shape of the active site, maximizing its interaction with the substrate.

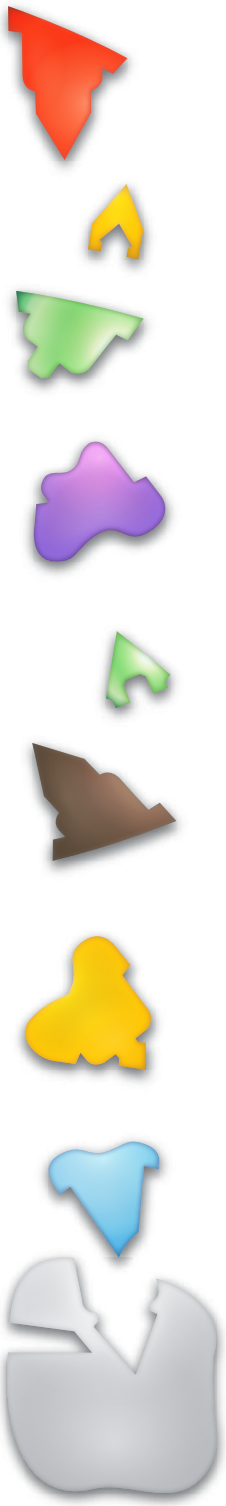
10. Describe how the foam catabolism model illustrates the induced fit model of enzyme-substrate interaction.

The enzyme shows flexibility as it reshapes slightly to allow for the substrate to dock into the active site.

11. Explain the difference between **catalysis** and **catabolism**.

Catabolism is defined as a metabolic process that breaks down complex molecules into simpler ones. Catalysis is the increase in the rate of a chemical reaction due to participation of a substance that can modify the rate of the reaction without being consumed in the process.

Enzyme Action Continues

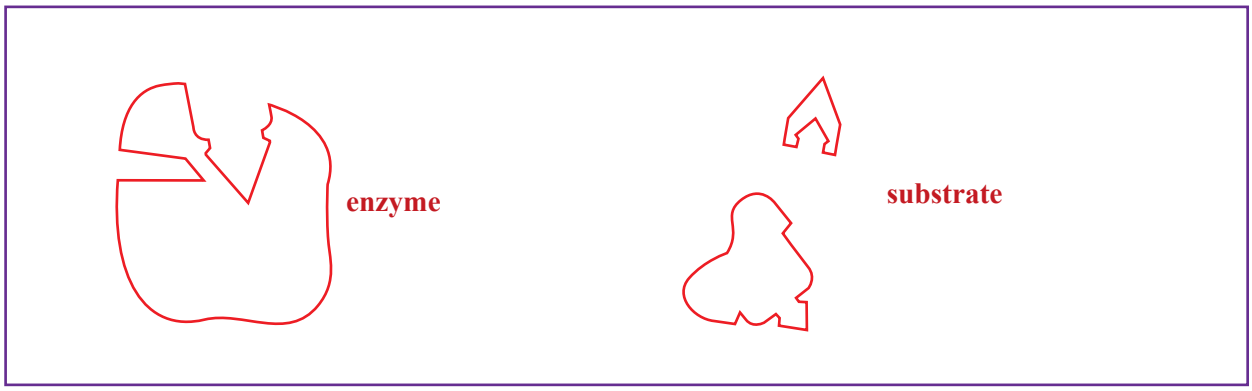


Anabolism

Enzymes may also bring substrates together to form a final product. This metabolic process is called **anabolism** (the building of complex molecules from simpler molecules).

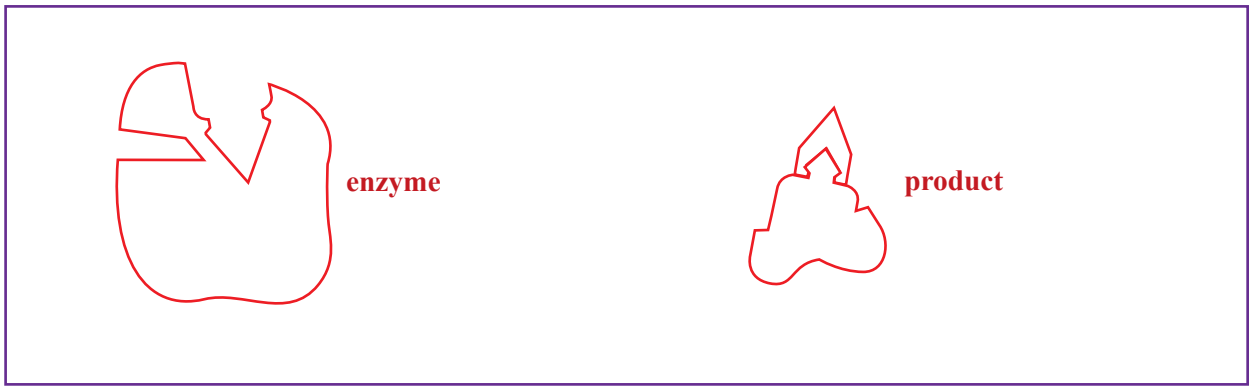
12. Use the gray foam piece and the orange foam pieces (C_1 and C_2) to simulate an anabolic process. The orange pieces should not be assembled prior to the anabolism action.

13. Sketch and label the **enzyme** and **substrate** prior to enzyme action in the space below.



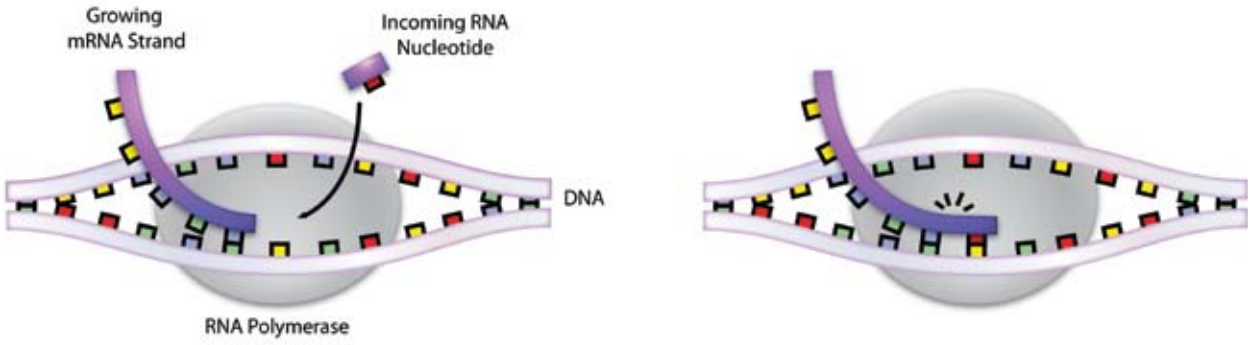
14. Place the small pointed orange piece (C_2) into the enzyme. Join the larger orange piece (C_1) to C_2. Note that the two pieces lock together to form a final product.

15. In the space below, sketch and label the **enzyme** and **products** after the enzyme has acted on the substrate.



Enzyme Action Continues

Note: A real life example of anabolism occurs when **RNA polymerase** links RNA nucleotides together by catalyzing the formation of a bond between the backbone sugar of one nucleotide to the backbone phosphate of another nucleotide during transcription.



16. Given what you now know about anabolism, identify the substrate in the above diagram.

The incoming RNA nucleotides.

17. Explain why the above process is an example of anabolism.

Anabolism is the process of bringing substrates together to form a product. The RNA polymerase brings the RNA nucleotides together to form an RNA molecule.

Lock and Key Model of Enzyme Action

In 1894 scientist Emil Fisher wrote, "To use a picture, I would like to say that enzyme and glucoside have to fit to each other like a lock and key in order to exert a chemical effect on each other." Fisher created a mental model of how an enzyme acts and referred to it as the ***Lock and Key Model of Enzyme Action***.

This model suggests that the enzyme and the substrate possess specific complementary geometric shapes that fit exactly into one another like a key into a lock.

18. Describe how the anabolic process you previously modeled illustrates the lock and key model of enzyme-substrate interaction.

The lock and key model suggests that the enzyme does not change shape to accommodate the substrate. In the anabolic activity, the enzyme model does not change shape when the substrate is bound.

Enzyme Action Continues

Note: Most enzymes catalyze either **catabolic** OR **anabolic** processes. There are a few enzymes that do both. ATP synthase and ATPase are the same protein but have different names because they function as enzymes in both catabolic and anabolic reactions.

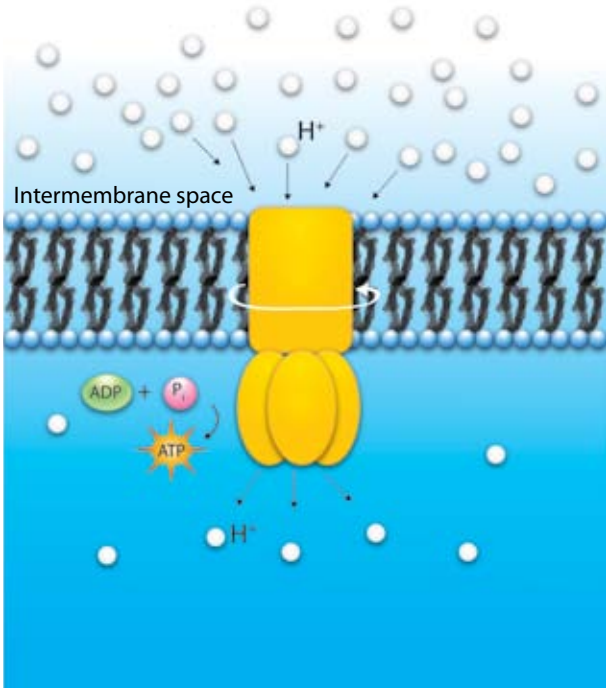


Diagram A — ATP Synthase

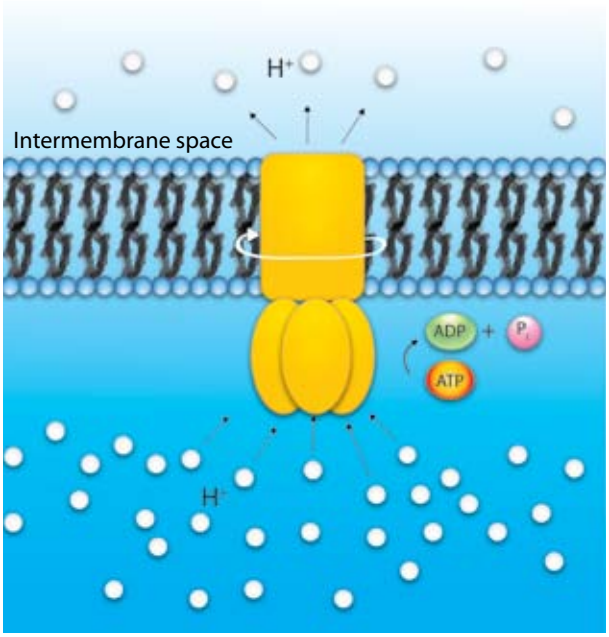


Diagram B — ATPase

19. Describe the action of the enzyme in diagram A. In your description, identify the substrate and enzyme.

Diagram A illustrates an anabolic process. The enzyme ATP synthase is putting together the substrates ADP and P_i to form ATP. Diagram B illustrates a catabolic process where the enzyme ATP synthase is helping to break apart the substrate ATP into the products ADP and P_i.

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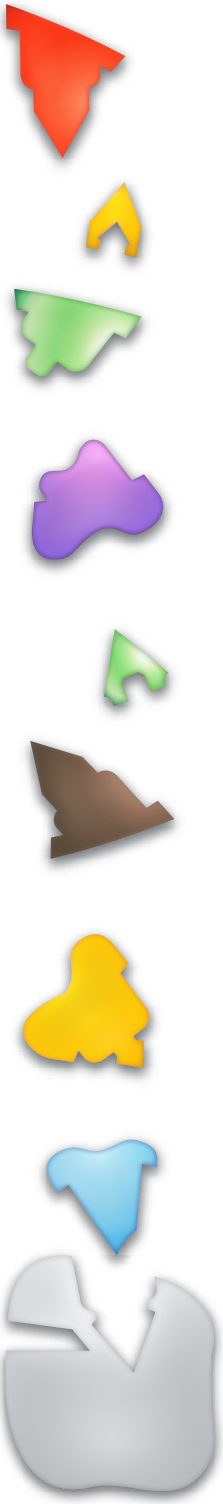
Enzyme Specificity



The reaction catalyzed by an enzyme is very specific. Most enzymes are proteins with unique three-dimensional configurations based on their amino acid sequence. The specificity of an enzyme can be attributed to the compatibility between the shape of the enzyme's active site and the shape of the substrate.

Model pieces needed

gray foam piece with stickers

red D foam piece with stickers

tan E foam piece with stickers

1. Place the enzyme model with the sticker side facing up. Write your observation about the active site of the enzyme below.

Answers will vary but may include: The active site has a negative charge on the left and a positive charge on the right. There is a yellow region deep inside the active site.

2. What might these specialized areas in the enzyme represent?

The charged areas represent charged amino acids found in the active site. The yellow region represents the hydrophobic amino acids in the active site.

3. What do the red D and tan E foam pieces represent?

The red and tan pieces represent possible substrates for the enzyme to act upon.

4. How do the specialized areas of the red D piece interact with the specialized areas of the enzyme?

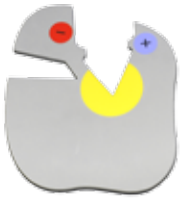
The positive charge of the enzyme matches with the negative charge of the red piece. The negative charge of the enzyme matches with the positive charge of the red piece. The yellow regions match together as well.

5. In order for enzymes to bind to the correct substrate, enzymes have specific active site configurations that allow for interaction with the substrate. Explain why the tan E substrate would not interact with the enzyme.

The tan substrate would not interact with the enzyme because the charged amino acids will tend to repel and the hydrophobic areas will not interact.

Enzyme Inhibition

Model pieces needed


gray foam piece with stickers


red D foam piece with stickers


purple (F) foam piece.


blue (G) foam piece.

Competitive Inhibition

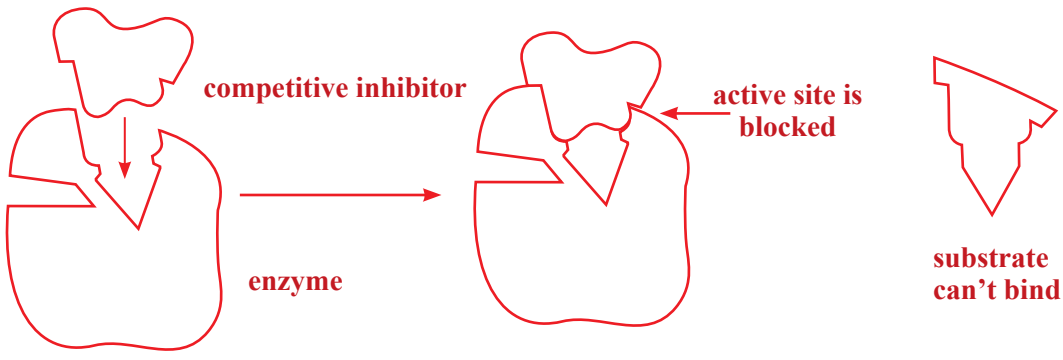
1. Place the gray A, red D, purple F and blue G foam pieces on your work surface. Which two pieces may fit into the active site?

the red and **the purple pieces**

2. Can the red D substrate bind to the active site if the purple F piece is bound to the enzyme? **No.**

A substance which binds in the active site and prohibits normal substrate interaction is called a **competitive inhibitor**.

3. Create a sketch using the foam models to illustrate **competitive inhibition**.



4. Predict what might happen in a cell if the concentration of competitive inhibitor exceeded that of the substrate.

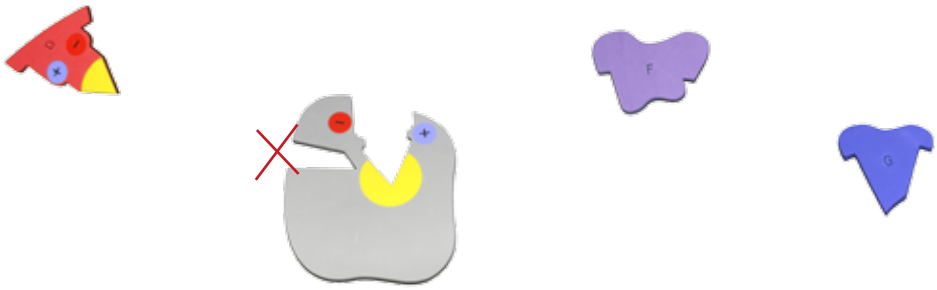
If the concentration of competitive inhibitor exceeded that of the substrate, the enzyme would not be able to bind the substrate and the reaction would slow down.

Enzyme Inhibition

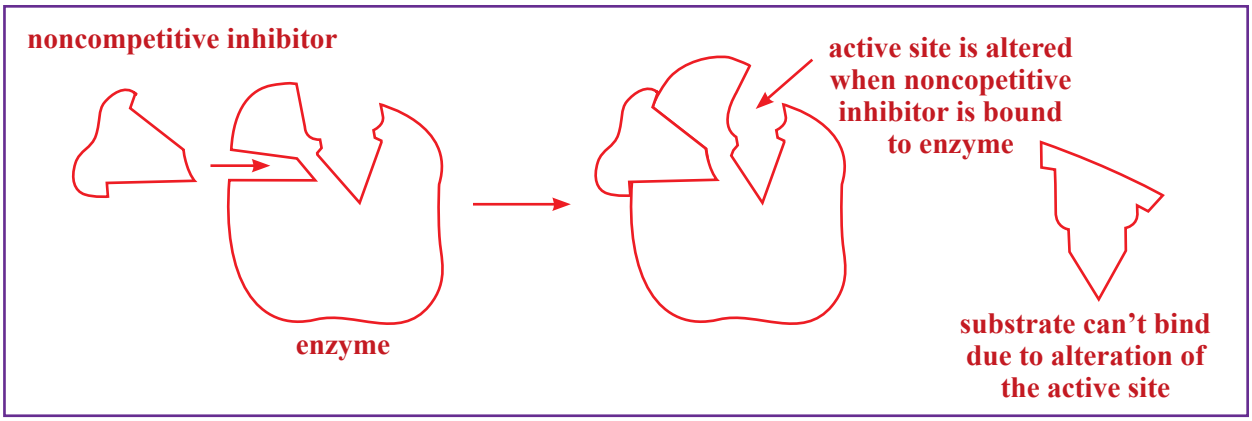
Noncompetitive Inhibition

A **noncompetitive inhibitor** impedes enzymatic action by binding to another part of the enzyme. This second site, known as the ***allosteric site***, is the place on an enzyme where a molecule that is not a substrate may bind, thus changing the shape of the enzyme and influencing its ability to be active.

5. In the diagram below, draw an "**X**" where the **blue G** piece may noncompetitively bind to the enzyme.



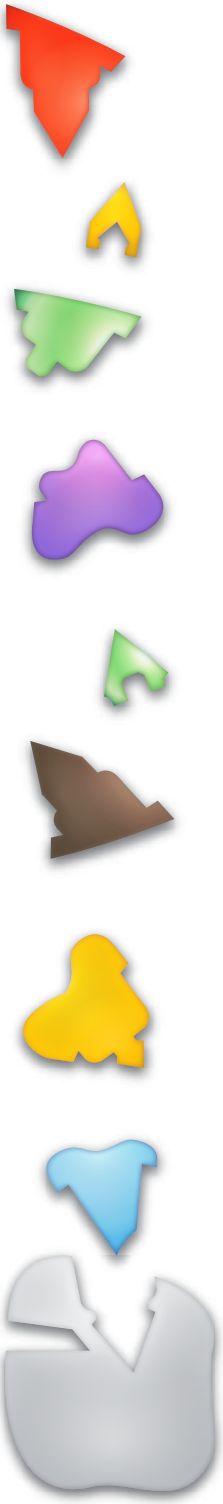
6. Sketch what happens to the shape of the enzyme when the blue piece is bound to the allosteric site.



7. How does this affect the binding of the substrate?

When the noncompetitive inhibitor is bound to the enzyme, the active site changes shape so that the substrate is unable to bind to the enzyme.

Activation Energy



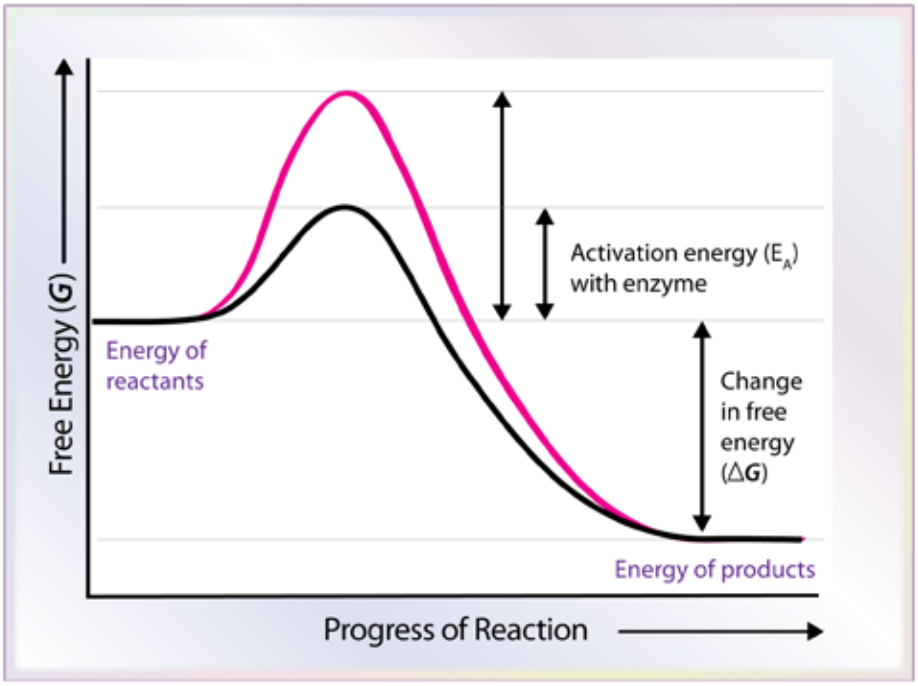
Activation energy may be defined as the minimum amount of energy required to get the reactants in a chemical reaction to the transition state, in which bonds are broken and new bonds are formed. The activation energy of a reaction is usually denoted by E_A. By now you know that enzymes are proteins that catalyze chemical reactions. Enzymes lower the activation energy needed to start a reaction.

You may use the foam pieces to simulate the activation energy needed in a reaction with and without an enzyme. Begin by connecting the green foam pieces. To illustrate the activation energy without the enzyme interaction, pull the apart the two green pieces with your hands.

Reconnect the green pieces. This time lock them into the active site on the gray A enzyme (without stickers) foam piece. With the help of the enzyme it takes less energy to pull the pieces apart. The activation energy has been lowered!

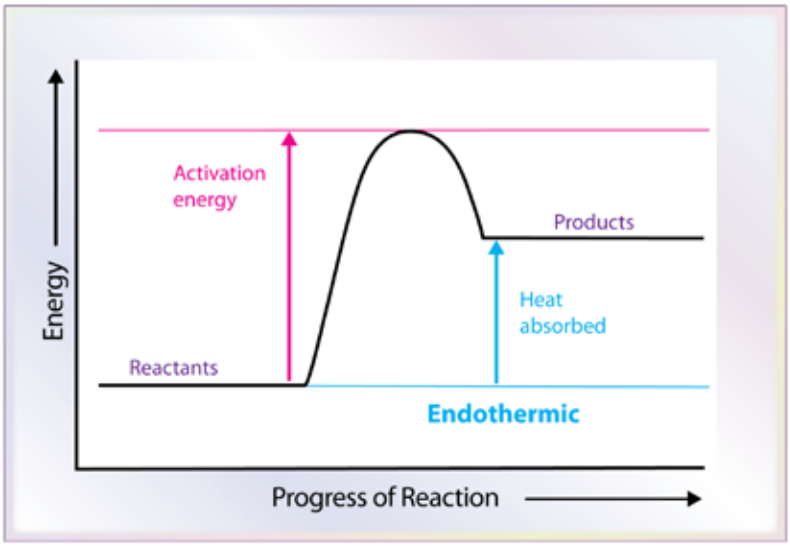
Notice in the graph below that the resulting products have less free energy than the reactants. In such a reaction, energy has been released and the reaction is said to be **exothermic**. A specific example of an exothermic reaction is the breakdown of hydrogen peroxide into hydrogen and oxygen. The enzyme used to facilitate this reaction is known as **catalase**.

$$2H_2O_2 \rightarrow 2H_2O + O_2$$



Activation Energy Continues

Conversely, in the graph below, the products have more free energy than the reactants. Reactions that absorb heat from the environment are known as **endothermic** reactions. A common example is a chemical ice pack which typically contains water and a packet of ammonium chloride. To activate the ice pack, the barrier separating the two substances must be physically broken so the two substances may react. Enzymes may also facilitate endothermic reactions.



1. Examine the graph below. Is the reaction depicted exothermic or endothermic? Explain your answer.

The reaction in the graph below is endothermic because the products have more energy than the reactants, indicating that energy has been taken in by the reaction.

The activation energy curve show below represents a non-enzyme catalyzed reaction.

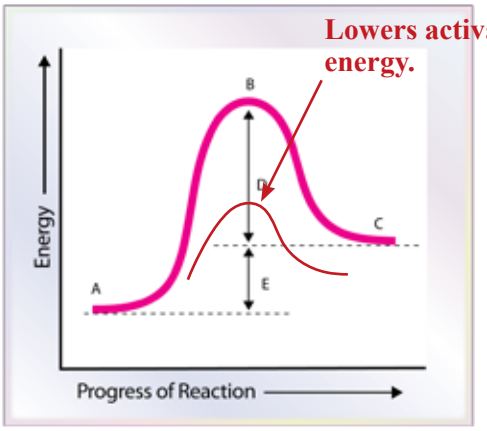
2. Draw a line on the graph indicating the activation energy in the presences of an enzyme.

3. Which letter depicts the activation energy without the enzyme present?

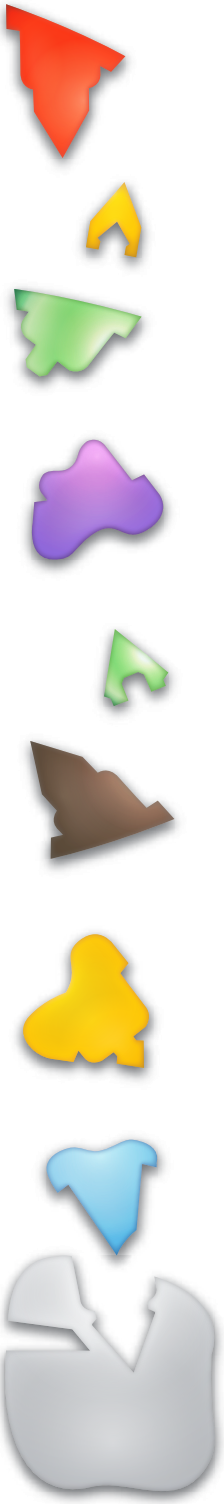
B

4. What does the letter 'E' represent?

Heat absorbed / Endothermic



Activation Energy Continues



Post Lab Questions

1. Predict what might happen if enzyme activity were not regulated within a cell's metabolic pathways.

Answers will vary but may include: If enzyme activity were not regulated within a cell's metabolic pathways, too much heat may be released in a reaction and the cell may sustain damage. Additionally, if the enzyme is not working to produce a product, those products may not be available for the next step in a cascade of reactions.

2. Contrast the action of a competitive inhibitor with that of a noncompetitive inhibitor.

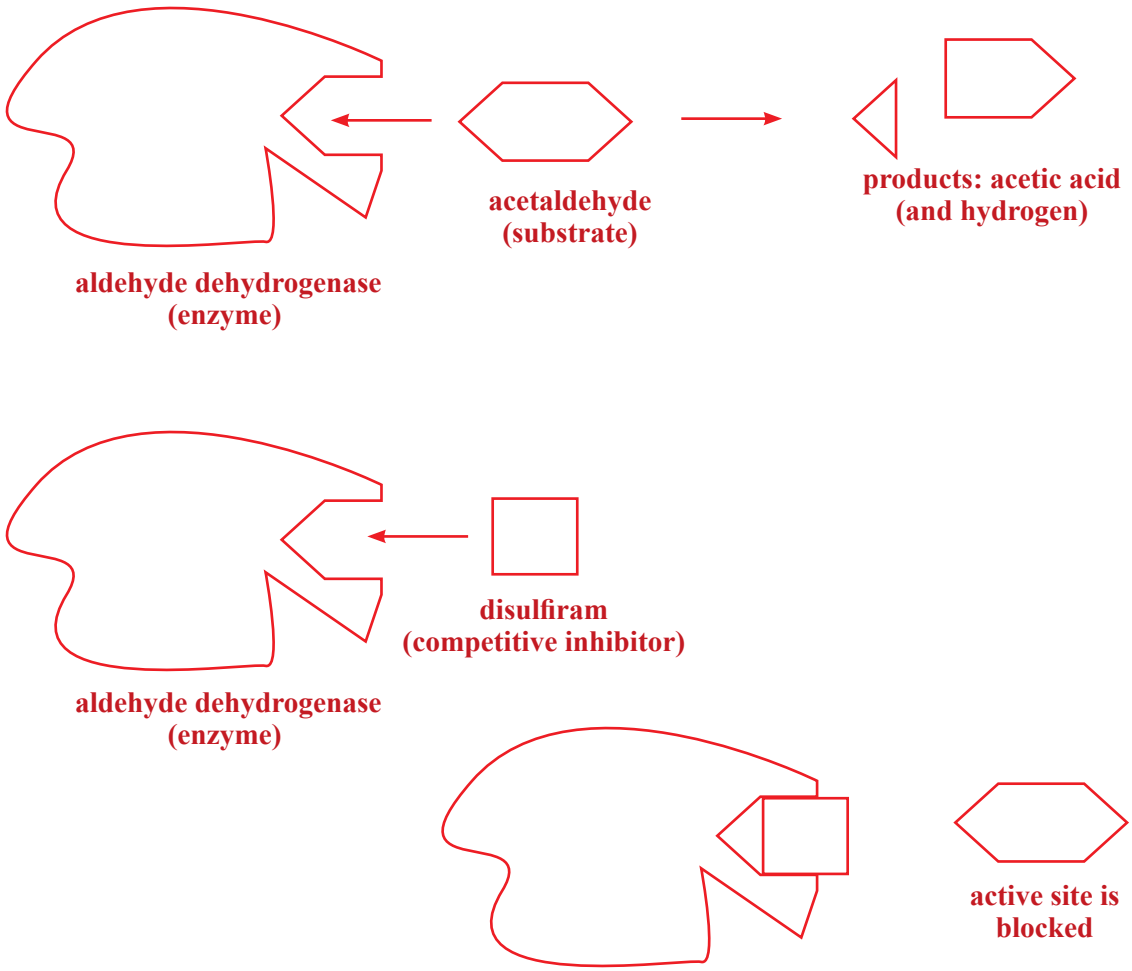
A competitive inhibitor binds in the active site preventing the substrate from interacting with the enzyme, while a noncompetitive inhibitor binds at another site on the enzyme which changes the active site preventing the substrate from interacting with the enzyme.

Activation Energy Continues

Ethanol is metabolized in the body into acetaldehyde. Normally, acetaldehyde does not accumulate in the body because aldehyde dehydrogenase rapidly oxidizes the acetaldehyde into acetic acid. The drug disulfiram inhibits aldehyde dehydrogenase, which causes an accumulation of acetaldehyde in the body with the subsequent unpleasant side effects of nausea and vomiting. Disulfiram is sometimes used to treat patients with a drinking habit.

3. Create a sketch to illustrate the action of the competitive inhibitor disulfiram. Be sure to label your diagram with the following terms, **aldehyde dehydrogenase**, **disulfiram**, **acetaldehyde**, **enzyme**, **competitive inhibitor**, **substrate** and **product**.

Example only: Student's answers will vary



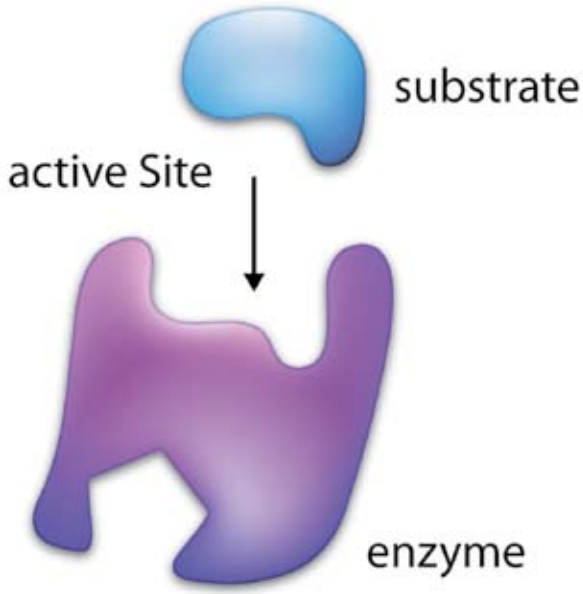
Activation Energy Continues

4. How is Koshland's theory of induced fit supported by noncompetitive inhibition of enzymes?

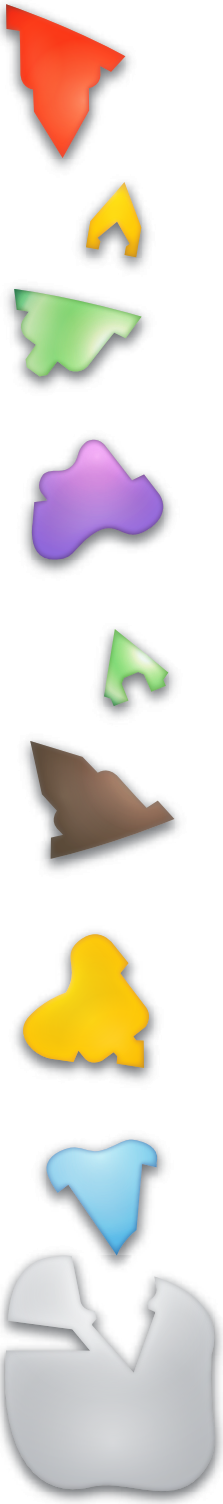
Koshland's theory of induced fit implies that the enzyme must change shape in order to interact with the substrate. Noncompetitive inhibition occurs when a substance binds to the enzyme at a site other than the active site while still causing a change in the active site.

5. Examine the model of the enzyme shown below. Design a competitive and noncompetitive inhibitor for this enzyme.

Answers will vary, but the competitive inhibitor should block the active site while the noncompetitive inhibitor blocks the allosteric site.



National Standards



Connections to: A Framework for K-12 Science Education

Practices, Crosscutting Concepts, and Core Ideas*

Dimension 1. Scientific and Engineering Practices

1. Asking Questions (for science) and Defining Problems (for engineering)
2. Developing and Using Models
6. Constructing Explanations (for science) and Designing Solutions (for engineering)

Dimension 2. Crosscutting Concepts

1. Patterns
2. Cause and Effect: Mechanism and Explanation
4. Systems and System Models
6. Structure and Function
7. Stability and Change

Dimension 3. Disciplinary Core Ideas

Physical Science

HS-PS1: Matter and its Interactions

HS-PS1-2: Construct and revise an explanation for the outcome of a simple chemical reaction based on the outermost electron states of atoms, trends in the periodic table, and knowledge of the patterns of chemical properties.

HS-PS1-4: Develop a model to illustrate that the release of absorption of energy from a chemical reaction system depends upon the changes in total bond energy.

HS-PS1-5: Apply scientific principles and evidence to provide an explanation about the effects of changing the temperature or concentration of the reacting particles on the rate at which a reaction occurs.

Life Science

LS 1: From Molecules to Organisms: Structures and Processes

HS-LS1-1: Construct an explanation based on evidence for how the structure of DNA determines the structure of proteins which carry out the essential functions of life through systems of specialized cells.

HS-LS1-2: Develop and use a model to illustrate the hierarchical organization of interacting systems that provide specific functions within multicellular organisms.

Engineering, Technology and Applications of Science

HS-ETS1: Engineering Design

HS-ETS1-4: Use a computer simulation to model the impact of proposed solutions to a complex real-world problem with numerous criteria and constraints on interactions within and between systems relevant to the problem.

*The NSTA Reader's Guide to A Framework for K-12 Science Education, National Research Council (NRC), 2011. A Framework for K-12 Science Education: Practices, Crosscutting Concepts, and Core Ideas. Washington, D.C.: National Academies Press.

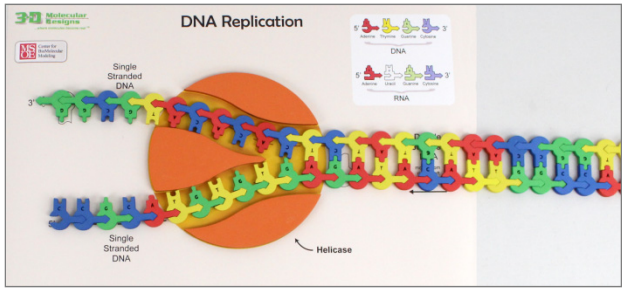
DNA Replication

The process by which a DNA molecule is copied

Template

Non-template

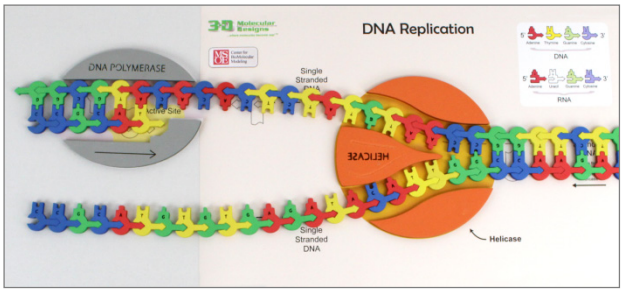
Diagram 1



Helicases unwind the DNA at the replication fork

Helicases are enzymes that untwist the double helix and separate the two strands.

Diagram 2



DNA polymerase catalyzes the synthesis of new DNA

Leading Strand: New DNA can elongate only in the 5′ → 3′ direction. The DNA strand that is made **continuously** is referred to as the leading strand. DNA polymerase moves toward the replication fork.

Diagram 3

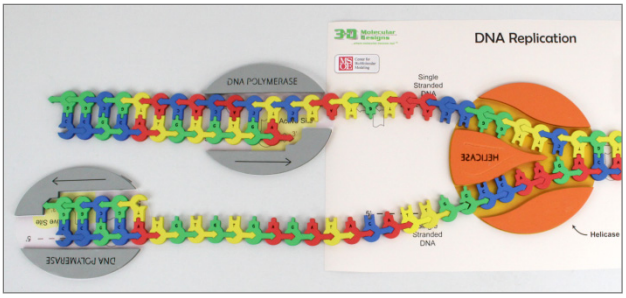
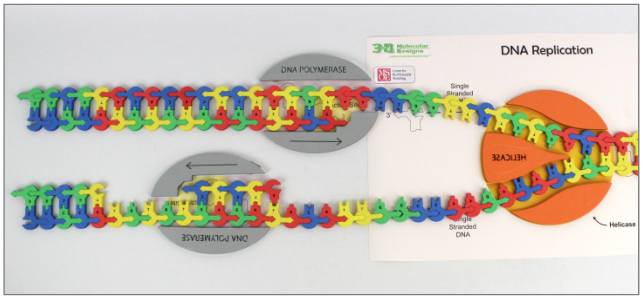


Diagram 4



Lagging Strand: DNA polymerase must move away from the fork instead of toward the fork as it did in the leading strand in order to maintain 5′ → 3′ DNA synthesis (diagram 3). The lagging strand is synthesized in a series of **discontinuous** fragments referred to as Okazaki fragments (diagram 4).

Note: Each of the two daughter molecules has one old strand from the parental molecule and one newly synthesized strand. This type of replication is referred to as **semiconservative replication**.

Flow of Genetic Information

DNA Replication

Pre-Lab:

1? State at least three reasons why a cell must undergo division.

Scenario: Imagine you are cutting a bagel (one of the most common household injuries) and you get a cut. The cut heals.

2? How do the new cells compare to the original (pre-cut) cells?

3? How does your body ensure that the new cells are the same?

4? How does DNA get into the new cells?

Lab

Student Introduction:

No molecular structure has gained world-wide notoriety more than the double helical structure of DNA. The famous *Nature* paper written by James Watson and Francis Crick in 1953 entitled, "Molecular Structure of Nucleic Acids" ends with the statement, "It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material." Since the release of the paper, the focus of the research of a number of scientists has been on elucidating the mechanism of DNA replication.

Modeling DNA Replication

In this lesson you will learn how a copy of DNA is replicated for each cell.

STEP 1 You will model a 2D representation of DNA replication using the foam pieces provided. Assemble the non-template strand of the abbreviated sequence of the beta-globin gene using the pattern shown below.

STEP 2 Base pair the nucleotides of the template strand in order to the non-template strand of DNA you have previously constructed to create a double stranded DNA model.

2a Record the template strand bases in the blank spaces provided above.

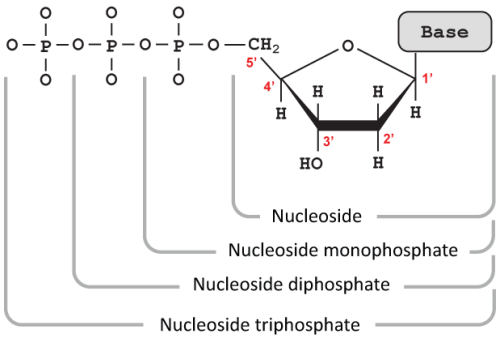
2b Examine the strands of DNA. What can you observe about the "arrow" ends of the model?

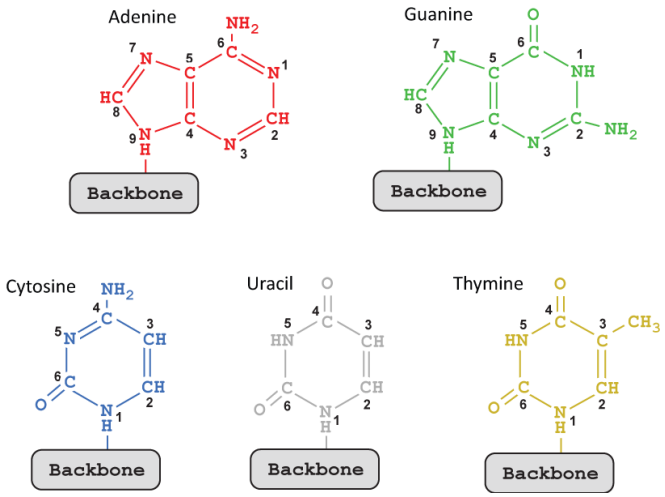
_____

_____

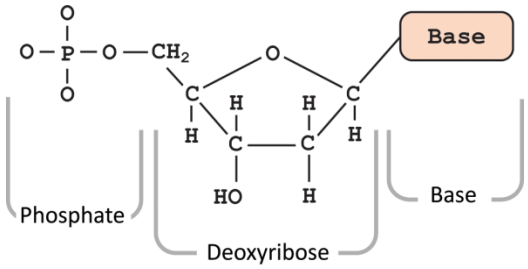
The arrow indicates the 3' end of the DNA molecule. Examine the diagrams below.

STEP 3: Examine the diagrams below.

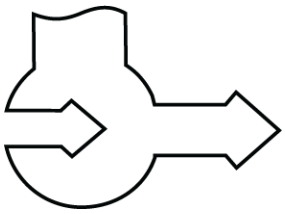




3a? Circle and label the 3' carbon and the 5' carbon in the DNA nucleotide shown in the diagram to the right. Primes are used in the numbering of the carbons on the sugar portion of the nucleotide to distinguish them from the nitrogen base carbons.



3b? Identify and label the nitrogen base, phosphate group, hydroxyl group and sugar in the representation pictured to the right. Label the locations of the 3' and 5' carbons.



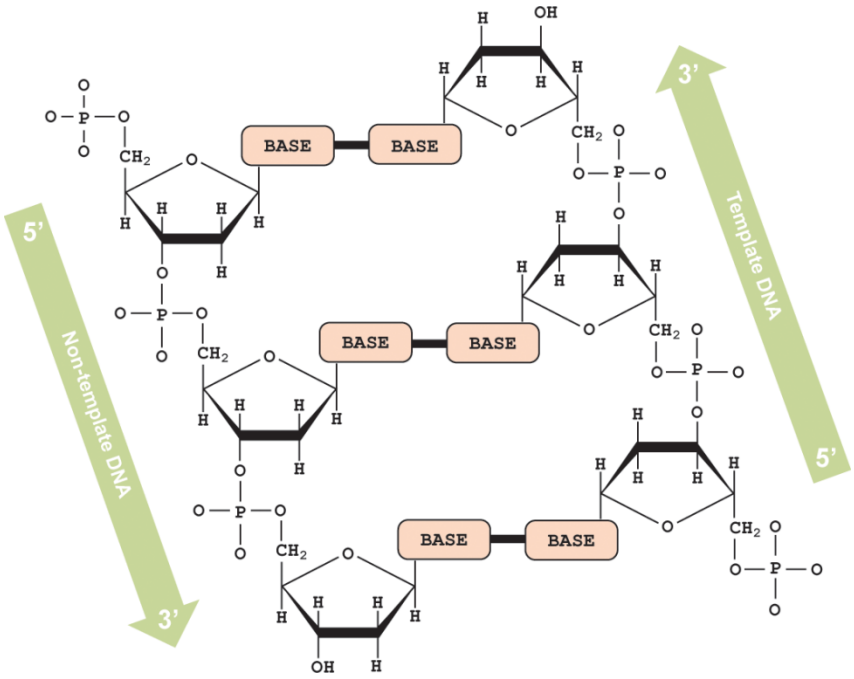
3c? How are the 3' and 5' carbons oriented in the strands of the DNA molecule you assembled?

_____

_____

STEP 4: Examine the detailed diagram of the DNA model below.

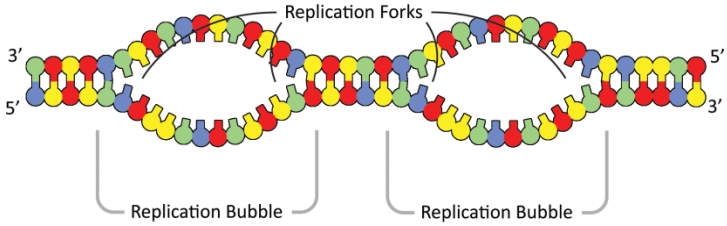
Double stranded DNA is composed of **<u>two anti-parallel strands</u>**! Each DNA strand has "**<u>directionality</u>**". The two sugar-phosphate backbones run in opposite 5' → 3' directions from each other. It is important to keep this directionality in mind as you model the process of DNA replication.

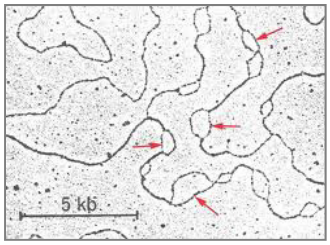


4a? Circle and label the 3' carbons and the 5' carbons in the DNA molecule above.

4b? What group is attached to the 3' carbon? What group is attached to the 5' carbon?

Replication of DNA begins at specific sites referred to as origins of replication. A eukaryotic chromosome may have hundreds or even a few thousand replication origins. Proteins that start DNA replication attach to the DNA and separate the two strands creating a replication "bubble". At each end of the **<u>replication bubble</u>** is a Y-shaped region where the parental strands of DNA are being unwound. This region is referred to as the **<u>replication fork</u>**.





http://kootation.com/origins-of-replication.html

STEP 5: Observe your teacher create a model of a DNA replication bubble using two toobers.

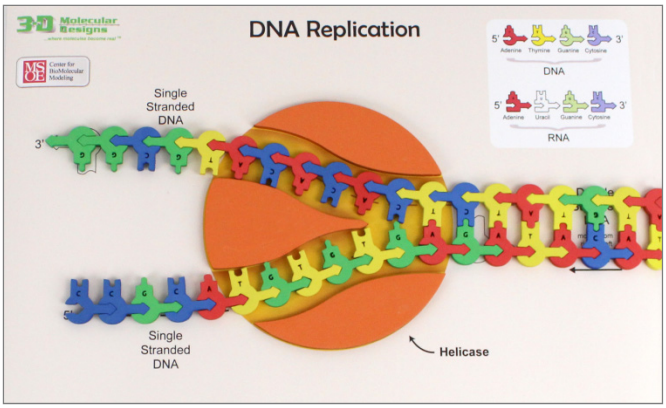
5a? Identify and label the replication bubble and replication forks in the model below.

5b? Looking at the toober model, what do you think might be the first step of replication?

5c? Nucleotides are added at an approximate rate of 50 nucleotides per second in eukaryotic cells. The human genome contains 6.4 billion nucleotides (3.2 billion base pairs) which must be copied. Calculate the length of time in days that it would take to copy the human genome. Show all calculations including units.

5d? Why do you think multiple replication bubbles form during the process of DNA replication?

STEP 6: Begin the process of DNA replication by feeding the strands of the constructed DNA into the top of the **helicase** enzyme on the replication mat. Be sure to position the 5' and 3' ends of the DNA appropriately as you place the DNA on the mat. Continue feeding the DNA through the enzyme until you have 11 bases emerging from the bottom of the helicase. Notice that helicase moves into the replication fork NOT away from it.



6a? What does the helicase appear to be doing?

_____

_____

6b? Identify which type of bond is broken.

_____

_____

6c? Why is the helicase able to break these bonds?

_____

_____

Note: Replication occurs on both sides of the replication fork simultaneously. For simplicity and clarification you will simulate replication on one side of the fork at a time.

STEP 7: **DNA polymerase** catalyzes the synthesis of new DNA by adding nucleotides to a preexisting chain. **New DNA can elongate only in the 5' → 3' direction.** The DNA strand that is made continuously is referred to as the **leading strand**.

Simulate replication in the **leading strand** by placing one DNA polymerase at the point of origin (refer to Diagram 2 on the Replication Placemat) and adding nucleotides in the active site to the parent strand. Continue adding nucleotides as you move the DNA polymerase until you reach the fork.

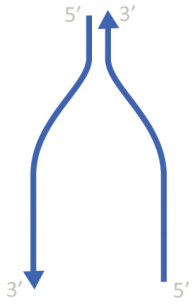
7a? As a new nucleotide is added to the growing DNA strand, which part of the new nucleotide forms a bond with the 3' OH group?

_____

_____

Additional Note: The 3' OH group is essential for adding a new nucleotide to the growing DNA strand. If this group is not present, for example, if there is a 3' H instead of a 3' OH, then DNA synthesis cannot continue. This is the basis for the Sanger Sequencing method used in determining the sequence of nucleotides.

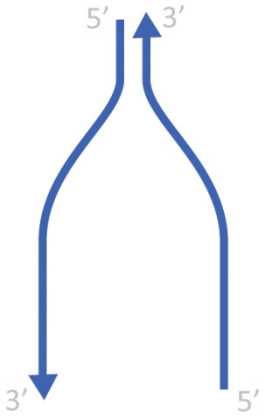
7b? Insert a sketch of the helicase on the diagram below and indicate the directionality of the newly replicated **leading strand** of DNA:



7c? Will you be able to synthesize the other strand of DNA in a continuous manner when using the model? Explain why or why not.

STEP 8: Place the second DNA polymerase at the fork on the other strand of DNA. Notice that the DNA polymerase must move away from the fork instead of toward the fork as it did in the leading strand. In order to accommodate the 5′ → 3′ synthesis of DNA, short fragments are made on the second strand referred to as the **lagging strand**. Continue adding nucleotides in the active site as you move the DNA polymerase away from the fork until you reach the end.

8a? Sketch and indicate the directionality of the fragments composing the **lagging strand** of DNA below:



STEP 9: Feed the next eleven nucleotides through the helicase. Continue sliding the DNA polymerase along the **leading strand**, adding more nucleotides as you progress.

STEP 10: The **lagging strand** requires that you move the DNA polymerase! Place the DNA polymerase back at the fork junction to create the next fragment. Move the DNA polymerase so that the bases may be added from the 5' → 3' direction. (Refer to the third diagram on the DNA Replication Placemat.) You have now created a second fragment of DNA on the lagging strand. These fragments are referred to as **Okazaki fragments** and are usually 100-200 nucleotides long in eukaryotic cells.

When you "bump" into the first fragment, you will need to remove the DNA polymerase and join the two fragments together with the appropriate nucleotide. The actual process of joining the Okazaki fragments together is a bit more complicated and involves several other molecules.

STEP 11: Complete the process of DNA replication with the remaining 11 nucleotides on both the leading and the lagging strands. DNA replication is considered to be a **semi-discontinuous** process.

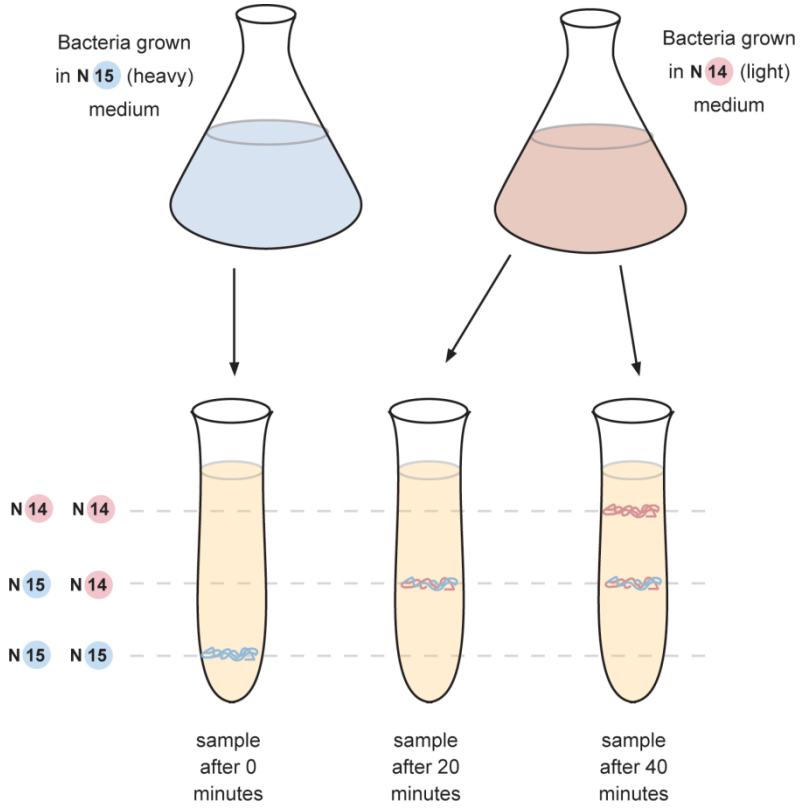
11a? Why is DNA replication considered to be a **semi-discontinuous** process?

11b? Create a sketch which models the semi-discontinuous process of DNA replication. Be sure to label the following aspects of your representation: leading and lagging strands, helicase, Okazaki fragments, parental strands, 3' ends and 5' ends.

11c? How do these two new strands compare to the original (parental) strand?

Three Models for the Process of DNA Replication:

In 1958 at the California Institute of Technology Matthew Meselson and Franklin Stahl devised an elegant series of experiments to discern which one of three models explained the mechanism of DNA replication. Meselson and Stahl cultured *E. coli* in a medium containing nucleotides labeled with a heavy isotope of nitrogen, ^{15}N. They transferred the bacteria to a medium with only ^{14}N, a lighter isotope. A sample was taken after the DNA had replicated once. Another sample was taken after the DNA replicated again. The DNA was extracted from the bacteria in the samples and then centrifuged to separate the DNA of different densities. Their results are shown below:



STEP 1: Obtain and assemble 11 nucleotide basepairs of the colored DNA foam pieces. Find the matching gray basepair pieces but DO NOT assemble them. These colored DNA strands represent the parental strands from *E. coli* grown in a medium tagged with ^{15}N nucleotides. The gray foam pieces represent the nucleotides used to synthesize new DNA.

You will create a physical representation of the three mechanisms of DNA replication; (1) conservative, (2) semiconservative, and (3) dispersive. Begin with modeling the first round of replication of the DNA after the bacteria were transferred to a medium with only ^{14}N.

You will use the foam DNA models to discern which mechanisms of replication would most likely explain Meselson and Stahl's results

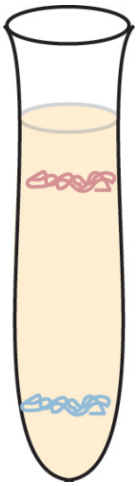
Conservative model:

In the conservative model of DNA replication the parental strands are used as templates for the new DNA molecule and somehow come back together to "conserve" the parental molecule.

STEP 2: Using the colored DNA parental strands you have just created and the gray nucleotides, model the end result of the conservative method of DNA replication. You should have 1 parental model made entirely of colored pieces and 1 daughter molecule with the same sequence of base pairs but made entirely of gray foam nucleotides.

2a? Sketch the new and old strands after one round of replication. It will be helpful if you have two different colored pens or pencils to create your sketches.

STEP 3: A sketch of a test tube showing the density gradient of ^{15}N tagged DNA after one round of conservative replication is shown below.



Semiconservative model:

In the semiconservative model of DNA replication, each of the two daughter molecules will have one old strand from the parental molecule and one newly made strand.

STEP 4: Now using the colored DNA parental strands you have created and the gray nucleotides, model the semiconservative method of DNA replication.

4a? Sketch the results of one round of DNA synthesis after the semiconservative method of replication.

4b? Sketch a test tube showing the density gradient of ^{15}N tagged DNA after one round of semi-conservative replication. Refer to the Meselson and Stahl experiment to help you create your sketch.

Dispersive model:

In the dispersive model of DNA replication, each strand of both daughter molecules contains a mixture of old and newly synthesized DNA.

STEP 5: Finally, using the colored DNA parental strands you have just created and the gray nucleotides, model the dispersive method of DNA replication.

5a? Sketch the results of one round of DNA synthesis after the dispersive method of replication.

5b? Sketch a test tube showing the density gradient of ^{15}N tagged DNA after one round of dispersive replication.

5c? Which of the methods can now be eliminated based on the results that Meselson and Stahl got after one round of replication? Why?

_____

_____

_____

STEP 6: Use the foam pieces to visualize what the newly synthesized strands of DNA would look like after a second round of replication in each of the methods. Sketch your results in the first column in the table below. In the second column, sketch what the DNA density gradient would look like in the test tube.

DNA Synthesized After A Second Round of Replication	DNA Density gradient
Conservative Model	
Semi-conservative Model	
Dispersive Model	

6a? Which method of DNA replication may now be eliminated after the second round of DNA replication based on the results of the Meselson and Stahl experiments? Why?

_____

_____

_____

6b? Based on the results of Meselson and Stahl's experiments, DNA is shown to replicate in a

_____ manner.

Post-Lab Questions:

1. What is the relationship of DNA replication to cell division?

2. Of the representations of DNA models (foam pieces, paper diagram, toobers), identify the strengths and weaknesses of each.

3. Based on what you have learned from this activity, explain why semi-conservative replication is the preferred process of DNA replication as opposed to dispersive or conservative.

For a detailed description suitable for IB or AP Biology:

http://www.youtube.com/watch?v=teV62zrm2P0
http://www.youtube.com/watch?v=-mtLXpgjHL0
(these descriptions include RNA primer)

For a general overview animation of continuous and discontinuous replication:

http://www.wehi.edu.au/education/wehitv/molecular_visualisations_of_dna/
http://www.dnalc.org/resources/3d/04-mechanism-of-replication-advanced.html

A group of videos on DNA replication:

http://www.youtube.com/watch?v=AGUuX4PGlCc&list=PL38E7B903667B4498

Links to the Next Generation Standards

Scientific and Engineering Practices:

- Asking Questions (for science) and Defining Problems (for engineering)
- Developing and Using Models
- Using Mathematics and Computational Thinking
- Constructing Explanations (for science) and Designing Solutions (for engineering)

Crosscutting Concepts:

- Patterns
- Cause and Effect: Mechanism and Explanation
- Scale, Proportion, and Quantity
- Structure and Function
- Systems and System Models
- Stability and Change

Disciplinary Core Ideas:

- LS 1: From Molecules to Organisms: Structures and Processes
- HS-LS1-1: Construct an explanation based on evidence for how the structure of DNA determines the structure of proteins which carry out the essential functions of life through systems of specialized cells.

- LS 2: Heredity: Inheritance and Variation of Traits
- HS-LS3-1: Ask questions to clarify relationships about the role of DNA and chromosomes in coding the instructions for characteristic traits passed from parents to offspring.
- HS-LS3-2: Make and defend a claim based on evidence that inheritable genetic variations may result from (1) new genetic combinations through meiosis, (2) viable errors occurring during replication, and/or (3) mutations caused by environmental factors.

- HS-ETS1: Engineering Design
- HS-ETS1-4: Use a computer simulation to model the impact of proposed solutions to a complex real-world problem with numerous criteria and constraints on interactions within and between systems relevant to the problem.

Students will:

- **Identify** the directionality of a DNA strand.
- **Explain** the implications of the anti-parallel structure of DNA on replication.
- **Model** the replication process of the leading and lagging strands of DNA.
- **Describe** the semi-conservative nature of DNA replication.
- **Describe** the semi-discontinuous process of DNA replication.
- **Explain** how a change in the DNA code may occur.

Prerequisite Knowledge and Skills:

- Hydrogen bonding and covalent bonding
- Cell structure
- DNA structure
- Cell cycle basics
- Prokaryotic and eukaryotic cell structure

Materials:

- DNA toober model
- Student Lab Packet
- DNA Replication Placemat, recommended one kit per group of three students

Flow of Genetic Information

DNA Replication

Pre-Lab:

1. State at least three reasons why a cell must undergo division.

(possible answers include: growth, repair, reproduction, the cell gets too big (surface area to volume ratio))

Scenario: Imagine you are cutting a bagel (one of the most common household injuries) and you get a cut. The cut heals.

2. How do the new cells compare to the original (pre-cut) cells?

(*answers may include: exactly the same, scar forms, cells are different ages)*

3. How does your body ensure that the new cells are the same?

(possible answers: DNA contains the information in the old cells as well as the new cells. The DNA is the same in each cell)

4. How does DNA get into the new cells?

(*answers will vary. Answers may not be accurate, but lead to discussions regarding DNA replication)*

Lab

Student Introduction:

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2a Record the template strand bases in the blank spaces provided above.

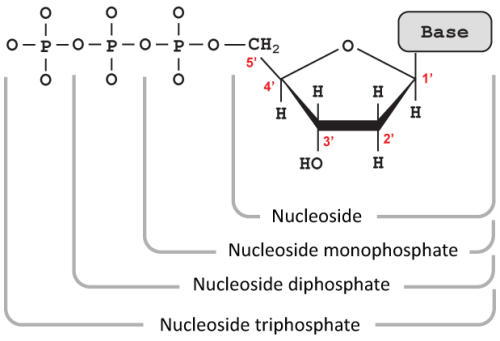
2b Examine the strands of DNA. What can you observe about the "arrow" ends of the model?
(The arrows are on opposite ends of the strands.)

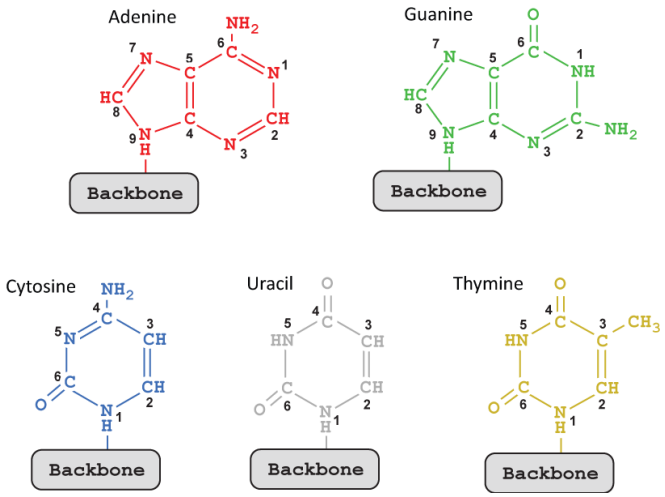
_____

_____

The arrow indicates the 3' end of the DNA molecule. Examine the diagrams below.

STEP 3: Examine the diagrams below.





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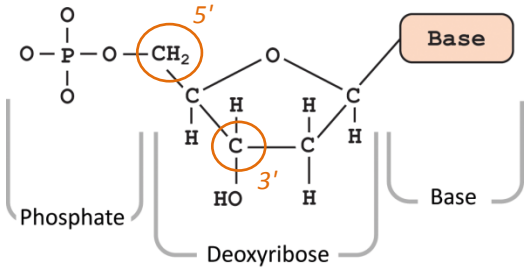
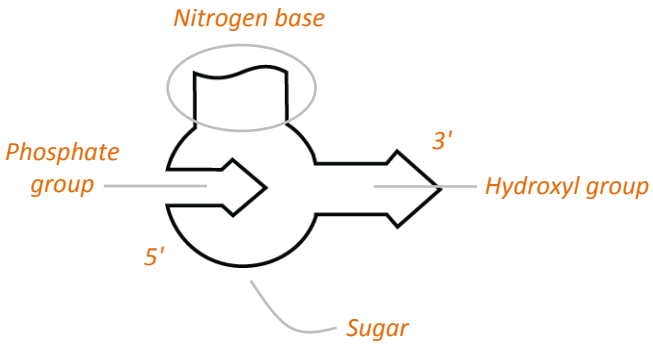


3b? Identify and label the nitrogen base, phosphate group, hydroxyl group and sugar in the representation pictured to the right. Label the locations of the 3' and 5' carbons.

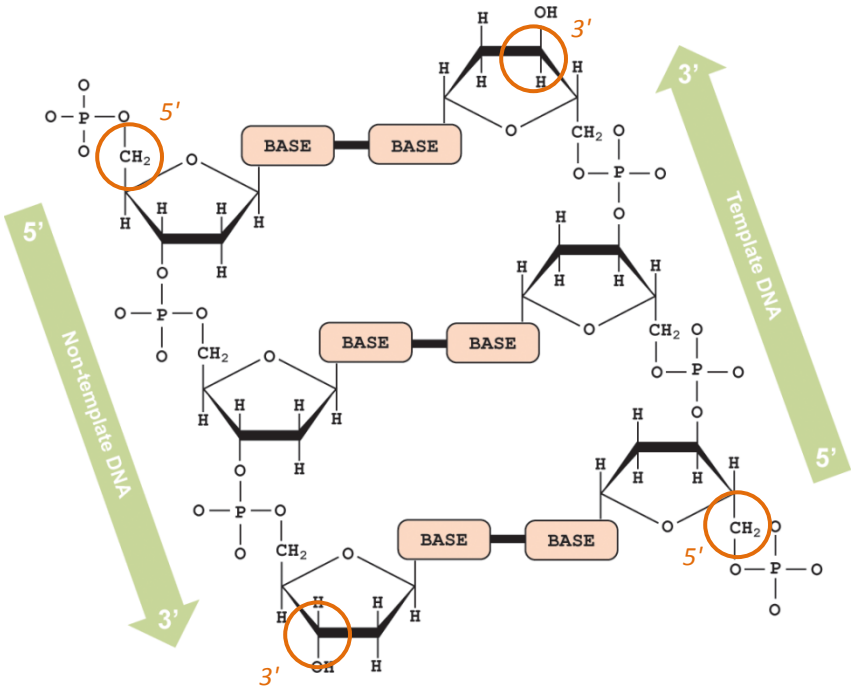


3c? How are the 3' and 5' carbons oriented in the strands of the DNA molecule you assembled?

(The 3' and 5' carbons are on opposite ends of each strand and the strands are "anitparallel" to each other)

STEP 4: Examine the detailed diagram of the DNA model below.

Double stranded DNA is composed of **<u>two anti-parallel strands</u>**! Each DNA strand has "**<u>directionality</u>**". The two sugar-phosphate backbones run in opposite 5' → 3' directions from each other. It is important to keep this directionality in mind as you model the process of DNA replication.

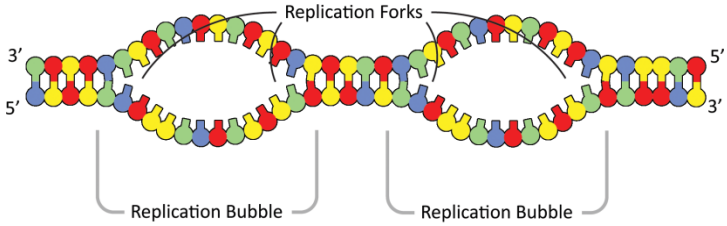


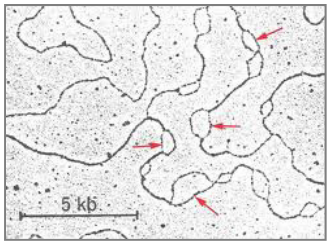
4a? Circle and label the 3' carbons and the 5' carbons in the DNA molecule above.

4b? What group is attached to the 3' carbon? What group is attached to the 5' carbon?

(The hydroxyl group is attached to the 3' carbon while the phosphate group is attached to the 5' carbon.)

Replication of DNA begins at specific sites referred to as origins of replication. A eukaryotic chromosome may have hundreds or even a few thousand replication origins. Proteins that start DNA replication attach to the DNA and separate the two strands creating a replication "bubble". At each end of the **<u>replication bubble</u>** is a Y-shaped region where the parental strands of DNA are being unwound. This region is referred to as the **<u>replication fork</u>**.

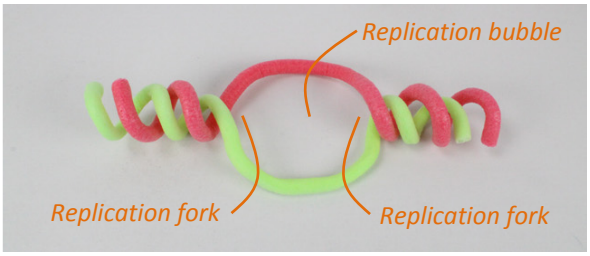




http://kootation.com/origins-of-replication.html

STEP 5: Observe your teacher create a model of a DNA replication bubble using two toobers.

5a? Identify and label the <u>replication bubble</u> and <u>replication forks</u> in the model below.



5b? Looking at the toober model, what do you think might be the first step of replication?

(The unwinding of DNA)

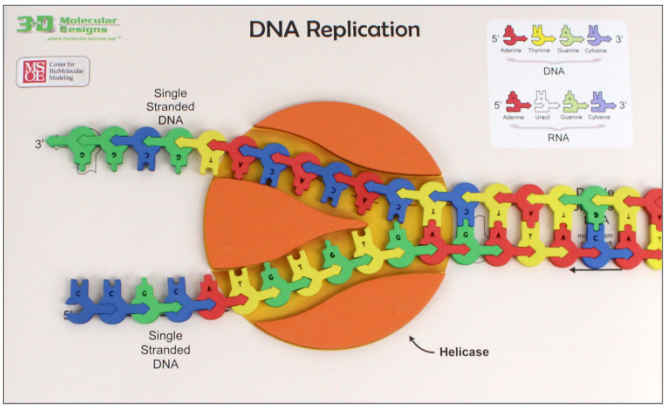
5c? Nucleotides are added at an approximate rate of 50 nucleotides per second in eukaryotic cells. The human genome contains 6.4 billion nucleotides (3.2 billion base pairs) which must be copied. Calculate the length of time in days that it would take to copy the human genome. Show all calculations including units.

(1.5 X 10^3 days) (6.4 X10^9 nucleotides X 1 second/50 nucleotides X 1hour/60 seconds X 1day/24 hours)

5d? Why do you think multiple replication bubbles form during the process of DNA replication?

(The replication process would be too slow if DNA replication occurred at a single bubble)

STEP 6: Begin the process of DNA replication by feeding the strands of the constructed DNA into the top of the **<u>helicase</u>** enzyme on the replication mat. Be sure to position the 5' and 3' ends of the DNA appropriately as you place the DNA on the mat. Continue feeding the DNA through the enzyme until you have 11 bases emerging from the bottom of the helicase. Notice that helicase moves into the replication fork NOT away from it.



6a? What does the helicase appear to be doing?

(Helicase appears to be separating the two DNA strands.)

6b? Identify which type of bond is broken.

(Hydrogen bond.)

6c? Why is the helicase able to break these bonds?

(Helicase is an enzyme that facilitates breaking the hydrogen bonds as shown by the "active site", depicted by the pointy orange wedge in the model).

Note: Replication occurs on both sides of the replication fork simultaneously. For simplicity and clarification you will simulate replication on one side of the fork at a time.

STEP 7: **DNA polymerase** catalyzes the synthesis of new DNA by adding nucleotides to a preexisting chain. **New DNA can elongate only in the 5' → 3' direction.** The DNA strand that is made continuously is referred to as the **leading strand**.

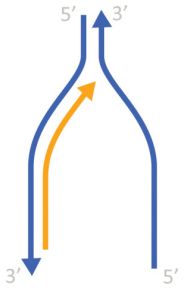
Simulate replication in the **leading strand** by placing one DNA polymerase at the point of origin (refer to Diagram 2 on the Replication Placemat) and adding nucleotides in the active site to the parent strand. Continue adding nucleotides as you move the DNA polymerase until you reach the fork.

7a? As a new nucleotide is added to the growing DNA strand, which part of the new nucleotide forms a bond with the 3' OH group?

(the phosphate group)

Additional Note: The 3' OH group is essential for adding a new nucleotide to the growing DNA strand. If this group is not present, for example, if there is a 3' H instead of a 3' OH, then DNA synthesis cannot continue. This is the basis for the Sanger Sequencing method used in determining the sequence of nucleotides.

7b? Insert a sketch of the helicase on the diagram below and indicate the directionality of the newly replicated **leading strand** of DNA:

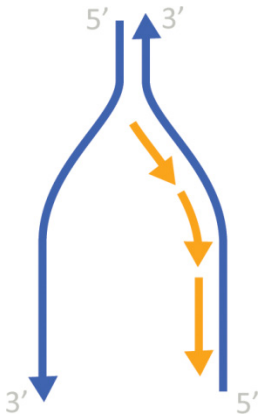


7c? Will you be able to synthesize the other strand of DNA in a continuous manner when using the model? Explain why or why not.

(DNA may be synthesized only in the 5′ → 3′ direction. Because DNA is anti-parallel, the other strand would be synthesized in the 3′ → 5′ direction if it were continuous synthesis.)

STEP 8: Place the second DNA polymerase at the fork on the other strand of DNA. Notice that the DNA polymerase must move away from the fork instead of toward the fork as it did in the leading strand. In order to accommodate the 5′ → 3′ synthesis of DNA, short fragments are made on the second strand referred to as the **lagging strand**. Continue adding nucleotides in the active site as you move the DNA polymerase away from the fork until you reach the end.

8a? Sketch and indicate the directionality of the fragments composing the **lagging strand** of DNA below:



STEP 9: Feed the next eleven nucleotides through the helicase. Continue sliding the DNA polymerase along the **leading strand**, adding more nucleotides as you progress.

STEP 10: The **lagging strand** requires that you move the DNA polymerase! Place the DNA polymerase back at the fork junction to create the next fragment. Move the DNA polymerase so that the bases may be added from the 5' → 3' direction. (Refer to the third diagram on the DNA Replication Placemat.) You have now created a second fragment of DNA on the lagging strand. These fragments are referred to as **Okazaki fragments** and are usually 100-200 nucleotides long in eukaryotic cells.

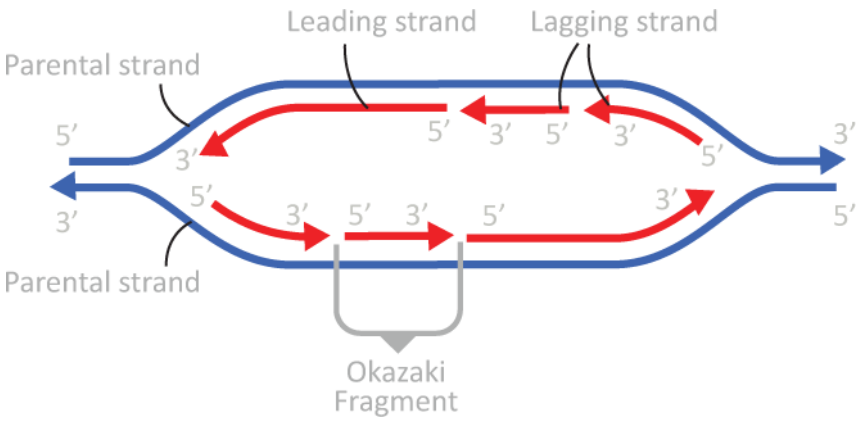
When you "bump" into the first fragment, you will need to remove the DNA polymerase and join the two fragments together with the appropriate nucleotide. The actual process of joining the Okazaki fragments together is a bit more complicated and involves several other molecules.

STEP 11: Complete the process of DNA replication with the remaining 11 nucleotides on both the leading and the lagging strands. DNA replication is considered to be a **semi-discontinuous** process.

11a? Why is DNA replication considered to be a **semi-discontinuous** process?

(DNA may be synthesized only in the 5' → 3' direction. Because DNA is anti-parallel, the other strand would be synthesized in the 3' → 5' direction if it were continuous synthesis.)

11b? Create a sketch which models the semi-discontinuous process of DNA replication. Be sure to label the following aspects of your representation: leading and lagging strands, helicase, Okazaki fragments, parental strands, 3' ends and 5' ends.

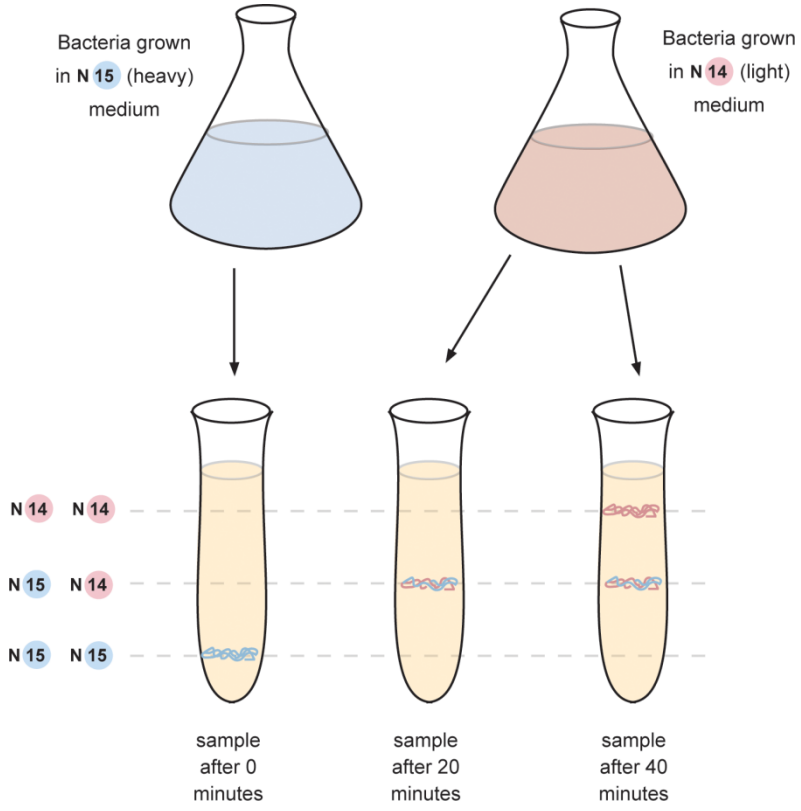


11c? How do these two new strands compare to the original (parental) strand?

(Answers may include the fact that the two daughter molecules are identical to the parent molecule, that each daughter molecule is composed of ½ parental (template) DNA and ½ new DNA)

Three Models for the Process of DNA Replication:

In 1958 at the California Institute of Technology Matthew Meselson and Franklin Stahl devised an elegant series of experiments to discern which one of three models explained the mechanism of DNA replication. Meselson and Stahl cultured *E. coli* in a medium containing nucleotides labeled with a heavy isotope of nitrogen, ^{15}N. They transferred the bacteria to a medium with only ^{14}N, a lighter isotope. A sample was taken after the DNA had replicated once. Another sample was taken after the DNA replicated again. The DNA was extracted from the bacteria in the samples and then centrifuged to separate the DNA of different densities. Their results are shown below:



STEP 1: Obtain and assemble 11 nucleotide basepairs of the colored DNA foam pieces. Find the matching gray basepair pieces but DO NOT assemble them. These colored DNA strands represent the parental strands from *E. coli* grown in a medium tagged with ^{15}N nucleotides. The gray foam pieces represent the nucleotides used to synthesize new DNA.

You will create a physical representation of the three mechanisms of DNA replication; (1) conservative, (2) semiconservative, and (3) dispersive. Begin with modeling the first round of replication of the DNA after the bacteria were transferred to a medium with only ^{14}N.

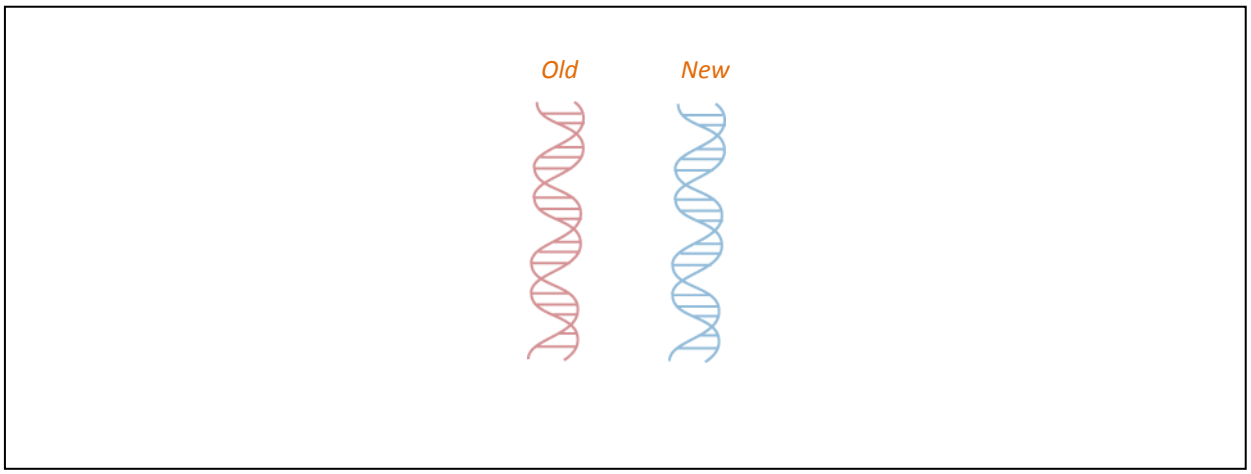
You will use the foam DNA models to discern which mechanisms of replication would most likely explain Meselson and Stahl's results

Conservative model:

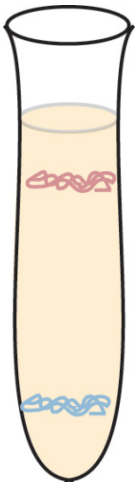
In the conservative model of DNA replication the parental strands are used as templates for the new DNA molecule and somehow come back together to "conserve" the parental molecule.

STEP 2: Using the colored DNA parental strands you have just created and the gray nucleotides, model the end result of the conservative method of DNA replication. You should have 1 parental model made entirely of colored pieces and 1 daughter molecule with the same sequence of base pairs but made entirely of gray foam nucleotides.

2a? Sketch the new and old strands after one round of replication. It will be helpful if you have two different colored pens or pencils to create your sketches.



STEP 3: A sketch of a test tube showing the density gradient of ^{15}N tagged DNA after one round of conservative replication is shown below.

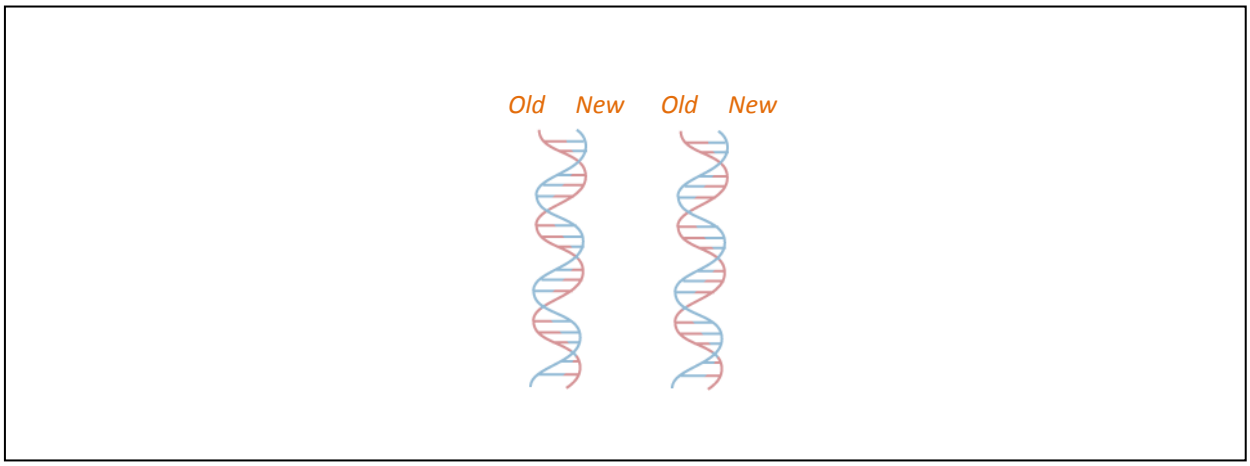


Semiconservative model:

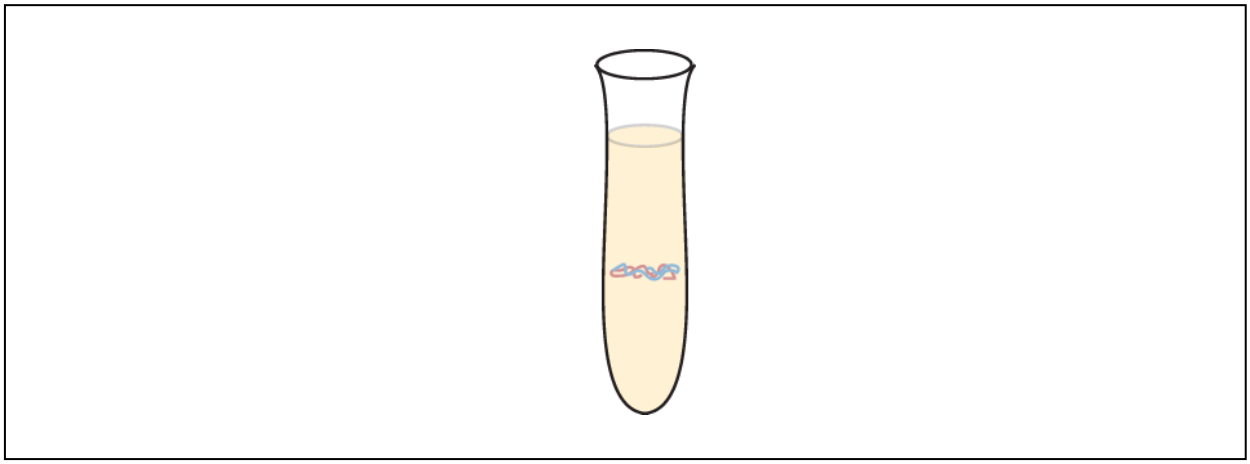
In the semiconservative model of DNA replication, each of the two daughter molecules will have one old strand from the parental molecule and one newly made strand.

STEP 4: Now using the colored DNA parental strands you have created and the gray nucleotides, model the semiconservative method of DNA replication.

4a? Sketch the results of one round of DNA synthesis after the semiconservative method of replication.



4b? Sketch a test tube showing the density gradient of ^{15}N tagged DNA after one round of semi-conservative replication. Refer to the Meselson and Stahl experiment to help you create your sketch.

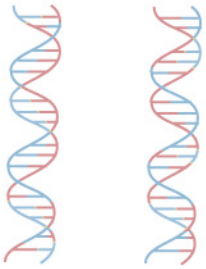


Dispersive model:

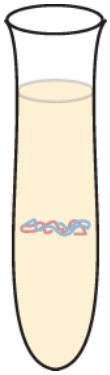
In the dispersive model of DNA replication, each strand of both daughter molecules contains a mixture of old and newly synthesized DNA.

STEP 5: Finally, using the colored DNA parental strands you have just created and the gray nucleotides, model the dispersive method of DNA replication.

5a? Sketch the results of one round of DNA synthesis after the dispersive method of replication.



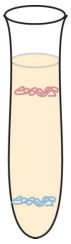
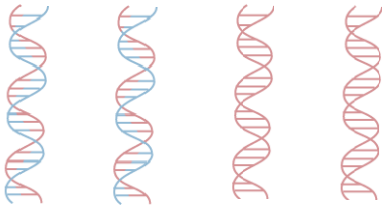
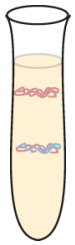
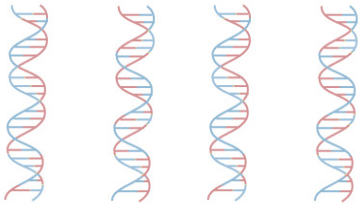
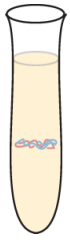
5b? Sketch a test tube showing the density gradient of ^{15}N tagged DNA after one round of dispersive replication.



5c? Which of the methods can now be eliminated based on the results that Meselson and Stahl got after one round of replication? Why?

(The conservative mechanism of DNA replication may be eliminated because it produces two bands in the density gradient test tube. Meselson and Stahl's experiment showed only one band after one round of replication.)

STEP 6: Use the foam pieces to visualize what the newly synthesized strands of DNA would look like after a second round of replication in each of the methods. Sketch your results in the first column in the table below. In the second column, sketch what the DNA density gradient would look like in the test tube.

DNA Synthesized After A Second Round of Replication	DNA Density gradient
Conservative Model 	
Semi-conservative Model 	
Dispersive Model 	

6a? Which method of DNA replication may now be eliminated after the second round of DNA replication based on the results of the Meselson and Stahl experiments? Why?

(The dispersive method may be eliminated after the second round of DNA replication because 1 band is shown in the density gradient while Meselson and Stahl's experiment showed two bands in the density gradient.)

6b? Based on the results of Meselson and Stahl's experiments, DNA is shown to replicate in a

(Semi-conservative) manner.

Post-Lab Questions:

1. What is the relationship of DNA replication to cell division?

(DNA replication is the process by which cells make a copy of DNA for the daughter cells.)

2. Of the representations of DNA models (foam pieces, paper diagram, toobers), identify the strengths and weaknesses of each.

(Various.)

3. Based on what you have learned from this activity, explain why semi-conservative replication is the preferred process of DNA replication as opposed to dispersive or conservative.

(Semi-conservative replication is an efficient, controlled process with directionality. The other two methods lack these properties. The other two methods would introduce far more error (mutation) into the process than does the semi-conservative method.)

For a detailed description suitable for IB or AP Biology:

http://www.youtube.com/watch?v=teV62zrm2P0
http://www.youtube.com/watch?v=-mtLXpgjHL0
(these descriptions include RNA primer)

For a general overview animation of continuous and discontinuous replication:

http://www.wehi.edu.au/education/wehitv/molecular_visualisations_of_dna/
http://www.dnalc.org/resources/3d/04-mechanism-of-replication-advanced.html

A group of videos on DNA replication:

http://www.youtube.com/watch?v=AGUuX4PGlCc&list=PL38E7B903667B4498

Links to the Next Generation Standards

Scientific and Engineering Practices:

- Asking Questions (for science) and Defining Problems (for engineering)
- Developing and Using Models
- Using Mathematics and Computational Thinking
- Constructing Explanations (for science) and Designing Solutions (for engineering)

Crosscutting Concepts:

- Patterns
- Cause and Effect: Mechanism and Explanation
- Scale, Proportion, and Quantity
- Structure and Function
- Systems and System Models
- Stability and Change

Disciplinary Core Ideas:

- LS 1: From Molecules to Organisms: Structures and Processes
- HS-LS1-1: Construct an explanation based on evidence for how the structure of DNA determines the structure of proteins which carry out the essential functions of life through systems of specialized cells.

- LS 2: Heredity: Inheritance and Variation of Traits
- HS-LS3-1: Ask questions to clarify relationships about the role of DNA and chromosomes in coding the instructions for characteristic traits passed from parents to offspring.
- HS-LS3-2: Make and defend a claim based on evidence that inheritable genetic variations may result from (1) new genetic combinations through meiosis, (2) viable errors occurring during replication, and/or (3) mutations caused by environmental factors.

- HS-ETS1: Engineering Design
- HS-ETS1-4: Use a computer simulation to model the impact of proposed solutions to a complex real-world problem with numerous criteria and constraints on interactions within and between systems relevant to the problem.

Students will:

- **Identify** the directionality of a DNA strand.
- **Explain** the implications of the anti-parallel structure of DNA on replication.
- **Model** the replication process of the leading and lagging strands of DNA.
- **Describe** the semi-conservative nature of DNA replication.
- **Describe** the semi-discontinuous process of DNA replication.
- **Explain** how a change in the DNA code may occur.

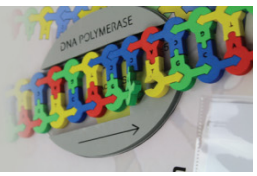
Prerequisite Knowledge and Skills:

- Hydrogen bonding and covalent bonding
- Cell structure
- DNA structure
- Cell cycle basics
- Prokaryotic and eukaryotic cell structure

Materials:

- DNA toober model
- Student Lab Packet
- DNA Replication Placemat, recommended one kit per group of three students

Transcription of DNA into RNA



DNA carries the instructions for making the proteins that are found in our bodies.

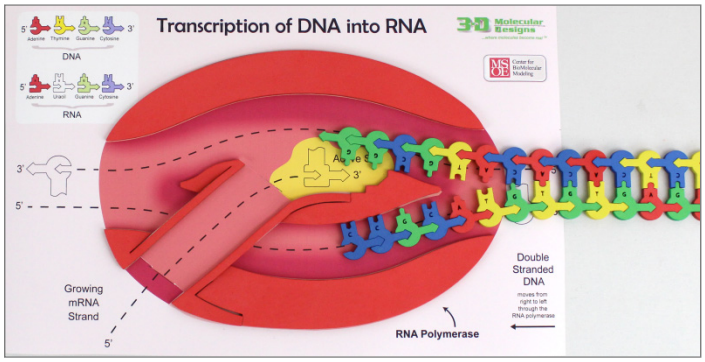
Template

Non-template

Transcription: Initiation

RNA polymerase binds to DNA.
DNA is transcribed by an enzyme called RNA polymerase. The enzyme pries apart the two DNA strands and starts transcribing the template strand (diagram 1). Specific nucleotide sequences tell RNA polymerase where to begin and where to end on the DNA. These sequences are not included in this model.

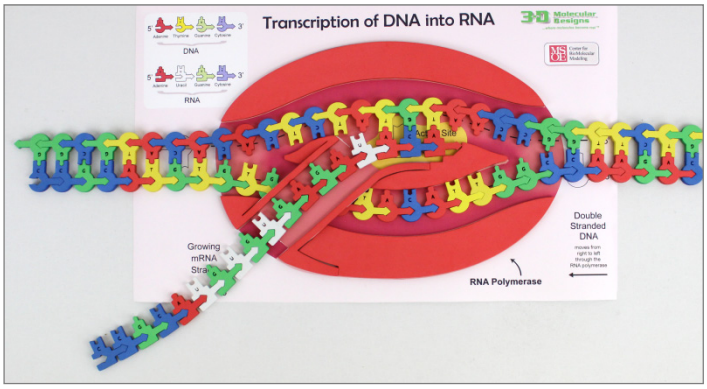
Diagram 1



Transcription: Elongation

The mRNA is synthesized.
RNA polymerase moves downstream breaking the hydrogen bonds between the DNA base pairs exposing 10-20 DNA nucleotides at a time. Messenger RNA is assembled in the 5′ → 3′ direction using the template DNA strand (diagram 2). When RNA polymerase transcribes DNA, guanine pairs with cytosine and adenine pairs with uracil.

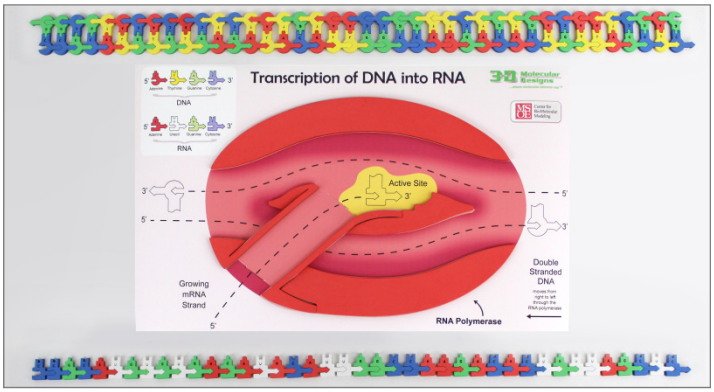
Diagram 2



Transcription: Termination

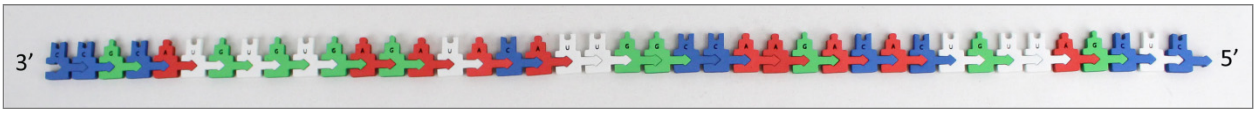
The mRNA transcript is released.
Eventually the single stranded mRNA transcript is released, and the polymerase detaches from the double stranded DNA (diagram 3).

Diagram 3



Translation of RNA into Proteins

mRNA carries the nucleotide sequence for synthesizing proteins.



Translation: Initiation

Ribosomal subunits bind to the mRNA.

The small ribosomal subunit binds to the mRNA. Note the orientation of the mRNA in this subunit shown in the diagram 1 to the right. An initiator tRNA, with the anticodoon UAC, base-pairs with the start codon, AUG. This tRNA carries the amino acid methionine. The large ribosomal subunit completes the intiation complex. In addition to a binding site for mRNA, each ribosome has three binding sites for tRNA. The **P site** (**p**eptidyl-tRNA binding site) holds the tRNA carrying the growing polypeptide chain. The **A site** (**a**minoacyl-tRNA binding site) holds the tRNA carrying the next amino acid to be added to the chain. Discharged tRNAs leave the ribosome from the **E site** (**e**xit site)

Diagram 1

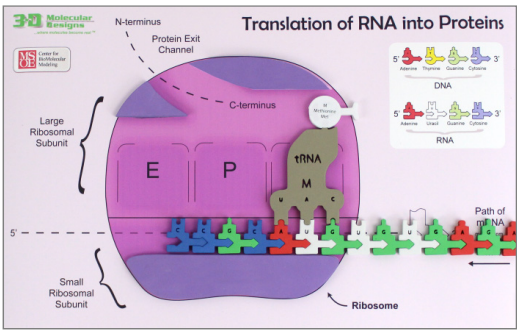
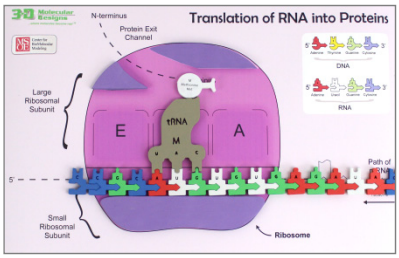


Diagram 2



Translation: Elongation

Amino acids are added to the growing protein.

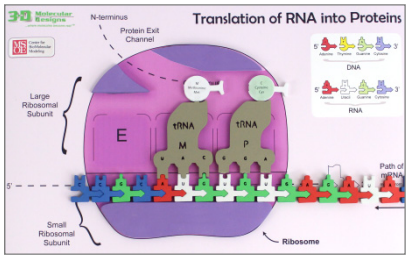
1. The initiator tRNA is in the P site; the A site is available to the tRNA carrying the next amino acid (diagram 2)

2. An rRNA molecule of the large ribosomal subunit catalyzes the formation of a peptide bond between the amino group of the new amino acid in the A site and the growing protein chain in the P site (diagram 3).

3. The ribosome translocates the tRNA in the A site to the P site. The empty tRNA in the P site is moved to the E site where it is released (diagram 4).

4. Elongation continues as each amino acid is added to the chain until the polypeptide is completed (diagram 5).

Diagram 3



Translation: Termination

The mRNA transcript is released.

Elongation continues until a stop codon in the mRNA reaches the A site of the ribosome. The mRNA codons UAG, UAA and UGA do not code for amino acids but act as signals to stop translation.

Diagram 4

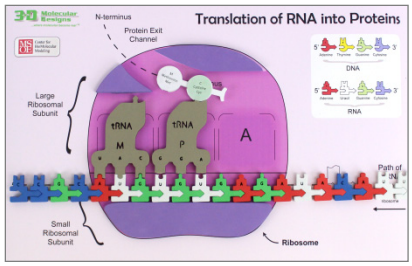
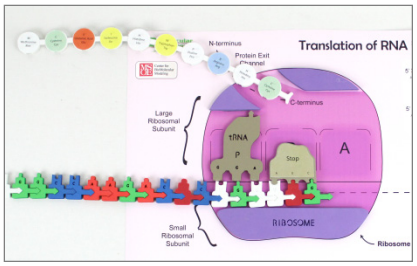


Diagram 5



Flow of Genetic Information

Transcription and Translation

PreLab

Student Introduction:

Almost all dynamic functions in a living organism depend on proteins. A wide variety of essential functions carried out by proteins have been identified including support, movement, transport, buffering, metabolic regulation, coordination and control and defense. More than 50% of the dry mass of an average cell is composed of protein. The current accepted number of proteins in the human body is approximately 25,000. Given the important role that these molecules play in an organism's survival, it is understandable that scientists focus a considerable amount of attention studying them. Central to their study is the question of how these biologically crucial molecules are produced in a cell. The molecular chain of command that dictates the directional flow of genetic information from DNA to RNA to protein was dubbed the "central dogma" by Francis Crick in 1956.

DNA carries all of the instructions for making the proteins that are found in our bodies. In fact, DNA is the universal code for the characteristics of simple organisms such as bacteria as well as for complex organisms such as plants or animals. DNA codes for the characteristics of all LIVING THINGS!! In this lesson you will learn how to interpret the DNA code to make proteins which determine these characteristics.

DNA has only four nitrogen bases; A, T, G, and C. But there are 20 amino acids that serve as the building blocks (monomers) for all proteins. How can only four letters code for all of these proteins? In order to accomplish this task DNA combines these four nitrogen bases into a three letter code called a **triplet code**.

1? How many possible combinations of these four base letters can be formed in total?

_____

_____

2? Given that there are more possible combinations for amino acids than amino acids themselves what does this imply about the number of codes for each amino acid?

_____

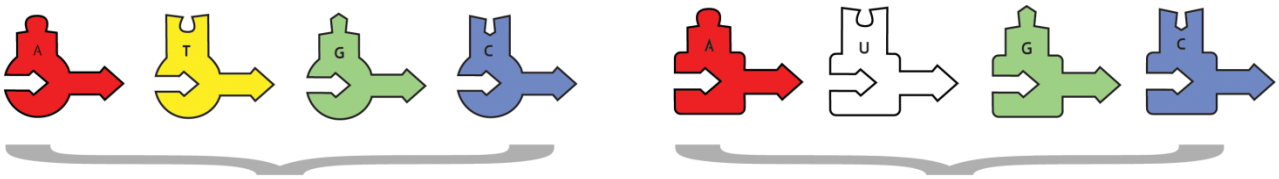
_____

Getting DNA to protein requires two major stages: (i) **transcription** and (ii) **translation**. The process by which a DNA template is used to produce a single-stranded RNA molecule is referred to as **transcription**.

In eukaryotic cells, DNA can found in the nucleus, chloroplasts, and mitochondria and cannot leave these structures. As a result, transcription occurs inside these organelles of eukaryotic cells.

3? Why can't DNA leave the nucleus?

Proteins are made on ribosomes (workbenches) that are outside of the nucleus in the cytoplasm. How does the information carried by DNA get to the ribosomes? Another molecule must carry this code from the DNA to the ribosome for the manufacture of proteins. In the process of protein synthesis there are two important types of nucleic acids; DNA and RNA (ribonucleic acid). Three different types of RNA (mRNA, tRNA, and rRNA) are major contributors to this process. The molecule that receives a copy of the DNA code in the nucleus and carries it to the ribosomes is called **messenger RNA** (mRNA).
The mRNA code is not identical to the DNA code.

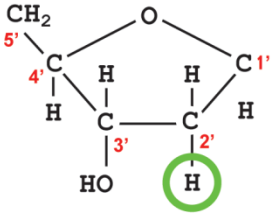


DNA RNA

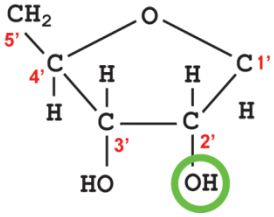
4? Examine the foam DNA pieces and compare them to the foam mRNA pieces. Identify any similarities and differences in the bases that comprise each nucleic acid.

Other differences between RNA and DNA are not readily visible in the model. The RNA backbone contains the sugar ribose which has an extra oxygen atom not found in the deoxyribose sugar of DNA. The model depicts this difference in the rounded shape of the DNA nucleotides as compared to the squared shape of the RNA nucleotides.

Deoxyribose
found in DNA



Ribose
found in RNA

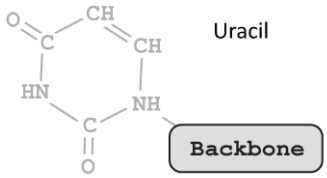


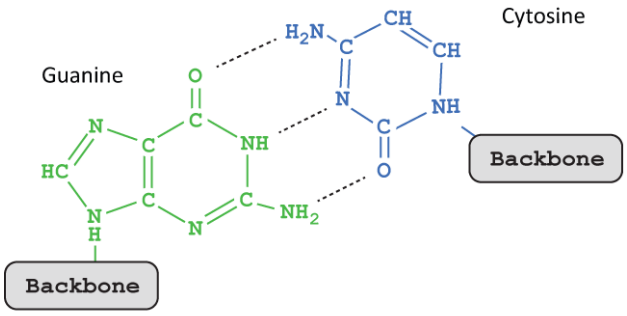
Transcription may be thought of in three stages: (1) initiation, (2) elongation and (3) termination. DNA acts as a blueprint for making mRNA. In eukaryotes, initiation begins with a collection of proteins called transcription factors mediating the binding of an enzyme **RNA polymerase** to the DNA. RNA polymerase breaks the hydrogen bonds between the two strands of DNA apart and joins the RNA nucleotides as they base-pair along the DNA template. When this happens only one side of the DNA will be used as a template for mRNA nucleotides to complementary base pair to the DNA.

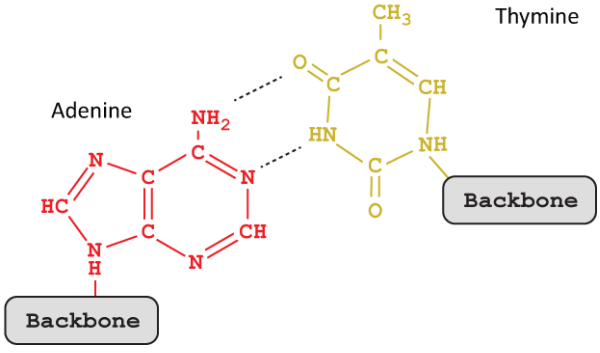
Base paring rules still apply with one exception. Guanine still pairs with cytosine while adenine pairs with uracil (recall that RNA contains the base uracil instead of the base thymine).

5? Complete the following chart by matching the correct RNA complementary base to the DNA base:

DNA Base	RNA Base
T	
G	
C	
A	
C	
A	







Lab

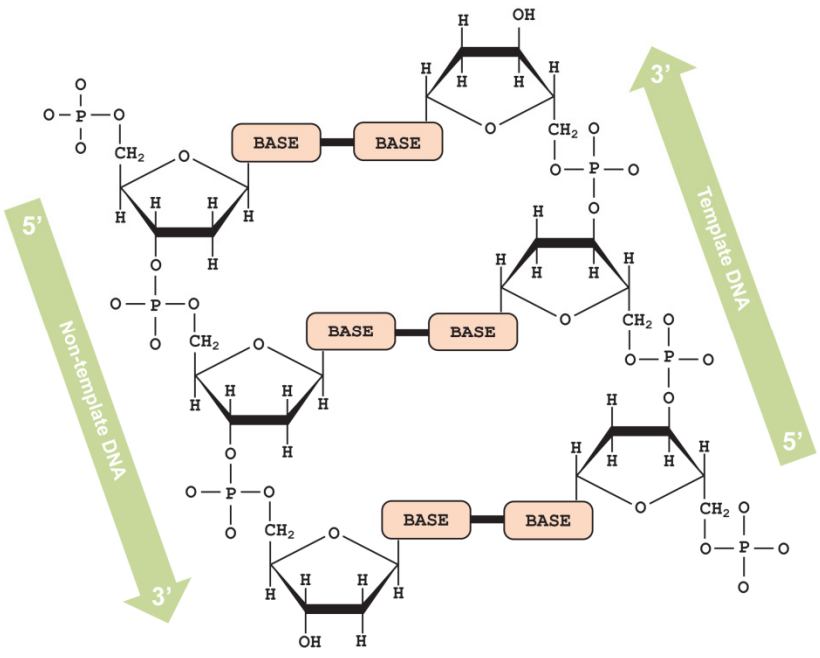
Modeling the Flow of Genetic Information

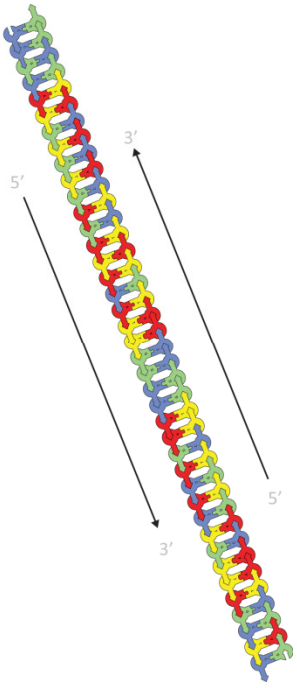
Note to Teacher: If you choose to pursue a more rigorous lesson, you may elect to introduce pre-mRNA, introns, exons, splicing and post transcriptional modification. The details of these processes are shown on the 3DMD Map of the Human β-Globin Gene.

Part I: Transcription

STEP 1: Using the rounded DNA foam pieces and following the code listed in question 1a or on the placemat, create a **non-template strand** of DNA. On the DNA backbone the sugar end is the 3' end (arrow end of the foam piece) and the phosphate end is the 5' end. In order for DNA to be interpreted correctly the 3' → 5' direction must be maintained.

Important Note! Refer to the diagram to ensure correct initiation of the protein synthesis process. Recall the antiparallel nature of the DNA molecule.





1a? Fill in the correct base pairs in the template strand below and build the DNA template strand.

STEP 2: Build the template strand of DNA to create a double stranded DNA model. Attach the two strands together following the rules of complementary DNA base pairing.

2a? Recalling from the lesson on DNA structure identify the type of bond that holds the two strands of DNA together.

_____

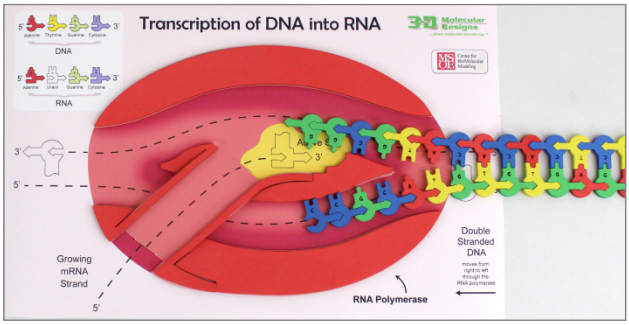
_____

STEP 3: Compare and contrast the foam model to the DNA Discovery Kit model or DNA Starter kit model on display.

3a? Identify and label the 3′ and 5′ ends in each of the models above.

3b? Identify two similarities and two differences between these models.

Transcription: Initiation



STEP 4: RNA polymerase assembles the mRNA only in its 5' → 3' direction. In order for this to properly occur, the template strand of DNA must be oriented in the top slot with the 3' end (arrow end) entering the polymerase first. (Please refer to the photo to ensure proper setup.)

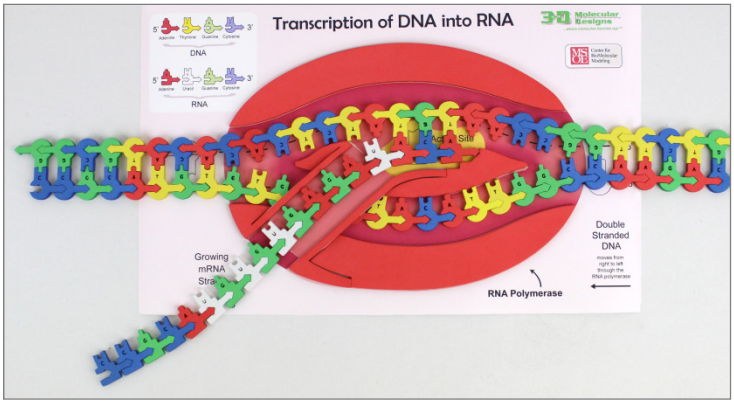
4a? Label the DNA template strand and non-template strand in the photo above.

Transcription: Elongation

STEP 5: Feed the DNA into the RNA polymerase (refer to diagram 1 on the Transcription Placemat).

5a? What will happen when RNA polymerase acts on DNA?

STEP 6: Sprinkle free RNA nucleotides around the enzyme. RNA polymerase uses the template strand of DNA to synthesize the mRNA. You will use the template strand of DNA to complementary base pair the correct sequence of mRNA nucleotides. Complete the base pairing process on your placemat.



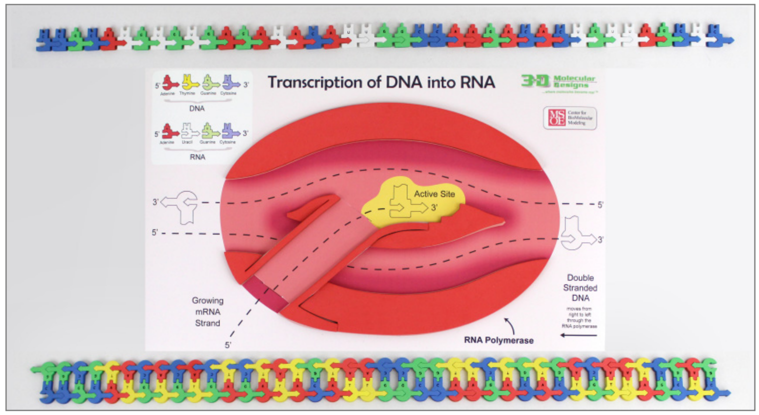
6a? Using your mRNA model record the correct sequence of mRNA base pairs:

5′ _ 3′

Note to Teacher: Reinforce differences between RNA structure and DNA structure.

Transcription: Termination

STEP 7: At this point the mRNA will separate from the DNA and may be processed into its final form. The template strand of DNA will rejoin with the nontemplate strand. Complete this step with your model. Refer to diagram 3 on the Transcription Placemat.

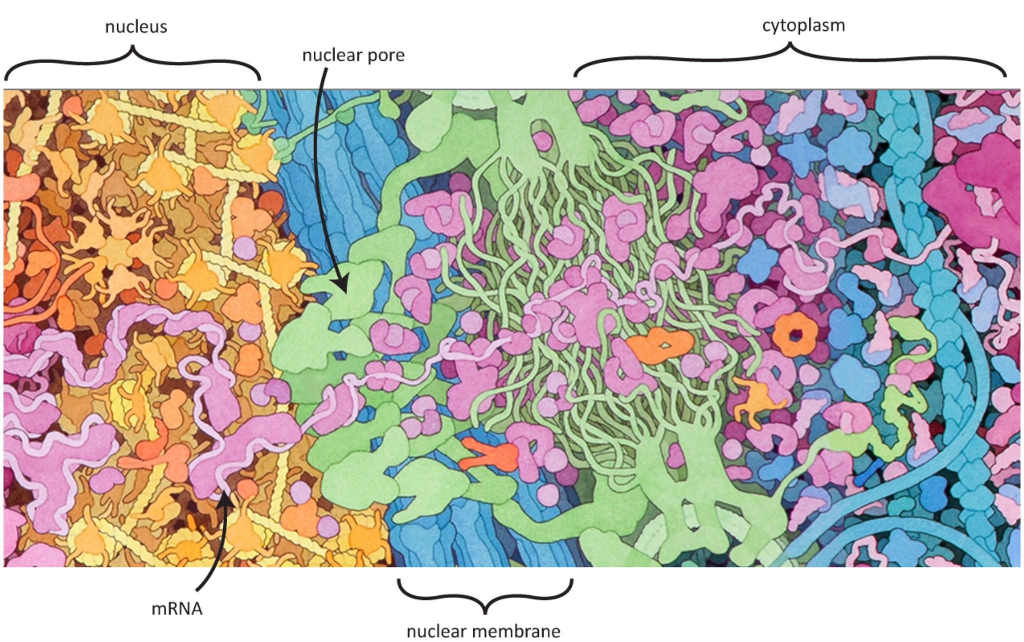


7a? What type of bond is broken when mRNA separates from DNA and what characteristic of this bond allows for this separation?

____

____

In eukaryotic cells the mRNA leaves the nucleus through nuclear pores after being processed into its final form.



Part II: Translation

Translation occurs in the cytoplasm of the cell and is defined as the synthesis of a protein (polypeptide) using information encoded in an mRNA molecule. Messenger RNA (mRNA) has the information for arranging the amino acids in the correct order to make a functional protein.

Translation of the mRNA occurs in groups of three nitrogenous bases called **codons**. The three nitrogen bases in one codon will indicate a specific amino acid. The order in which the amino acids are put together depends on the sequence of bases in the mRNA. Typically one mRNA strand will result in a protein (polypeptide strand) that can be 100 – 1000's of amino acids long.

7b? What part of the mRNA nucleotide contains the information to make a protein?

_____

_____

The identity of the amino acids in the protein sequence can be determined using the mRNA strand you created above. Starting from the 5' end of the mRNA every three bases determines a particular amino acid.

STEP 8: Use the table to the right to determine the identity of the correct amino acid for each codon in your mRNA strand.

8a? Identify the three letter and one letter abbreviation for each amino acid in the table below.

	U	C	A	G	
U	UUU → Phe F	UCU → Ser S	UAU → Tyr T	UGU → Cys C	U
	UUC → Phe F	UCC → Ser S	UAC → Tyr T	UGC → Cys C	C
	UUA → Leu L	UCA → Ser S	UAA → Stop	UGA → Stop	A
	UUG → Leu L	UCG → Ser S	UAG → Stop	UGG → Trp W	G
C	CUU → Leu L	CCU → Pro P	CAU → His H	CGU → Arg R	U
	CUC → Leu L	CCC → Pro P	CAC → His H	CGC → Arg R	C
	CUA → Leu L	CCA → Pro P	CAA → Gln Q	CGA → Arg R	A
	CUG → Leu L	CCG → Pro P	CAG → Gln Q	CGG → Arg R	G
A	AUU → Ile I	ACU → Thr T	AAU → Asn N	AGU → Ser S	U
	AUC → Ile I	ACC → Thr T	AAC → Asn N	AGC → Ser S	C
	AUA → Ile I	ACA → Thr T	AAA → Lys K	AGA → Arg R	A
	AUG → Met M	ACG → Thr T	AAG → Lys K	AGG → Arg R	G
G	GUU → Val V	GCU → Ala A	GAU → Asp D	GGU → Gly G	U
	GUC → Val V	GCC → Ala A	GAC → Asp D	GGC → Gly G	C
	GUA → Val V	GCA → Ala A	GAA → Glu E	GGA → Gly G	A
	GUG → Val V	GCG → Ala A	GAG → Glu E	GGG → Gly G	G

5' → 3'

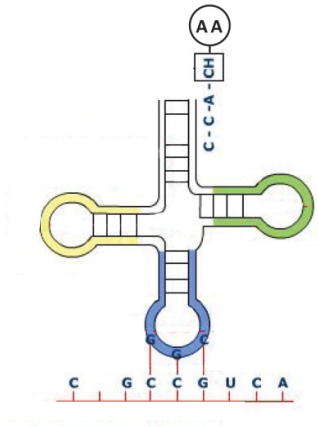
Codon	*AUG*	*UGU*	*GAG*	*AUA*	*CAU*	*UGG*	*CCA*	*AGA*	*CAC*	*UGU*	*UAG*
Amino Acid Abbreviations											

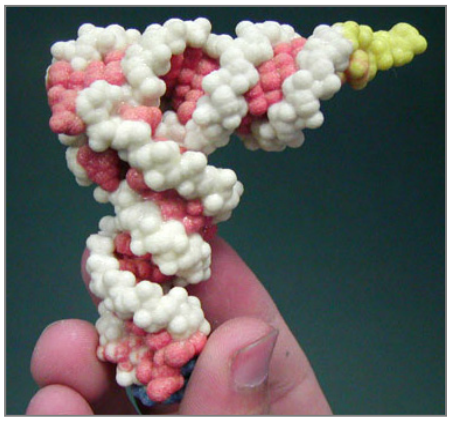
Note: Translation may also be thought of in three stages: (1) initiation, (2) elongation and (3) termination.

Translation: Initiation

Although this particular model does not illustrate the entire initiation process, the initiation stage of translation brings together mRNA, a second type of RNA called transfer RNA (tRNA) and the two subunits of a ribosome.

Two functional portions of the tRNA are necessary for protein synthesis to continue. One functional part of tRNA is a series of three nitrogen bases referred to as an **anticodon**. This anticodon complementary base pairs with the codon of the mRNA. The other functional part of tRNA attaches to a specific amino acid.





8b? On the preceding diagrams, label the 5' and 3' ends, anticodon, amino acid binding site of each tRNA model.

Note to Teacher: You may elect to include the following interesting note:

If one tRNA anticodon variety existed for each mRNA codon specifying an amino acid, there would be 61 tRNAs. In fact, there are only about 45, implying that some tRNAs must be able to bind to more than one codon. Such flexibility is possible because the rules for base pairing between the third nucleotide base of the mRNA codon and the corresponding tRNA anticodon are relaxed. Flexible base pairing at this codon position is referred to as wobble. For example, a tRNA with the anticodon 3'-CGU-5' can base pair with either the mRNA codon 5'-GCA-3' or 5'-GCG-3' both of which code for alanine.

8c? What amino acid is associated with the tRNA that will bind to the mRNA start codon AUG?

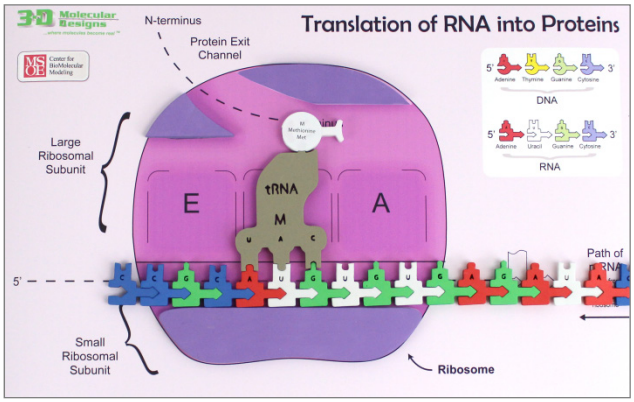
_____

8d? In the table below insert the mRNA codons from ?13 above and record the tRNA anticodons:

5′ ——————————————————————→ 3′

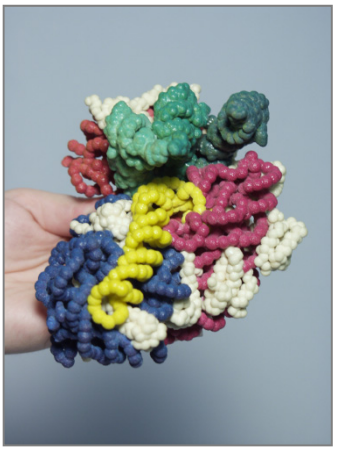
mRNA codons	*AUG*	*UGU*	*GAG*	*AUA*	*CAU*	*UGG*	*CCA*	*AGA*	*CAC*	*UGU*	*UAG*
tRNA anticodons											
Amino acids											

STEP 9: Bond the appropriate amino acids to each of the tRNAs identified in the table above. The amino acids have different colors which represent their various chemical properties such as acidic, basic, hydrophobic, and hydrophilic. Refer to Diagram 1 on the Translation Placemat.



9a? Draw your own illustration of the model and label the, anticodon and the amino acid on the mRNA or tRNA in the space below.

While the tRNA-amino acid complex is being assembled in the cytoplasm, mRNA moves towards the ribosome. Ribosomal subunits are made in the nucleolus of eukaryotic cells. The resulting ribosomal subunits are exported via nuclear pores to the cytoplasm. Approximately one third of the mass of a ribosome is made up of protein while the rest is composed of a third type of RNA, ribosomal ribonucleic acid (rRNA).



The ribosome consists of two separate parts; the large and small subunits which are unattached when not in use. First, the small ribosome subunit binds to both mRNA and a specific initiator tRNA bearing the amino acid methionine. The attachment of the large ribosomal subunit completes the translation initiation complex.

The large and small subunits join to form a functional ribosome only when they attach to an mRNA. Each ribosome has three binding sites for tRNA. The **P site** (peptidyl-tRNA binding site) holds the tRNA carrying the growing polypeptide chain). The **A site** (aminoacyl-tRNA binding site) holds the tRNA carrying the next amino acid to be added to the chain. Discharged tRNAs leave the ribosome from the **E site** (exit site).

In the next part of this activity you will model the elongation and termination processes of translation.

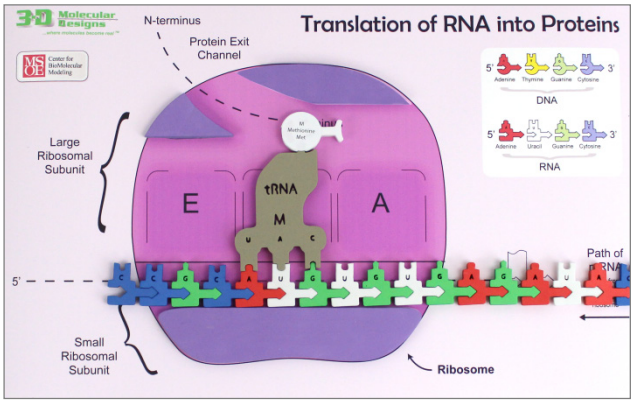
9b? Which end of the mRNA strand attaches to the small ribosomal subunit?

_____

_____

Refer to your place mat to ensure the mRNA is in the proper orientation in your ribosome.

STEP 10: Slide your mRNA into the small ribosomal subunit. Now attach the first tRNA-amino acid complex to the mRNA in the P site.



10a? Referring to the previous amino acid codon table you completed, record which tRNA anticodon and accompanying amino acid will attach first in this P site.

_____

_____

Translation: Elongation

STEP 11: The anticodon of another tRNA base pairs with the mRNA in the A site. Complete this process using your model.

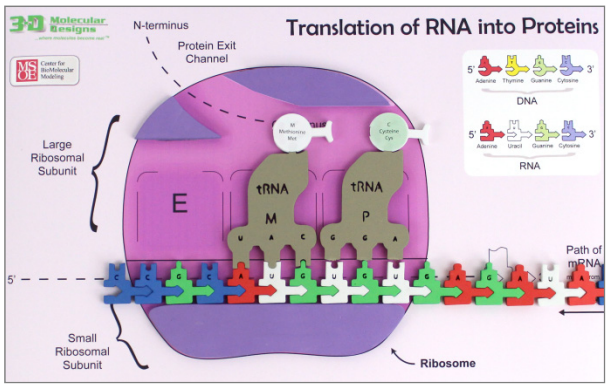
11a? Which tRNA-amino acid complex will attach into the A site at this time?

_____

_____

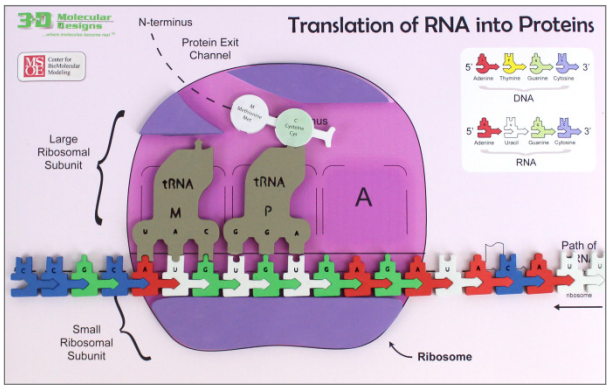
STEP 12: An rRNA found in the large ribosmal subunit catalyzes the formation of a peptide bond between the amino group of the amino acid in the A site and the carboxyl end of the amino acid in the P site.

Simulate the peptide bond formation with your model.



12a? Label the peptide bond in the photo to the right.

STEP 13: The ribosome translocates the tRNA in the A site to the P site. The tRNA in the P site is simultaneously moved to the E site where it is released.



STEP 14: Separate your tRNA in the E site from mRNA and return the tRNA to the cytoplasm.

14a? What characteristic allows tRNA to separate from mRNA at the ribosome?

_____

_____

14b? Why would tRNA get recycled for use in future translation?

_____

_____

14c? Which mRNA codon is now located in the A site? _____

STEP 15: With the A site now available for another tRNA-amino acid complex these steps can continue. Remember that the growing polypeptide transfers from the P site to the A site. Demonstrate this process using all of your tRNA-amino acid complexes in the appropriate order.

The mRNA is translated in one direction from its 5' → 3' end.

Translation: Termination

This developing polypeptide will exit the ribosome through the opening in the large ribosomal subunit. A stop codon is also present to indicate the end of the protein.

15a? Using the reference mRNA Codon/Amino Acid Chart list the various stop codons.

_____

15b? What is the order of amino acids in your polypeptide?

Met M									

15c? Compare the amino acid sequence of the poly peptide you created to the sequence predicted in question 13. How do your sequences compare?

15d? When you reach the end of the mRNA strand in your modeling of the translation process, describe what has happened to the polypeptide.

For Further Exploration

15e? What will happen next to the polypeptide?

15f? As you have followed this process of translation what steps are now left to be completed? What will happen to the mRNA, tRNA, and the ribosome at the end of this process?

15g? How long did this process of translation take for you and your lab group? Do you think the cell could operate at this rate?

mRNA, tRNA, and ribosomes can be reused over and over. The same protein can be made again if needed, or a new piece of mRNA can be translated. Ribosomes add new amino acids to the polypeptide at a rate of 20 amino acids per second (at 37^{o} C).

15h ? At this rate, how long would it take to make a protein such as actin 375 amino acids long?

15i ? Develop a new model summarizing the entire process of transcription and translation with your lab group. You will be asked to communicate and share your model with the class.

Links to the Next Generation Standards

Scientific and Engineering Practices:

- Asking Questions (for science) and Defining Problems (for engineering)
- Developing and Using Models
- Analyzing and Interpreting Data
- Using Mathematics and Computational Thinking
- Constructing Explanations (for science) and Designing Solutions (for engineering)

Crosscutting Concepts:

- Patterns
- Cause and effect: Mechanism and Explanation
- Scale, Proportion, and Quantity
- Structure and Function
- Systems and System Models
- Stability and Change

Disciplinary Core Ideas:

- LS 1: From Molecules to Organisms: Structures and Processes
- HS-LS1-1: Construct an explanation based on evidence for how the structure of DNA determines the structure of proteins which carry out the essential functions of life through systems of specialized cells.

- LS 2: Heredity: Inheritance and Variation of Traits
- HS-LS3-1: Ask questions to clarify relationships about the role of DNA and chromosomes in coding the instructions for characteristic traits passed from parents to offspring.
- HS-LS3-2: Make and defend a claim based on evidence that inheritable genetic variations may result from (1) new genetic combinations through meiosis, (2) viable errors occurring during replication, and/or (3) mutations caused by environmental factors.
- HS-LS3-3: Apply concepts of statistics and probability to explain the variation and distribution of expressed traits in a population.

- HS-ETS1: Engineering Design
- HS-ETS1-4: Use a computer simulation to model the impact of proposed solutions to a complex real-world problem with numerous criteria and constraints on interactions within and between systems relevant to the problem.

Students will:

- Identify different types of RNA.
- **Demonstrate** how a molecule of messenger RNA is created from the template of DNA using the model.
- Compare and **contrast** the structures of RNA and DNA.
- Explain the structure and function of codons and anticodons in the formation of proteins.
- Model the flow of genetic information from DNA → RNA → protein (also known as the Central Dogma).
- Explain how changing the DNA code, a mutation, may ultimately change the sequence of amino acids in the protein.

Prerequisite Knowledge and Skills:

- Hydrogen bonding and covalent bonding
- Cell structure
- DNA structure
- Structure of amino acids and proteins
- Prokaryotic and eukaryotic cell structure

Materials:

- One DNA Discovery Kit, assembled for display
- Student Lab Packet
- Protein Synthesis Kit, recommended one kit per group of four students

Flow of Genetic Information

Transcription and Translation

PreLab

Student Introduction:

Almost all dynamic functions in a living organism depend on proteins. A wide variety of essential functions carried out by proteins have been identified including support, movement, transport, buffering, metabolic regulation, coordination and control and defense. More than 50% of the dry mass of an average cell is composed of protein. The current accepted number of proteins in the human body is approximately 25,000. Given the important role that these molecules play in an organism's survival, it is understandable that scientists focus a considerable amount of attention studying them. Central to their study is the question of how these biologically crucial molecules are produced in a cell. The molecular chain of command that dictates the directional flow of genetic information from DNA to RNA to protein was dubbed the "central dogma" by Francis Crick in 1956.

DNA carries all of the instructions for making the proteins that are found in our bodies. In fact, DNA is the universal code for the characteristics of simple organisms such as bacteria as well as for complex organisms such as plants or animals. DNA codes for the characteristics of all LIVING THINGS!! In this lesson you will learn how to interpret the DNA code to make proteins which determine these characteristics.

DNA has only four nitrogen bases; A, T, G, and C. But there are 20 amino acids that serve as the building blocks (monomers) for all proteins. How can only four letters code for all of these proteins? In order to accomplish this task DNA combines these four nitrogen bases into a three letter code called a **triplet code**.

1? How many possible combinations of these four base letters can be formed in total?

(64, calculation: 4 different bases, in groups of three, $4^3 = 64$)

2? Given that there are more possible combinations for amino acids than amino acids themselves what does this imply about the number of codes for each amino acid?

(*Some but not all amino acids may be coded for in more than one way. Therefore there is redundancy in the code.)*

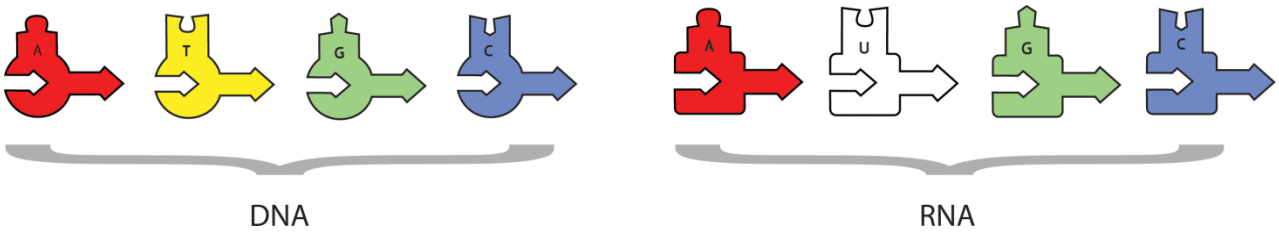
Getting DNA to protein requires two major stages: (i) **transcription** and (ii) **translation**. The process by which a DNA template is used to produce a single-stranded RNA molecule is referred to as **transcription**.

In eukaryotic cells, DNA can found in the nucleus, chloroplasts, and mitochondria and cannot leave these structures. As a result, transcription occurs inside these organelles of eukaryotic cells.

3? Why can't DNA leave the nucleus?

(DNA is too large; its information is too valuable to risk exposure to harmful chemicals in the cytoplasm.)

Proteins are made on ribosomes (workbenches) that are outside of the nucleus in the cytoplasm. How does the information carried by DNA get to the ribosomes? Another molecule must carry this code from the DNA to the ribosome for the manufacture of proteins. In the process of protein synthesis there are two important types of nucleic acids; DNA and RNA (ribonucleic acid). Three different types of RNA (mRNA, tRNA, and rRNA) are major contributors to this process. The molecule that receives a copy of the DNA code in the nucleus and carries it to the ribosomes is called **messenger RNA** (mRNA).
The mRNA code is not identical to the DNA code.

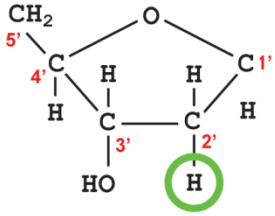


4? Examine the foam DNA pieces and compare them to the foam mRNA pieces. Identify any similarities and differences in the bases that comprise each nucleic acid.

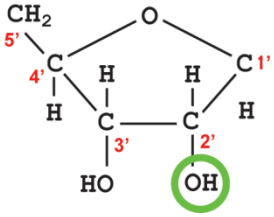
(DNA and RNA both contain adenine, guanine and cytosine. RNA contains the base uracil while DNA contains the base thymine.)

Other differences between RNA and DNA are not readily visible in the model. The RNA backbone contains the sugar ribose which has an extra oxygen atom not found in the deoxyribose sugar of DNA. The model depicts this difference in the rounded shape of the DNA nucleotides as compared to the squared shape of the RNA nucleotides.

Deoxyribose
found in DNA



Ribose
found in RNA

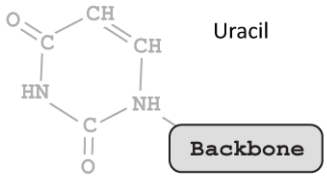


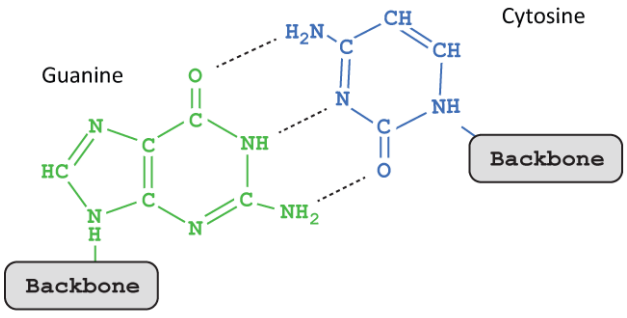
Transcription may be thought of in three stages: (1) initiation, (2) elongation and (3) termination. DNA acts as a blueprint for making mRNA. In eukaryotes, initiation begins with a collection of proteins called transcription factors mediating the binding of an enzyme **RNA polymerase** to the DNA. RNA polymerase breaks the hydrogen bonds between the two strands of DNA apart and joins the RNA nucleotides as they base-pair along the DNA template. When this happens only one side of the DNA will be used as a template for mRNA nucleotides to complementary base pair to the DNA.

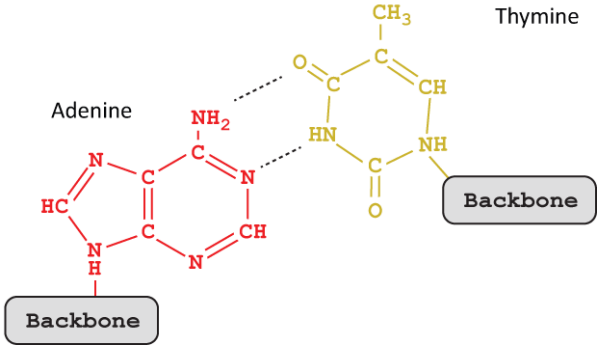
Base paring rules still apply with one exception. Guanine still pairs with cytosine while adenine pairs with uracil (recall that RNA contains the base uracil instead of the base thymine).

5? Complete the following chart by matching the correct RNA complementary base to the DNA base:

DNA Base	RNA Base
T	*(A)*
G	*(C)*
C	*(G)*
A	*(U)*
C	*(G)*
A	*(U)*







Lab

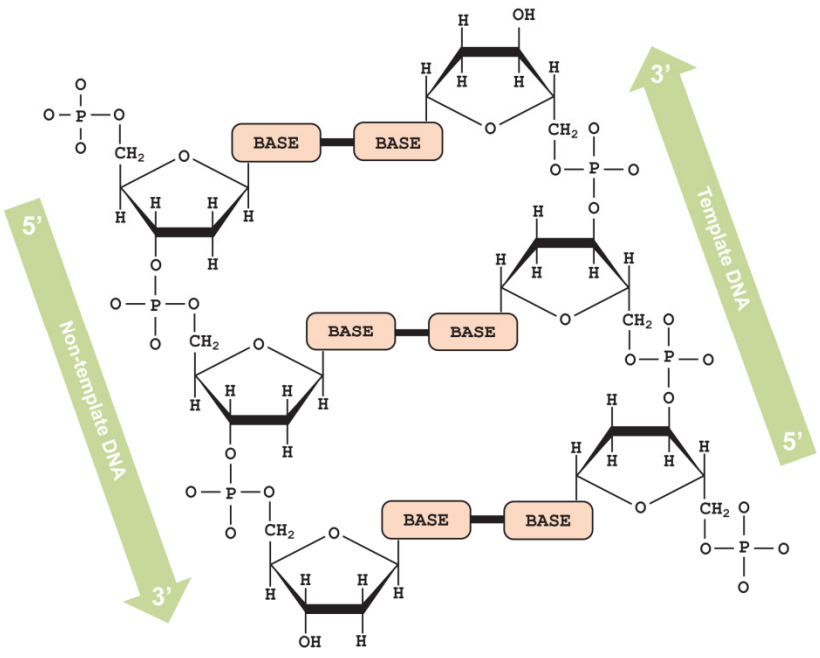
Modeling the Flow of Genetic Information

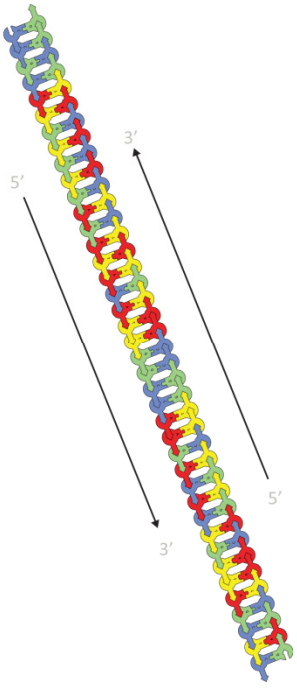
Note to Teacher: If you choose to pursue a more rigorous lesson, you may elect to introduce pre-mRNA, introns, exons, splicing and post transcriptional modification. The details of these processes are shown on the 3DMD Map of the Human β-Globin Gene.

Part I: Transcription

STEP 1: Using the rounded DNA foam pieces and following the code listed in question 1a or on the placemat, create a **non-template strand** of DNA. On the DNA backbone the sugar end is the 3' end (arrow end of the foam piece) and the phosphate end is the 5' end. In order for DNA to be interpreted correctly the 3' → 5' direction must be maintained.

Important Note! Refer to the diagram to ensure correct initiation of the protein synthesis process. Recall the antiparallel nature of the DNA molecule.





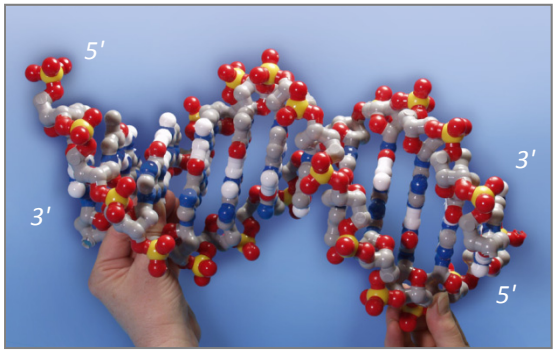
1a Fill in the correct base pairs in the template strand below and build the DNA template strand.

STEP 2: Build the template strand of DNA to create a double stranded DNA model. Attach the two strands together following the rules of complementary DNA base pairing.

2a Recalling from the lesson on DNA structure identify the type of bond that holds the two strands of DNA together.

(Hydrogen bonds)

STEP 3: Compare and contrast the foam model to the DNA Discovery Kit model or DNA Starter kit model on display.



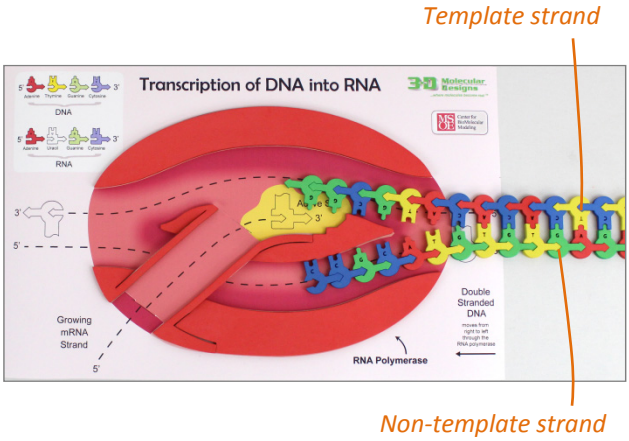
3a Identify and label the 3’ and 5’ ends in each of the models above.

3b? Identify two similarities and two differences between these models.

(Similarities: Base pairing rules are consistent, DNA is antiparallel; Differences: foam model is two dimensional and does not show the detail of the three dimensional model, major and minor grooves are missing from the foam model, cannot see the sugar phosphate backbone, foam model does not show the twisted ladder structure.)

Transcription: Initiation

STEP 4: RNA polymerase assembles the mRNA only in its 5' → 3' direction. In order for this to properly occur, the template strand of DNA must be oriented in the top slot with the 3' end (arrow end) entering the polymerase first. (Please refer to the photo to ensure proper setup.)



4a? Label the DNA template strand and non-template strand in the photo above.

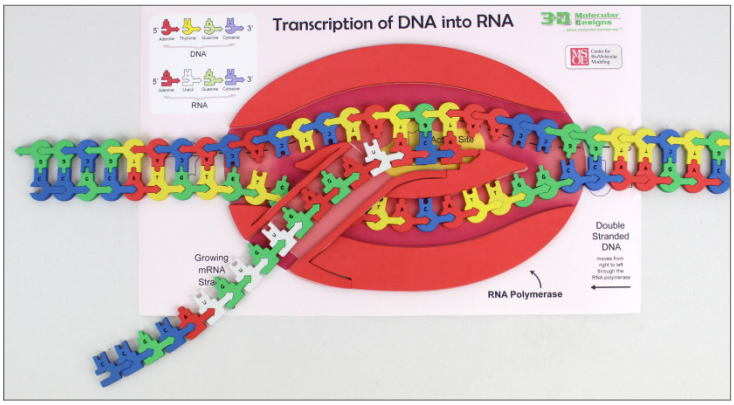
Transcription: Elongation

STEP 5: Feed the DNA into the RNA polymerase (refer to diagram 1 on the Transcription Placemat).

5a? What will happen when RNA polymerase acts on DNA?

(RNA polymerase breaks the hydrogen bonds between the DNA base pairs to open up the DNA)

STEP 6: Sprinkle free RNA nucleotides around the enzyme. RNA polymerase uses the template strand of DNA to synthesize the mRNA. You will use the template strand of DNA to complementary base pair the correct sequence of mRNA nucleotides. Complete the base pairing process on your placemat.



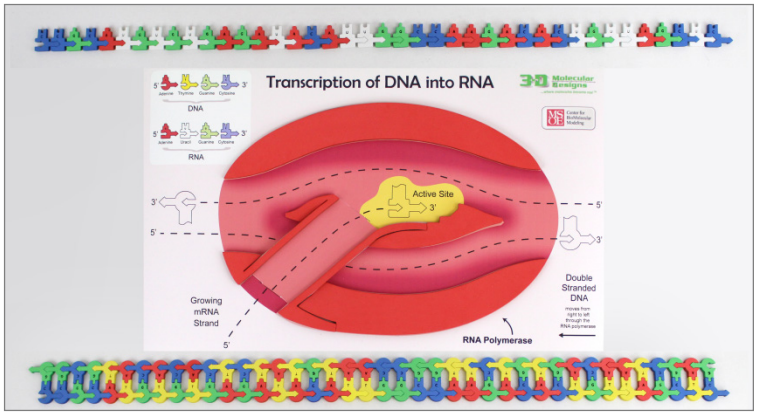
6a? Using your mRNA model record the correct sequence of mRNA base pairs:

5′ C C G C A U G U G U G A G A U A C A U U G G C C A A G A C A C U G U U A G C U C 3′

Note to Teacher: Reinforce differences between RNA structure and DNA structure.

Transcription: Termination

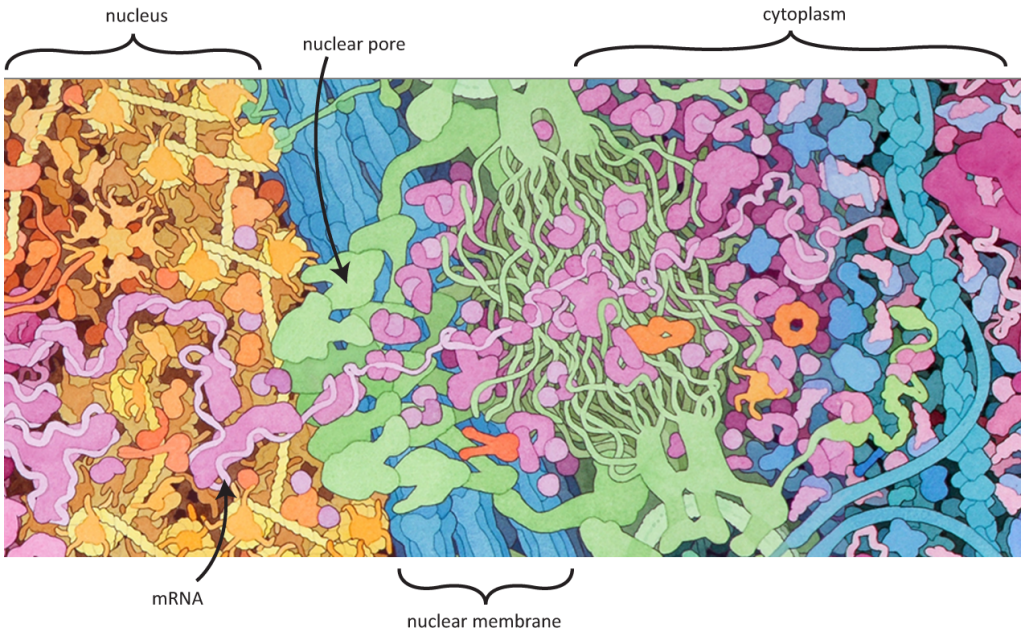
STEP 7: At this point the mRNA will separate from the DNA and may be processed into its final form. The template strand of DNA will rejoin with the nontemplate strand. Complete this step with your model. Refer to diagram 3 on the Transcription Placemat.



7a? What type of bond is broken when mRNA separates from DNA and what characteristic of this bond allows for this separation?

(hydrogen bond, weak bond)

In eukaryotic cells the mRNA leaves the nucleus through nuclear pores after being processed into its final form.



Part II: Translation

Translation occurs in the cytoplasm of the cell and is defined as the synthesis of a protein (polypeptide) using information encoded in an mRNA molecule. Messenger RNA (mRNA) has the information for arranging the amino acids in the correct order to make a functional protein.

Translation of the mRNA occurs in groups of three nitrogenous bases called **codons**. The three nitrogen bases in one codon will indicate a specific amino acid. The order in which the amino acids are put together depends on the sequence of bases in the mRNA. Typically one mRNA strand will result in a protein (polypeptide strand) that can be 100 – 1000's of amino acids long.

7b? What part of the mRNA nucleotide contains the information to make a protein?

(the order of the various nitrogen bases, the codon)

STEP 8: Use the table to the right to determine the identity of the correct amino acid for each codon in your mRNA strand.

8a? Identify the three letter and one letter abbreviation for each amino acid in the table below.

	U	C	A	G	
U	UUU → Phe F	UCU → Ser S	UAU → Tyr T	UGU → Cys C	U
	UUC → Phe F	UCC → Ser S	UAC → Tyr T	UGC → Cys C	C
	UUA → Leu L	UCA → Ser S	UAA → Stop	UGA → Stop	A
	UUG → Leu L	UCG → Ser S	UAG → Stop	UGG → Trp W	G
C	CUU → Leu L	CCU → Pro P	CAU → His H	CGU → Arg R	U
	CUC → Leu L	CCC → Pro P	CAC → His H	CGC → Arg R	C
	CUA → Leu L	CCA → Pro P	CAA → Gln Q	CGA → Arg R	A
	CUG → Leu L	CCG → Pro P	CAG → Gln Q	CGG → Arg R	G
A	AUU → Ile I	ACU → Thr T	AAU → Asn N	AGU → Ser S	U
	AUC → Ile I	ACC → Thr T	AAC → Asn N	AGC → Ser S	C
	AUA → Ile I	ACA → Thr T	AAA → Lys K	AGA → Arg R	A
	AUG → Met M	ACG → Thr T	AAG → Lys K	AGG → Arg R	G
G	GUU → Val V	GCU → Ala A	GAU → Asp D	GGU → Gly G	U
	GUC → Val V	GCC → Ala A	GAC → Asp D	GGC → Gly G	C
	GUA → Val V	GCA → Ala A	GAA → Glu E	GGA → Gly G	A
	GUG → Val V	GCG → Ala A	GAG → Glu E	GGG → Gly G	G

5′ → 3′

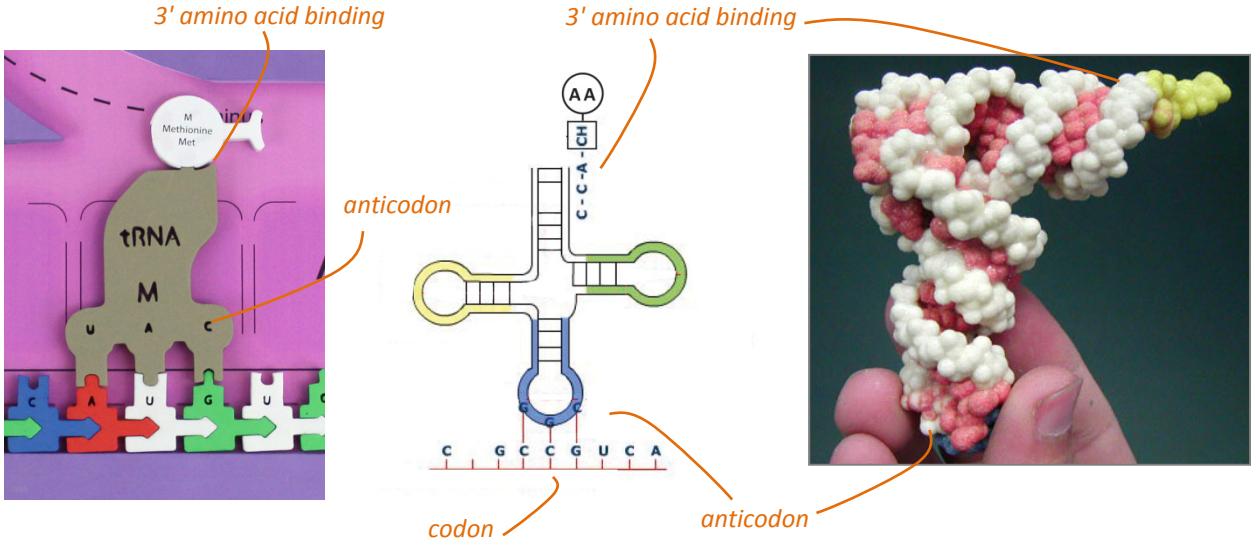
Codon	*AUG*	*UGU*	*GAG*	*AUA*	*CAU*	*UGG*	*CCA*	*AGA*	*CAC*	*UGU*	*UAG*
Amino Acid Abbreviations	*Met M (Start)*	*Cys C*	*Glu E*	*Ile I*	*His H*	*Trp W*	*Pro P*	*Arg R*	*His H*	*Cys C*	*STOP*

Note: Translation may also be thought of in three stages: (1) initiation, (2) elongation and (3) termination.

Translation: Initiation

Although this particular model does not illustrate the entire initiation process, the initiation stage of translation brings together mRNA, a second type of RNA called transfer RNA (tRNA) and the two subunits of a ribosome.

Two functional portions of the tRNA are necessary for protein synthesis to continue. One functional part of tRNA is a series of three nitrogen bases referred to as an **anticodon**. This anticodon complementary base pairs with the codon of the mRNA. The other functional part of tRNA attaches to a specific amino acid.



8b On the preceding diagrams, label the 5' and 3' ends, anticodon, amino acid binding site of each tRNA model.

Note to Teacher: You may elect to include the following interesting note:

If one tRNA anticodon variety existed for each mRNA codon specifying an amino acid, there would be 61 tRNAs. In fact, there are only about 45, implying that some tRNAs must be able to bind to more than one codon. Such flexibility is possible because the rules for base pairing between the third nucleotide base of the mRNA codon and the corresponding tRNA anticodon are relaxed. Flexible base pairing at this codon position is referred to as wobble. For example, a tRNA with the anticodon 3'-CGU-5' can base pair with either the mRNA codon 5'-GCA-3' or 5'-GCG-3' both of which code for alanine.

8c What amino acid is associated with the tRNA that will bind to the mRNA start codon AUG?

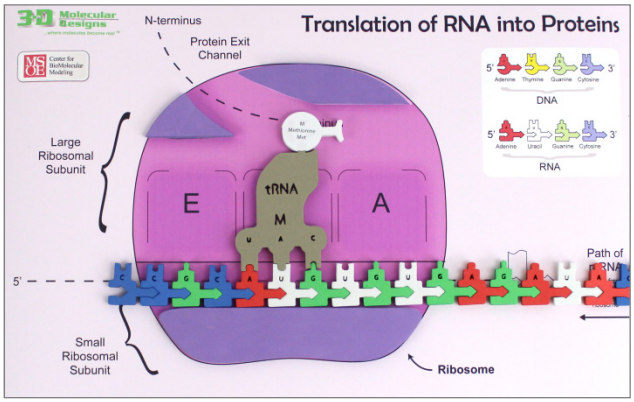
(methionine)

8d? In the table below insert the mRNA codons from ?13 above and record the tRNA anticodons:

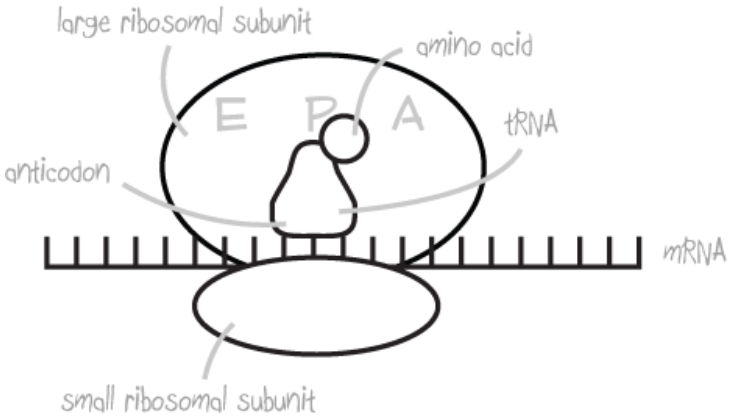
5′ ⟶ 3′

mRNA codons	AUG	UGU	GAG	AUA	CAU	UGG	CCA	AGA	CAC	UGU	UAG
tRNA anticodons	UAC	ACA	CUC	UAU	GUA	ACC	GGU	UCU	GUG	ACA	
Amino acids	M	C	E	I	H	W	P	R	H	C	STOP

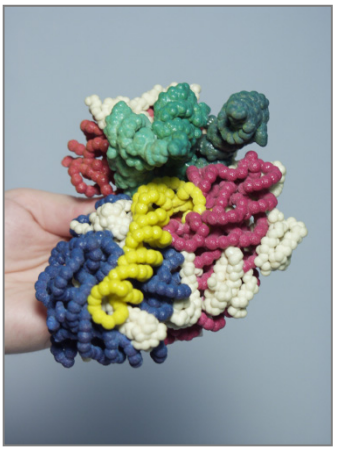
STEP 9: Bond the appropriate amino acids to each of the tRNAs identified in the table above. The amino acids have different colors which represent their various chemical properties such as acidic, basic, hydrophobic, and hydrophilic. Refer to Diagram 1 on the Translation Placemat.



9a? Draw your own illustration of the model and label the, anticodon and the amino acid on the mRNA or tRNA in the space below.



While the tRNA-amino acid complex is being assembled in the cytoplasm, mRNA moves towards the ribosome. Ribosomal subunits are made in the nucleolus of eukaryotic cells. The resulting ribosomal subunits are exported via nuclear pores to the cytoplasm. Approximately one third of the mass of a ribosome is made up of protein while the rest is composed of a third type of RNA, ribosomal ribonucleic acid (rRNA).



The ribosome consists of two separate parts; the large and small subunits which are unattached when not in use. First, the small ribosome subunit binds to both mRNA and a specific initiator tRNA bearing the amino acid methionine. The attachment of the large ribosomal subunit completes the translation initiation complex.

The large and small subunits join to form a functional ribosome only when they attach to an mRNA. Each ribosome has three binding sites for tRNA. The **P site** (peptidyl-tRNA binding site) holds the tRNA carrying the growing polypeptide chain). The **A site** (aminoacyl-tRNA binding site) holds the tRNA carrying the next amino acid to be added to the chain. Discharged tRNAs leave the ribosome from the **E site** (exit site).

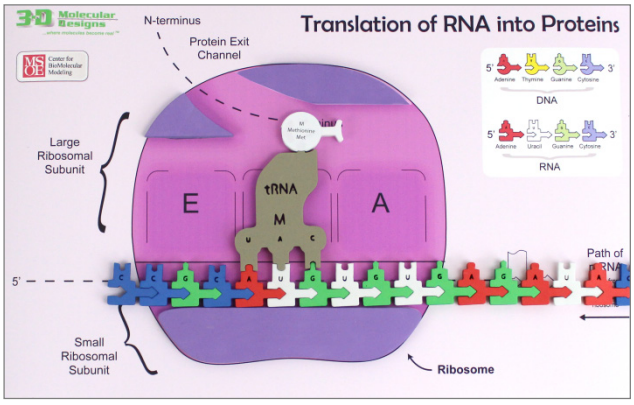
In the next part of this activity you will model the elongation and termination processes of translation.

9b? Which end of the mRNA strand attaches to the small ribosomal subunit?

(the part with the start codon, 5' end)

Refer to your place mat to ensure the mRNA is in the proper orientation in your ribosome.

STEP 10: Slide your mRNA into the small ribosomal subunit. Now attach the first tRNA-amino acid complex to the mRNA in the P site.



10a? Referring to the previous amino acid codon table you completed, record which tRNA anticodon and accompanying amino acid will attach first in this P site.

(UAC, or methionine)

Translation: Elongation

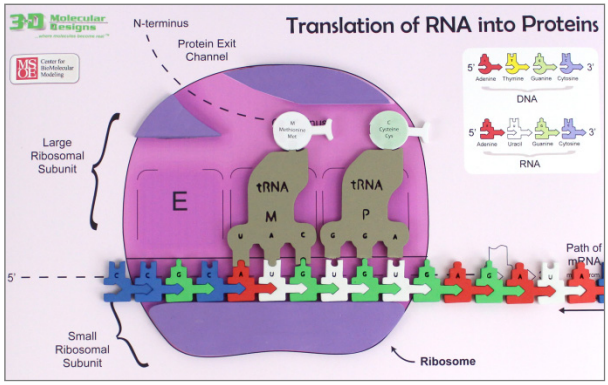
STEP 11: The anticodon of another tRNA base pairs with the mRNA in the A site. Complete this process using your model.

11a? Which tRNA-amino acid complex will attach into the A site at this time?

(UGU or cysteine)

STEP 12: An rRNA found in the large ribosmal subunit catalyzes the formation of a peptide bond between the amino group of the amino acid in the A site and the carboxyl end of the amino acid in the P site.

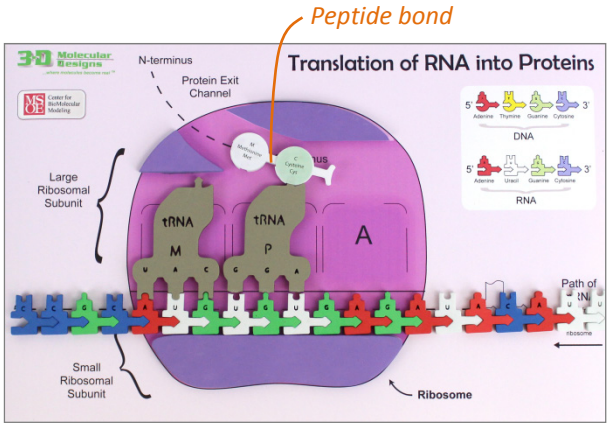
Simulate the peptide bond formation with your model.



12a? Label the peptide bond in the photo to the right.

STEP 13: The ribosome translocates the tRNA in the A site to the P site. The tRNA in the P site is simultaneously moved to the E site where it is released.

STEP 14: Separate your tRNA in the E site from mRNA and return the tRNA to the cytoplasm.



14a? What characteristic allows tRNA to separate from mRNA at the ribosome?

(the hydrogen bond formation between codon and anticodon allows tRNA to separate from mRNA as the hydrogen bond is a weak bond)

14b? Why would tRNA get recycled for use in future translation?

(tRNA picks up the correct amino acid in the cytoplasm. These amino acids are products of protein digestion.)

14c? Which mRNA codon is now located in the A site? *(UGU)*

STEP 15: With the A site now available for another tRNA-amino acid complex these steps can continue. Remember that the growing polypeptide transfers from the P site to the A site. Demonstrate this process using all of your tRNA-amino acid complexes in the appropriate order.

The mRNA is translated in one direction from its 5' → 3' end.

Translation: Termination

This developing polypeptide will exit the ribosome through the opening in the large ribosomal subunit. A stop codon is also present to indicate the end of the protein.

15a? Using the reference mRNA Codon/Amino Acid Chart list the various stop codons.

(UGA, UAA, UAG)

15b? What is the order of amino acids in your polypeptide?

Met M	*Cys* *C*	*Glu* *E*	*Ile* *I*	*His* *H*	*Trp* *W*	*Pro* *P*	*Arg* *R*	*His* *H*	*Cys* *C*

15c? Compare the amino acid sequence of the poly peptide you created to the sequence predicted in question 13. How do your sequences compare?

(the sequence of amino acids match)

15d? When you reach the end of the mRNA strand in your modeling of the translation process, describe what has happened to the polypeptide.

(polypeptide is emerging from the opening in the large ribosomal subunit, polypeptide is longer, eleven amino acids long)

For Further Exploration

15e? What will happen next to the polypeptide?

(polypeptide will separate from the mRNA strand and leave the ribosome through the opening in the large subunit; it may be a functional protein at this time or may require further modification in which case it will be transported to the rough ER.)

15f? As you have followed this process of translation what steps are now left to be completed? What will happen to the mRNA, tRNA, and the ribosome at the end of this process?

(the last tRNA will separate, the mRNA will leave the ribosome, and the large and small ribosomal subunits will separate and could be reused later)

15g? How long did this process of translation take for you and your lab group? Do you think the cell could operate at this rate?

(varies; no, too slow for cellular processes)

mRNA, tRNA, and ribosomes can be reused over and over. The same protein can be made again if needed, or a new piece of mRNA can be translated. Ribosomes add new amino acids to the polypeptide at a rate of 20 amino acids per second (at 37^{o} C).

15h ? At this rate, how long would it take to make a protein such as actin 375 amino acids long?

(approximately 20 seconds, 375 amino acids x 1 sec/20 amino acids = 18.75 sec., actin is used in muscle contractions and found in the cytoskeleton)

15i ? Develop a new model summarizing the entire process of transcription and translation with your lab group. You will be asked to communicate and share your model with the class.

(various answers)

Links to the Next Generation Standards

Scientific and Engineering Practices:

- Asking Questions (for science) and Defining Problems (for engineering)
- Developing and Using Models
- Analyzing and Interpreting Data
- Using Mathematics and Computational Thinking
- Constructing Explanations (for science) and Designing Solutions (for engineering)

Crosscutting Concepts:

- Patterns
- Cause and effect: Mechanism and Explanation
- Scale, Proportion, and Quantity
- Structure and Function
- Systems and System Models
- Stability and Change

Disciplinary Core Ideas:

- LS 1: From Molecules to Organisms: Structures and Processes
- HS-LS1-1: Construct an explanation based on evidence for how the structure of DNA determines the structure of proteins which carry out the essential functions of life through systems of specialized cells.

- LS 2: Heredity: Inheritance and Variation of Traits
- HS-LS3-1: Ask questions to clarify relationships about the role of DNA and chromosomes in coding the instructions for characteristic traits passed from parents to offspring.
- HS-LS3-2: Make and defend a claim based on evidence that inheritable genetic variations may result from (1) new genetic combinations through meiosis, (2) viable errors occurring during replication, and/or (3) mutations caused by environmental factors.
- HS-LS3-3: Apply concepts of statistics and probability to explain the variation and distribution of expressed traits in a population.

- HS-ETS1: Engineering Design
- HS-ETS1-4: Use a computer simulation to model the impact of proposed solutions to a complex real-world problem with numerous criteria and constraints on interactions within and between systems relevant to the problem.

Students will:

- Identify different types of RNA.
- **Demonstrate** how a molecule of messenger RNA is created from the template of DNA using the model.
- Compare and **contrast** the structures of RNA and DNA.
- Explain the structure and function of codons and anticodons in the formation of proteins.
- Model the flow of genetic information from DNA → RNA → protein (also known as the Central Dogma).
- Explain how changing the DNA code, a mutation, may ultimately change the sequence of amino acids in the protein.

Prerequisite Knowledge and Skills:

- Hydrogen bonding and covalent bonding
- Cell structure
- DNA structure
- Structure of amino acids and proteins
- Prokaryotic and eukaryotic cell structure

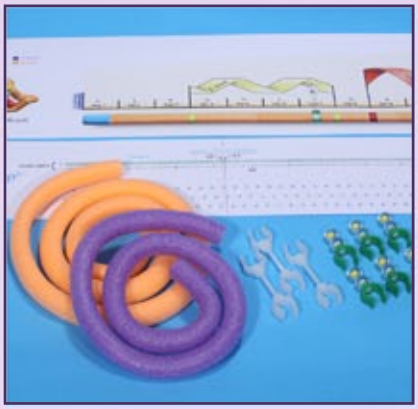
Materials:

- One DNA Discovery Kit, assembled for display
- Student Lab Packet
- Protein Synthesis Kit, recommended one kit per group of four students

Insulin mRNA to Protein Kit©

A 3DMD Paper BioInformatics and Mini-Toober Folding Activity©

Student Handout



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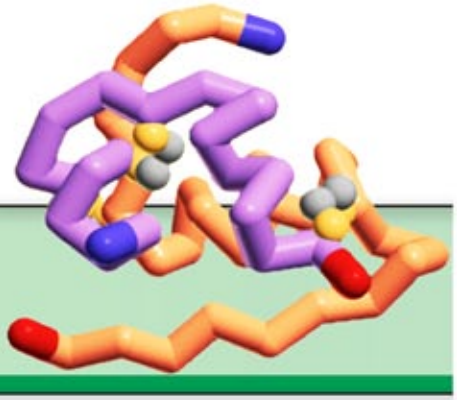


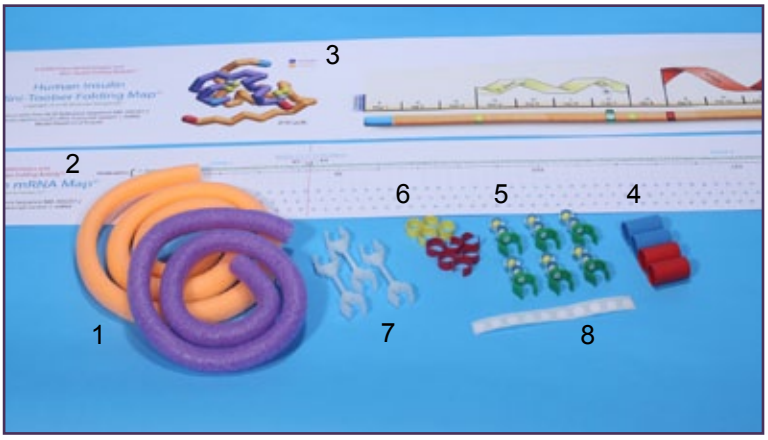
Insulin mRNA to Protein Kit©

Contents

Becoming Familiar with the Data 3
Identifying the A-Chain and the B-Chain of Insulin 5
Preproinsulin: The Precursor Form of Insulin 8
Folding a Physical Model of Insulin 12
Insulin in Review 14

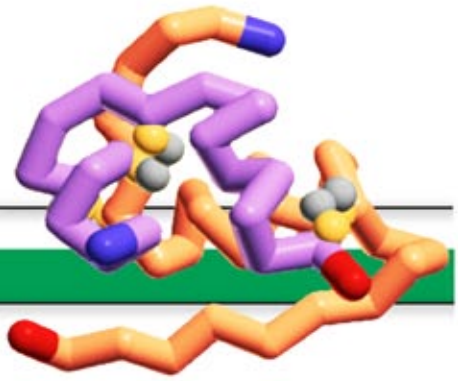
Parts

1. Mini-Toobers (orange and purple)
2. Insulin mRNA Map
3. Insulin Mini-Toober Folding Map
4. Endcaps
5. Cysteine with Plastic Clips
6. Plastic Markers
7. Support Posts
8. White Dots



Why Is Insulin Important?

Insulin is a protein (peptide hormone) that plays a major role in glucose homeostasis – the regulation of your blood sugar levels. After you eat insulin is normally released into your blood, triggering your liver, muscle, and fat cells to take up glucose from your bloodstream. Once inside these cells, the glucose can be used to fuel the production of ATP (adenosine triphosphate). ATP is frequently called the universal molecular currency because it transfers energy in our cells. *See the animation at* 3dmoleculardesigns/Teacher-Resources.htm *for more information on the role insulin plays in regulating blood sugar and the uptake of insulin.*



Insulin Paper BioInformatics Activity

In this activity, you will explore the steps involved in the synthesis of the insulin, starting with insulin mRNA. Specifically, you will consider how this mRNA is translated by the ribosome into a precursor form of insulin, and how the precursor is *processed* to create the final, functional protein. As the final step in this activity, you will create a physical model of insulin by folding two mini toobers (foam-covered wires) into the precise 3-D shape of the A-chain and the B-chain of this protein.

Becoming Familiar with the Data

A gene encoded within the DNA of a chromosome is transcribed into mRNA in the nucleus of a cell. The mRNA is then transported into the cytoplasm*, where a ribosome reads the code and builds a protein (translation). This activity focuses on how the insulin mRNA is translated into the insulin protein.

1. Unroll your Insulin mRNA Map and look at the green-colored sequence of letters at the top of the map.

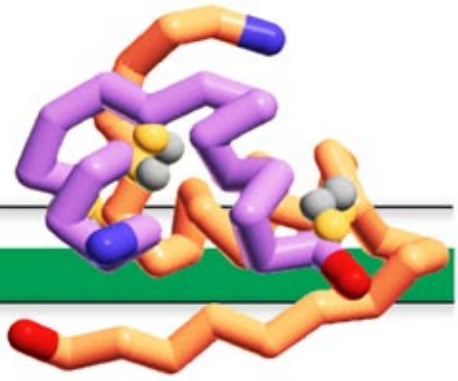
a. What different letters appear in this sequence?

_____

b. What do these letters represent?

_____

_____



The Standard Genetic Code

When RNA polymerase initially transcribes the insulin gene into messenger RNA, two introns – totaling 966 additional nucleotides – are included in the precursor form of the insulin mRNA. These intron sequences are removed from the mRNA in a splicing reaction as the mRNA is being transported out of the nucleus of the cell. You might want to discuss why almost all eukaryotic genes contain introns.

Second Letter

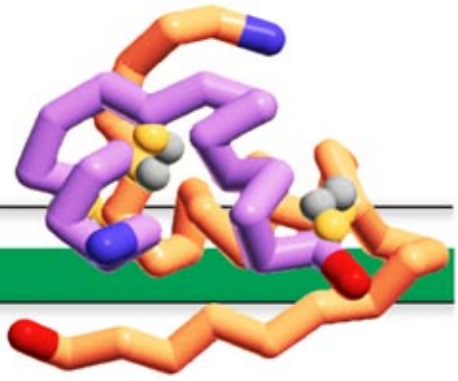
First Letter	U	C	A	G	Third Letter
U	UUU → Phe	UCU → Ser	UAU → Tyr	UGU → Cys	U
	UUC → Phe	UCC → Ser	UAC → Tyr	UGC → Cys	C
	UUA → Leu	UCA → Ser	UAA → Stop	UGA → Stop	A
	UUG → Leu	UCG → Ser	UAG → Stop	UGG → Trp	G
C	CUU → Leu	CCU → Pro	CAU → His	CGU → Arg	U
	CUC → Leu	CCC → Pro	CAC → His	CGC → Arg	C
	CUA → Leu	CCA → Pro	CAA → Gln	CGA → Arg	A
	CUG → Leu	CCG → Pro	CAG → Gln	CGG → Arg	G
A	AUU → Ile	ACU → Thr	AAU → Asn	AGU → Ser	U
	AUC → Ile	ACC → Thr	AAC → Asn	AGC → Ser	C
	AUA → Ile	ACA → Thr	AAA → Lys	AGA → Arg	A
	AUG → Met	ACG → Thr	AAG → Lys	AGG → Arg	G
G	GUU → Val	GCU → Ala	GAU → Asp	GGU → Gly	U
	GUC → Val	GCC → Ala	GAC → Asp	GGC → Gly	C
	GUA → Val	GCA → Ala	GAA → Glu	GGA → Gly	A
	GUG → Val	GCG → Ala	GAG → Glu	GGG → Gly	G

- translation start codon
- translation stop codon
- hydrophobic amino acids
- hydrophilic non-charged amino acids
- - charged amino acids
- + charged amino acids
- cysteine

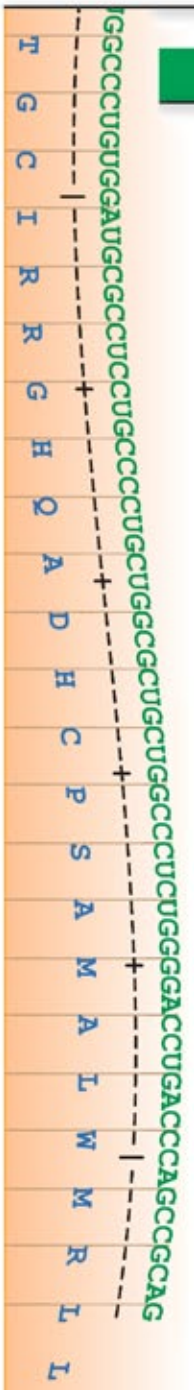
Translation Reading Frames

2. Look at the three **blue sequences** at the bottom of the Insulin mRNA map.

a. What different letters appear in these blue sequences? How many different letters appear in these sequences?



Translation Reading Frames (continued)



b. What do these letters represent?

_____

_____

c. What is the relationship between the green letters at the top of the strip to the blue letters at the bottom?

_____

_____

d. Why are there three blue sequences?

_____

_____

e. What do you think the asterisks (*) represent in the blue sequences?

_____

_____

Identifying the A-Chain and the B-Chain

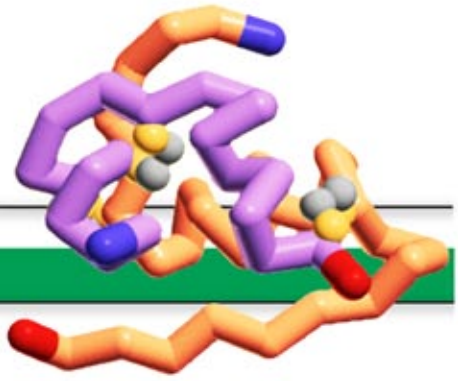
The insulin protein actually consists of two separate chains, known as the **A-chain** and the **B-chain**. The amino acid sequences of the two chains are shown below:

A-Chain

G I V E Q C C T S I C S L Y Q L E N Y C N

B-Chain

F V N Q H L C G S H L V E A L Y L V C G E R G F F Y T P K T



Identifying the A-Chain and the B-Chain (continued)

3. Locate, highlight and label the A-chain and the B-chain amino acid sequences on your Insulin mRNA Map.

   a. What do you notice about the location of the A-chain and B-chain amino acid sequences within the bioInformatics map?

_____

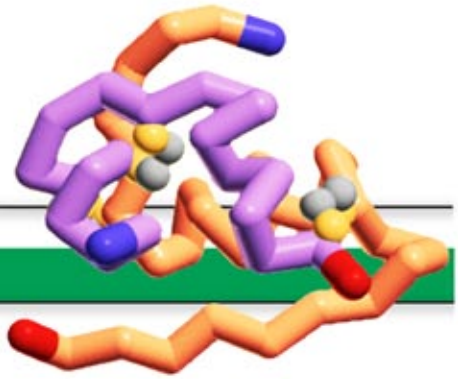
_____

Note: The subunit composition of insulin (two chains) was known before the sequence of the gene was determined. Unfortunately, when the gene was sequenced and the two chains were named, it was discovered that the B-chain was encoded before the A-chain – which has been confusing biology students ever since!

Translating mRNA into Protein

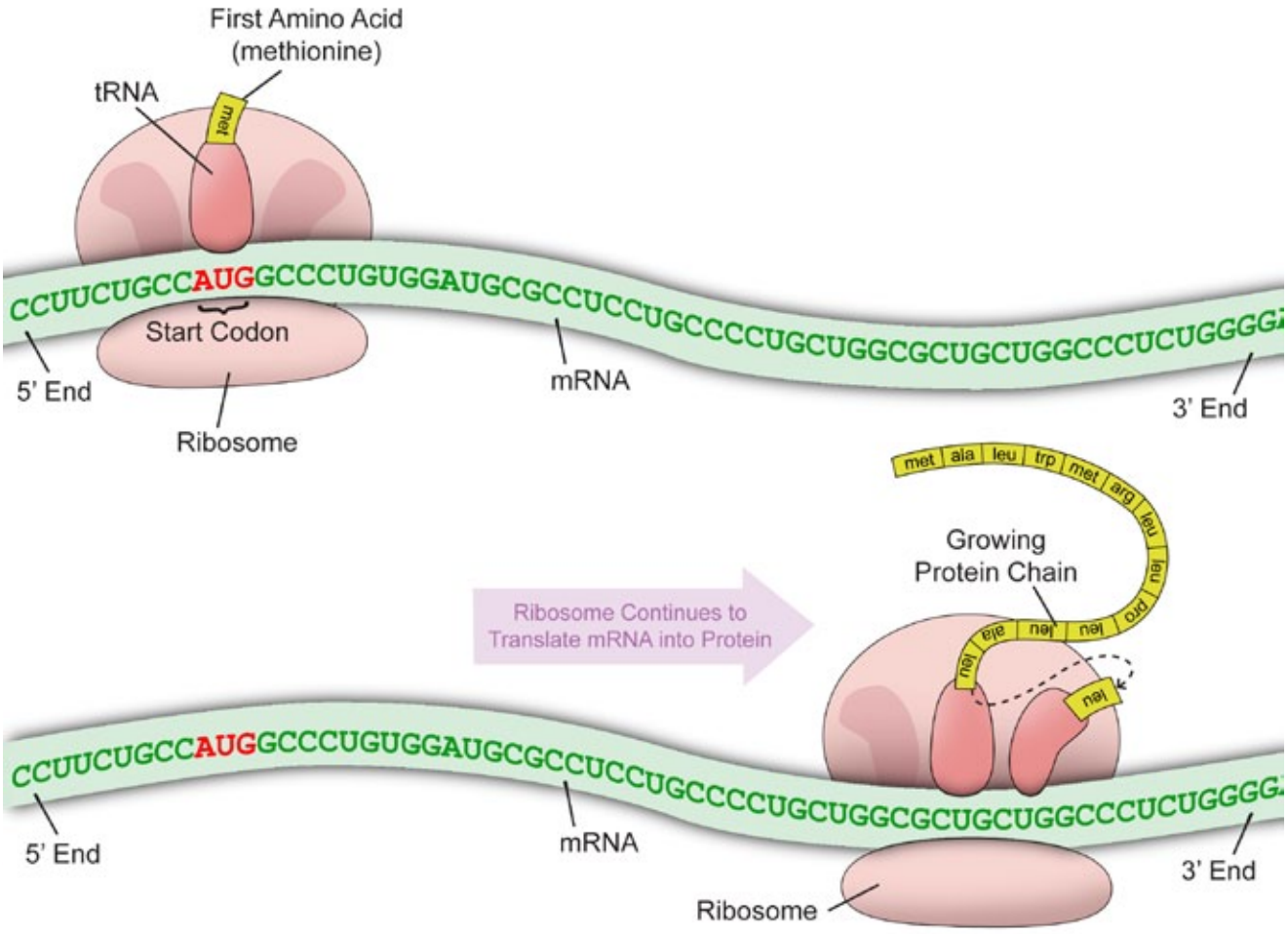
To translate mRNA into protein, the ribosome recognizes an AUG codon – and begins decoding the mRNA as it moves from left to right (5' to 3') down the mRNA sequence. As a result, all proteins begin with the amino acid methionine (Met, M) at their N-terminal end.

In humans and other eukaryotes the ribosome begins synthesizing proteins at the first AUG codon from the 5' end of the mRNA.



Translating mRNA Into Protein

Protein Synthesis of Insulin Protein



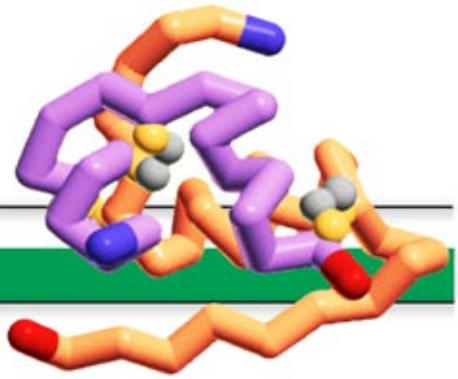
4. Highlight the protein that is synthesized by a ribosome. The ribosome binds to the first AUG located downstream (to the right) of the 5' end of the mRNA to begin synthesis.

a. Where does the protein stop?

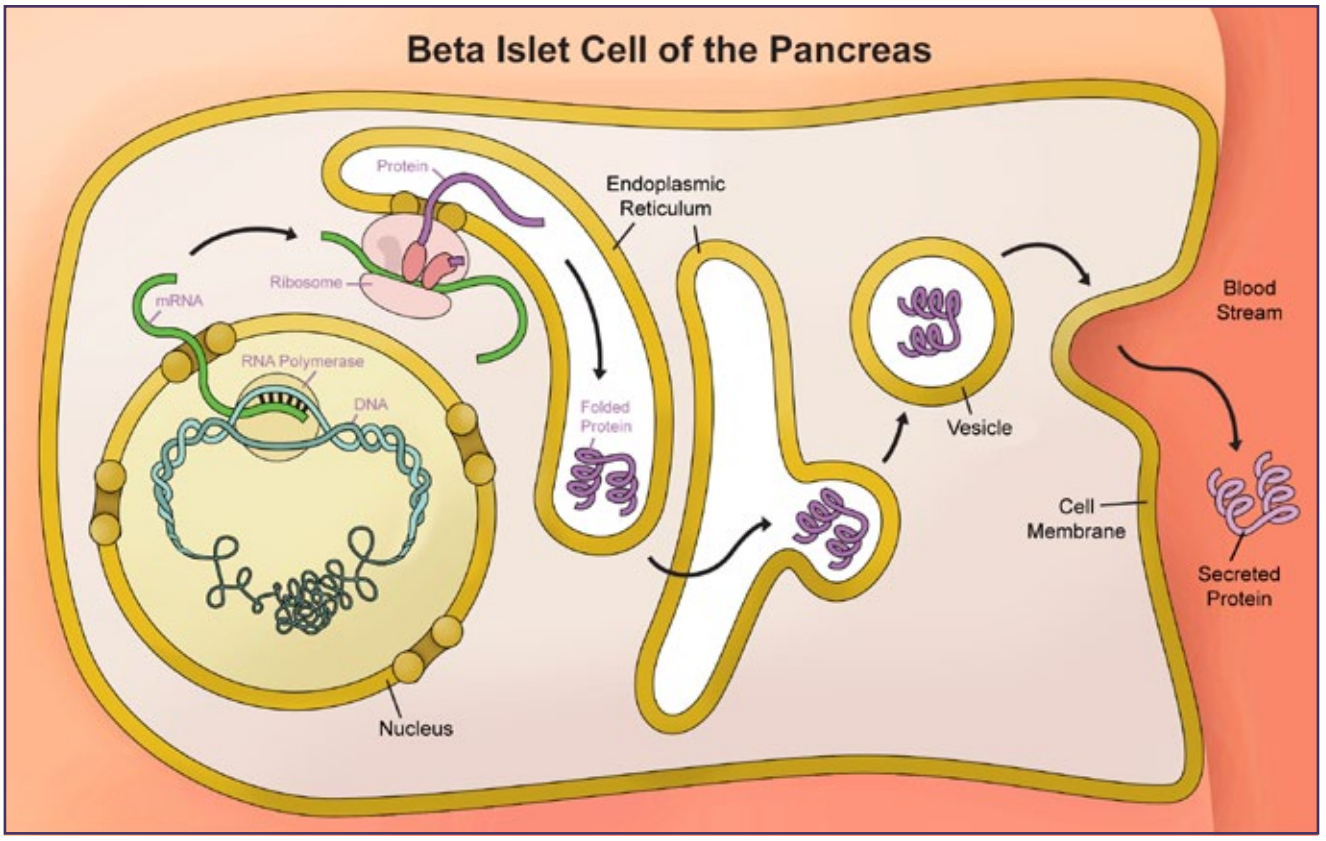
_____

b. How many amino acids are in the insulin protein?

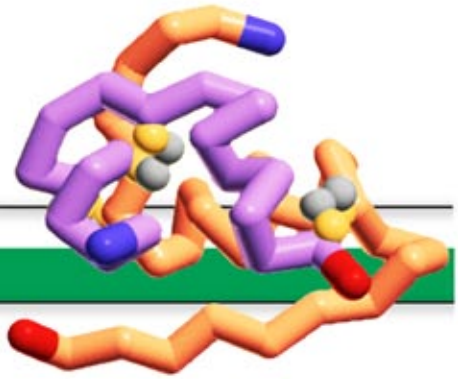
_____



Preproinsulin - the Precursor Form of Insulin



Insulin is synthesized in beta islet cells of the pancreas. Following a meal, it is secreted from these cells into the bloodstream. Proteins that are destined to be released from the cell travel through the endoplasmic reticulum and Golgi apparatus of pancreatic cells to the cell surface where they can be secreted.



Preproinsulin - the Precursor Form of Insulin

Precursor Insulin

The precursor (inactive) form of insulin is known as *preproinsulin*. The first 24 amino acids of preproinsulin make up the **endoplasmic reticulum*** (ER) **signal sequence**. As the protein is being synthesized, this signal sequence begins to emerge from the ribosome. Other proteins in the cell recognize this peptide and dock the ribosome onto the ER. As the rest of the protein is synthesized, it is directed through this membrane, into the lumen of the ER. From there, the preproinsulin is further processed (cleaved into four pieces) as it moves through the ER to the Golgi, and to the cell surface.

5. Locate, highlight and label the ER Signal Sequence on your Insulin Bioinformatics map.

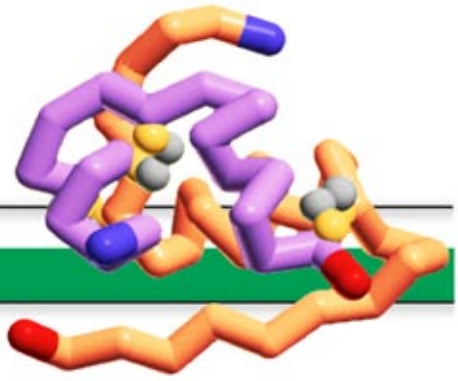
Signal Peptide

M A L W M R L L P L L A L L A L W G P D P A A A

a. Referring to the Standard Genetic Code table, *categorize* the chemical properties of each of the 24 amino acids that make up the ER Signal Peptide (hydrophobic, hydrophilic, positive charge, or negative charge). What is notable about the chemical properties of the amino acids that make up the ER Signal Peptide?

_____

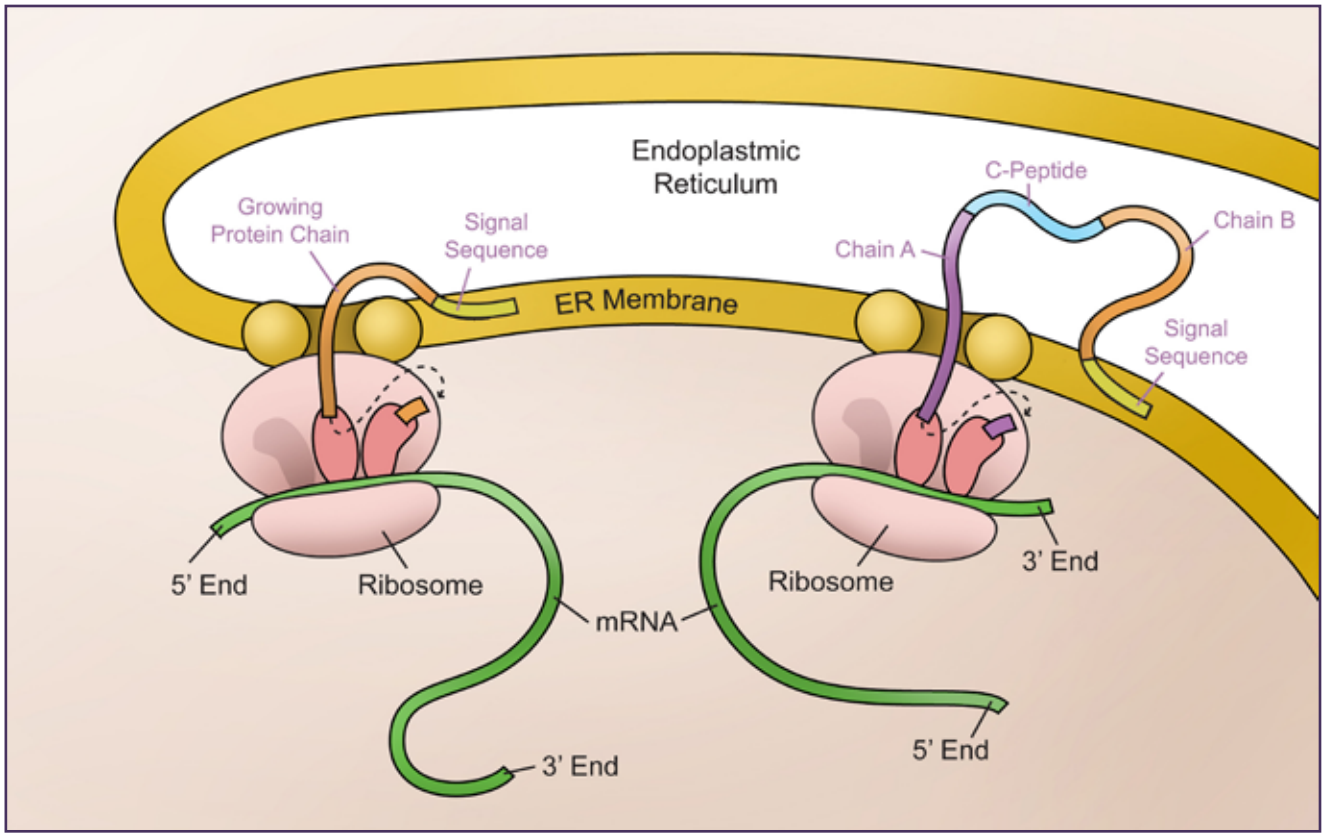
_____



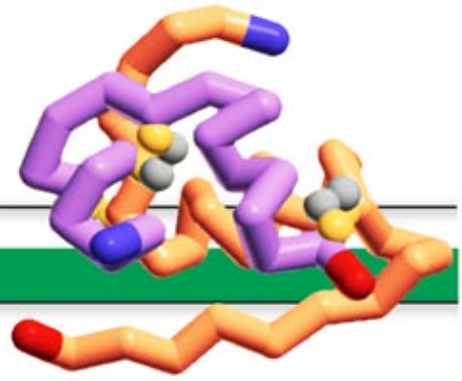
Preproinsulin to Proinsulin

Soon after the ribosome that is synthesizing preproinsulin is docked onto the ER, a protease in the ER cuts the precursor protein between amino acids 24 and 25 (Alanine, Ala, A and Phenylalanine, Phe, F). The 24 amino acid signal peptide is rapidly degraded, while the remaining 86 amino acid proinsulin begins its journey toward the Golgi and cell surface.

Proinsulin consists of the B-chain (30 amino acids) and the A-chain (21 amino acids), separated by the 35 amino-acid C-peptide.



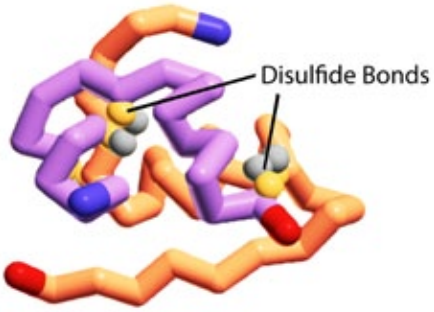
As proinsulin spontaneously folds into its final 3-D shape in the ER, another protease cuts the protein at two sites: between amino acids 54 and 55 (Threonine, Thr, T and Arginine, Arg, R) and between amino acids 89 and 90 (Agrinine, Arg, R and Glycine, Gly, G). As the C-peptide is released from the folded B-chain and A-chain complex, it floats away and is degraded.



Preproinsulin to Proinsulin (continued)

6. Locate, highlight, and label the C-peptide on your Insulin BioInformatics Map.

   a. Since the C-peptide is cut out of proinsulin to create the final mature insulin (B-chain and A-chain) what role do you think the C-peptide might play in the biosynthesis of the mature insulin protein?



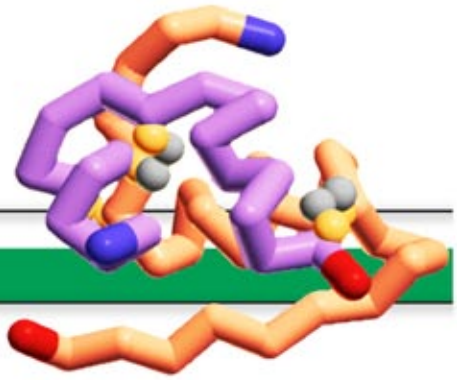
As with many secreted proteins that must function in the harsh environment outside the cell, insulin is stabilized by two covalent disulfide bonds that join the B-chain to the A-chain. Each chain contributes one cysteine amino acid (Cys, C) to each disulfide bond.

Cys7 of the B-chain forms a disulfide bond with Cys7 of the A-chain.

Cys19 of the B-chain forms a disulfide bond with Cys20 of the A-chain.

A third disulfide bond forms between Cys6 and Cys11, both from the A-chain.

7. Circle each Cys on your Insulin mRNA to Protein© map that participates in disulfide bond formation, and connect (with a line) the pairs that interact to form each disulfide bond.



Folding the Physical Model of Insulin

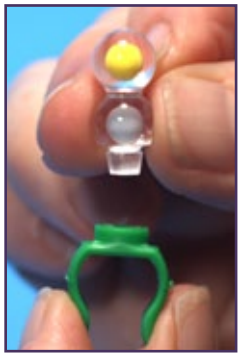
Like all proteins, insulin folds into a specific 3-D shape, following basic principles of chemistry. It is this 3-D shape that allows it to bind to the insulin receptor protein on the surface of liver, muscle, and fat cells to trigger the uptake of glucose from the bloodstream.

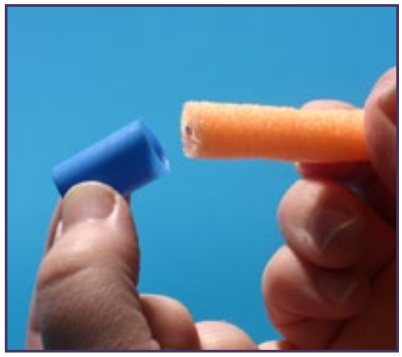
In this final activity, you will shape two mini-toobers into the 3-D shape of the insulin protein.

1. Gather all of the parts you need (see contents photo on page 2).
   - Insulin mini-toober folding map
   - Orange and purple mini toobers
   - Bag with parts for mini toobers
     - Cysteine sidechains and plastic clips
     - Support posts
     - White dots
     - Plastic markers
     - Endcaps

As you proceed with the directions (2) through (6) below you can work with the two chains at the same time or you can complete the B-chain (orange mini toober) and then repeat with the A-chain (purple mini toober).

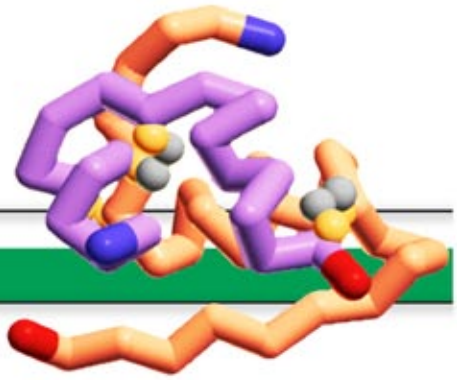
2. Insert each cysteine into a green plastic clip





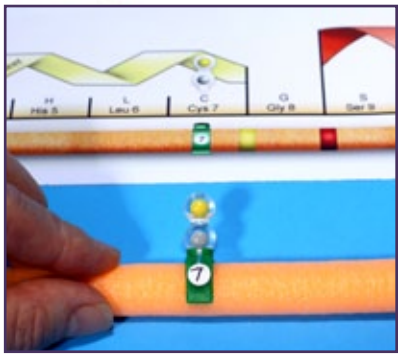
3. Unroll your Insulin Mini Toober Folding Map and identify the **N-terminus (blue)** and the **C-terminus (red)** of each protein chain by putting one red and one blue end cap onto the ends of each mini toober.

4. Using the map, locate the cysteine amino acids on each protein chain. Write the number of each of the six cysteines on the white dots and add these numbered dots to six plastic clips.

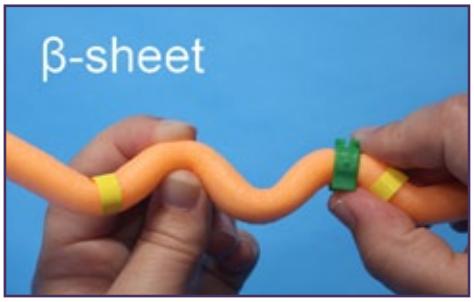


Folding the Physical Model Of Insulin (continued)

5. Carefully align each mini toober with the corresponding chain on the Insulin Mini Toober Folding Map matching the end caps to the images of the end caps on the map. Add the appropriately numbered plastic clips to the mini toober. The plastic clips represent the alpha-carbon of each cysteine amino acid.

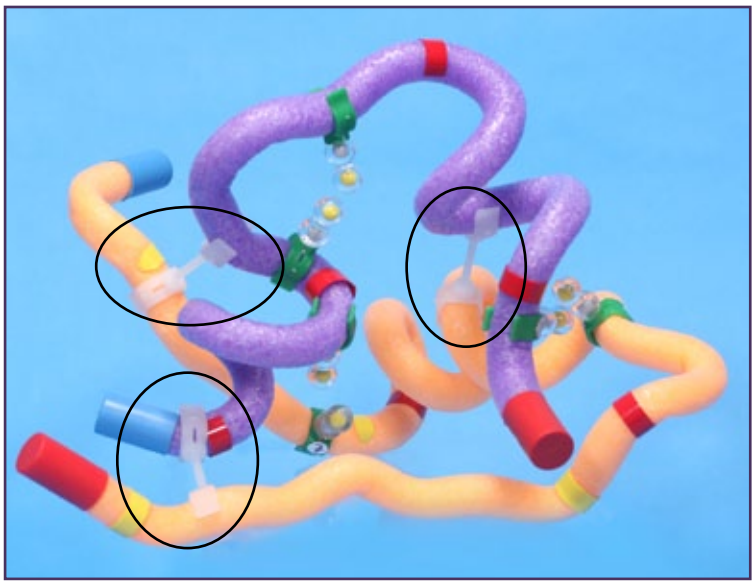


6. Indicate where the α-helicies are on each protein chain by placing the red plastic markers at the beginning and the end of each α-helix. Indicate where the β-sheets are on each protein chain – by placing the yellow plastic markers on the mini-toober at the beginning and the end of each β-sheet shown on the map.



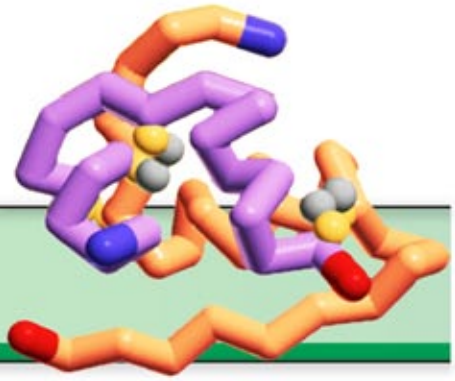
7. Fold the mini toobers to create the α-helicies (right-handed) and the β-sheet strands (extended zig-zag) in each protein chain. See photos above.

   To fold the overall 3-D shape of each protein chain, use the online Jmol visualization tool at 3dmoleculardesigns/Teacher-Resources.htm and/or the images at the end of the map to fold your insulin.



8. Assemble the two chains into the final insulin model by positioning the chains as shown in the photo using the images on the map and/or the Jmol visualization tool.

Hint: The three pairs of cysteine amino acids that form covalent disulfide bonds should be close to each other in the final model. Use the three plastic support posts to stabilize the protein, as shown in the photo.



Insulin In Review

- The insulin gene is located on the short arm of chromosome 11 in humans.
- The insulin gene is transcribed into an insulin mRNA molecule in the nucleus of the beta islet cells of the pancreas.
- *Insulin* mRNA is transported to the cytoplasm of the cell where a ribosome recognizes the first AUG near the 5'-end of the mRNA and begins translating the protein, starting with methionine.
- The ribosome synthesizes a precursor form of insulin, known as preproinsulin.
- Preproinsulin is processed to become mature, functional insulin as it proceeds through the endoplasmic reticulum and Golgi apparatus, moving toward the cell membrane where it can be secreted from the cell.
- When there are high levels of sugar in the blood, insulin is released from the beta cells. It binds to receptors on the surface of liver, muscle, and fat cells. This binding results in a series of reactions within the cell, (called a signal cascade), leading to the fusion of vesicles containing glucose transporter proteins (GLUTS) with the membrane. The GLUTS transport glucose into the cells, where it is stored.