# Briny (Archae)bacteria

## Finding life in an environment full of salt

UF | Center for Precollegiate Education and Training
UNIVERSITY of FLORIDA

# Contributions

Author: William J. Furiosi II
Reviewers: Jessica Furiosi

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Many thanks go out to the Maupin-Furlow Lab of UF's Department of Microbiology & Cell Science with their help in exposing me to a new field of life and allowing me to participate in the molecular biology aspect of their research.

Please direct inquiries regarding UF CPET programs to Julie Bokor at julie@cpet.ufl.edu. For inquiries about the lesson, please contact William Furiosi at william_furiosi@scps.k12.fl.us.

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Cover Images

From "The Unbelievable Pink Lakes Of Las Coloradas In Mexico," by M. Karsten, 2016 (https://expertvagabond.com/las-coloradas-pink-lake/). [Permission pending.]

From "Haloferax volcanii grown in laboratory conditions and imaged using a phase contrast microscope," by Yeijneun, 2013 (https://commons.wikimedia.org/wiki/File:Haloferax_volcanii.png). CC BY-SA 3.0.

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# Author's Note

If the suffix "-ology" means the study of, then why does archaeology involve the study of humanity rather than the study of archaea? Archaea are a phylogenetically distinct prokaryotic domain of life described first by Woese & Fox (1977). They are believed to have first originated on Earth more than 2.5 billion years ago (Gribaldo & Brochier-Armanet, 2006), but it wasn't until 30 years ago where we became alerted to their presence. It seems a bit anthropocentric to name archaeology for humans when archaea have us beaten us to existence on this planet by orders of magnitude.

I graduated in 2013 from the University of Central Florida with a degree that up until the last semester of my senior year was known as Molecular & Microbiology. In my four years of undergraduate coursework, only 23 pages of my textbooks were reserved for archaebacteria despite being one of the main three branches on the tree of life. I am appreciative of my time in the Maupin-Furlow Lab at UF because it gave me a greater glimpse into a domain that I previously knew so little about. It was there I learned about *Haloferax volcanii*, a halophile endemic to the Dead Sea and presumably other marine environments whose salt concentrations approach saturation. I observed the characteristic pink colonies and tinted liquid cultures of *H. volcanii,* which instantly brought me back to my time in Rio Lagartos, Yucatan, Mexico. The waters were a vibrant pink (see cover), just like the cultures. It led me to contemplate whether the pink was due to brine shrimp as I had been told, or if archaea were the culprit. Based on that curiosity and seeing the versatility of archaea in the lab, I decided to make it a focus of my lesson presented here.

*My wife, Jessica, and myself at Rio Lagartos in Winter 2017. Could it be archaea that cause the pinkish hue?*

We care about eukaryotes because they are responsible for what we eat and much of what we see with our naked eye. Bacteria are at the forefront of our minds as we strive to maintain cleanliness and avoid feeling ill. Unfortunately, because archaea aren't omnipresent like bacteria and eukaryotes, they tend to be forgotten. Hopefully this lesson brings some further recognition to other forms of life around us, both large and small, a

better appreciation for a sorely underrepresented domain, and avenues for improving and adapting to an ever-changing world.

# Introduction

What causes the waters adjacent to Rio Lagartos to be pink? Is it because of chemicals added to the water for salt purification? Or could it be the brine shrimp that the flamingos feed upon? Or is there something microscopic lurking past our field of view? At time of publication, there appears no empirical evidence supporting one versus the other. Students will, therefore, design an experiment to identify the organisms in the water.*

> *"Archaea is one of the few remaining organisms on this Earth that significant discoveries can be made at the bench by a high school student."* – Dr. Julie Maupin-Furlow

Just like archaea is relatively new to the scientific community, so is the use of archaea within the classroom. Students will ultimately operate off the hypothesis that the archaeal halophile *Haloferax volcanii* is the culprit. The lessons presented can be carried out in either a sequential, interdisciplinary format or (and likely more effectively) as an overarching theme for an AP Biology course that continuously gets revisited through each discrete unit. *H. volcanii* will lead students on a journey through its unique (and, in some cases, conserved) biochemical requirements, its evolutionary relatedness to the other domains, its ecological impacts, and its industrial potential for human society. Along the way, students will hear about new applications, discuss the significance and relevance of archaea, model proteins, and analyze genuine scientific data.

After engaging in this lesson, students will hopefully experience a similar moment of shock and excitement that Woese had when he first observed archaeal rRNA while working at the University of Illinois at Urbana-Champaign.

Plated cultures of *H. volcanii* H26. Morphologically, the species is reddish-pink due to the high levels of carotenoids in its membrane.

*Because carrying out the experiment can find itself exceeding the scope and available time for a traditional AP Biology curriculum, in the extension portion of this lesson, students may have the opportunity to answer that question.

# Curriculum Tips

## Lesson Plan Format:

All lessons in this curriculum unit are formatted in the same manner. In each lesson, you will find the following components:

Lesson Summary: Overview the content addressed and the student learning outcomes.

Standards: List of specific benchmarks codes addressed in the lesson according to three agencies: Next Generation State Standards (NGSS), Florida's Next Generation Sunshine State Standards (NGSSS), and the College Board's AP Biology Essential Knowledge statements (EK).

Key Question(s): Identifies the questions students should be able to answer following the lesson.

Learning Objectives: Focuses on what students will know, feel, or be able to do at the conclusion of the lesson.

Overall Time Estimate: Estimated time needed to accomplish the lesson in class with advanced preparation considerations addressed separately.

Materials: Items and quantities needed to complete the lesson.

Background Information: Provides accurate, up-to-date information from reliable sources about the lesson topic.

Required Student Background Knowledge: Knowledge students should have or information that should be presented prior to beginning the lesson.

Advanced Preparation: Explains what needs to be done to get ready for the lesson.

Vocabulary: Key words necessary for comprehension and accomplishing learning objectives. Collected together in a glossary at the end of the lesson.

Procedure and Discussion Questions with Time Estimates: The procedure details the steps of implementation with suggested time estimates. The times will likely vary depending on the class.

Assessment Suggestions: Formative assessment suggestions have been given. Teachers should feel free to create additional formative and summative assessment pieces as necessary

Modifications or Extensions: There are many activities and reading sources available to augment and enhance the curriculum, especially those endorsed by or aligned to the AP Biology curriculum.

Resources / References: Complete citations of references and resources used to design the lesson are included in APA style. Additional resources, most often in the form of YouTube videos, are provided with embedded links.

Teacher Masters: Versions of the student pages with answers or the activity materials for preparation.

# Lesson Summaries

## Lesson One: Pretty and Pink

Why are the waters of Rio Lagartos pink? Students will design an experiment to answer the question, learn about archaea and chemical functional groups through lecture, read a scientific magazine article about the wonders of Archaea, and will compare skeletal structures between the three domains of life.

## Lesson Two: Keeping Active

What is it about active sites of enzymes that allow them to catalyze chemical reactions? Students will be shown how research into enzymatic function is important for various industries, especially health. Students will explore the relationship of structure and function through a hands-on manipulative activity of substrates and active sites. They will deepen their knowledge by investigating the chemical interactions of amino acid side chains with substrates. Lastly, they will connect their understanding of structure to function by analyzing how enzyme activity is quantified and affected by changes to an enzyme's environment.

## Lesson Three: Finding Similarities

Some gold standards for measuring evolutionary relatedness is looking at nucleotide and amino acid sequence alignments. This lesson reveals another method of showing evolutionary relatedness – protein structure alignment. Students will BLAST nucleotide and amino acid sequences for the inorganic pyrophosphatase (PPA) of five species: *H. volcanii*, *P. furiosus*, *E. coli*, *S. cerevisiae*, and *H. sapiens*. After measuring evolutionary relatedness, they will spend an additional day modeling 3D protein structures of PPA in each species and level of similarity between each.

SUBJECT: Advanced Placement (AP) Biology, Biotechnology, Genetics

GRADE LEVEL/ABILITY: HS 9-12, Honors/AP

# Lesson Sequencing Guide

Two lesson sequences are presented for this unit:

1. Continuous: Carrying out each lesson continuously in sequence would be beneficial by providing students with a primer for evolutionary biology and ecology if using the "Small-to-Big" method or for an ecological review and evolutionary primer in the big-small-big method.
    a. "Small-to-Big" method involves starting with biochemistry and proceeding to ecology.
    b. "Big-small-big" method involves starting with ecology, proceeding to biochemistry, before building up again to macrobiology.
2. Thematically Discontinuous: Carrying out each lesson as an extension for each content area would be beneficial as the lessons each are complex and analytical in nature.
    a. This method is likely the most effective implementation of this lesson, using archaea as a theme for major units, such as ecology, evolution, and biochemistry independently.
    b. Students would require background knowledge in most instances before being able to adequately perform the lessons, so using them as thematic capstones would provide students with the best opportunity for learning.

Biochemistry

| Day 1 | Day 2 |
|---|---|
| Lesson 1<br>*Pretty & Pink*<br><br>Introducing the archaeal domain and comparing biochemical structures of membranes | Lesson 2<br>*Keeping Active*<br><br>Exploring the relationship of structure and function of enzymes with an evolutionarily conserved protein |

Evolution

| Day 3 | Day 4 |
|---|---|
| Lesson 3<br>*Finding Similarities*<br>Sequence Homology<br><br>Comparing the evolutionary relatedness using DNA and amino acid sequences of PPA | Lesson 3 (cont.)<br>*Finding Similarities*<br>3D Structure<br><br>Comparing evolutionary relatedness of proteins at the 3D structural level of PPA |

| | | | |
|---|---|---|---|
| 50 minutes | 50 minutes | 50 minutes | 50 minutes |

## Future Considerations and Extensions

Later in this lesson plan, *Haloferax volcanii* will be presented as a model archaeon for scientific research. Similarly, it can serve as a model organism for an entire year of Advanced Placement Biology. The potential for *H. volcanii* to be used thematically in the course has not escaped my notice, and some ideas for development and extension are proposed below.

### Future Considerations

Biochemistry

| Lesson 1 |
|---|
| *Pumping Ion*<br><br>Analyzing how archaeal halophiles manage high salt environments compared to other species.<br><br>50 minutes |

Ecology

| Lesson 1 |
|---|
| *So Salty*<br><br>Relating the role of archaea in nutrient cycles and connecting cycle disturbances with climate change.<br><br>50 minutes |

## Extensions

Biotechnology

| Lesson 1 | Lesson 2 | Lesson 3 | Lesson 4 |
|---|---|---|---|
| *Microscopy & Culturing* | *Identification* | *UV Mutagenesis* | *Gene Knockouts* |
| Students will attempt to culture *H. volcanii* from an unknown sample and via it under a microscope. | Using archaeal primers, utilize PCR and electrophoresis to identify archaeal species in the sample. | Identify growth phases using spectrometry and perform UV mutagenesis assay on known cultures. | Using transposon mutagenesis, auxotroph mutants, and other selectable markers, attempt gene identification. |
| Multi-day | Multi-day | 50 minutes | Multi-day |

# Vocabulary

Critical terms for content understanding (A-P)

Acidic: Side chains that contain carboxylic acid functional groups that are protonated and can donate protons at neutral pH (in other words, they have a low pKa). They are extremely hydrophilic. Side chains that are acidic include glutamic acid and aspartic acid.

Active site: Location on an enzyme where catalysis of chemical reactions occurs and is mediated by constituent amino acid residues (most often their side chains) within the active site.

Activity: A measure of an enzymes ability to catalyze reactions.

Amino acid: Monomeric building block of proteins consisting of an N-terminal amine group, a C-terminal carboxylic acid group, and a side chain that carries its own unique chemical properties.

Archaea: One of the three domains of life discovered in 1977 by Carl Woese & George Fox that differs from bacteria and eukaryotes predominantly because of its ribosomal RNA (rRNA). Other differences include membrane structure, gene structure, and metabolic pathways. They are also known as archaeabacteria or archaeon when referring to a single cell.

BLAST: Basic Local Alignment Search Tool, a tool using computer algorithms to regions of similarity among nucleotide or amino acid sequences.

Cladogram: Graphical representation of evolutionary relatedness among species.

Domain: Highest taxonomic division distinguished by similarities in rRNA including archaea, eukarya, and bacteria as the three major branches on the tree of life.

Enzyme: A biological macromolecule that speeds of chemical reactions, consisting primarily of proteins, but also inclusive of RNA molecules.

Functional group: Subunit of a molecule that has distinct chemical properties.

Homology: Measure of the relatedness or similarity between two items, such as nucleotide sequences, physical structures, proteins, amino acid sequences, etc.

Hydrophilic: Water-loving chemical functional groups because of the polar nature of their bonds and/or their ability to form hydrogen bonds with water molecules. Side chains of amino acids that are hydrophilic include serine, threonine, asparagine, and glutamine.

PDB: Protein Data Bank, the repository by the National Center for Biotechnology Information, with structures and information of known proteins.

# Vocabulary

Critical terms for content understanding (P-Z)

Phylogenetic tree: Graphical representation of evolutionary relatedness among species, similar to a cladogram, but contains more information about the degree of change and time needed for the change to occur.

pI: The isoelectric point of a protein at which a molecule carries no net electrical charge and is based on the chemical nature of functional groups on amino acid side chains and related modifications. Important in determining the functionality of proteins in specific aqueous environments.

Protein: Macromolecule responsible for numerous functions in living organisms, including enzymes and providing structure, that are comprised of amino acid monomers.

Pyrophosphatase: An enzyme responsible for catalyzing the breakdown of pyrophosphate ($PP_i$) into two inorganic phosphate ions. The reaction releases a significant amount of energy (-19 kJ/mol free energy change). Because it is often coupled with endergonic, or energy-requiring, reactions like DNA synthesis, it is highly conserved.

RMSD: Root mean square difference is a statistical function that calculates the distances of amino acid residues between two superimposed structures. The function provides a numerical output that is interpreted as the confidence in structural alignment. The lower the value, the more aligned the two structures are. Values <2.000 indicate strong alignment.

Side chains: The functional group attached to the alpha-carbon of an amino acid that bestows the amino acid's chemical properties. Denoted by the letter "R" in amino acid diagrams.

Tree of life: Model illustrating the relationship of organisms beginning with the origin of life Earth and branching out from the last universal common ancestor.

# Standards

## AP Biology Curriculum Framework: Essential Knowledge Statements

| Standard | Lesson | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| E.K.1.B.1<br>Organisms share many conserved core processes and features that evolved and are widely distributed among organisms today. | | ✓ | | |
| E.K.1.B.2<br>Phylogenetic trees and cladograms are graphical representations (models) of evolutionary history that can be tested. | | | ✓ | ✓ |
| E.K.2.B.1<br>Cell membranes are selectively permeable due to their structure. | ✓ | | | |
| E.K.4.A.1<br>The subcomponents of biological molecules and their sequence determine the properties of that molecule. | ✓ | ✓ | | |
| E.K.4.B.1<br>Interactions between molecules affect their structure and function. | ✓ | ✓ | | |

## AP Biology Curriculum Framework: Science Practices

| Standard | Lesson | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| Science Practice 1<br>The student can use representations and models to communicate scientific phenomena and solve scientific problems. | ✓ | ✓ | ✓ | ✓ |
| Science Practice 3<br>The student can engage in scientific questioning to extend thinking or to guide investigations within the context of the AP course. | ✓ | | | |

# Standards

## Next Generation State Standards

| Standard | Lesson | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| HS-LS1-1 From Molecules to Organisms: Structures and Processes 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 From Molecules to Organisms: Structures and Processes Develop and use a model to illustrate the hierarchical organization of interacting systems that provide specific functions within multicellular organisms. | ✓ | ✓ | | |
| HS-LS1-6 From Molecules to Organisms: Structures and Processes Construct and revise an explanation based on evidence for how carbon, hydrogen, and oxygen from sugar molecules may combine with other elements to form amino acids and/or other large carbon-based molecules. | ✓ | ✓ | | |
| HS-LS4-1 Biological Evolution: Unity and Diversity Communicate scientific information that common ancestry and biological evolution are supported by multiple lines of empirical evidence. | | | ✓ | ✓ |

Next Generation Sunshine State Standards

| Standard | Lesson | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| SC.912.N.1.1<br>Define a problem based on a specific body of knowledge, for example: biology, chemistry, physics, and earth/space science, and do the following… | ✓ | | | |
| SC.912.N.3.5<br>Describe the function of models in science, and identify the wide range of models used in science. | | ✓ | ✓ | ✓ |
| SC.912.L.14.3<br>Compare and contrast the general structures of plant and animal cells. Compare and contrast the general structures of prokaryotic and eukaryotic cells. | ✓ | | | |
| SC.912.L.15.4<br>Describe how and why organisms are hierarchically classified and based on evolutionary relationships. | | | ✓ | ✓ |
| SC.912.L.15.6<br>Discuss distinguishing characteristics of the domains and kingdoms of living organisms. | ✓ | | ✓ | ✓ |
| SC.912.L.16.10<br>Evaluate the impact of biotechnology on the individual, society and the environment, including medical and ethical issues. | | ✓ | | |
| SC.912.L.18.1<br>Describe the basic molecular structures and primary functions of the four major categories of biological macromolecules. | ✓ | | | |
| SC.912.L.18.4<br>Describe the structures of proteins and amino acids. Explain the functions of proteins in living organisms. Identify some reactions that amino acids undergo. Relate the structure and function of enzymes. | | ✓ | ✓ | ✓ |
| SC.912.L.18.11<br>Explain the role of enzymes as catalysts that lower the activation energy of biochemical reactions. Identify factors, such as pH and temperature, and their effect on enzyme activity. | | ✓ | | |

# Background

Life is described according to two general distinctions: those without nuclei (prokaryotes) and those with nucleic (eukaryotes). Up until 1977, it was believed that prokaryotes consisted only of bacteria. Carl Woese revolutionized our understanding of taxonomy when a colleague provided him with a microorganism that was unlike any Woese had sequenced previously. Using ribosomal RNA as a basis of evolutionary relatedness, he subdivided prokaryotes into eubacteria (true bacteria) and archaeabacteria. Now, rather than kingdoms being the highest taxonomic level, it has since been replaced with three domains: archaea, bacteria, and eukarya.

Archaea are believed to be evolutionary relics of life on Earth. Structurally speaking, their ribosomal RNA shares greater similarities to eukaryotes than bacteria. Archaeal RNA polymerases also follow the same trend. Archaea have an unusual lipid structure composed of isoprenoid polymers rather than fatty acids and are attached via ether bonds (instead of esters) to phosphoglycerol. Some archaeal species have monolayer membranes, while others have bilayers. Metabolically, many prefer conditions resembling early Earth and are found in some of the most extreme environments; however, more research is showing that archaea are more abundant worldwide than previously thought. Up until the last thirty years, an entire domain of life was outside the purview of scientists because of their unique habitats and lack of pathogenesis. Consequently, archaea are believed to have had little pressure to change from their original niches, which is one explanation for why there are no known pathogenic archaeal species (Schaechter, Ingraham, & Neidhart, 2006).

The best studied phyla of archaea are Crenarchaeota (thermoacidophiles) and Euryarchaeota (halophiles and methanogens). There are numerous other phyla presented, including Korarchaeota, Nanoarchaeota, and the Asgardian archaeaota (Lokiarchaeota, Thorarchaeota, Odinarchaeota, and Heimdallarchaeota). The other phyla are less-well studied due to their extremophilic nature and the subsequent difficulty in culturing them. Below are some of the qualifications to be extremophiles, adapted from Schaechter, Ingraham, & Neidhart (2006) and Oren (2002).

| Class | Typically grows at: |
|---|---|
| Thermophile | >50°C |
| Extreme thermophile | >70°C |
| Acidophile | pH<3.0 |
| Halophile | >100 g/L salt |
| Barophile | >40MPa |
| Psychrophile | 5°C |

The archaea utilized as a model in these lessons, *Haloferax volcanii*, is a halophile. According to Oren (2002), halophiles exist in all three domains. Many halophiles are also photosynthetic even in the highest of salt concentrations, especially bacteria and eukaryotes. One genus of eukaryotes is a green alga called *Dunaliella*. *D. salina and D. bardawil* diverge from the typically green appearance because both contain large amounts of beta carotene, which give the characteristic red colors found in certain salterns. There is also a species of fungus that prefers growing in high salt environments, and despite occasionally being a contaminant in halophile cultures, it has not been well-studied.

*Haloferax volcanii* is facultative aerobe widely considered to be a model archaeon because of its mesophilic temperature preferences (42°C), ability to be cultured, nonpathogenic nature, and resistance to contamination because of its halophilic requirements (21% NaCl). The organism was discovered in 1940 by Bernard Volcani during his studies of the Dead Sea, but was first presented as a species by Mullakhanbhai & Larsen in 1975. The requirements for growth are minimal, many of which are even found on grocery store shelves: NaCl (table salt), $MgSO_4 \cdot 7H_2O$ (Epsom salt), $MgCl_2 \cdot 6H_2O$, KCl (Nu-Salt), $CaCl_2 \cdot 2H_2O$ (antacid), agar, yeast extract, and tryptone (Kouassi, Waldron, Tripepi, & Pohlschroder, 2017). It grows optimally in salt concentrations greater than 2M NaCl at 45°C with a doubling time every 4 hours. In liquid cultures, *H. volcanii* appears a pale, yellowish-pink, and on solid media, it bears the color as observed in the Introduction of this lesson. It respires preferentially on glycerol, especially the remains of *Dunaliella* after seasonal increase in salt concentration causes the eukaryotes to lyse. It preferentially grows with oxygen as a terminal electron acceptor, but in anoxic conditions, it can act as a denitrifier. In terms of its genome, it the DS2 (Dead Sea 2) strain was fully sequenced in 2010 (Hartman et al., 2010). Four years later, a comprehensive transposon insertion mutant library was created in order to aid gene discovery (Kiljunen et al., 2014). Extensions to this lesson are quite evident due to the growing understanding of *H. volcanii*Unlike many halophiles who use a salt-out strategy, where they pump salt out of their cells and maintain osmostasis through the production of organic solutes (like glycerol in the case of *D. salina*), *H. volcanii* maintains osmostasis by pumping KCl into the cell balance the high salt concentration in the external environment. These mechanisms are addressed in a lesson regarding osmolarity.

Another mechanism addressed as a lesson is the research completed by the Maupin-Furlow Lab on inorganic pyrophosphatase in *H. volcanii* (HvPPA). PPA is an enzyme found abundantly in living organisms and assumes the role of hydrolyzing inorganic pyrophosphate ($PP_i$) to two inorganic phosphate ions ($P_i$). While ATP can be broken down into ADP, other reactions occur where ATP is broken down directly into AMP with $PP_i$ as an additional product. Hydrolysis of pyrophosphate is highly exergonic (energy-releasing) and is therefore coupled with other endergonic reactions (energy requiring) such as DNA synthesis or the formation of other macromolecules. Pyrophosphate can be concern in biotechnology as a contaminant and its breakdown can lead

to the supply of energy for unintended side reactions. Furthermore, according to Heikinheimo et al. (1996), high levels of inorganic pyrophosphate can be toxic to the cell, thus emphasizing life's universal need for it.

# Lesson 1: Pretty and Pink

Introduction to Archaea

## LESSON SUMMARY:

Why are the waters of Rio Lagartos pink? Students will design an experiment to answer the question, learn about archaea and chemical functional groups through lecture, read a scientific magazine article about the wonders of Archaea, and will compare skeletal structures between the three domains of life.

STANDARDS:

| AP Biology | NGSS | NGSSS |
|---|---|---|
| E.K.2.B.1 | HS-LS1-2 | SC.912.N.1.1 |
| E.K.4.A.1 | HS-LS1-6 | SC.912.L.14.3 |
| E.K.4.B.1 | | SC.912.L.15.6 |
| Science Practice 1 | | SC.912.L.18.1 |
| Science Practice 3 | | |

KEY QUESTION(S):

What are archaea?

How do they relate to bacteria and eukaryotes?

LEARNING OBJECTIVES:

The student will be able to...

1.Define and identify functional groups in biochemical molecules.

2. Describe archaea.

3. Explain the similarities and differences between archaea, bacteria, and eukarya.

OVERALL TIME ESTIMATE:

Advanced Preparation: 20 minutes to read and assemble *Scientific American* articles, laminate the membrane molecules, and print Experimental Design Graphic Organizer and Membrane Comparison Activity sheets.

Lesson: 50 minutes

MATERIALS:

*Essential*

Experimental Design Graphic Organizer (1 per group of 4)

Membrane Comparison Activity (1 sheet and 1 set of 9 molecules per group of 4)
*Scientific American* article titled, “Archaea are more wonderful than you know” (1 for each student; electronic access would save paper)
7 colored vis-à-vis markers (blue, red, green, yellow, purple, orange, black) (1 set per group of 4)

## BACKGROUND INFORMATION:

Ria Lagartos Biosphere Reserve, or Rio Lagartos, is a UNESCO biosphere reserve in Yucatan, Mexico. Translated in English to “lizard river,” it was named by Spanish conquistador Francisco Hernandez because of the numerous crocodiles he saw during an expedition. As a nature reserve, it’s known for its unique biodiversity, but it’s also known for the unique coloration of its waters. Las Coloradas are lakes within the biosphere that are strikingly pink. Explanations about its coloration range from the presence of brine shrimp (and the pink flamingos frequenting there), chemicals in the water for purification of the salts, or, as hypothesized for this lesson, archaebacteria.

In 1977, Carl Woese was approached by a fellow researcher with a microorganism that he wanted Woese to identify. Using a new sequencing method he developed for ribosomal RNA, he noticed that it was unlike any bacteria he had seen before – thus, the three-domain system was born. Archaea are distinct from other prokaryotes and eukaryotes because of its rRNA sequence; it actually is more closely related in structure to eukaryotes than bacteria. Furthermore, their membranes have unique structures made of ether linkages between lipids and glycerol molecules rather than ester linkages found in the other domains. The membranes can be bilayers or in some cases monolayers, with opposite layers chemically bonded together. The nature of these linkages is believed to be less reactive than ester-linkages and also better adapted to extreme environments (so their cytosolic contents don’t leak out). The hydrocarbon chains also aren’t simple, linear chains. Their subunits are actually isoprene (  ) polymers. Speaking of extreme environments, likely the biggest reason archaea haven’t been discovered earlier is because of their presence in environments humans typically cannot tolerate, such as anaerobic regions, deep sea thermal vents, or acidic bodies of water; however, in recent years, archaea are found to be more ubiquitous in moderate locals than previously thought. Additionally, because no archaea have been known to cause disease, they have managed to stay out of the human eye for much longer than expected.

Functional groups are the subunits responsible for chemical reactivity in a molecule. The AP Biology Curriculum Framework includes a learning objective specifically mentioning the use of models to predict and justify how changes in structure affect the function (Learning Objective 4.3). While specific molecular structures of macromolecules are beyond the scope of the curriculum, teachers would be remiss not to facilitate recognition of important molecular components and connect it to function. Furthermore, understanding membrane composition is key to understanding their semipermeable nature and how they help to suit specific organisms to their environments (especially in the case of archaea).

## REQUIRED STUDENT BACKGROUND KNOWLEDGE:

Students should have previously covered the structural and chemical differences of the four main classes of macromolecules: lipids, carbohydrates, proteins, and nucleic acids. This lesson serves as an extension to that concept by justifying how macromolecules are used to distinguish between different forms of life.

Students should also have prior knowledge of the components to experimental design: hypothesis, variables (independent, dependent, and controlled), control versus experimental groups, and expectations on data collection.

Background knowledge on cell structure, specifically members are beneficial. Most students in an AP Biology classroom should have had prior background experience that cellular membranes are phospholipids (phosphate head connected to a lipid), but a refresher on this fact might be helpful.

## ADVANCED PREPARATION:

For reusability, it would be helpful to laminate the membrane molecules ahead of time. Students could then annotate the laminations with vis-à-vis markers, and wash it off when finished. Otherwise, printing separate sets each time would allow the students to write on them and keep them for future review. Place the sets equally throughout the classroom (preferably on lab benches) with 7 vis-à-vis marker colors provided: blue, red, orange, purple, green, yellow, and black.

Print copies for the Experimental Design Graphic Organizer and Membrane Comparison Activity (1 per group of 4).

The *Scientific American* article can be given as a handout or digitally. If given as a handout, 1 copy per student is needed; otherwise, provide a link or a PDF version of the article students if providing digitally. Also, make sure the article link is still valid and accessible via the medium you wish to use. The accompanying questions can be assembled into a handout, displayed via projector, or written on the whiteboard. If choosing either of the latter, have students take out a sheet of paper to jot their answers down in order to facilitate discussion.

## VOCABULARY:

Archaea: One of the three domains of life discovered in 1977 by Carl Woese & George Fox that differs from bacteria and eukaryotes predominantly because of its ribosomal RNA (rRNA). Other differences include membrane structure, gene structure, andmetabolic pathways. They are also known as archaeabacteria or archaeon when referring to a single cell.

Tree of life: Model illustrating the relationship of organisms beginning with the origin of life Earth and branching out from the last universal common ancestor.

Domain: Highest taxonomic division distinguished by similarities in rRNA including archaea, eukarya, and bacteria as the three major branches on the tree of life.

Functional group: Subunit of a molecule that has distinct chemical properties.

## PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES:

*Why Is It Pink?* (15 – 20 minutes)

1. Hook the students by displaying the slide with the pictures of Rio Lagartos from the Expert Vagabond blog and/or personal images. If the teacher has been to any high saline lakes, such as Great Salt Lake, the Dead Sea, or others, providing personal experience will greatly help increase engagement.
2. As the images are shown, ask the students, "Why is it pink?" Elicit feedback and possible mechanisms about what could cause the coloration. List them on a whiteboard.
3. Arrange the students in groups of 4 and instruct them to spend 10 minutes designing an experiment to identify what causes the colors of the lake.
    a. Students will want to establish a hypothesis before attempting to create methods, and they will want to clearly identify variables and experimental groups.
    b. Each group should use the Experimental Design Graphic Organizer to help organize their plan.
    c. Move around the room to field questions as necessary and/or to give further details needed to refine their study.
    d. While walking around the room, formatively assess the groups' progress. If unable to get to all groups, collect the graphic organizers for later assessment.

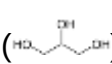
*What Are Archaea?* (10 – 15 minutes) [POTENTIAL MODIFICATION]

1. Transition the students to reading the *Scientific American* article titled, "Archaea are more wonderful than you know."
    a. Students may stay in their groups to facilitate the next activity and intragroup discussion, or they can return to their seats to read independently.
    b. If using handouts, distribute one to each student and encourage them to mark it up as they read.
    c. If using digital versions, ensure the students have access to the link and their devices are functioning prior to the start of this segment.
2. Provide the students with the questions ahead of time, either through a handout or via overhead display. Allow the students to read the article, then discuss the answers to the questions below.

- Why is it difficult to distinguish bacteria species from animal species?
- When did Carl Woese discover the new domain, archaea?
- What ultimately distinguishes the three domains of life?
- How are archaea similar to "Us"?
- How are archaea dissimilar to bacteria?
- Identify 1-3 words you're unsure about their meaning.
- What's one concept that stood out to you from the article?
- Make a prediction about archaea that we will eventually discover in the future and justify your claim.

3. MODIFICATION: If time is a concern, have the students read the article ahead of time and prepare their answers for discussion during class. This component may also be used as an online discussion, but would likely be best suited for in-class discussion prior to the start of the Membrane Comparison Activity.

*Membrane Comparison Activity* (15 – 20 minutes)

1. Arrange the students again into groups of four if they were working independently. Direct them to tables or benches where the laminated cutouts and markers are already placed for the Membrane Comparison Activity.
2. Students will do the following with the cutouts:
    a. Circle functional groups with specific colors according to the handout.
    b. Identify intermolecular force potential (FON elements can hydrogen bond, polar molecules form dipole interactions, and C-H structures [and all others] can form dispersion forces]
    c. Identify which type of molecules can pass through the portion of the structure: hydrophobic or hydrophilic.
3. Once they finish the annotation segment, students will then want to identify which molecule belongs to which domain based on their readings and prior knowledge of cellular membranes of eukaryotes and bacteria.
    a. Students should provide a brief set of reasoning, such as "ester bonds = eukaryotes/bacteria" to justify their position.
4. The remaining time should be spent answering the extension questions. Encourage group discussion and collaboration. If there is not enough time remaining, have students complete this for homework. Because it is a group assignment, encourage students to take pictures with their phones if they need to complete it for homework.
    a. Tip: Encourage students to utilize prefixes and suffixes to help them understand structures. For example, phospholipids should have phosphates and lipids in them. Glycerol (  ) is why the lipid test you have at the doctor's is for triglycerides (three fatty-acid chains on one glycerol). Cholesterols are found only in the eukaryotic domain, and lipopolysaccharides (lipids attached to multiple sugars) are the endotoxic component of gram-negative bacteria. Students will certainly be exposed to vocabulary on the AP exam that they will have never heard before, so encouraging them to practice morphemic analysis will greatly help their comprehension later in the year.

## ASSESSMENT SUGGESTIONS:

*Experimental Design*

Formatively assess students' experimental designs by walking around the room offering critiques. If unable to view each group or need more time for more detailed observation, collect their designs. Verbalize and/or annotate possible revisions as experimental design is a key understanding requirement for any science course.

*Membrane Comparison Activity*

Collect the completed handout and compare to the teacher master copy.

Engage in discussion on extensions questions, asking students to provide answers and to defend their points of view with evidence provided from the article and from the molecule activity.

## MODIFICATIONS or EXTENSIONS:

Based on students reading skills, the article may be assigned as a homework assignment prior to the start of the lesson. It would save approximately 10 minutes of time that could be transferred to the Membrane Comparison Activity, especially because students will likely have greater difficulty with that section of the lesson than the article.

Students may be able to carry their design experiments with help from individuals residing in Rio Lagartos, individually created samples comprised of brine shrimp and *Haloferax volcanii* cultures (ATCC29605), or by receiving samples from archaea researchers. There are previously designed lessons involving culturing and biotechnology work involving archaeal halophiles by Dassarma et. al (2016) and Kouassi, Waldron, Tripepi, & Pohlschroder (2017).

## RESOURCES/REFERENCES:


Dassarma, P., Tuel, K., Nierenberg, S. D., Phillips, T., Pecher, W. T., Dassarma, S. (2016). Inquiry-driven teaching & learning using the archaeal microorganisms *Halobacterium* NRC-1. *American Biology Teacher*, *78*(1), 7-13.

Frazer, J. (2013). Archaea are more wonderful than you know. *Scientific American*. Retrieved from https://blogs.scientificamerican.com/artful-amoeba/archaea-are-more-wonderful-than-you-know/

Gribaldo, S., & Brochier-Armanet, C. (2006). The origin and evolution of Archaea: a state of the art. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *361*(1470), 1007–1022

Jain, S., Caforio, A., & Driessen, A. J. M. (2014) Biosynthesis of archaeal membrane ether lipids. *Frontiers in Microbiology*, *5*, 1 – 15. doi: 10.3389/fmicb.2014.00641

Karsten, M. (2016). The unbelievable pink lakes of Las Coloradas in Mexico. *Expert Vagabond*. Retrieved from https://expertvagabond.com/las-coloradas-pink-lake

Kouassi, J. E., Waldron, I., Tripepi, M., & Pohlschroder, M. (2017). Laboratory activity to promote student understanding of UV mutagenesis & DNA repair. *Journal of Microbiology & Biology Education, 18*(1), 1-3. doi: 10.1128/jmbe.v18i1.1202

Woese, C. R., & Fox, G. E. (1977). Phylogenetic structure of the prokaryotic domain: the primary kingdoms. *PNAS*, *74*(11), 5088–5090

*Articles & Photos*

- "Archaea are more wonderful than you know," *Scientific American*: https://blogs.scientificamerican.com/artful-amoeba/archaea-are-more-wonderful-than-you-know/?print=true
- "The unbelievable pink lakes of Las Coloradas in Mexico," Expert Vagabond: https://expertvagabond.com/las-coloradas-pink-lake

*Supplemental Videos*

- Bozeman Science – Biological Molecules
- Crash Course – Biological Molecules: You Are What You Eat
- Crash Course – In Da Club: Membranes & Transport

# Experimental Design Graphic Organizer

| | |
|---|---|
| Background | **Problem or Question**<br>*Identify the knowledge gap or reason for conducting research on this topic.* |
| | **Background Information**<br>*Provide key information about the knowledge gap exists and why there needs to be research on this topic.* |
| | **Hypothesis**<br>*Identify the expected outcome of your investigation what you hope to discover or accomplish.* |
| Variables & Design | **Independent Variable(s)**<br>*Identify what you are manipulating and how it can be quantified.* |
| | **Dependent Variable(s)**<br>*Identify what you are measuring and how it can be quantified.* |
| | **Other Variables**<br>*Identify the other variables that could impact the outcome of the experiment and what value you will hold them constant.* |
| | **Methods**<br>*Describe your methods concisely in key bullet points, being specific to what is being manipulated, measured, and recorded. Identify control group and experimental groups.* |
| | **Materials**<br>*Identify important materials and equipment necessary to accomplish your methods.* |
| Results | **Data Organization**<br>*Describe how you intend on recording your data (usually a data table or spreadsheet) and how you intend on graphically representing it* |

# Experimental Design Graphic Organizer

## Background

### Problem or Question

*Identify the knowledge gap or reason for conducting research on this topic.*

What is causing the water of Las Coloradas to turn pink?

### Background Information

*Provide key information about the knowledge gap exists and why there needs to be research on this topic.*

Brine shrimp are pink in large populations and are the cause of flamingos pink-colored feathers.

*Haloferax volcanii* are pink when cultured in high salt concentrations.

Potassium permanganate is rumored to be responsible for purifying the salterns and has a pinkish/purple color.

Algae high in carotenoids can appear reddish/pinkish in color depending on the size of the population.

### Hypothesis

*Identify the expected outcome of your investigation what you hope to discover or accomplish.*

*H. volcanii* and/or other halophilic archaeal species are causing the pink color.

## Variables & Design

### Independent Variable(s)

*Identify what you are manipulating and how it can be quantified.*

Species of organisms being cultured.

### Dependent Variable(s)

*Identify what you are measuring and how it can be quantified.*

Qualitative coloration and presence of *H. volcanii* on plates.

### Other Variables

*Identify the other variables that could impact the outcome of the experiment and what value you will hold them constant.*

Subculture nutrients (according to Kouassi, Waldron, Tripepi, & Pohlschroder (2017).)

Temperature (42°C)

Salt concentration according to growth protocol (2.5 – 5M)

### Methods

*Describe your methods concisely in key bullet points, being specific to what is being manipulated, measured, and recorded. Identify control group and experimental groups.*

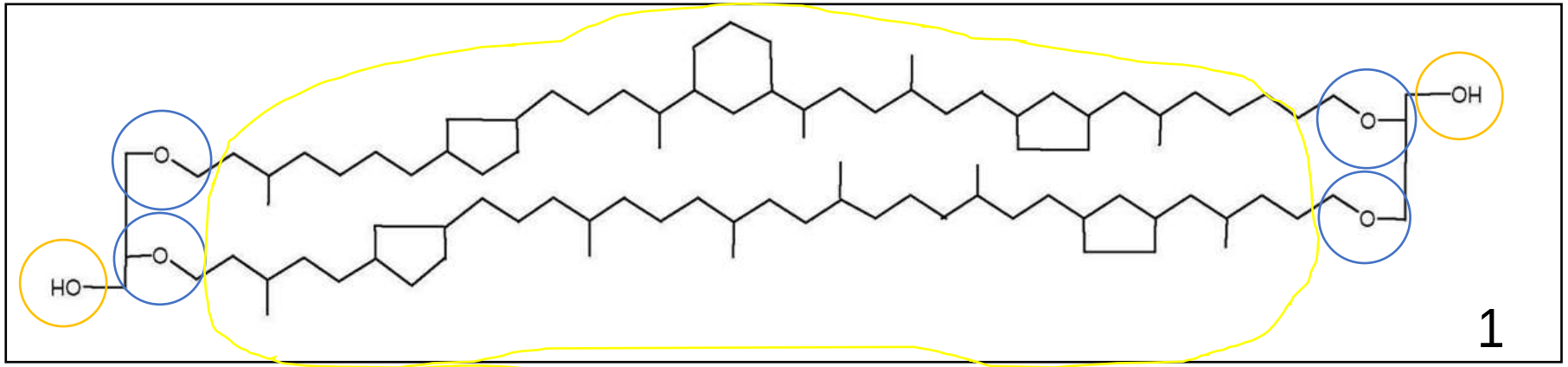
Prepare growth media according to plan listed above.

Transfer portion of sample to growth media and incubate for 5-7 days.

Control Groups: + control for *H. volcanii*, - control (no culture)

Experimental Groups: 5 plates of cultured sample

| | |
|---|---|
| | Materials<br>*Identify important materials and equipment necessary to accomplish your methods.*<br>Nutrient media according to Kouassi, Waldron, Tripepi, & Pohlschroder (2017).<br>Sample from Rio Lagartos |
| Results | Data Organization<br>*Describe how you intend on recording your data (usually a data table or spreadsheet) and how you intend on graphically representing it*<br>Growth as a +/-<br>Coloration as a +/-<br><br>If culture fails, attempt with other culturing methods for other species. |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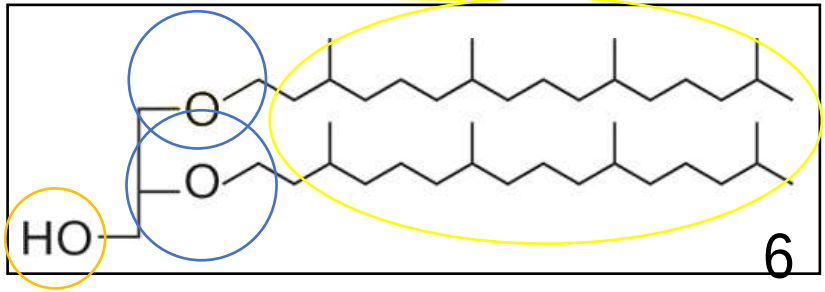
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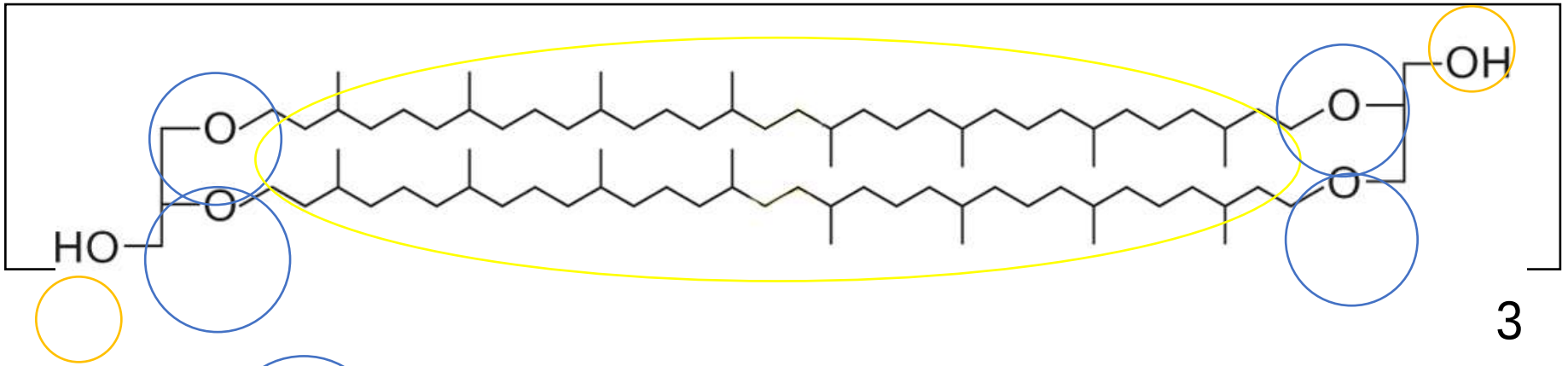
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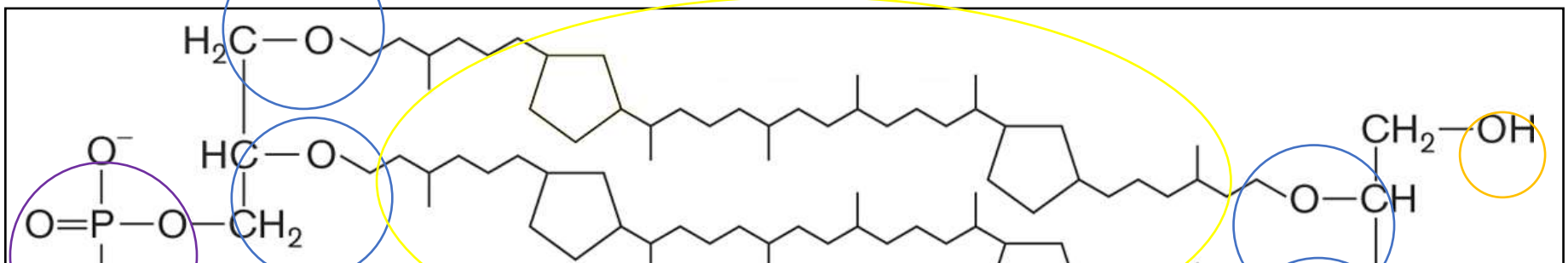
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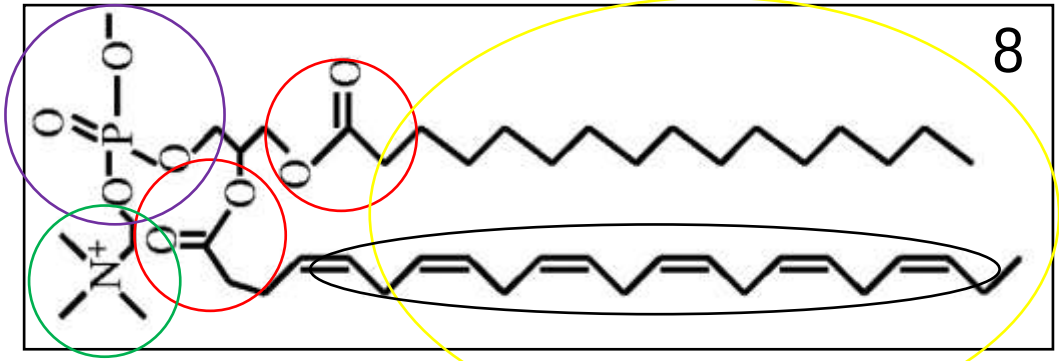
O—CH



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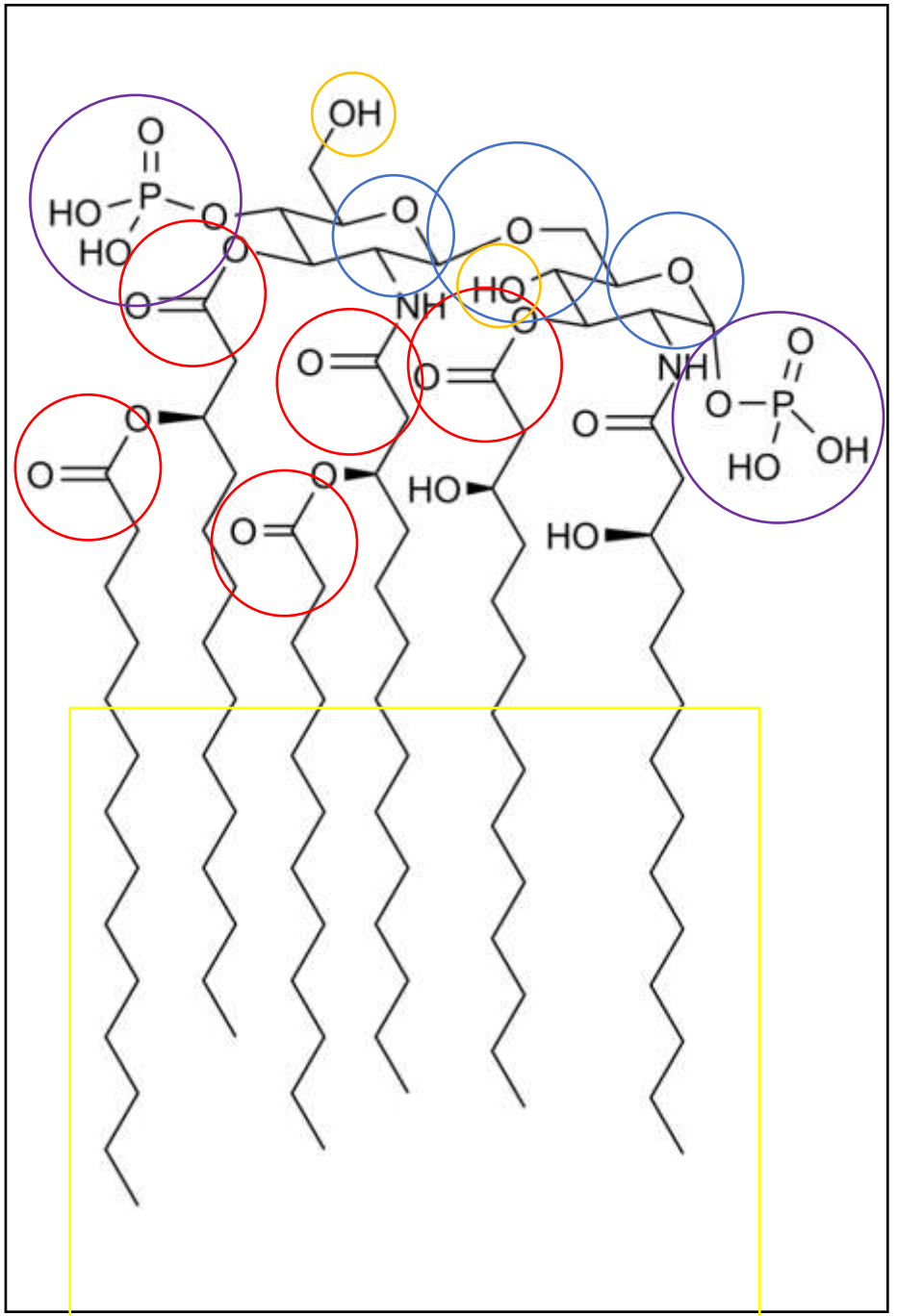

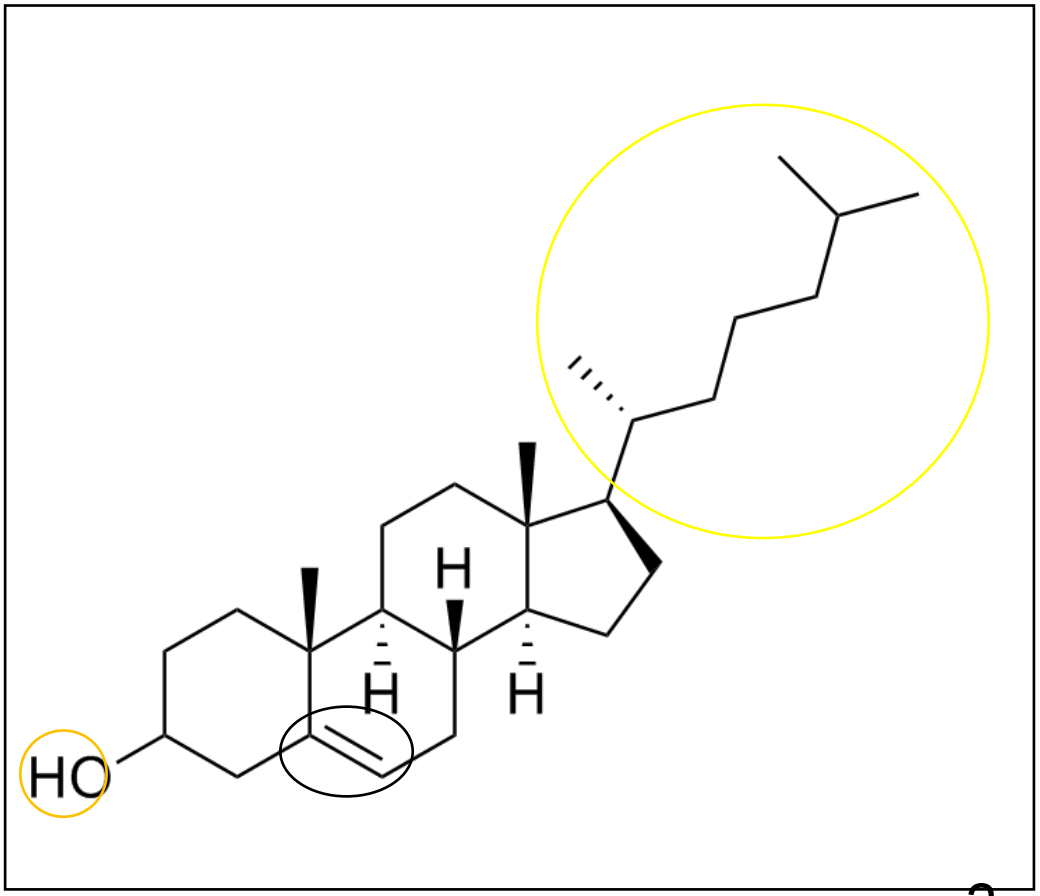

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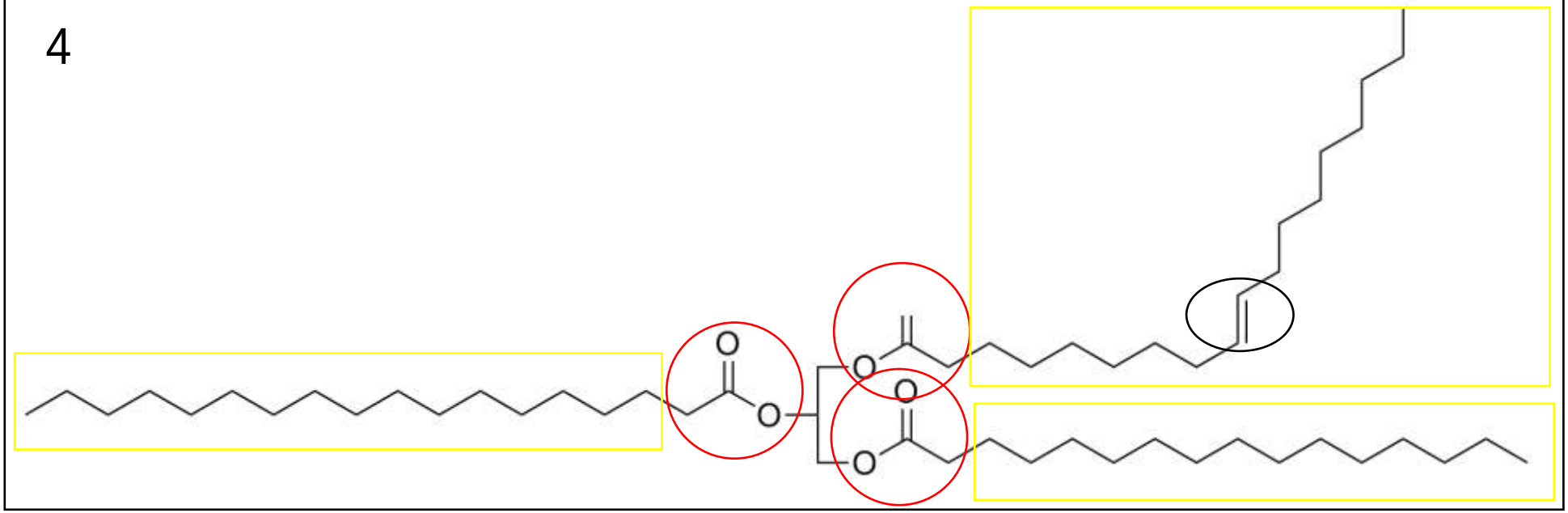


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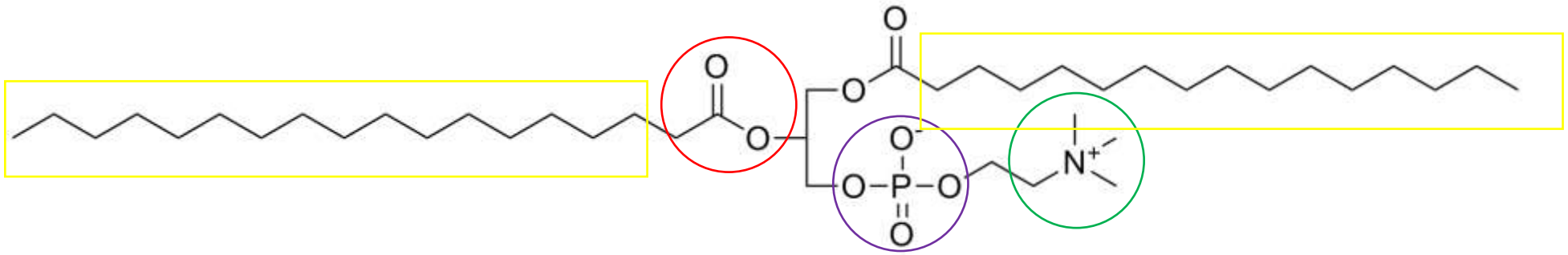
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# Membrane Comparison Activity

Circle the following functional groups on each molecule using the colors indicated below. Also, indicate the intermolecular force (IMF) potential and identify which type of molecules (hydrophobic/hydrophilic) could pass through regions containing these groups.

| Functional Group | Color | IMF Potential | Ability to Pass Hydrophobic/Hydrophilic |
|---|---|---|---|
| Ether | Blue | | |
| Ester | Red | | |
| Phosphate | Purple | | |
| Amine | Green | | |
| Hydrocarbon Chain (~>5 carbons) | Yellow | | |
| Unsaturated Bond | Black | | |
| Hydroxyl Group | Orange | | |

Identify the domain that each membrane component corresponds to (some can have more than one) and provide a brief justification why.

| Molecule | Domain | Reasoning |
|---|---|---|
| 1 | | |
| 2 | | |
| 3 | | |
| 4 | | |
| 5 | | |
| 6 | | |
| 7 | | |
| 8 | | |
| 9 | | |

Extension Questions

The structures for the archaeal lipids in this activity are missing a functional group that should be present in the membranes. What is missing? What functional group should they attach to?

Describe the most significant chemical difference between archaeal membranes and other domains of life.

Structure determines function. Predict what the difference in lipid structure provides functionally for archaea versus bacteria and eukaryotes.

# Membrane Comparison Activity

Circle the following functional groups on each molecule using the colors indicated below. Also, indicate the intermolecular force (IMF) potential and identify which type of molecules (hydrophobic/hydrophilic) could pass through regions containing these groups.

| Functional Group | Color | IMF Potential | Ability to Pass Hydrophobic/Hydrophilic |
|---|---|---|---|
| Ether | Blue | London, Dipole, H Bond | Hydrophilic |
| Ester | Red | London, Dipole, H Bond | Hydrophilic |
| Phosphate | Purple | London, Dipole, H Bond | Hydrophilic |
| Amine | Green | London, Dipole, H Bond | Hydrophilic |
| Hydrocarbon Chain (~>5 carbons) | Yellow | London | Hydrophobic |
| Unsaturated Bond | Black | London | Hydrophobic |
| Hydroxyl Group | Orange | London, Dipole, H Bond | Hydrophilic |

Identify the domain that each membrane component corresponds to (some can have more than one) and provide a brief justification why.

| Molecule | Domain | Reasoning |
|---|---|---|
| 1 | Archaea | Ether linkage to hydrocarbon chain; isoprene chain |
| 2 | Eukarya | Cholesterol only found in animals (and modified version in plants) |
| 3 | Archaea | Ether linkage to hydrocarbon chain; isoprene chain; monolayer |
| 4 | Eukarya | Triglycerides are found primarily in eukaryotes (although new evidence shows rare presence in bacteria) |
| 5 | Bacteria | Lipopolysaccharide layer found in Gram-negative bacteria |
| 6 | Archaea | Ether linkage to hydrocarbon chain; isoprene chain; monolayer |
| 7 | Bacteria/Eukarya | Ester linkages & phospholipid |
| 8 | Bacteria/Eukarya | Ester linkages & phospholipids |
| 9 | Archaea | Ether linkage to hydrocarbon chain; isoprene chain; monolayer |

Extension Questions

The structures for the archaeal lipids in this activity are missing a functional group that should be present in the membranes. What is missing? What functional group should they attach to?

Archaeal lipids are missing phosphate groups and they should attach to the free –OH (hydroxyl) group.

Describe the most significant chemical difference between archaeal membranes and other domains of life.

The biggest chemical difference is the ether bond between the glycerol and the fatty acid in archaea versus esters in bacteria and eukarya. The fatty acid chains are also made of isoprenoids rather than simple, linear hydrocarbons.

Structure determines function. Predict what the difference in lipid structure provides functionally for archaea versus bacteria and eukaryotes.

Ethers are less reactive than esters, which would allow archaea to survive in more extreme environments. It prevents leaking of their cytoplasm, especially in high temperatures, which isn't true for other domains.

Teacher Notes

Lesson 1: Pretty and Pink – Introduction to Archaea

# Lesson 2: Keeping Active

Exploring the active site of inorganic pyrophosphatase

## LESSON SUMMARY:

What is it about active sites of enzymes that allow them to catalyze chemical reactions? Students will be shown how research into enzymatic function is important for various industries, especially health. Students will explore the relationship of structure and function through a hands-on manipulative activity of substrates and active sites. They will deepen their knowledge by investigating the chemical interactions of amino acid side chains with substrates. Lastly, they will connect their understanding of structure to function by analyzing how enzyme activity is quantified and affected by changes to an enzyme's environment.

STANDARDS:

| AP Biology | NGSS | NGSSS |
|---|---|---|
| E.K.1.B.1 | HS-LS1-2 | SC.912.N.3.5 |
| E.K.4.A.1 | HS-LS1-6 | SC.912.L.16.10 |
| E.K.4.B.1 | | SC.912.L.18.4 |
| Science Practice 1 | | SC.912.L.18.11 |

KEY QUESTION(S):

How do enzymes catalyze reactions?

How does protein structure determine its function?

What are benefits and limitations of models in science?

LEARNING OBJECTIVES:

The student will be able to...

1. Compare and contrast the change in models over time.
2. Model and describe how substrates and enzymes interact to catalyze chemical reactions.
3. Analyze the optimum conditions for enzymatic activity.

OVERALL TIME ESTIMATE:

Advanced Preparation: 20 minutes to print copies of the Side Chain Analysis Activity, Enzyme Active Site/Substrate Manipulative, and Enzyme Activity Graphical Analysis. The substrates for the manipulative should be printed on transparency, and the enzymes should be laminated. Markers or other colored utensils should also be placed at stations accompanying the manipulatives. The first page of the Enzymatic Activity Graphical Analysis handout should be printed single-sided and potentially laminated for student annotations and repeat usability. The data analysis questions should be printed double sided.

Lesson: 50 minutes

MATERIALS:

*Essential*

Set of three colored writing utensils (blue, red, and black) (1 set per group of 4)

Laminated enzyme active site and printed substrates on transparency paper (1 set per group of 4)

Side Chain Analysis Activity Handout (1 per group of 4)

Enzymatic Activity Graphical Analysis Handout (1 per group of 4)

## BACKGROUND INFORMATION:

Proteins are one of the four major classes of organic macromolecules and are comprised of amino acid monomers. A reaction between two amino acids results in dehydration (loss of water) between the carboxylic group of one amino acid and the amine group of another in order to form a covalent, peptide bond. Proteins ultimately have four levels of structure that bestow its function: primary, secondary, tertiary, and quaternary structure. Primary structure is the linear amino acid sequence as determined by the gene sequence and is held together by covalent, peptide bonds. Secondary structure occurs when the carboxylic acid and amine groups hydrogen bond together to form the initial three-dimensional structure. The two predominant forms of secondary structure occur: alpha-helices and beta-sheets. Tertiary structure occurs when the amino acid side chains begin to exhibit intermolecular attractions (also called van der Waals forces), such as London dispersion forces, dipole-dipole interactions, or hydrogen bonding. Additionally, tertiary structure occurs through covalent disulfide bridges between two cysteine thiol side chains or two amino acids forming salt-bridges due to opposite charge attractions. Lastly, quaternary structure occurs when multiple polypeptides attract via intermolecular forces. All told,

the change in conformation (shape) helps to lower the energy and increase stability of the molecule. Depending on the structure, proteins can assume various functions including structural or enzymatic roles within or outside of the cell.

Enzymes are biological catalysts that are predominantly protein in structure, but can sometimes exist in the form of RNA. Enzymes lower the activation energy of chemical reactions, therefore making them more thermodynamically and energetically favorable to occur. The primary driver of the lowering of activation energy is an enzyme's active site. Within an active site, the amino acids participating will increase strain on the bonds of the substrate, thereby making it easier for the reaction to occur. The active site is heavily dependent on environmental conditions, such as proper water or salt content, pH levels, or temperature. An enzyme can be regulated in numerous ways, either by limiting its production (lessening gene expression), competitively inhibiting its active site with another substrate, or modifying the shape of its active site via allosteric inhibition..

Inorganic pyrophosphatase is an enzyme found abundantly in living organisms and assumes the role of hydrolyzing inorganic pyrophosphate ($PP_i$) to two inorganic phosphate ions ($P_i$). Pyrophosphate is often present as a product of ATP breakdown into AMP. Hydrolysis of pyrophosphate is highly exergonic (energy-releasing) and is therefore coupled with other endergonic reactions (energy requiring) such as DNA synthesis or the formation of other macromolecules. Pyrophosphate can be concern in biotechnology as a contaminant and its breakdown can lead to the supply of energy for unintended side reactions. Furthermore, according to Heikinheimo et al. (1996), high levels of inorganic pyrophosphate can be toxic to the cell, thus emphasizing the need for it. Related to this lesson, McMillan, Hewopit, & Maupin-Furlow (2016) have elucidated the activity of *H. volcanii* inorganic pyrophosphatase activity under high levels of organic solvent. This lesson helps to set the stage on why understanding protein structure is important, especially in unique environments and applications.

## REQUIRED STUDENT BACKGROUND KNOWLEDGE:

Students should be able to recognize the relative structure and reactivity of key functional groups, such as amine, hydroxyl, carbonyl, carboxyl, and hydrophobic groups.

Students should enter this activity with prior knowledge of the four levels of protein structure (primary, secondary, tertiary, and quaternary) and should be able to describe and model the chemical attractions leading to each.

Students should also know the major functions of proteins, especially enzyme function, and be able to describe the thermodynamics related to chemical reactions and enzyme activity.

## ADVANCE PREPARATION:

The DNA base substrates should be printed on transparency paper (1 set per group of 4). The two sides are mirror images to each other, so cut along the outside border and fold in half at the center. Meanwhile, print and laminate the enzyme (1 set per group of 4). Three colored writing untensils (blue, red, black) (1 set per group of 4) should be put out at each lab bench along with the manipulatives.

Print copies of the Side Chain Analysis Activity (1 per group of 4).

## VOCABULARY:

Protein: Macromolecule responsible for numerous functions in living organisms, including enzymes and providing structure, that are comprised of amino acid monomers.

Amino acid: Monomeric building block of proteins consisting of an N-terminal amine group, a C-terminal carboxylic acid group, and a side chain that carries its own unique chemical properties.

Side chains: The functional group attached to the alpha-carbon of an amino acid that bestows the amino acid's chemical properties. Denoted by the letter "R" in amino acid diagrams.

Acidic: Side chains that contain carboxylic acid functional groups that are protonated and can donate protons at neutral pH (in other words, they have a low pKa). They are extremely hydrophilic. Side chains that are acidic include glutamic acid and aspartic acid.

Hydrophilic: Water-loving chemical functional groups because of the polar nature of their bonds and/or their ability to form hydrogen bonds with water molecules. Side chains of amino acids that are hydrophilic include serine, threonine, asparagine, and glutamine.

pI: The isoelectric point of a protein at which a molecule carries no net electrical charge and is based on the chemical nature of functional groups on amino acid side chains and related modifications. Important in determining the functionality of proteins in specific aqueous environments.

Enzyme: A biological macromolecule that speeds of chemical reactions, consisting primarily of proteins, but also inclusive of RNA molecules.

Active site: Location on an enzyme where catalysis of chemical reactions occurs and is mediated by constituent amino acid residues (most often their side chains) within the active site.

Activity: A measure of an enzymes ability to catalyze reactions.

Pyrophosphatase: An enzyme responsible for catalyzing the breakdown of pyrophosphate ($PP_i$) into two inorganic phosphate ions. The reaction releases a significant amount of energy (-19 kJ/mol free energy change). Because it is often coupled with endergonic, or energy-requiring, reactions like DNA synthesis, it is highly conserved.

## PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES:

*Pyrophosphatase Introduction* (10 minutes)

1. Explain that discoveries in nature are a vehicle for improving society. Some examples include:
    a. *Taq* polymerase for polymerase chain reaction (PCR) from the bacteria *Thermus aquaticus* at Yellowstone National Park
    b. Insulin produced in *E. coli*, yeasts, or in plants for human injection via recombinant DNA technology
    c. Monoclonal antibodies produced by goats, rabbits, or other animals against hCG hormones for pregnancy testing
2. Currently, research is being done to discover enzymes that can handle extreme conditions, such as those in high salt or those with little water. McMillan, Hepowit, & Maupin-Furlow (2016) have characterized the pyrophosphatase enzyme in the halophile, *H. volcanii*.

a. Pyrophosphatase is required for cleaving pyrophosphate ($PP_i$), which is produced in the hydrolysis of ATP into AMP. It is highly conserved because all organisms use nucleotide triphosphates, like ATP, to power endergonic reactions. In doing so, $PP_i$ is going to be a byproduct of those reactions, so every organism would need this enzyme.
b. It could be useful to prevent unintended reactions during PCR and other biotechnology applications.

*Active Site & Side Chain Analysis* (20 minutes)

1. [5 minutes] Arrange students in groups of 4 and have them attempt to fit the substrates into the enzyme's active site using the Enzyme Active Site/Substrate Manipulative Activity.
    a. Walk around the classroom observing and correcting students attempts to fit the nucleotides into the active site.
    b. Remind students that hydrophobicity, hydrophilicity, and intermolecular force potential matter in how substrates interact with active sites.
2. [15 minutes] Once finished with the interactive portion, transition students to working on the Side Chain Analysis Activity.
    a. Students will again be analyzing the chemical structure of enzymes, but this time with a greater eye on the chemical properties of the amino acid side chains.
    b. Students will compare an adapted active site model (without substrates, water, and cofactors) with a proposed active site model from 1992 and 1996.

*Enzymatic Activity Graphical Analysis* (20 minutes)

1. With students still in groups, give each group the Enzymatic Activity Graphical Analysis handout.
2. [5-10 minutes] Have students discuss the experimental design and the results, and annotate each (especially if laminated) before looking at the questions.
3. [10-15 minutes] Once discussed, students should transition to answering the questions.
    a. Remind students to connect this assignment to the Enzyme Active Site/Substrate Manipulative Activity as well as the Side Chain Analysis Activity.

b. Walk around the room reminding students about the impacts of protein structure on protein structure, especially how the presence of salt and water is needed in the correct quantities to stabilize the active site.
c. TIP: The most trouble portion of the analysis will likely be the control groups question, and it is an important focus of science and AP Biology curriculum. Remind students that control groups are used as a basis of comparison to the treatment groups, but they must explain HOW its used as a basis of comparison.

## ASSESSMENT SUGGESTIONS:

*Enzyme Active Site/Substrate Manipulative*

Formatively assess students' comprehension of matching structures and intermolecular forces by going around the room and observing their trial-and-error attempts.

*Side Chain Analysis Activity*

Student responses can be collected and formatively assessed, or provide immediate feedback during the activity by walking around with the key.

*Enzyme Activity Graphical Analysis*

The big takeaway of understanding amino acid side chains, intermolecular forces, and protein structure is to ultimately explain how it impacts function. The Enzyme Activity Graphical Analysis can be used as a formative assessment to determine how well students made the aforementioned connection or as a summative assessment.

## MODIFICATIONS or EXTENSIONS:

During the Enzyme Active Site/Substrate Manipulative Activity, on the laminated enzymes, students can draw in their molecules and annotate hydrogen bond interactions using vis-à-vis markers.

AP Biology Investigation 13 focuses on enzyme activity and has been a focal lab in the AP Biology curriculum since its inclusion in the 2001 AP Biology Lab Manual. In the investigation, students will utilize peroxidase from turnips in order to determine optimum temperatures, pH,

or other environmental conditions they decide. The investigation extends students graphical analysis from this lesson to devising their own graphs specific to enzyme activity.

## RESOURCES/REFERENCES:


The College Board. (2012). *AP Biology investigative labs: An inquiry-based approach.* New York, NY: The College Board.

Cooperman, B. S., Baykov, A. A., & Lahti, R. (1992). Evolutionary conservation of the active site of soluble inorganic pyrophosphatase. *Trends in Biochemical Sciences*, *17*, 262 – 266.

McMillan, L. J., Hepowit, N. L., & Maupin-Furlow, J. A. (2016). Archaeal inorganic pyrophosphatase displays robust activity under high-salt conditions and in organic solvents. *Applied and Environmental Microbiology*, *82*(2), 538-548.

Heikinheimo, P., Lehtonen, J., Baykov, A., Lahti, R., Cooperman, B. S., & Goldman, A. (1996). The structural basis for pyrophosphatase catalysis. *Structure*, *4*(12), 1491 – 1508. doi: 10.1016/S0969-2126(96)00155-4


*Supplemental Videos*

The following video is incredibly helpful to have students watch before beginning the lesson and in each subsequent protein-related lesson.

RCSB Protein Data Bank – What is a Protein?

The following three videos give general overviews of proteins, with Khan Academy focusing more on the chemistry structural specifics.

RCBS Protein Data Bank – What is a Protein? 3D Shape & Function

Bozeman Science - Proteins

Khan Academy – Four levels of protein structure

The three following videos explain, in increasing complexity of detail, the nature of enzymes.

Amoeba Sisters – Enzymes (Updated) – Basic

Bozeman Science – Enzymes – Intermediate

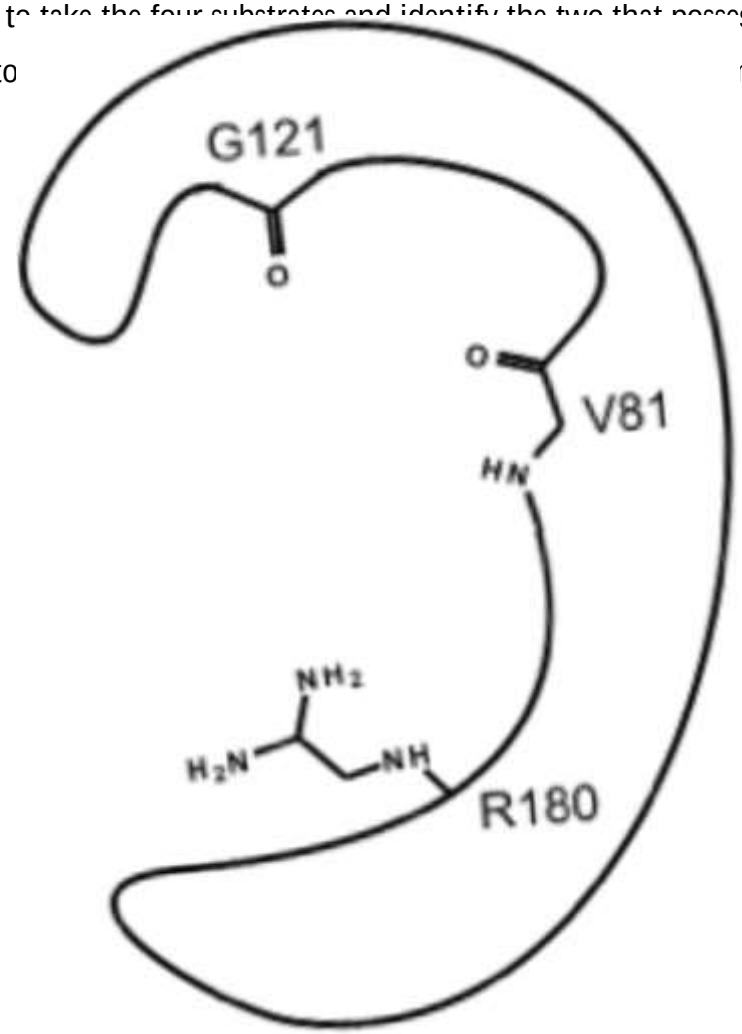
Khan Academy – Enzymes | Energy and enzymes – Advanced

For teachers engaging in the extension activity, the following video walks through the older AP Biology (2001-2012) version of the enzyme catalysis lab.

Bozeman Science – Lab 2: Enzyme Catalysis

# Enzyme Active Site/Substrate Manipulative

Below is the active site of an enzyme. There are three amino acid residues shown below: Glycine (G121), valine (V81), and arginine (R180). For glycine and valine, the contribution to the active site is actually their backbone (carbonyl group and secondary amine group) rather than side chains. Your task is to take the four substrates and identify the two that possess the best fit in the active site. Be sure to [illegible] nage fits.

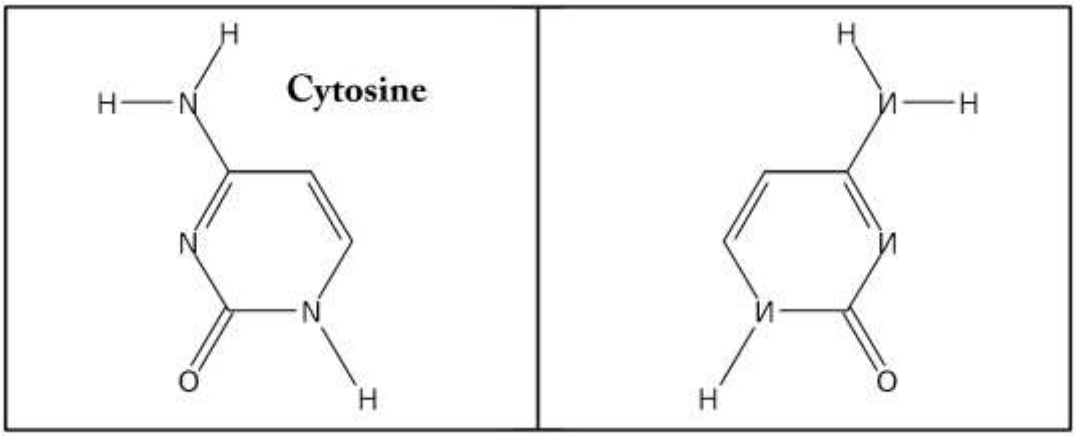


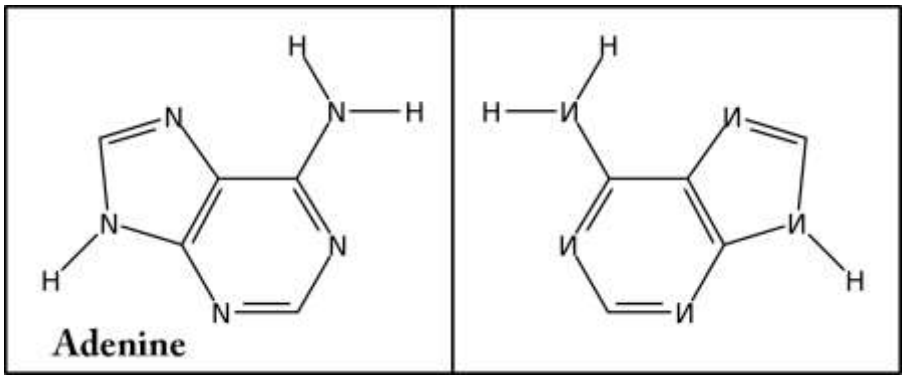
## Questions and Thoughts to Consider

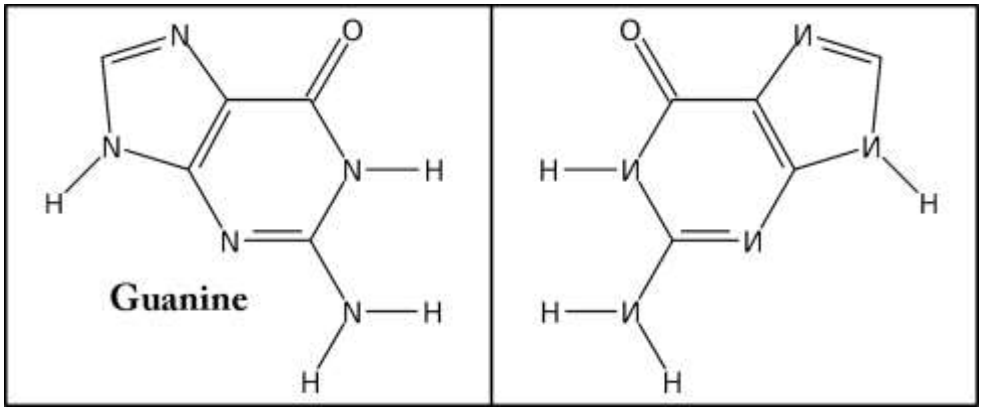
When manipulating the molecules, try to visualize the fact that there are many enzymes in a cell with likely even more substrate molecules available. The molecules are randomly floating around in solution until their fit and attractive forces match appropriately with each other.

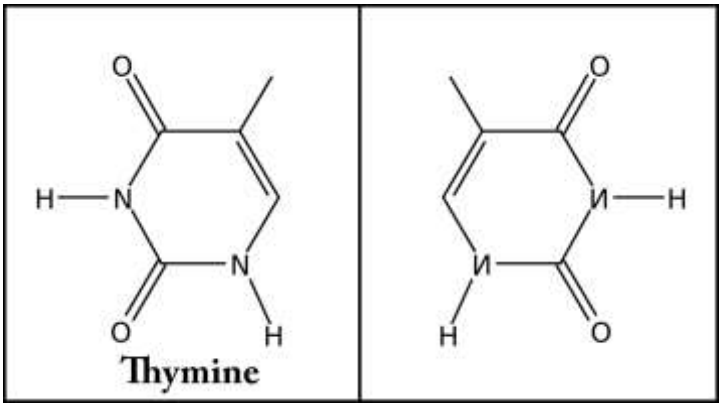
Which two substrates fit the best?

What was it that made them fit? Shape? Intermolecular force potential? Hydrophobicity/hydrophilicity? A mixture of all three?



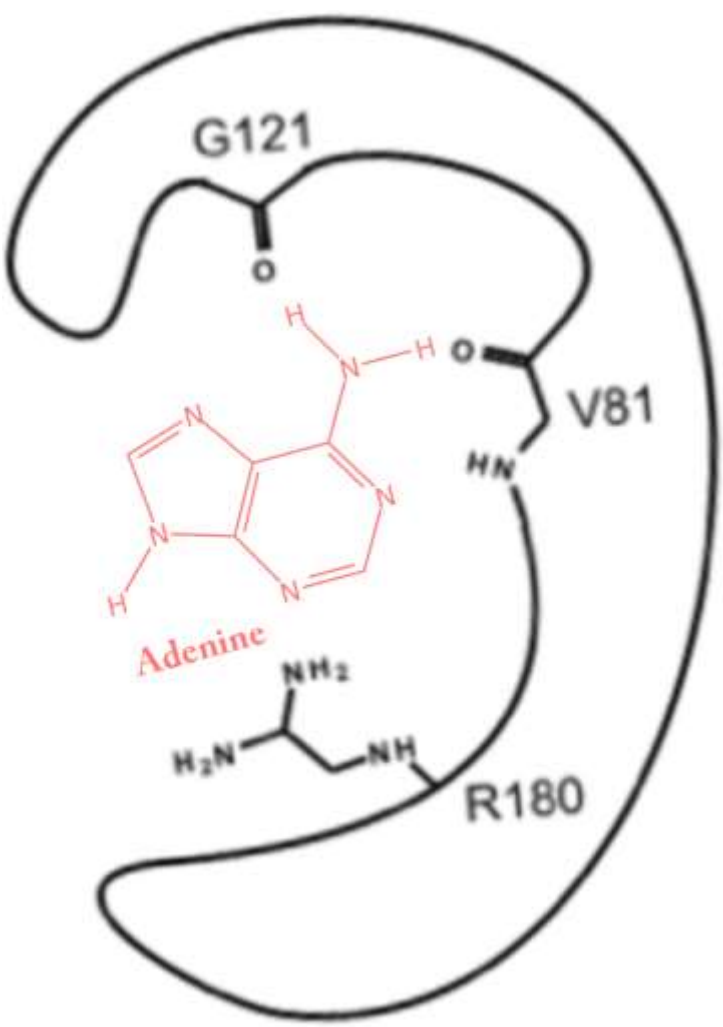



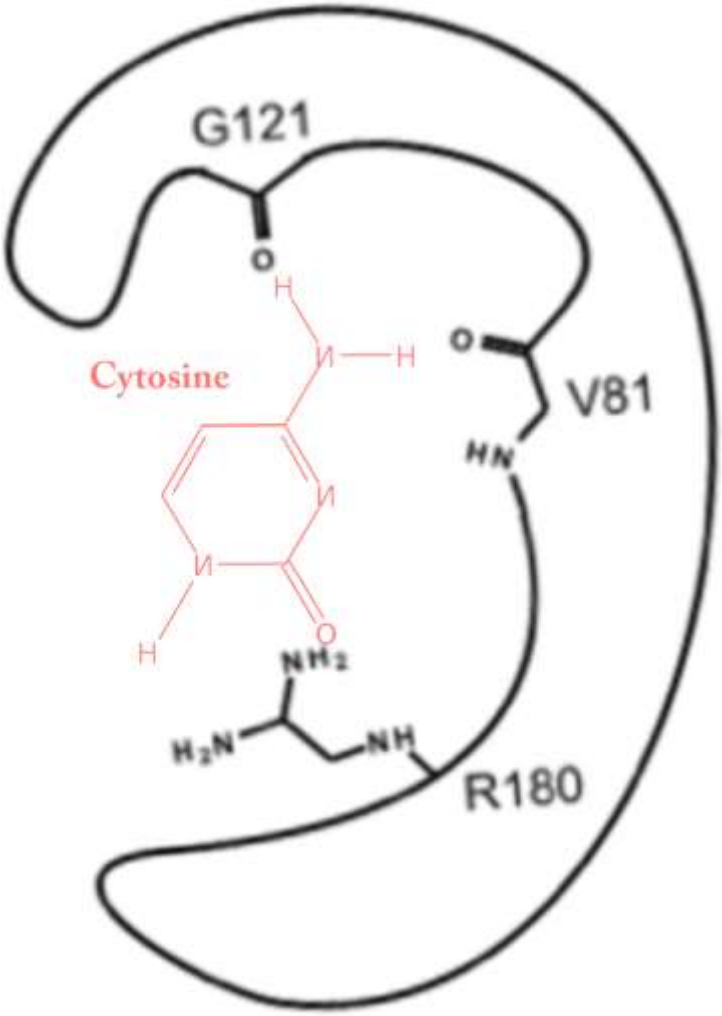



Enzyme Active Site/Substrate Manipulative

Suggested Student Responses

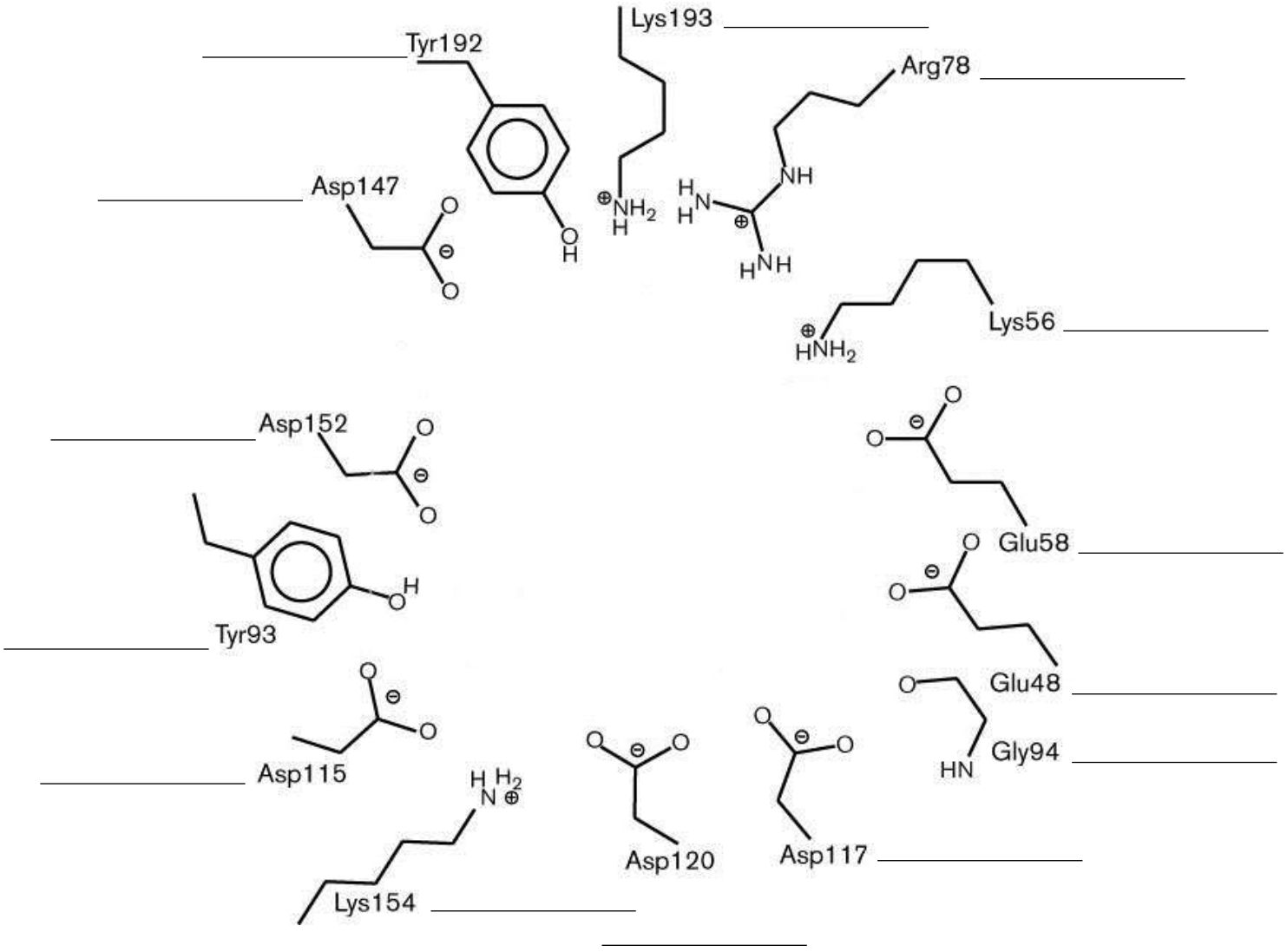



The following diagrams represent the best fits of substrates. In fact, adenine is a substrate for this enzyme. Hydrogen bonds are implied between adjacent hydrogen, oxygen, and nitrogen atoms due to their proximity. Guanine also has a potential fit, but the hydrogen of the secondary amine adjacent the carbonyl group wouldn't hydrogen bond with the secondary amine of the active site.

# Side Chain Analysis Activity

The following figure represents the active site structure of *H. volcanii's* inorganic pyrophosphatase adapted from Heikinheimo et al. (1996). The side chain residues of amino acids interact with inorganic pyrophosphate (not shown in this figure) in order to catalyze the reaction to two phosphate ions. Analyze the structure, paying attention to the side chains and their chemical properties, and answer the questions below.




## Analysis

Write the name of each amino acid on the line adjacent to it. Predict what the numbers mean.

Identify the number of side chains based on the following properties in the accompanying table:

*Special Cases* _____

*Polar, Uncharged Side Chains* _____

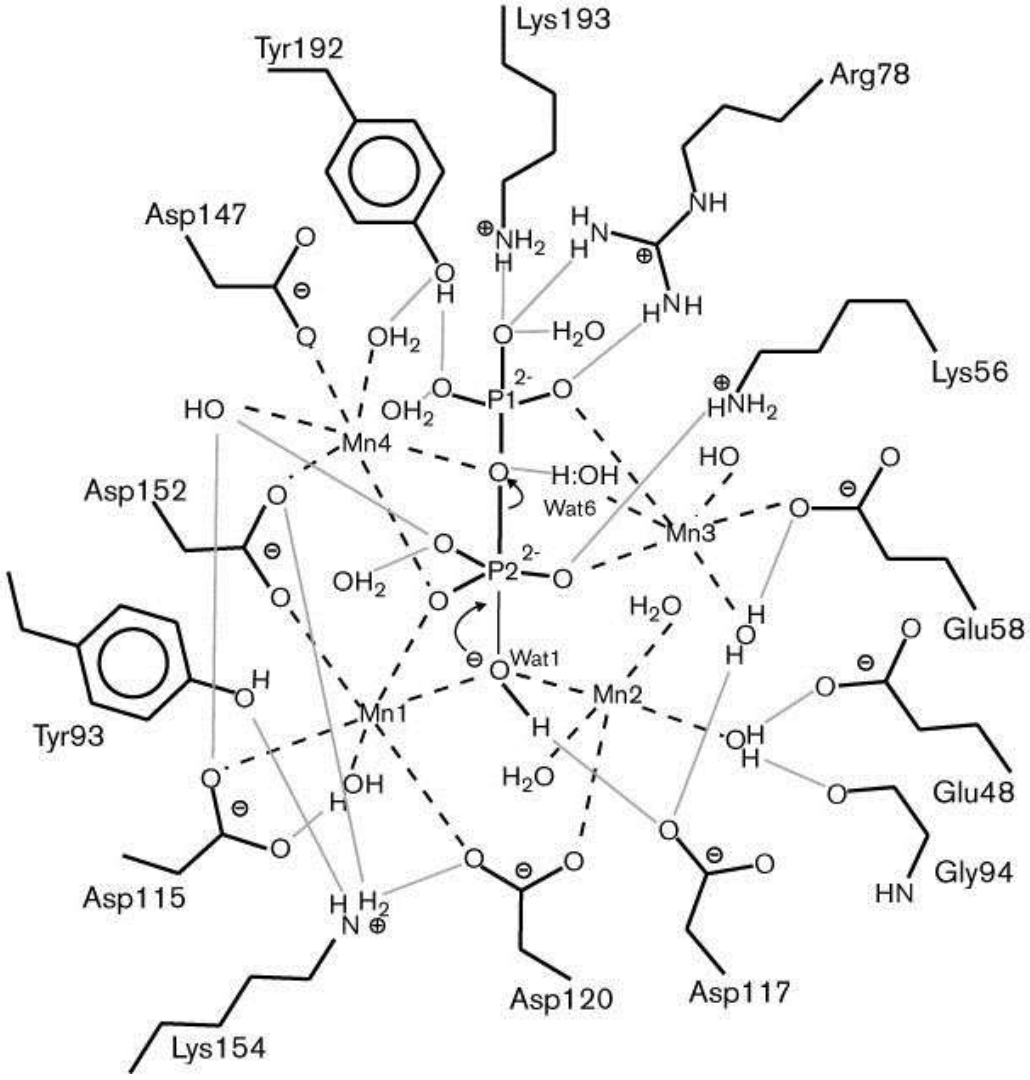
*Polar, Electrically Charged Side Chains* _____

*Hydrophobic Side Chains* _____

Predict what common molecule is necessary to stabilize the active site based on their side chains.

Side Chain Analysis Activity

The following figure represents the active site structure of *H. volcanii's* inorganic pyrophosphatase with pyrophosphate stabilized inside (Heikinheimo et al., 1996). Again analyze the structure, this time paying attention to interactions between the side chains, substrate, and accompanying substances.

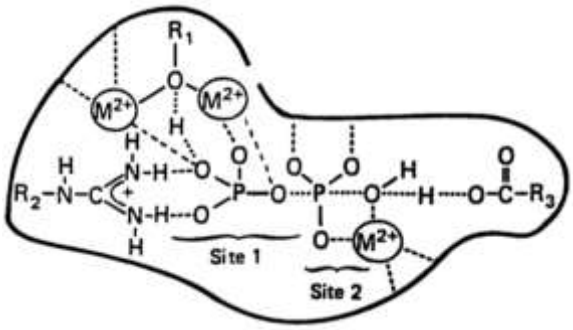


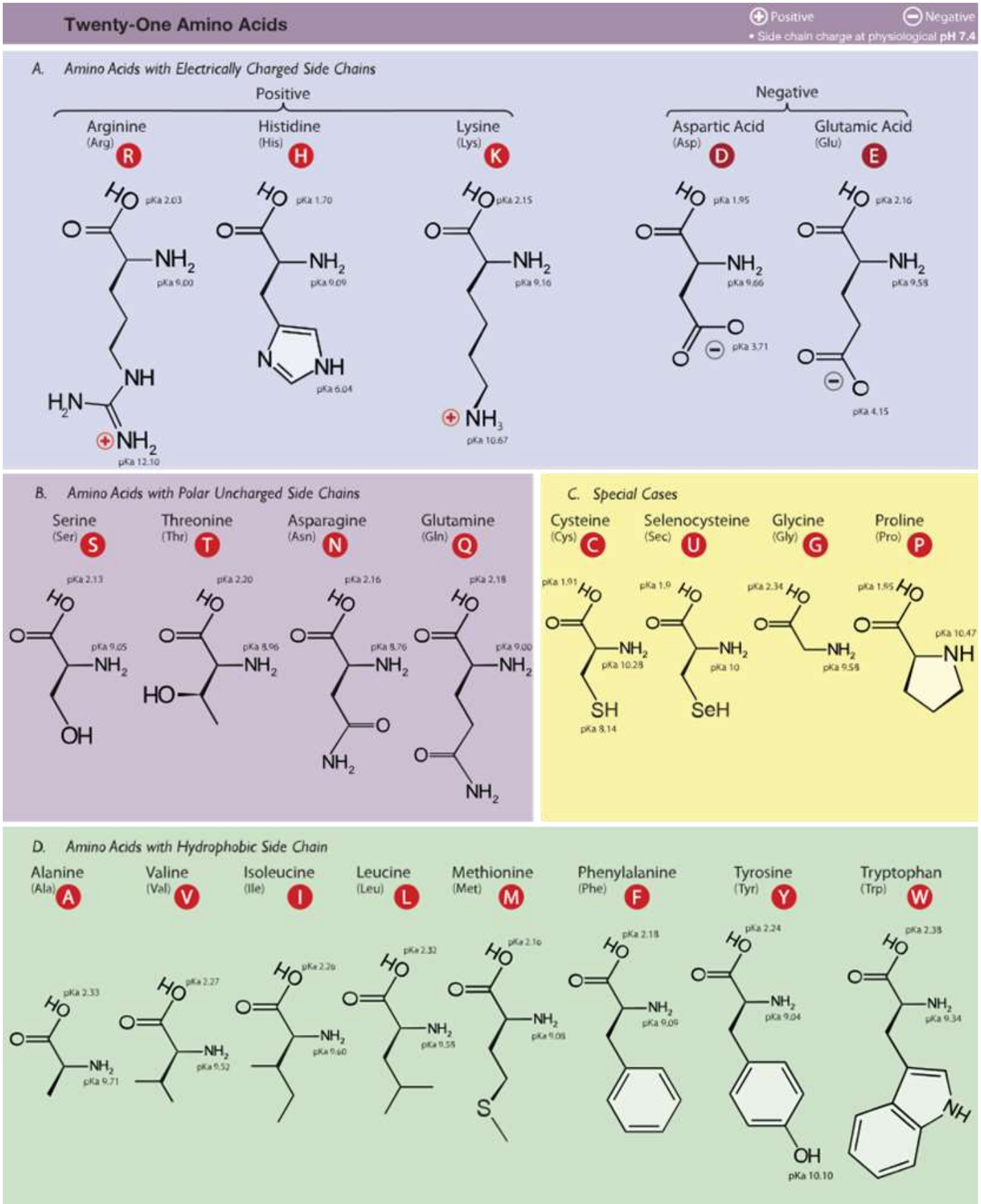

Analysis

Circle the following according to each color listed:

| Pyrophosphate ($PP_i$) | Water | Metal Ions |
|---|---|---|
| Red | Blue | Black |

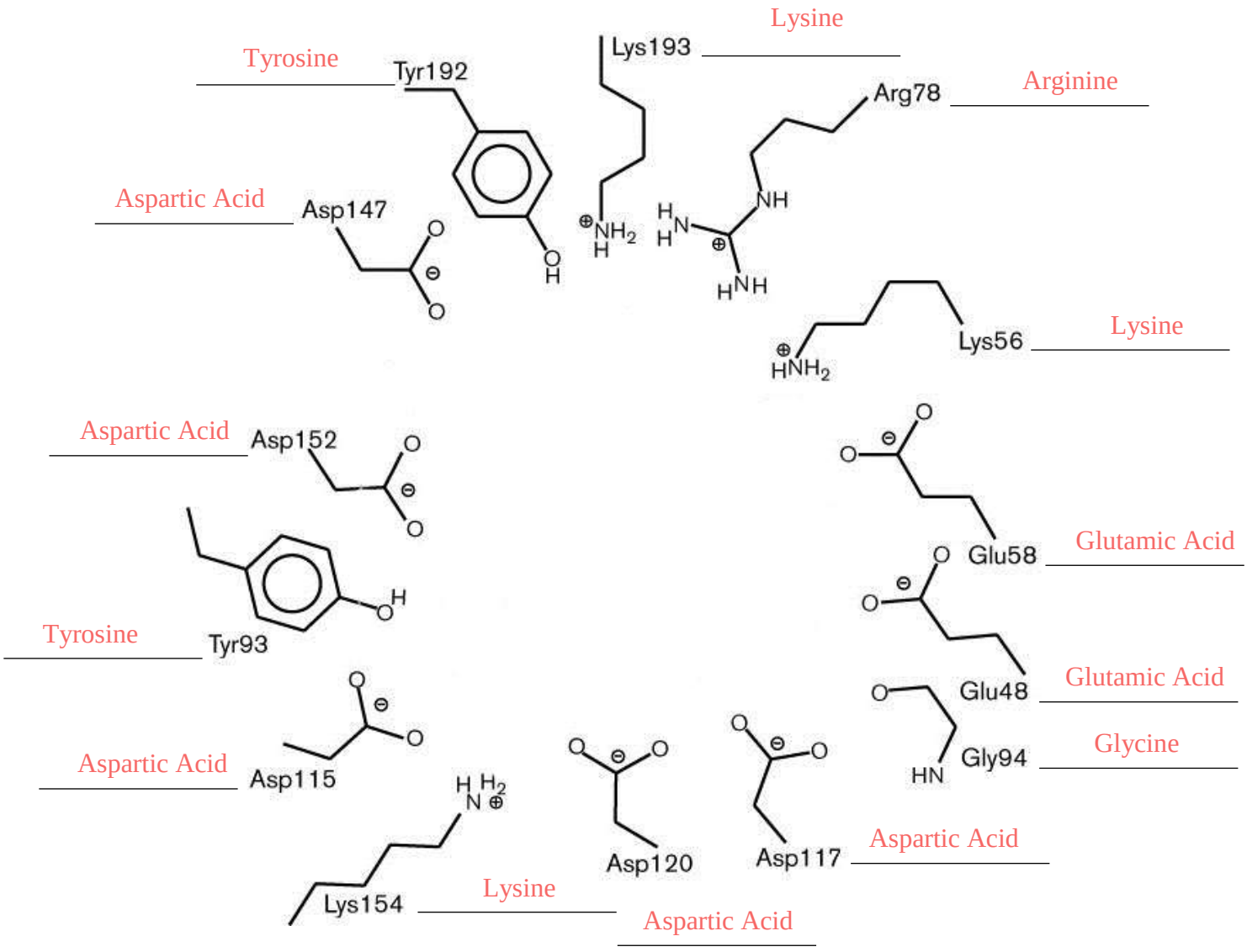
The figure to the left is a proposed model in 1992 from the same researchers who published the model above in 1996. Explain the model changed over time by describing its similarities and differences.


R1
M2+
M2+
H
H
N-H
O
O
O
O
O
O
H
O
R2-N-C
N-H
O
P
O
P
O
H
O-C-R3
H
O
M2+
Site 1
Site 2


Twenty-One Amino Acids
Positive
Negative
• Side chain charge at physiological pH 7.4
A. Amino Acids with Electrically Charged Side Chains
Positive
Arginine (Arg) R
Histidine (His) H
Lysine (Lys) K
Negative
Aspartic Acid (Asp) D
Glutamic Acid (Glu) E
B. Amino Acids with Polar Uncharged Side Chains
Serine (Ser) S
Threonine (Thr) T
Asparagine (Asn) N
Glutamine (Gln) Q
C. Special Cases
Cysteine (Cys) C
Selenocysteine (Sec) U
Glycine (Gly) G
Proline (Pro) P
D. Amino Acids with Hydrophobic Side Chain
Alanine (Ala) A
Valine (Val) V
Isoleucine (Ile) I
Leucine (Leu) L
Methionine (Met) M
Phenylalanine (Phe) F
Tyrosine (Tyr) Y
Tryptophan (Trp) W

Side Chain Analysis Activity

The following figure represents the active site structure of *H. volcanii's* inorganic pyrophosphatase adapted from Heikinheimo et al. (1996). The side chain residues of amino acids interact with inorganic pyrophosphate (not shown in this figure) in order to catalyze the reaction to two phosphate ions. Analyze the structure, paying attention to the side chains and their chemical properties, and answer the questions below.




Analysis

Write the name of each amino acid on the line adjacent to it. Predict what the numbers mean.

The numbers refer to the positions of the amino acids on the protein's primary sequence.

Identify the number of side chains based on the following properties in the accompanying table:

| *Special Cases* | |
|---|---|
| | 1 |
| | 0 |

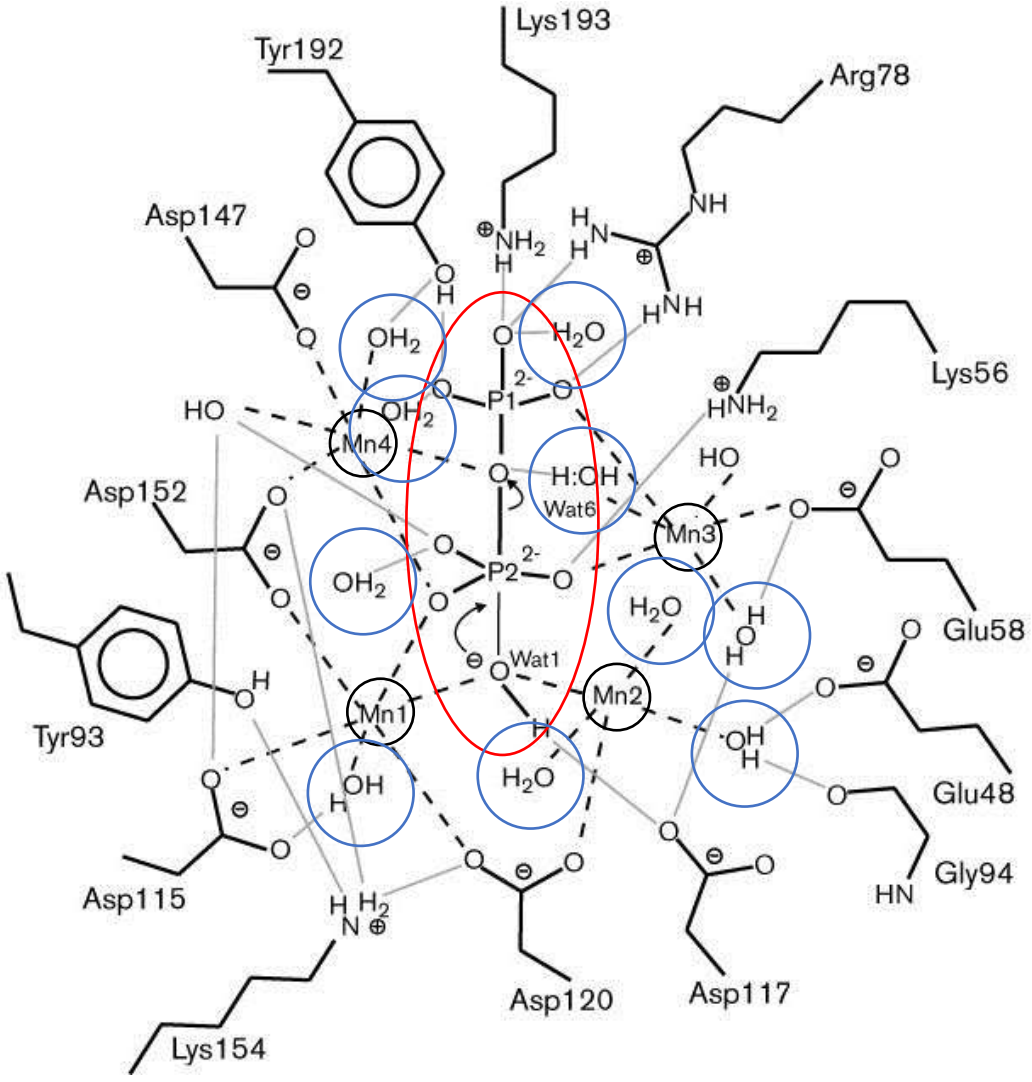
| | |
|---|---|
| *Polar, Uncharged Side Chains* | 11 |
| *Polar, Electrically Charged Side Chains* | 2 |
| *Hydrophobic Side Chains* | |

Predict what common molecule is necessary to stabilize the active site based on their side chains.

Given the great potential for H bonding, water is necessary to stabilize the active site.

Side Chain Analysis Activity

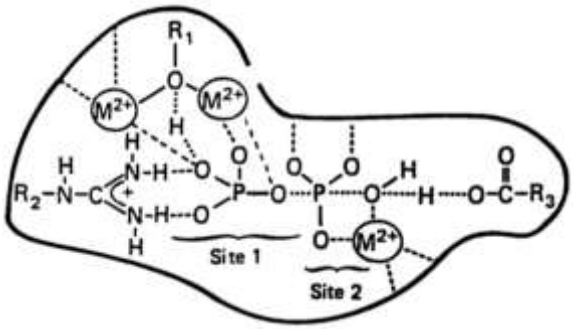
The following figure represents the active site structure of *H. volcanii's* inorganic pyrophosphatase with pyrophosphate stabilized inside (Heikinheimo et al., 1996). Again analyze the structure, this time paying attention to interactions between the side chains, substrate, and accompanying substances.




Analysis

Circle the following according to each color listed:

| Pyrophosphate ($PP_i$) | Water | Metal Ions |
| --- | --- | --- |
| Red | Blue | Black |




The figure to the left is a proposed model in 1992 from the same researchers who published the model above in 1996. Explain the model changed over time by describing its

The model changes to include far more amino acid side chains within the active sites, more metal ion interactions, and a greater focus on the inclusion of water. The model retained the need for metal ion stability (going from 3 to 4) and also the presence of

# Enzymatic Activity Graphical Analysis

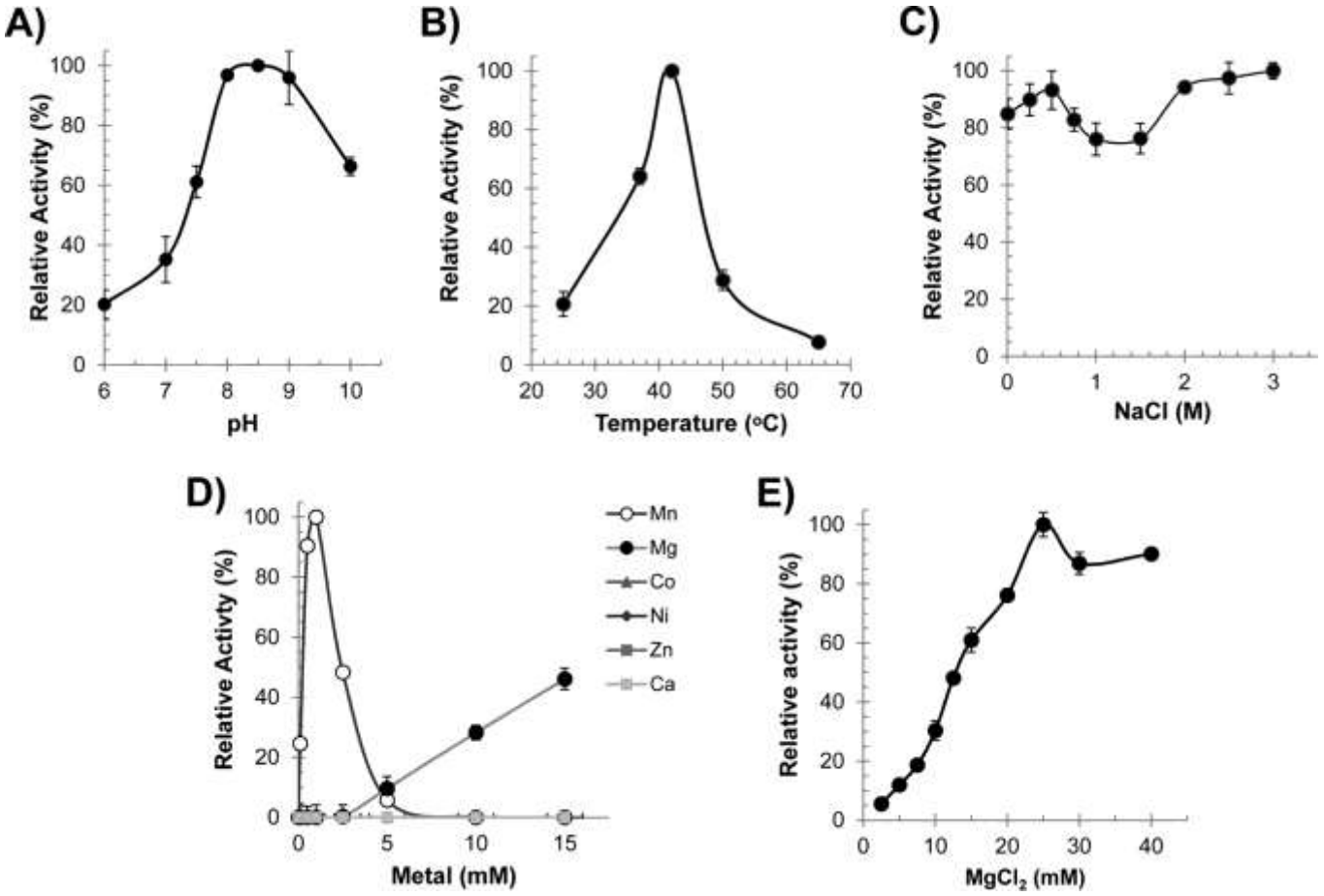
McMillan, Hepowit, & Maupin-Furlow (2016) characterized the enzymatic properties of inorganic pyrophosphatase in *Haloferax volcanii* (HvPPA), a halophile isolated from the Dead Sea. Inorganic pyrophosphatase mediates the following reaction:

$$PP_i + H_2O \rightarrow 2P_i + 2H^+ \qquad \Delta G^{\circ f} = -19.2\ kJ/mol$$

The researchers carried out a series of experiments to identify optimum conditions for five different environments: pH, temperature, salt, metal ion, and magnesium ions. In experiments A to C, HvPPA was equilibrated for 10 minutes at the pH, temperature, and NaCl concentrations before 0.25 mM $PP_i$ was added.

Each experiment was carried out according to the conditions in the table below:

| Group | A | B | C | D | E |
|---|---|---|---|---|---|
| pH | IV | 8 | 8 | 8 | 8 |
| Temp. | 42°C | IV | 42°C | 42°C | 42°C |
| NaCl | 3M | 3M | IV | 2M | 2M |
| $MgCl_2$ | 2.5mM | 2.5mM | 2.5 mM | IV | IV |




Data Analysis

Identify the optimum condition in each of the 5 graphs.

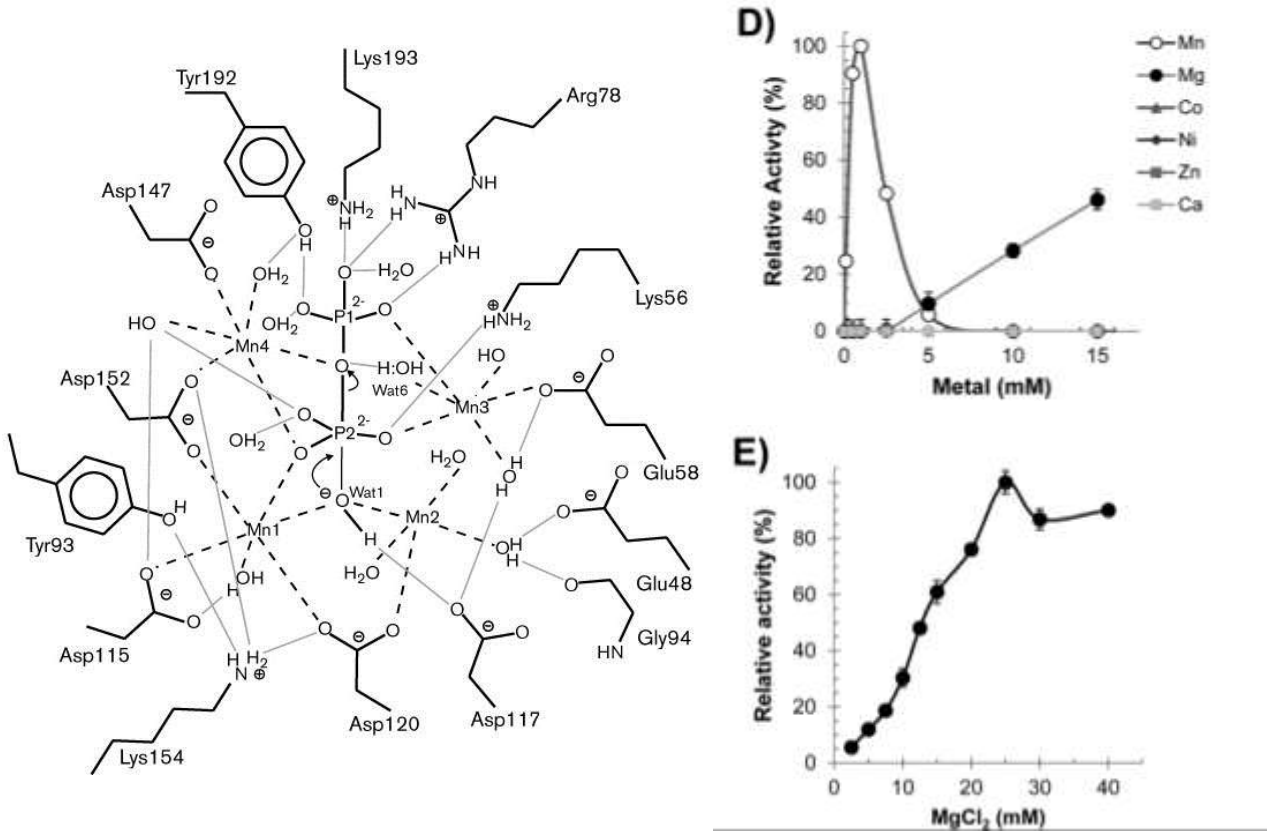
| Group | A | B | C | D | E |
|---|---|---|---|---|---|
| Condition | | | | | |

Predict the effects on the structure of the enzyme at temperatures and pH greater than 65°C and pH 10.

Chemically and thermodynamically speaking, explain why enzyme activity is low, but not the lowest, at 25°C.

Predict how the breakdown of inorganic pyrophosphate would be impacted without functional inorganic pyrophosphatase.

Look at the enzyme active site from the previous activity. Use the model to explain why activity increases with increasing metal ion and $MgCl_2$ concentrations.




Propose ONE possible control treatment for each of the five experiments, and describe how each control treatment would increase the validity of the results.

Enzymatic Activity Graphical Analysis

McMillan, Hepowit, & Maupin-Furlow (2016) characterized the enzymatic properties of inorganic pyrophosphatase in *Haloferax volcanii* (HvPPA), a halophile isolated from the Dead Sea. Inorganic pyrophosphatase mediates the following reaction:

$$PP_i + H_2O \rightarrow 2P_i + 2H^+ \qquad \Delta G^{\circ f} = -19.2\ kJ/mol$$

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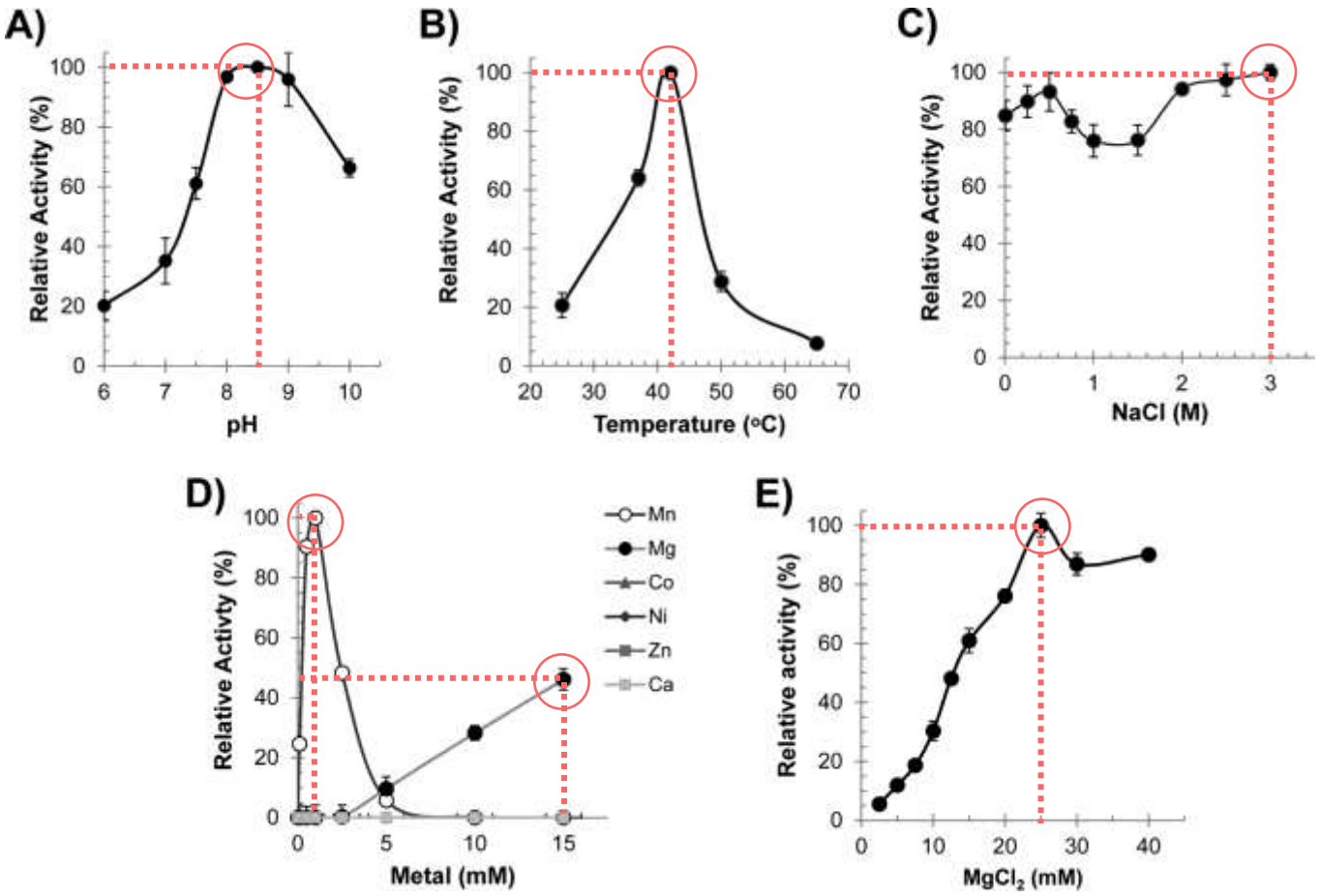
Each experiment was carried out according to the conditions in the table below:

| Group | A | B | C | D | E |
|---|---|---|---|---|---|
| pH | IV | 8 | 8 | 8 | 8 |
| Temp. | 42°C | IV | 42°C | 42°C | 42°C |
| NaCl | 3M | 3M | IV | 2M | 2M |
| $MgCl_2$ | 2.5mM | 2.5mM | 2.5 mM | IV | IV |




Data Analysis

Identify the optimum condition in each of the 5 graphs.

| Group | A | B | C | D | E |
|---|---|---|---|---|---|
| Condition | pH 8.5 | 42°C | 3M NaCl | 1 mM Mn<br>15 mM Mg | 25 mM<br>$MgCl_2$ |

Predict the effects on the structure of the enzyme at temperatures and pH greater than 65°C and pH 10.

At temperatures greater than 65°C and pH 10, the structure of the enzyme likely denatures. Intermolecular interactions, especially at the active site, may be disrupted by the lessening of H+

ions. Furthermore, because intermolecular forces are weaker than covalent bonds, higher temperatures can lead to the loss of secondary, tertiary, and quaternary structure.

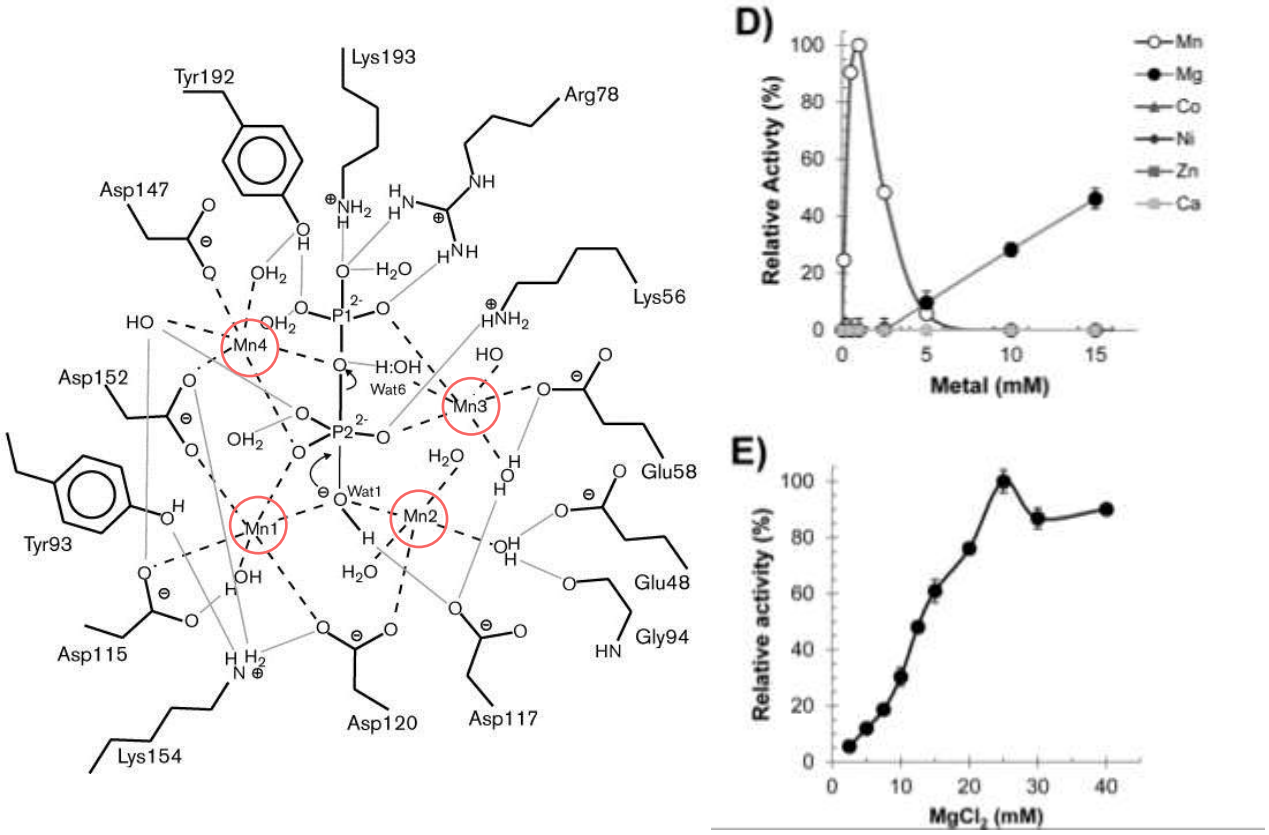
Chemically and thermodynamically speaking, explain why enzyme activity is low, but not the lowest, at 25°C.

At lower temperatures, molecules move around less which decreases the likelihood that a substrate and enzyme would interact. Also, macromolecules can become less flexible at lower temperatures. Because active sites and enzymes require movement to change conformation, activity lessens with lower temperatures. Activity isn't as low at lower temperatures as they are at higher temperatures because the added energy to the system leads to denaturing of the proteins.

Predict how the breakdown of inorganic pyrophosphate would be impacted without functional inorganic pyrophosphatase.

Because the Gibb's free energy value is negative ($\Delta G^{\circ}{}_{f}$=-19.2 kJ/mol), the breakdown of inorganic pyrophosphate into inorganic phosphate is spontaneous and will occur naturally without the help of an enzyme. It likely occurs much slower in nature without an enzyme than with one.

Look at the enzyme active site from the previous activity. Use the model to explain why activity increases with increasing metal ion and $MgCl_2$ concentrations.




In the proposed active site mechanism, there are at least four metal ions (circled above) involved in stabilizing the catalysis of inorganic pyrophosphate. In order for HvPPA to function, it needs multiple Mn and Mg ions to function. Consequently, increasing metal ion concentration for Mn and Mg lead to increasing activity because it provides more ions in solution to facilitate catalysis.

Propose ONE possible control treatment for each of the five experiments, and describe how each control treatment would increase the validity of the results.

A: A sample without functional HvPPA enzyme to show that there isn't enzymatic activity in the environments provided.

B: A sample held at 42°C because 42°C is the optimum growth temperature for *H. volcanii*.

C: A sample held at 0 M of NaCl to confirm that salt is necessary.

D: A sample without $PP_i$ to confirm that $PP_i$ is the only substrate in solution for the reaction.

E: A sample without $MgCl_2$ to confirm that $MgCl_2$ is necessary.

[Multiple answers can be acceptable. This gives a diversity of answers that may or may not be acceptable for each group.]

# Lesson 3 & 4: Finding Similarities

Investigating evolutionary relationships at levels higher than DNA or protein sequences

## LESSON SUMMARY:

Some gold standards for measuring evolutionary relatedness is looking at nucleotide and amino acid sequence alignments. This lesson reveals another method of showing evolutionary relatedness – protein structure alignment. Students will BLAST nucleotide and amino acid sequences for the inorganic pyrophosphatase (PPA) of five species: *H. volcanii*, *P. furiosus*, *E. coli*, *S. cerevisiae*, and *H. sapiens*. After measuring evolutionary relatedness, they will spend an additional day modeling 3D protein structures of PPA in each species and level of similarity between each.

STANDARDS:

| AP Biology | NGSS | NGSSS |
|---|---|---|
| E.K.1.B.2 | HS-LS1-1 | SC.912.N.3.5 |
| Science Practice 1 | HS-LS4-1 | SC.912.L.15.4 |
| | | SC.912.L.15.6 |
| | | SC.912.L.18.4 |

KEY QUESTION(S):

How does pyrophosphatase in *H. volcanii* compare with other organisms in the tree of life?

Can similarities exist when amino acids and nucleotide sequences differ greatly?

LEARNING OBJECTIVES:

The student will be able to...

1. Compare 3D structure of proteins using modeling software.
2. Create cladograms and data tables showing evolutionary relatedness.

OVERALL TIME ESTIMATE:

Advanced Preparation: 60-120 minutes. Time needed to install Chimera on each classroom/student computer will vary depending on the computer, but each installation takes <5 minutes. It's advisable to provide the sequences and Protein Data Bank (PDB) files on an online course management system or

preload them on each computer ahead of the lesson. Copies of the Sequence and Structural Homology of Inorganic Pyrophosphatase should also be printed (1 per pair). The majority of the time should be invested by the teacher to explore Chimera and NCBI BLAST, become familiar with its functions, and predict potential pitfalls unique to his/her own classes.

Lesson: 100 minutes (2 50-minute classes)

MATERIALS:

*Essential*

UCSF's Chimera protein modeling software

Inorganic pyrophosphatase (PPA) PDB files

PPA nucleotide sequences for *H. volcanii*, *P. furiosus*, *E. coli*, *S. cerevisiae,* and *H. sapiens*

PPA amino acid sequences for *H. volcanii*, *P. furiosus*, *E. coli*, *S. cerevisiae*, and *H. sapiens*

Sequence and Structural Homology of Inorganic Pyrophosphatase Handout

## BACKGROUND INFORMATION:

The Basic Local Alignment Search Tool was produced by the National Center for Biotechnology Information in 1990 (Madden, 2013). The tool has progressed to have stronger computational algorithms and more options for analysis. Coupled with the growing number of nucleotide and amino acid sequences collected, BLAST is a powerful bioinformatics tool and often the start of many researchers genomic and proteomic work. BLAST operates by using algorithms to identify similar or identical sequences between a query string and reference sequence. The confidence in that match is then statistically determined and reported both numerically and graphically for the user. Sequences that have an E-value $1 \times 10^{-4}$ are said to be homologous or related to the reference sequence (Lessick, 2010). Sequence identity (instances where aligned sequences are identical) are also graphically shown with the two sequences aligned on top of one another. Identical sequences mean the same nucleotide or amino acid is found in the same location between the two sequences. For amino acid sequences, positive matches (+) indicate chemically similar amino acids occupy that position (Lessick, 2010). Given impossibility of physically comparing homology between two distinctly different organisms, such as felids and Gram-negative bacteria, BLAST is a useful tool in identifying less visible similarities through nucleotide and amino acid sequence alignments.

Chimera, on the other hand, helps to provide further insight about structural similarities when sequence similarities aren't evident. Chimera is a molecular modeling software developed by the University of California-San Francisco in 2004 (Petterson et al., 2004). For this lesson, the functions of focus are Chimera's ability to model Protein Data Bank files for visualization and structural comparison. Molecules can be visualized in ball-and-stick, space-filling, and ribbon structures. Specific amino acids, atoms, side chains, or higher levels of protein structure can be selected and contrasted against the rest of the molecule through the various selection tools available. This lesson focuses on the MatchAlign tool that takes two protein models and superimposes them together. From the superimposition, a root mean square difference in amino acid locations is calculated to determine structural homology. Four of the five sequences used in this lesson are from known protein structures accessible by the UniProt and Protein Data Bank databases. The *H. volcanii* protein structure has not yet been elucidated, but in this lesson, it a model was created using Protein Homology/analogY Recognition Engine V2.0 (Phyre$^2$) (Kelley, Mezulis, Yates, Wass, & Sternberg, 2015). The engine uses existing protein structures to model inputted sequences of not-yet-elucidated structures. For *H. volcanii*, the model was based off the *Arabidopsis thaliana* inorganic pyrophosphatase, which may bias the results towards similarity to eukaryotic origins.

## REQUIRED STUDENT BACKGROUND KNOWLEDGE:

Students should understand evolutionary relatedness, whether through sequences or structures. Students also know how to represent those similarities and differences through tables, cladograms, and phylogenetic trees.

Students should have prior knowledge about protein structure, especially secondary and tertiary structure. Chimera will expose students to 3D models of proteins, so the ability to recognize structural features, especially alpha-helices and beta-pleated sheets, will help them comprehend structural homology better.

## ADVANCE PREPARATION:

The University of California – San Francisco's Chimera protein modeling software should be installed ahead of time on each computer used. At least an hour should be spent by the teacher familiarizing him

or herself with the software. Upload the sequences and Protein Data Bank (PDB) files on an online course management system or preload them on each computer ahead of the lesson.

Print copies of the Sequence and Structural Homology of Inorganic Pyrophosphatase (1 per pair).

NCBI's BLAST website can be a very powerful tool, but also confusing given the number of features and options that can be manipulated. It is highly recommended to walk through a video tutorial and the student section of this lesson before implementing it in the classroom.

## VOCABULARY:

BLAST: Basic Local Alignment Search Tool, a tool using computer algorithms to regions of similarity among nucleotide or amino acid sequences.

Cladogram: Graphical representation of evolutionary relatedness among species.

Homology: Measure of the relatedness or similarity between two items, such as nucleotide sequences, physical structures, proteins, amino acid sequences, etc.

PDB: Protein Data Bank, the repository by the National Center for Biotechnology Information, with structures and information of known proteins.

Phylogenetic tree: Graphical representation of evolutionary relatedness among species, similar to a cladogram, but contains more information about the degree of change and time needed for the change to occur.

RMSD: Root mean square difference is a statistical function that calculates the distances of amino acid residues between two superimposed structures. The function provides a numerical output that is interpreted as the confidence in structural alignment. The lower the value, the more aligned the two structures are. Values <2.000 indicate strong alignment.

## PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES:

*Have a BLAST* (50 minutes)

1. [5 minutes] Explain to the students that they will be resuming their investigation of inorganic pyrophosphatase by exploring the homology of the enzyme among five species: three microbes, yeast, and humans. Fun fact: *Pyrococcus furiosus* was used only because of its resemblance to my last name.
    a. Ask students the following questions:
        i. "How we can determine evolutionary relatedness between these organisms?"
        ii. "At the molecular level, what can help use quantity and describe those similarities?"
        iii. "Why can't we use physical structures to describe homology in this case?"
    b. Elicit thoughts on potential similarity between the five species with inorganic phosphatase and have them defend their viewpoints with evidence.
2. [10 minutes] Arrange students in pairs at computers or tablets and have students watch the following video during class or for homework before class starts: Johns Hopkins University – NCBI BLAST Tutorial. The teacher can also walk the students through the first BLAST alignment search with little impact on student experience (they will be doing numerous BLAST alignments).
3. [25-30 minutes] Students will first identify common names of the species and the domain in which they are found. Students will then follow the instructions on the assignment in order to tabulate the homology between the five species for both nucleotides and amino acids. Walk around the room providing feedback and answering any questions the students may have. Below are some sample questions the students might have or you might want to ask (sample answers are in parentheses):
    - Why is there so little identity between the nucleotide sequences?
        - (Intronic regions in the case of *H. sapiens*, true lack of genetic between the five drastically different species, especially because of the environments *H. volcanii* and *P. furiosus* inhabit.).
    - What might be a better alternative to nucleotide BLAST?

        - (mRNA or amino acid sequences because they have introns removed in eukaryotes and have less redundancy [64 codons for 20 amino acids]).
    - What is the extent of similarity between yeast and humans? Is it the whole sequence or only a portion?
        - (It's <100 nucleotides compared to the sequence that's nearly 1000 nucleotides for yeast and 25000 for humans).
    - Why is the human sequence so much longer than the other four species?
        - (Introns. Only about 1/25$^{th}$ of the entire sequence is coding.)
    - Predict why protein sequences between species X and Y are [insert value].
        - (Answers will vary, but likely stem from environment and domain.)
4. [5-10 minutes] Students should answer the first two questions before leaving class or entering class the next day.

*Modeling with Chimera* (50 minutes)

1. [5 minutes] Have the class hypothesize whether there should be any homology between the different PPAs.
    a. Encourage students to use their evidence from the previous day to support or refute their claims.
    b. Given the ubiquity of nucleotide triphosphates and their use as energy sources within living organisms, the need for inorganic pyrophosphatase should hypothetically show homology.
2. [35 minutes] Arrange the students again in pairs with computers (tablets will not work for this lesson) and have them watch the following video: RCSB Protein Data Bank – UCSF Chimera: Structure Comparisons. Instead of having the students watch the video, the software could be modeled in the next step below.
3. Load Chimera and demonstrate protein visualization with the software, showing some or all of the following functions:
    a. Model the rotational keys needed to orient the protein in the proper viewing area.
    b. Different model views (ball-and-stick, ribbon structure, and space-filling models).
    c. Show how to select specific amino acids, their R groups, or the sequences.
    d. Remind students about protein structure, specifically how alpha-helices and beta-pleated sheets form.

e. Walk through one alignment comparison and explain how the RMSD value can reveal information about structural homology.

4. Allow the students to work in pairs on filling out protein structural homology table and have them answer the rest of the questions on the assignment. Walk around the class providing feedback and answering/asking probing questions, such as the following below:
    - How does your RMSD data compare to the sequence alignments? How do their alignments visually compare? Why might there be a difference?
    - Where is the active site on each of the PPAs?
    - What portion of the sequence do you think is retaining homology? The outer portion? The inner portion? The active site?

## ASSESSMENT SUGGESTIONS:

*Sequence and Structural Homology of Inorganic Pyrophosphatase*

Formatively assess students' understanding of various levels of homology by walking around the classroom and observing their use of the BLAST and Chimera tools. Because of the open-ended nature of the assignment, student understanding can be assessed by either collecting the assignment or leading a classroom discussion. Students likely will have different cladogram interpretations and the opportunity to present the information in a whole-class setting may lead to some revision of knowledge and interesting discussions.

## MODIFICATIONS or EXTENSIONS:

To tie in with the previous lessons' focus on functional groups and enzyme active sites, students can explore the enzyme's structure using Chimera. *H. volcanii*'s active site, according to McMillan, Hepowit, & Maupin-Furlow (2016) includes the following amino acid residues: K31, E33, R45, Y57, D67, D69, D72, D99, D104, K142, E145, and K148. Displaying those R-groups Chimera is possible using the Atom Specification tutorial.

Students can also change the views from ribbon models to ball-and-stick models or space-filling models using the Molecule Display tutorial.

AP Investigation 3 has an inquiry activity with hypothesis testing and revision based on fossil remains and four collected DNA samples. The late Kim Foglia created a more scaffolded and guided version titled Lab 21 – Using Bioinformatics to Investigate Evolutionary Relationships; Have a BLAST!

## RESOURCES/REFERENCES:


The College Board. (2012). *AP Biology investigative labs: An inquiry-based approach.* New York, NY: The College Board.

Kelley, L. A., Mezulis, S., Yates, C. M., Wass, M. N., & Sternberg, M. J. E. (2015). The Phyre2 web portal for protein modeling, prediction and analysis. *Nature Protocols*, *10*, 845-858. doi: 10.1038/nprot.2015.053

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Madden, T. (2013). The BLAST sequence analysis tool . In *NCBI Handbook*. Bethesda, MD: National Center for Biotechnology Information.

McMillan, L. J., Hepowit, N. L., & Maupin-Furlow, J. A. (2016). Archaeal inorganic pyrophosphatase displays robust activity under high-salt conditions and in organic solvents. *Applied and Environmental Microbiology*, *82*(2), 538-548.

Pettersen, E. F., Goddard, T.D., Huang, C.C., Couch, G.S., Greenblatt, D. M., Meng, E. C., & Ferrin, T.E. (2004). UCSF Chimera - a visualization system for exploratory research and analysis. *Journal of Computational Chemistry*, *25*(13), 1605 - 1612.


*Chimera Tutorials*

The following links are written tutorials by UCSF regarding the Chimera modeling software. Start here if you prefer text. For those who prefer videos, skip to the next set of links below.

UCSF - Getting Started

UCSF - Match-Align

The following links are YouTube video tutorials by RCSB Protein Data Bank that visually walks through basic use of Chimera for this lesson. You may want to post these ahead of time on an online course management system so students are familiar with the software before they begin.

RCSB Protein Data Bank – UCSF Chimera: Basics

RCSB Protein Data Bank – UCSF Chimera: Structure Analysis

RCSB Protein Data Bank – UCSF Chimera: Structure Comparisons

*NCBI BLAST Tutorials*

The following links provide increasing levels of understanding related to using the BLAST tool. For conceptual understanding, students will be best served by the first link. For a full walkthrough explaining the steps of NCBI BLAST searches, the second link is much more helpful; however, because we are aligning two sequences, some of the steps do not apply.

Bozeman Science – Comparing DNA Sequences

Johns Hopkins University – NCBI BLAST Tutorial

NCBI – Webinar: A Practical Guide to NCBI BLAST

*Supplemental Videos*

The following videos help provide students with greater understanding of cladograms, phylogenetic trees, and the basis of evolutionary relatedness.

Bozeman Science - Cladograms

# Sequence and Structural Homology of Inorganic Pyrophosphatase

Because the genetic code links life on Earth, evolutionary relatedness can be determined through aligning DNA and even amino acid sequences. An often overlooked aspect is that structures of proteins can share homology as well. In this activity, you will BLAST nucleotide and amino acid sequences of five species to determine the relatedness of inorganic pyrophosphatase. After BLASTing, you'll use Chimera, a protein modeling software, in order to determine how each sequence aligns compared to the others.

Table 1: Nucleotide Sequence Identity

| Scientific Name | Common Name (if available) | Domain |
|---|---|---|
| *Haloferax volcanii* | | |
| *Pyrococcus furiosus* | | |
| *Escherichia coli* | | |
| *Saccharomyces cerevisiae* | | |
| *Homo sapiens* | | |

## Nucleotide BLAST

Step 1: Go to NCBI Standard Nucleotide BLAST. Check the box for "Align two or more sequences."

Step 2: Paste the first PPA nucleotide FASTA sequence into the "Enter Query Sequence" top box labeled "Enter accession number(s), gi(s), or FASTA sequence(s)" and the second PPA sequence in the "Enter Subject Sequence" bottom box labeled "Enter accession number(s), gi(s), or FASTA sequence(s)."

Step 3: Under "Program Selection," click the "More dissimilar sequences (discontinuous megablast)" option.

Step 4: Click "BLAST."

Step 5: Look at identity ("Ident") under the results to determine sequence portion with the greatest homology. Complete the table below with one-to-one comparisons.

Table 2: Nucleotide Sequence Identity

| Species | *H. volcanii* | *P. furiosus* | *E. coli* | *S. cerevisiae* | *H. sapiens* |
|---|---|---|---|---|---|
| *H. volcanii* | | | | | |
| *P. furiosus* | | | | | |
| *E. coli* | | | | | |
| *S. cerevisiae* | | | | | |
| *H. sapiens* | | | | | |

NSS: No significant similarity.

- : Indicates it is a comparison of the same organism.

## Protein BLAST

Step 1: Go to NCBI Standard Protein BLAST. Check the box for "Align two or more sequences."

Step 2: Paste the first PPA protein FASTA sequence into the "Enter Query Sequence" top box labeled "Enter accession number(s), gi(s), or FASTA sequence(s)" and the second PPA sequence in the "Enter Subject Sequence" bottom box labeled "Enter accession number(s), gi(s), or FASTA sequence(s)."

Step 3: Under "Program Selection," click the "blastp (protein-protein BLAST)" option.

Step 4: Click "BLAST."

Step 5: Look at identity ("Ident") under the results to determine sequence portion with the greatest homology. Complete the table below with one-to-one comparisons.

Table 3: Protein Sequence Identity

| Species | *H. volcanii* | *P. furiosus* | *E. coli* | *S. cerevisiae* | *H. sapiens* |
|---|---|---|---|---|---|
| *H. volcanii* | | | | | |
| *P. furiosus* | | | | | |
| *E. coli* | | | | | |
| *S. cerevisiae* | | | | | |
| *H. sapiens* | | | | | |

NSS: No significant similarity.

- : Indicates it is a comparison of the same organism.

## 3D Protein Modeling

Step 1: Open Chimera, click File -> Open and open the first PDB model.

Step 2: Click File -> Open again, and open the second PDB protein model.

Step 3: Click Tools -> Structural Comparison -> MatchMaker. Select one model as the reference structure and the other model as the structure to match, and click "OK."

Step 4: Look at the bottom of the screen at the RMSD value, specifically "Across all pairs" and record below. If the value disappears, click Favorites -> Reply Log to get the information.

Table 4: Root Mean Square Difference (RMSD) Between Protein Structures

| Species | *H. volcanii* | *P. furiosus* | *E. coli* | *S. cerevisiae* | *H. sapiens* |
|---|---|---|---|---|---|
| *H. volcanii* | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| *P. furiosus* | | | | | |
| *E. coli* | | | | | |
| *S. cerevisiae* | | | | | |
| *H. sapiens* | | | | | |

NSS: No significant similarity.

- : Indicates it is a comparison of the same organism.

P: pruned (number of amino acids pruned in parentheses)

A: all (number of amino acids total compared in parentheses)

Analysis

Construct a cladogram using the amino sequence homology data.

Predict why there are so few similar sequences among the nucleotide BLAST searches and compare to why there is greater sequence homology among amino acids.

Infer the difference between the pruned amino acid sequences and the “all amino acids” compared in Chimera.

Explain why there is minimal sequence homology with amino acids, but greater homology structurally speaking. Predict the conserved portion of the protein in your answer.

A RMSD value less than 2.0 indicates strong similarity between structures. Justify the importance of inorganic pyrophosphatase in life based on RMSD values.

# Sequence and Structural Homology of Inorganic Pyrophosphatase

Because the genetic code links life on Earth, evolutionary relatedness can be determined through aligning DNA and even amino acid sequences. An often overlooked aspect is that structures of proteins can share homology as well. In this activity, you will BLAST nucleotide and amino acid sequences of five species to determine the relatedness of inorganic pyrophosphatase. After BLASTing, you'll use Chimera, a protein modeling software, in order to determine how each sequence aligns compared to the others.

Table 1: Nucleotide Sequence Identity

| Scientific Name | Common Name (if available) | Domain |
|---|---|---|
| *Haloferax volcanii* | NA | Archaea |
| *Pyrococcus furiosus* | NA | Archaea |
| *Escherichia coli* | NA | Bacteria |
| *Saccharomyces cerevisiae* | Yeast | Eukarya |
| *Homo sapiens* | Humans | Eukarya |

## Nucleotide BLAST

Step 1: Go to NCBI Standard Nucleotide BLAST. Check the box for "Align two or more sequences."

Step 2: Paste the first PPA nucleotide FASTA sequence into the "Enter Query Sequence" top box labeled "Enter accession number(s), gi(s), or FASTA sequence(s)" and the second PPA sequence in the "Enter Subject Sequence" bottom box labeled "Enter accession number(s), gi(s), or FASTA sequence(s)."

Step 3: Under "Program Selection," click the "More dissimilar sequences (discontinuous megablast)" option.

Step 4: Click "BLAST."

Step 5: Look at identity ("Ident") under the results to determine sequence portion with the greatest homology. Complete the table below with one-to-one comparisons.

Table 2: Nucleotide Sequence Identity

| Species | *H. volcanii* | *P. furiosus* | *E. coli* | *S. cerevisiae* | *H. sapiens* |
|---|---|---|---|---|---|
| *H. volcanii* | - | NSS | NSS | NSS | NSS |
| *P. furiosus* | | - | NSS | NSS | NSS |
| *E. coli* | | | - | NSS | NSS |
| *S. cerevisiae* | | | | - | 75% |
| *H. sapiens* | | | | | - |

NSS: No significant similarity.

- : Indicates it is a comparison of the same organism.

## Protein BLAST

Step 1: Go to NCBI Standard Protein BLAST. Check the box for "Align two or more sequences."

Step 2: Paste the first PPA protein FASTA sequence into the "Enter Query Sequence" top box labeled "Enter accession number(s), gi(s), or FASTA sequence(s)" and the second PPA sequence in the "Enter Subject Sequence" bottom box labeled "Enter accession number(s), gi(s), or FASTA sequence(s)."

Step 3: Under "Program Selection," click the "blastp (protein-protein BLAST)" option.

Step 4: Click "BLAST."

Step 5: Look at identity ("Ident") under the results to determine sequence portion with the greatest homology. Complete the table below with one-to-one comparisons.

Table 3: Protein Sequence Identity

| Species | *H. volcanii* | *P. furiosus* | *E. coli* | *S. cerevisiae* | *H. sapiens* |
|---|---|---|---|---|---|
| *H. volcanii* | - | 49% | 40% | 29% | 30% |
| *P. furiosus* | | - | 43% | 27% | NSS |
| *E. coli* | | | - | 31% | 27% |
| *S. cerevisiae* | | | | - | 53% |
| *H. sapiens* | | | | | - |

NSS: No significant similarity.

- : Indicates it is a comparison of the same organism.

## 3D Protein Modeling

Step 1: Open Chimera, click File -> Open and open the first PDB model.

Step 2: Click File -> Open again, and open the second PDB protein model.

Step 3: Click Tools -> Structural Comparison -> MatchMaker. Select one model as the reference structure and the other model as the structure to match, and click "OK."

Step 4: Look at the bottom of the screen at the RMSD value, specifically "Across all pairs" and record below. If the value disappears, click Favorites -> Reply Log to get the information.

Table 4: Root Mean Square Difference (RMSD) Between Protein Structures

| Species | *H. volcanii* | *P. furiosus* | *E. coli* | *S. cerevisiae* | *H. sapiens* |
|---|---|---|---|---|---|
| *H. volcanii* | - | P: 0.772 (165) | P: 0.840 (150) | P: 0.891 (134) | P: 0.999 (131) |

| | | | | | |
|---|---|---|---|---|---|
| | | A: 0.938 (172) | A: 1.747 (172) | A: 4.837 (166) | A: 4.452 (173) |
| *P. furiosus* | | - | P: 0.715 (156)<br>A: 1.345 (167) | P: 0.856 (130)<br>A: 4.701 (166) | P: 0.913 (130)<br>A: 3.272 (165) |
| *E. coli* | | | - | P: 0.826 (99)<br>A: 5.612 (170) | P: 0.934 (94)<br>A: 6.552 (166) |
| *S. cerevisiae* | | | | - | P: 0.868 (227)<br>A: 2.104 (273) |
| *H. sapiens* | | | | | - |

NSS: No significant similarity.

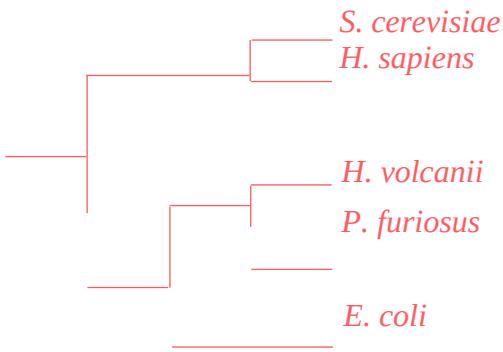
- : Indicates it is a comparison of the same organism.

P: pruned (number of amino acids pruned in parentheses)

A: all (number of amino acids total compared in parentheses)

Analysis

Construct a cladogram using the amino sequence homology data.




Predict why there are so few similar sequences among the nucleotide BLAST searches and compare to why there is greater sequence homology among amino acids.

Nucleotide sequences involve introns in eukaryotes, which may lead to less alignment, but more importantly there is redundancy built into the genetic code. There are 64 codons for 20 amino acid/start/stop sequences. One codon might code for lysine, while a different codon might code for the same amino acid. Despite coding for the same amino acids, accumulation of differences might build up enough to lack significant identity. Furthermore, similar amino acids can have similar functions and be substituted with minimal effect on final protein structure or function.

Infer the difference between the pruned amino acid sequences and the “all amino acids” compared in Chimera.

Pruned amino acids are those amino acids remaining after those deemed to be outside the range of best fit (<2.0 RMSD) are removed. Consequently, the pruned RMSD will always be closer to 0 than the RMSD for the entire structure. It is still helpful to see how many amino acids were retained after pruning compared to the entire sequence length in order to see how much of the protein fits well.

Explain why there is minimal sequence homology with amino acids, but greater homology structurally speaking. Predict the conserved portion of the protein in your answer.

There are sets of amino acids with R-groups that have similar chemical properties. When folding, they retain similar structural details despite having different identies. The conserved portion is likely the active site.

A RMSD value less than 2.0 indicates strong similarity between structures. Justify the importance of inorganic pyrophosphatase in life based on RMSD values.

In each protein structure, significant portions of the polypeptide are retained after pruning. The smallest retained amount is between *E. coli* and humans with 94/166 retained. Likely the retained portion with the lowest RMSD is the active site, as even in the halophile *H. volcanii*, the active site is still highly conserved (McMillan, Hepowit, & Maupin-Furlow, 2016). The most significant differences in structure are the exterior of the protein where each organism has specific environmental conditions to contend with. Consequently, PPA is important for life based on the low RMSD values.

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*Future Lessons & Extension Resources*

Kiljunen, S., Pajunen, M. I., Dilks, K., Storf, S., Pohschroder, M., & Savilahti, H. (2014). Generation of comprehensive transposon insertion mutant library for the model archaeon, *Haloferax volcanii*, and its use for gene discovery. *BMC Biology*, *12*(103). doi: 10.1186/s12915-014-0103-3

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CONTENT ASSESSMENT: Provide a pre/post test for the curriculum, and provide an answer key for teachers.

# Content Area Expert Evaluation

Thank you for reviewing *Briny (Archaea)bacteria* curriculum. Please review the entire curriculum and then complete the questions below. You are welcome to insert comments directly in the manual as well as in the section provided below. Comments and suggestions are greatly appreciated!

Reviewer name: _____

Date reviewed: _____ Email: _____

Employer: _____Department/Division: _____

Job title: _____

Part I: For each item below, please indicate your response to each question as it relates to the curriculum overall by circling Yes (Y), No (N), or Undecided (U).

| | | | |
|---|---|---|---|
| Is the science content in the curriculum accurate? | Y | N | U |
| Is the science content in the curriculum current? | Y | N | U |
| Is the science content in the curriculum important for science literacy? | Y | N | U |
| Is the content in the curriculum related to major biological concepts? (e.g., molecular genetics) | Y | N | U |
| Is the content coverage in the curriculum thorough and complete? | Y | N | U |
| Are potential misconceptions adequately addressed? | Y | N | U |

| | | | |
|---|---|---|---|
| Is the content in the curriculum properly sequenced for a novice? | Y | N | U |
| Are there additional concepts that should be included? (If yes, please elaborate below.) | Y | N | U |

Part II: Please include below any comments or suggestions about the curriculum.

General comments about the overall curriculum:

Comments regarding individual lessons:

| | |
|---|---|
| Lesson 1:<br>Pretty & Pink | |
| Lesson 2:<br>Keeping Active | |
| Lessons 3 & 4:<br>Finding Similarities | |
| Future Lessons | |

| | |
|---|---|
| Extensions | |

# Teacher Feedback Form

Thank you for reviewing *Briny (Archaea)bacteria* curriculum. Please review the entire curriculum and then complete the questions below. You are welcome to insert comments directly in the manual as well as in the section provided below. Comments and suggestions are greatly appreciated!

Teacher name: _____

Subjects taught: _____ Grade levels taught: _____

School: _____ Email: _____

Part I: Evaluation of the entire curriculum.

Section A: For each item below, please indicate your response to each question as it relates to the curriculum overall by marking Strongly Agree (SA), Agree (A), Undecided (U), Disagree (D), or Strongly Disagree (SD).

| | SA | A | U | D | SD |
|---|---|---|---|---|---|
| 1. Are the experimental procedures appropriate for your stude | | | | | |
| 2. Are the topics addressed important for your course objectiv | | | | | |
| 3. Are the topics addressed relevant to your students' lives? | | | | | |
| 4. Are the topics addressed interesting to your students? | | | | | |
| 5. Is the depth of coverage of topics appropriate? | | | | | |
| 6. Is the overall quality of the curriculum satisfactory? | | | | | |
| 7. Is the content in the curriculum properly sequenced? | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| 8. Is the content in the curriculum adaptable for a range of stu ability levels? | | | | | |

Section B: Please provide additional comments pertaining to the curriculumoverall.

Are there any topics/sections that should be added to/deleted from the curriculum? If so, please explain.

Additional comments:

Part II: Evaluation of individual lessons.

Section A: For each question below, please indicate your response for each specific lesson by marking High (H), Moderate (M), Low (L), or Not Applicable (NA).

| | Lesson 1: Pretty and Pink | | | | Lesson 2: Keeping Active | | | | Lessons 3 & 4: Finding Similarities | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | H | M | L | NA | H | M | L | NA | H | M | L | NA |
| 1. Is the amount of teacher background information sufficient? | | | | | | | | | | | | |
| 2. Do the time estimates seem reasonable? | | | | | | | | | | | | |
| 3. Is the amount of advance preparation reasonable? | | | | | | | | | | | | |
| 4. Is the procedure clearly stated? | | | | | | | | | | | | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 5. Is the suggested assessment sufficient? | | | | | | | | | | | | |

Section B: Please provide additional comments pertaining to each specific lesson

| Lesson | Are there any topics, sections, or resources that should be added or deleted? If so, please explain. | Additional Comments |
|---|---|---|
| Lesson 1:<br>Pretty & Pink | | |
| Lesson 2:<br>Keeping Active | | |
| Lessons 3 & 4:<br>Finding Similarities | | |
| Future Lessons | | |
| Extensions | | |

# Student Feedback Form

Name: _____ Date: _____ Grade Level: _____ Sex: M F

School Name: _____ Teacher's Name: _____ Subject: _____

Part II:

Section A: Evaluation of individual lessons.

| | Lesson 1: Pretty and Pink | | | | Lesson 2: Keeping Active | | | | Lessons 3 & 4: Finding Similarities | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | H | M | L | NA | H | M | L | NA | H | M | L | NA |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. Is the amount of teacher background information sufficient? | | | | | | | | | | | | |
| 2. Do the time estimates seem reasonable? | | | | | | | | | | | | |
| 3. Is the amount of advance preparation reasonable? | | | | | | | | | | | | |
| 4. Is the procedure clearly stated? | | | | | | | | | | | | |
| 5. Is the suggested assessment sufficient? | | | | | | | | | | | | |

For each question below, please indicate your response for each specific lesson by marking High (H), Moderate (M), Low (L), or Not Applicable (NA).

Section B: Please provide additional comments pertaining to each specific lesson

| Lesson | Are there any topics, sections, or resources that should be added or deleted? If so, please explain. | Additional Comments |
|---|---|---|
| Lesson 1:<br>Pretty & Pink | | |

| | | |
|---|---|---|
| Lesson 2:<br>Keeping Active | | |
| Lessons 3 & 4:<br>Finding Similarities | | |
| Future Lessons | | |
| Extensions | | |