Yellowstone Science

The Mystery of Protein Thermostability

Interview with Dr. John F. Burger

Roosevelt's 1903 Visit

A Ride to the Infernal Regions

Resource information available on the website includes the digital Yellowstone Atlas, a data-rich, visual display of information; the Resource Almanac, a one-page description of why the resource is important and its status and trends; an in-depth overview essay that details the background of the resource; a description of science and management projects; and links to articles, management documents, references, and other pertinent websites.

Science in the Greater Yellowstone

WITH THIS ISSUE, *Yellowstone Science* enters its 15th year in publication. Almost 250 articles on studies concerning the park's natural and cultural resources have been published in that time. The National Park Service has a research mandate and is required by law to use the highest quality science information to support park management decision-making. Yet researchers produce more than 200 papers, manuscripts, books, and book chapters *each year*. Park management is at a huge disadvantage when it comes to absorbing that volume of information. How can the park best collect, summarize, and make science information accessible?

We have been discussing this issue in the Yellowstone Center for Resources, as have many others throughout the National Park Service (NPS). The NPS has created 32 inventory and monitoring networks nationwide, challenged with the responsibility of preparing Vital Signs Monitoring plans. Park vital signs are selected physical, chemical, and biological elements and processes of park ecosystems that represent the overall health or condition of the park, known or hypothesized effects of stressors, or elements that have important human values. Vital signs monitoring is a key component in the Service's strategy to provide scientific data and information needed for management decision-making and education as well as to understand and measure performance regarding the condition of watersheds, landscapes, marine resources, and biological communities.

The NPS has also developed Research Learning Centers to facilitate research efforts and provide educational opportunities. They are places where science and education come together to preserve and protect areas of national significance. They have been designed as public-private partnerships that involve a wide range of people and organizations including researchers, universities, educators, and community groups.

Working together, the three park units in the Greater Yellowstone Inventory and Monitoring Network (Yellowstone and Grand Teton national parks and Bighorn Canyon National Recreation Area) have formed a Greater Yellowstone Science Learning Center, where information on natural and cultural resource topics can be made available. During the last year, with financial support from Canon U.S.A. through the Yellowstone Park Foundation, NPS staff have created a prototype website with new information products for a variety of resource topics.

We are looking for feedback on this new endeavor. Please visit the Greater Yellowstone Science Learning Center website at www.greateryellowstonescience.org, and send us your thoughts. Comments can be sent to Tami_Blackford@nps.gov or PO Box 168, Yellowstone National Park, WY 82190. We appreciate your input, and hope you enjoy this issue of *Yellowstone Science*.

[signature]

www.greateryellowstonescience.org

on the cover:
Grand Prismatic Spring.
NPS Photo by Jim Peaco.

NPS

Old Faithful erupting behind President Gerald Ford, 1976.

FEATURES

DEPARTMENTS

NEWS & NOTES

2007 Winter Elk Count

The Northern Yellowstone Cooperative Wildlife Working Group conducted its annual winter survey of the northern Yellowstone elk population on December 30, 2006. A total of 6,738 elk were counted during good survey conditions. Approximately two-thirds of the observed elk were located within Yellowstone National Park, and one-third was located north of the park boundary. Biologists used three fixed-wing aircraft to count elk through the entire northern range during the one-day survey. The northern Yellowstone elk herd winters between the northeast entrance of Yellowstone National Park and Dome Mountain/Dailey Lake in the Paradise Valley.

This year's count of 6,738 elk was similar to the count of 6,588 elk in March 2006, but significantly lower than the 9,545 elk counted in January 2005. "This decrease in counted elk likely reflects the continuing effects of predation by wolves and other large carnivores, as well as decreased detection of elk within Yellowstone due to anti-predation behaviors such as smaller group sizes, increased dispersion of groups, and increased use of forested habitats, making them more difficult to locate," according to P.J. White, biologist for Yellowstone National Park.

"It appears that elk distribution has changed in recent years with elk numbers north of Yellowstone Park leveling off at between 3,200–4,000 elk, while elk numbers wintering inside the park may be decreasing," according to Tom Lemke, biologist for Montana Fish, Wildlife and Parks (FWP).

"In an effort to reduce hunter mortality on female elk, FWP has reduced the number of antlerless Late Elk Hunt permits over the last several years. For the last two years, only 100 antlerless permits have been issued," said Lemke. "At the current level of harvest, recreational hunting has very little impact on elk numbers in a population of several thousand animals. Hunting has basically been removed as a significant factor regulating northern Yellowstone elk numbers."

The State Elk Plan calls for a winter population objective of 3,000–5,000 elk north of Yellowstone with 2,000–3,000 of those animals wintering on or near the state-owned Dome Mountain Wildlife Management Area (WMA). In the last four years, an estimated total of 3,200–4,000 elk have wintered in the area with 2,100–2,800 elk using the Dome Mountain WMA. By the end of this winter, biologists expect elk numbers north of the park to remain within the management objectives. In contrast, during the late 1990s, 5,300–8,600 elk wintered north of the park with 3,500–4,500 elk in the Dome Mountain area. Wintering such large numbers of elk could lead to long-term habitat decline and increase the likelihood of game damage problems on private land.

"From a winter elk management perspective we are currently meeting State Elk Plan population objectives. The number of elk wintering north of Yellowstone Park has been within State Elk Plan objectives since 2003," added Lemke.

The working group will continue to monitor trends of the northern Yellowstone elk population and evaluate the relative contribution of various components of mortality, including predation, environmental factors, and hunting. The working group was formed in 1983 to cooperatively preserve and protect the long-term integrity of the northern Yellowstone winter range for wildlife species by increasing our scientific knowledge of the species and their habitats, promoting prudent land management activities, and encouraging an interagency approach to answering questions and solving problems. The working group is comprised of resource managers and biologists from Montana Fish, Wildlife and Parks; Yellowstone National Park; Gallatin National Forest; and U.S. Geological Survey–Northern Rocky Mountain Science Center.

John Varley Named Big Sky Institute Director

John Varley, former director of the Yellowstone Center for Resources at Yellowstone National Park, has been named executive director of the Big Sky Institute at Montana State University.

Varley, 65, began a three-year appointment on January 16. He follows Lisa Graumlich who resigned to become director of the School of Natural Resources at the University of Arizona in Tucson.

"We are delighted that John will be bringing his unique combination of gifts and experience to a leadership position at MSU," Provost and Vice President for Academic Affairs David Dooley said. "MSU, in part through its 'University of the Yellowstone' initiative and the Big Sky Institute, is poised to achieve new levels of excellence and John will help us attain our goals."

Varley, who moved to Bozeman after retiring from the National Park Service in February 2006, said, "I wasn't looking for another job. They thought I had some skills I could bring to MSU. It looked very exciting to me."

Varley said he and Graumlich have worked together closely over the years,

including work on a joint Tanzania–Yellowstone wildlife research project.

In his new position, Varley said he will look at the Big Sky Institute's educational and science mission. He will continue to help develop the concept of MSU as the University of the Yellowstone.

Varley became director of the Yellowstone Center for Resources in 1993. During the 10 years before that, he was chief of the Division of Research at Yellowstone. He was a supervisory fisheries biologist in Idaho from 1980 to 1983, a fisheries biologist in Yellowstone from 1972 to 1980, and a fisheries research biologist in Utah from 1967 to 1972. He has been an adjunct professor at Ricks College in Rexburg, Idaho, the University of Wyoming in Laramie, Wyoming, and MSU in Bozeman, Montana.
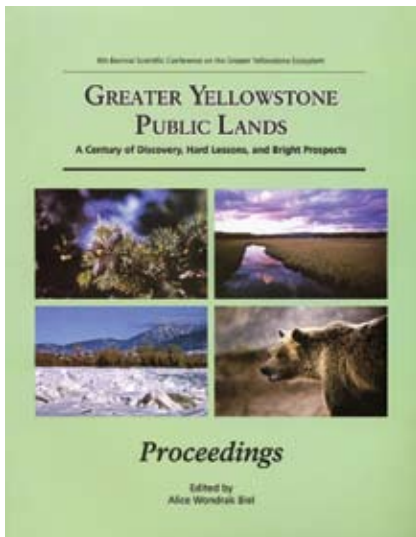
Eighth Biennial Conference Proceedings Available

The proceedings from the Eighth Biennial Science Conference on the Greater Yellowstone Ecosystem, *Greater Yellowstone Public Lands: A Century of Discovery, Hard Lessons, and Bright Prospects*, is now available from the Yellowstone Center for Resources. The conference, which took place in October 2005, focused on the mandates, "cultures," relationships, and accom-

plishments of the numerous local, state, and federal management agencies responsible for Greater Yellowstone's public lands.

If you would like to receive a copy of the proceedings, please contact Virginia Warner at virginia_warner@nps.gov, or 307-344-2230. An electronic version is also available at www.nps.gov/yell/naturescience/conferencearchive.htm.

Gerald R. Ford, 1913–2006: Park Ranger, 38th President of the United States

Gerald R. Ford holds a special place in the heart of the National Park Service family. He will be remembered for his many accomplishments as president of the United States and his compassion in healing the nation's wounds following the war in Vietnam. For the National Park Service, he is considered one of our own; he is the only American president to have served as a park ranger in the National Park Service.

In 1936, Gerald Ford worked as a seasonal park ranger at Yellowstone National Park. Ford later recalled that time as one of the greatest summers of his life. According to his supervisor at Yellowstone, Canyon District Ranger Frank Anderson, Ford was "a darned good ranger." While serving in Yellowstone, one of Ford's assignments was as an armed guard on the bear-feeding truck. The National Park Service no longer feeds bears, but Ford always remembered that duty and often regaled his family with stories about the bear-feeding truck. During his summer at Yellowstone, Ford also worked in the Canyon Hotel and Lodge meeting and greeting VIPs, though he felt it was "undemocratic and un-American to give special attention to VIPs." According to Wayne Repogle, Ford's roommate that summer, one of the duties that Ford particularly enjoyed was the early morning check. From 5 AM to 7 AM each day, every automobile in camp had to be checked for make, model, state, and license number. Repogle

President Gerald Ford in Yellowstone National Park, summer 1936.

indicated that the rangers had to run most of the time to get 150 to 200 licenses listed in two hours. As a football player, Ford was very fit and saw this duty as an opportunity to stay in shape. Repogle stated that Ford genuinely enjoyed "everything we rangers had to do."

As President of the United States, Ford oversaw an era when the National Park Service, under the leadership of Director Gary Everhardt, tightened the criteria for national parklands. Previously, for an area to be recommended for inclusion in the National Park System, it had to be considered nationally significant and lend itself to administration, preservation, and public use. The new emphasis would also consider whether the area was assured of adequate protection outside the National Park System and whether it would be available for public appreciation and use under such protection. During his time in office, President Ford added eighteen new areas to the National Park System.

The National Park Service family extends its heartfelt condolences to the Ford family at this difficult time and remembers one of its own fondly. We respect him as one of the pioneers in the field of rangering, and as a president that cared deeply for the National Park Service.

A New Prehistoric Source for Stone Tools

by Robin Szamuhel

In summer 2006, the archeology team revisited 248 sites to evaluate their current condition. The core team was composed of two volunteers (John Reynolds and myself) and an intern (Brian Quinn), with assistance from Yellowstone archeologist Ann Johnson. During these site visits, we came upon a stone source for prehistoric tools whose importance had been previously unappreciated. This site is enormous in both its size and its potential contribution to major archeological research questions in Yellowstone prehistory. With the help of aerial photographs and repeated visits to the site, the archeology team has been assessing the total size of the raw material source, and it appears to be more than 2,250,000 square meters.

During our initial survey, as we were looking for erosion or disturbances that might be damaging the site, we observed deep cuts in the bedrock made by early peoples, indicating quarrying activity or areas where stone had been removed. We could see where early people had dug into a cliff to follow a particularly good vein of raw material. There was also waste material, the stone left behind by prehistoric diggers. The debris on the ground was a spectrum of colors—translucent white, blue, green, purple, orange, red, brown, and everything in between. Someone had gone to the trouble of digging several large, fist- and head-sized chunks out of the bedrock, but the raw material had been left at the site. The color of these chunks was dramatic, with veins of crystalline material, speckles of black or white, or bands of different colors running through them. The colors and quality of the raw material will help us revise the interpretation of what raw materials were being brought into the park as tools, and what was being obtained locally.

Multiple dense concentrations of

Colorful piece of raw material.

flakes suggest workshop areas where people broke down chunks into smaller pieces to manufacture tools. Across the site, evidence of quarrying and manufacturing activities is so apparent that a vivid picture of prehistoric activity can be imagined. For example, a certain pile of flakes and debris suggests a prehistoric person perched on an adjacent outcrop, using a hammerstone to remove flake after flake from a chunk of translucent chalcedony in order to make a tool kit to take back to the camp.

We know that chert, chalcedony, and obsidian are three major lithic materials used by prehistoric people in Yellowstone to manufacture tools. Obsidian Cliff is the largest established material source in the park, and is the largest source of obsidian used in Yellowstone from the earliest occupations around 12,000 years ago to the time of first contact with Europeans. Obsidian Cliff obsidian has been found as far east as Ohio, as far northwest as Washington state, and as far south as Texas.

This new site has been named Robin's Quarry by park archeologist Ann Johnson. While not as large as Obsidian Cliff, the site may be comparable in its local importance as a source of raw
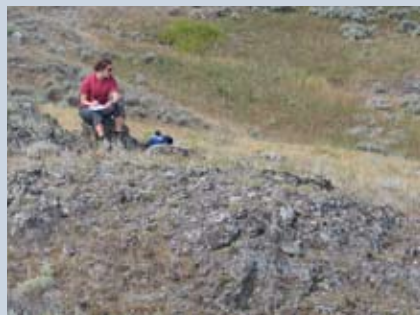
Volunteer Robin Szamuhel.

material. The amazing and distinctive colors found at this site are recognizable as material previously observed at several occupation sites along the Yellowstone River and on the south shore of Yellowstone Lake. The possible local sourcing of these materials is significant to our interpretations of trade and migration patterns. If the material being harvested from this quarry site is the same material left behind as projectile points, hide scrapers, or flakes discarded by someone retouching a knife's edge, we can follow and assess the pattern of a group's movement in the park, and perhaps even possible trade patterns with neighboring groups. For example, if materials or tools from the quarry are located beyond the usual migration area of a group, this may suggest that trade was occurring, or that our concept of a group's seasonal round (yearly travels) may have to be revised.

It is important to note that while sourcing obsidian can be done by non-destructively examining the chemical composition of the material, chert cannot be sourced in the same manner. Therefore, establishing the source of a piece of chert presents an interesting challenge, and tracing chert tools and flakes back to this site will be a complex endeavor for the archeology team.

While much remains to be studied in order to understand the importance of this raw material source, the evidence suggests a site of great magnitude, and we are excited about its research potential. As a science, archeology relies on fact and physical evidence to re-create prehistoric lifeways and patterns, and depends on the formulation and testing of hypotheses. With the data from this site, we have an opportunity to pursue questions that had heretofore not been formulated, and to address the increasingly complex questions about selection of raw material for stone tools and their use through time.

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Unfolding the Mystery of Protein Thermostability

John Peters, Brian Bothner, and Susan Kelly

Figure 1. Graduate student Josh Spuhler measures temperature and pH in some of Yellowstone's hottest thermal features while looking for organisms—and ultimately proteins—that thrive in temperatures over 80°C (176°F).

VISITORS TO Yellowstone National Park are amazed and delighted by the many thermal features—the geysers, bubbling mud pots, and steam vents; the clouds of steam carrying the familiar "rotten eggs" odor of hydrogen sulfide gas; and the multicolored waters of the pools. Even more amazing is the sight of the algae mats and the awareness that a whole array of microbial life lives in these inhospitable environments. The visitor cannot help but wonder, how does anything live here? "Protein thermostability" seems a dry term to describe such wonders, but it is one of the keys to life in Yellowstone's extreme environments.

The organisms in Yellowstone's thermal features are *thermophiles*, heat-loving microorganisms that not only survive, but thrive, at temperatures above 45°C (113°F). Hyperthermophiles prefer temperatures even hotter; above 80°C (176°F) (Figure 1). In response to the extreme conditions, these organisms have evolved mechanisms to protect their structure from the effects of high temperatures. A key mechanism is the development of proteins that are *thermostable*, or able to operate effectively at high temperatures. Unraveling the mysteries behind the thermostability of proteins is a fascinating area of research that not only illuminates one of nature's wonders, but also offers scientific knowledge of industrial and biotechnological utility.

Building Blocks of the Cell: Polymers and Proteins

The fascinating complexity of the cell has awed philosophers and inspired scientists for hundreds of years. A cell is composed of small and large molecules, each with a distinct purpose. The molecules themselves are lifeless; however, when

they occur in the right combination and under the proper conditions we know life emerges—the exact mechanics of how is of continuing and current interest, especially in light of the search for extraterrestrial life.

It is the very large molecules within the cell—*macromolecules*—that make up the cell structure, store information, and are responsible for the processes of life. Biological macromolecules are *polymers*; that is, they are composed of repeating or similar subunits linked into a chain by chemical bonds and further assembled into complex structures. Polymers can exist in long or short chains, and both biological organisms and synthetic chemists have used this property to their advantage, in that large-scale structures can be built from small molecules simply by making the chains very long. What makes biological polymers different from synthetic ones is that they are made up of many types of repeating subunits, rather than just one or two. Figure 2 illustrates the difference. Nylon and the common plastic polyethylene are both polymers, but they are composed of only one kind of very simple subunit; for example, the repeating ethylene unit in polyethylene. The three biological polymers collagen, protein, and cellulose, on the other hand, are shown to be complex molecules made up of large subunits that are themselves complex. Furthermore, long chains of biological polymers can adopt a variety of three-dimensional shapes, which in turn underlie a variety of functions. So, despite the fact that both synthetic and biological polymers are made up of carbon, nitrogen, oxygen, and hydrogen, the properties of the
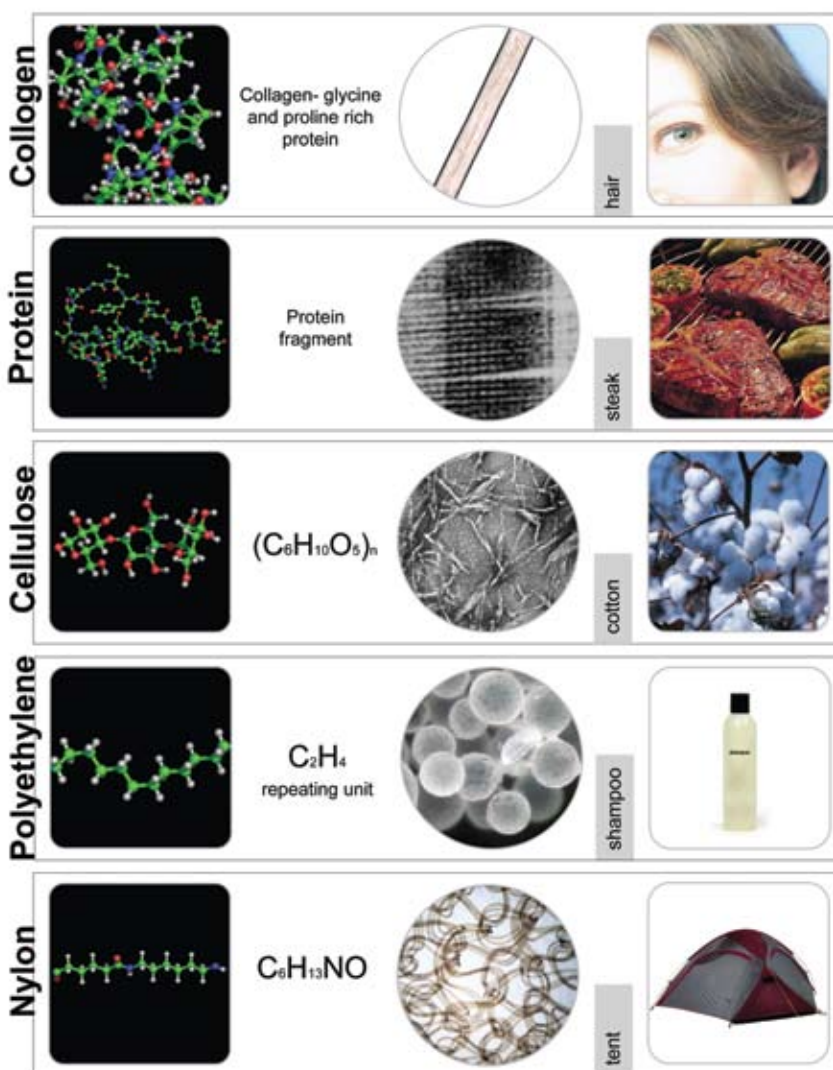

Figure 2. Biological and chemical polymers. Biology has made good use of polymers. Many of the most important biomolecules are polymers, including proteins (collagen), nucleic acids, lipids, and carbohydrates (cellulose). Like biomolecules, the synthetic polymers nylon and polyethylene are composed of repeating molecular units, but these are simpler and less varied than the subunits of biopolymers. Molecular components that make up the biological and synthetic polymers shown here include oxygen (red), nitrogen (blue), hydrogen (grey), and carbon (green).

resulting molecules are very different. The greater variety of repeating units give biomolecules the flexibility, adaptability, and variety essential to the processes of life.

Four major types of biopolymers are found in every living organism: polysaccharides, lipids, nucleic acids, and proteins. *Polysaccharides* are chains of carbohydrates (sugar, for example) and have highly diverse functional roles and sizes. In humans the polysaccharide glycogen, made up of repeating units of glucose, is used for short-term energy storage. The polysaccharide cellulose, the most abundant natural polymer on earth, is a structural component of plant cell walls. *Lipids,* like

other polymers, are composed of a hydrocarbon chain. When attached to an acidic molecule, this chain forms a fatty acid, which is the major energy storage molecule in animals. The primary role of *nucleic acids* is to store genetic information. Deoxyribonucleic acid (DNA) stores heritable information that is passed from one generation to the next, while ribonucleic acid (RNA) stores information transiently as cellular components are assembled. RNA and DNA are polymers made up of four different units that can be linked in any order, thereby providing the basis for genetic diversity. The fourth and final class of biopolymers is the *proteins*, linear chains of 20 possible

different amino acids linked together by peptide bonds. Proteins come in an enormous range of shapes, sizes, and structures, and it is their structure which underlies the vast range of functions they can adopt. The ability of Yellowstone thermophiles to withstand heat is intimately related to the structure of their proteins.

Functional Roles of Proteins

Proteins are the biological molecules responsible for the structure and function of cells and organisms. Just as there are thousands of genes in an organism, there are thousands of corresponding proteins, each with a unique function. Life, as we know it, depends on the orchestration of numerous types of proteins. As the primary agents of biological function, proteins carry out an array of jobs that includes chemical conversion, regulation, transport, and cell structure.

Each of the 20 different amino acids that make up proteins has its own chemical properties. As the composition and sequence of a protein varies, its physical character will change, leading to a nearly endless supply of diversity. For example, if three different amino acids are linked together, 8,000 (20^3) different sequences are possible. A small protein of 100 amino acids,

More than 70 research groups, including scientists from the Thermal Biology Institute, study some of Yellowstone's 14,000 estimated thermal features.

therefore, can have 20^{100} different possible combinations—an enormous number! In fact, there is not nearly enough observable matter in the universe to make even a single copy of each possible sequence. The amino acid sequence dictates how the long chain folds up into a globular structure, and the resulting three-dimensional structure determines the function of a given protein in a cell.

The largest functional class of proteins is the *enzymes*, or biological catalysts. A *catalyst* is a molecule that speeds up, or catalyzes, the rate of chemical reactions in a *substrate,* the molecule acted upon. Enzymes are known to catalyze 4,000 different biochemical reactions. As biological catalysts, enzymes accelerate the rate of chemical reactions essential to life without undergoing any chemical change themselves. Since the enzyme remains unchanged, it can direct many iterations of the same chemical conversion of a given substrate. A single enzyme molecule can be responsible for thousands of conversions per second, making enzymes the most efficient catalysts known. Most enzymes are highly *specific*, acting upon only one substrate, but others have a very broad range of activity. An enzyme is usually named according to either its substrate or the chemical reaction it catalyzes, with the suffix *_ase* added. Lactase, for example, is a common digestive enzyme that breaks down lactose, the sugar in milk, and may be taken as a supplement by lactose-intolerant people. As another example, DNA polymerase catalyzes the reaction by which the DNA chain or polymer is built.

A second functional group is the *regulatory* proteins, which alter the function of other proteins without undergoing chemical modification themselves. Hormones such as insulin are a good example of this class. Regulatory proteins often bind to other protein molecules and change their shape, thus altering their function. The job of certain regulatory proteins is to bind to DNA, thereby altering gene expression. A third group of proteins help *transport* molecules; for example, proteins in the cell membrane are responsible for bringing nutrients into the cell, while others direct intracellular transport, moving nutrients, metabolites, hormones, or even regulatory proteins around the cell. Finally, proteins provide the *structural* scaffolding of the cell, which is a more passive function than their other, highly dynamic processes. Structural proteins are often composed of multiple polymers that are assembled in a hierarchal fashion. Common examples of structural proteins are keratin, found in hair, fingernails, and horns; and collagen, which holds us together via skin, tendons, and cartilage.

Structure "Fold" Defines Function

The function of a protein in general, and of an enzyme specifically, is defined by its three-dimensional structure or what is sometimes termed the "fold." Protein structure is most often described in terms of levels of structural complexity. The first level, called the primary structure, refers to the sequence of amino acids in a continuous chain. The chain of amino acids, or *polypeptide*, can vary in length from about 50 to more than 2,000 amino acids linked together by peptide bonds. A functional protein is a polypeptide consisting of one or more of these chains folded in a well-defined way. Proteins are synthesized within the aqueous environment of a cell, and it is the presence of water that helps direct the folding of an extended polypeptide chain into a compact functional protein. Some

of the amino acids that make up the polypeptide chain are *hydrophilic*, meaning that they bond well with water molecules, while others are *hydrophobic* and reject water molecules. The protein is driven toward its final structure by the propensity of the hydrophobic amino acids to aggregate and prevent interactions with water by burying themselves in the protein interior, a phenomenon referred to as the *hydrophobic effect*.

The final active form of the protein is also held together by other forces such as electrostatic interactions, which are the attractive forces of positive and negative charges. An additional interaction that is observed infrequently but which contributes significantly to the stability of a protein is *disulfide bonding*, occurring when a pair of amino acids bond together via two sulfur atoms. The hydrophobic effect as well as these other interactions together stabilize the protein fold. The variety of interactions afforded by the diversity of amino acid structures can create regions of the protein that are rigid and other regions that are more flexible. Given that there are 20 common amino acids, the number of different combinations or sequences possible for an average-sized protein (~400 amino acids) is astronomical, introducing the possibility of different structures that could contribute to thermostability.

Enzymes Increase the Rate of Chemical Reactions

Sustaining a living cell requires an array of coordinated chemical events. When we consume carbohydrates, proteins, and/or fats in our diet these relatively large molecules are broken down to produce energy and synthesize components of new cells via complex pathways of reactions. These processes can occur spontaneously, but without an appropriate catalyst they would occur too slowly to sustain life. The rate of the reaction, or how fast a reactant is converted to a product, is determined by *activation energy*, the quantity of energy needed to get the reaction started; that is, to overcome the *activation barrier* (Figure 3). Enzymes catalyze biological reactions by lowering the activation barrier. The site at which rate enhancement or catalysis occurs on the enzyme is called the *active site*. This site is often chemically complementary to the reactant, and the elaborate protein architecture of the enzyme allows for this complementary site to be maintained but also allows for structural changes that lower the energy of key intermediate states. The structure of an enzyme defines its function and specificity in a biochemical reaction.

Enzymes are amazingly efficient biological catalysts, but they work optimally at defined temperatures. For example, most enzymes present in our bodies are most efficient near normal body temperature, 37ºC (98.6ºF). These enzymes are inactivated and destroyed if exposed to high temperatures because the overall three-dimensional structure or fold of the protein is permanently altered under extreme conditions. Enzymes in other life forms, such as the thermophiles of Yellowstone's thermal springs or those associated with deep sea thermal vents, have adapted to environments where temperatures can approach the boiling point of water. In response to the extreme conditions, thermophilic enzymes have evolved thermostability as a mechanism to protect their structure from the effects of high temperatures.

Chemistry and Life at High Temperatures

Life is very different in high-temperature environments. All life depends on a flow of electrons for energy production, but the mechanisms by which life in Yellowstone's extreme environments obtain energy is often very different from those of more familiar plants and animals, largely because of the unusual chemical environment. The superheated water in Yellowstone's thermal springs percolates throughout an extensive plumbing system, leaching metals and minerals from the subsurface rock. Frequent earthquake activity further mixes these mineral-rich waters, bringing them into contact with other rocks and minerals, so the composition of water in thermal springs varies dramatically. Most life exists in a neutral pH range, and some Yellowstone hot springs do maintain a neutral pH. However, many others exist at distinctly high pH (basic) or low pH (acidic) values (Figure 4). Essentially, many
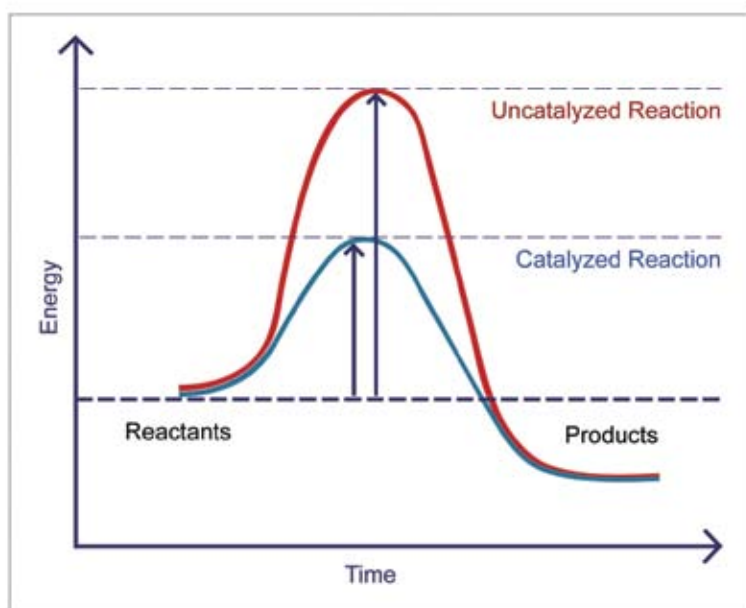

Figure 3. A reaction coordinate representing the energy over the course of a chemical reaction showing the difference in activation energy in uncatalyzed (red) and catalyzed (blue) reactions.
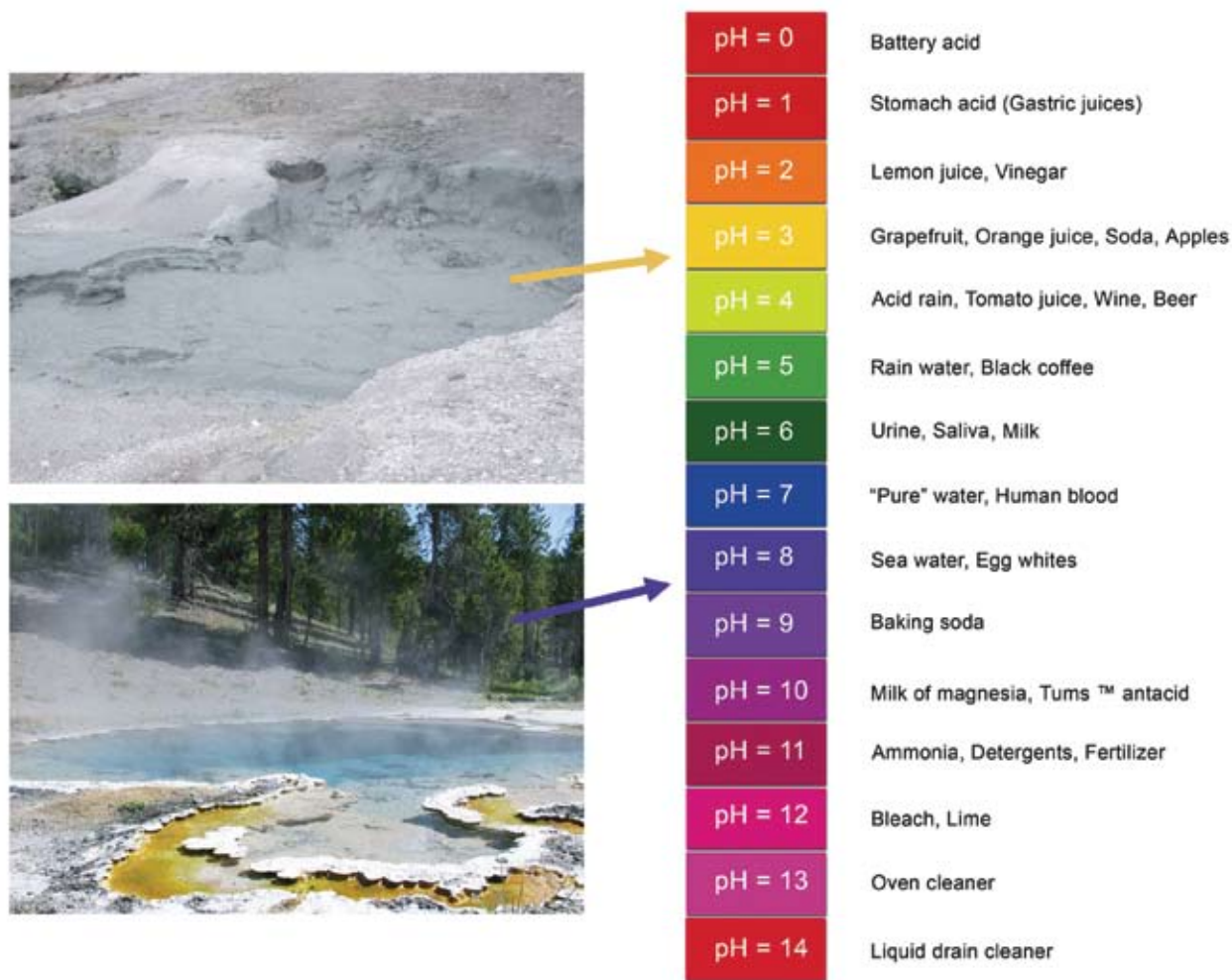
Figure 4. Yellowstone thermal features exhibit an astonishing range of chemical environments. The pH scale (1–14), is a measurement of the acidity of a solution and is defined in terms of the concentration of hydrogen ions (H+). A pH lower than 7 is considered acidic, while a pH higher than 7 is considered basic or alkaline; pH of 7 is neutral. On the pH scale, a shift up by one number represents a ten-fold decrease in hydrogen concentration. For example, a shift in pH from 2 to 3 (toward relatively less acidity) represents a decrease in total concentration of hydrogen by ten times. Thermal Biology Institute (TBI) researchers study features across a wide pH range—from less than pH 3 in some Norris Geyser Basin features (top) to as high as pH 10; Octopus Spring, pH 8, shown here (bottom).

Yellowstone thermophiles are living in pools of boiling acid.

Animals typically gain energy by *oxidation*, a process that strips electrons from organic compounds such as carbohydrates, proteins, and fats (the *electron donors)*, and *respiration*, the process by which electrons are finally transferred to oxygen, the final *electron acceptor*. This type of energy metabolism for sustaining life requires oxygen, but in many of the thermal environments in the park oxygen is not present at all, or only in vanishingly small amounts. Furthermore, organic carbon, an essential element of typical electron donors, is usually not abundant in thermal environments. Consequently, to survive in hot springs thermophilic organisms have evolved alternative mechanisms for energy production in which inorganic compounds such as hydrogen and hydrogen sulfide gases can serve

as electron donors, and metals like iron and arsenic can serve as terminal electron acceptors.

Nonetheless, despite the unusual alternative mechanisms that thermophiles have developed to survive in extreme environments, there remains a common thread among all living things. All life is dependent on coordinated sets of chemical reactions catalyzed by enzymes, but high temperature affects catalysis in two important ways. First, the rate of most chemical reactions is accelerated at higher temperatures, whether enzymes are present or not. However, enzymes are still required to achieve the reaction rates required to sustain life. Second, high temperatures affect the stability of proteins. Proteins that aren't adapted to high temperatures become nonfunctional very rapidly when exposed to heat. Heat is energy, and enough
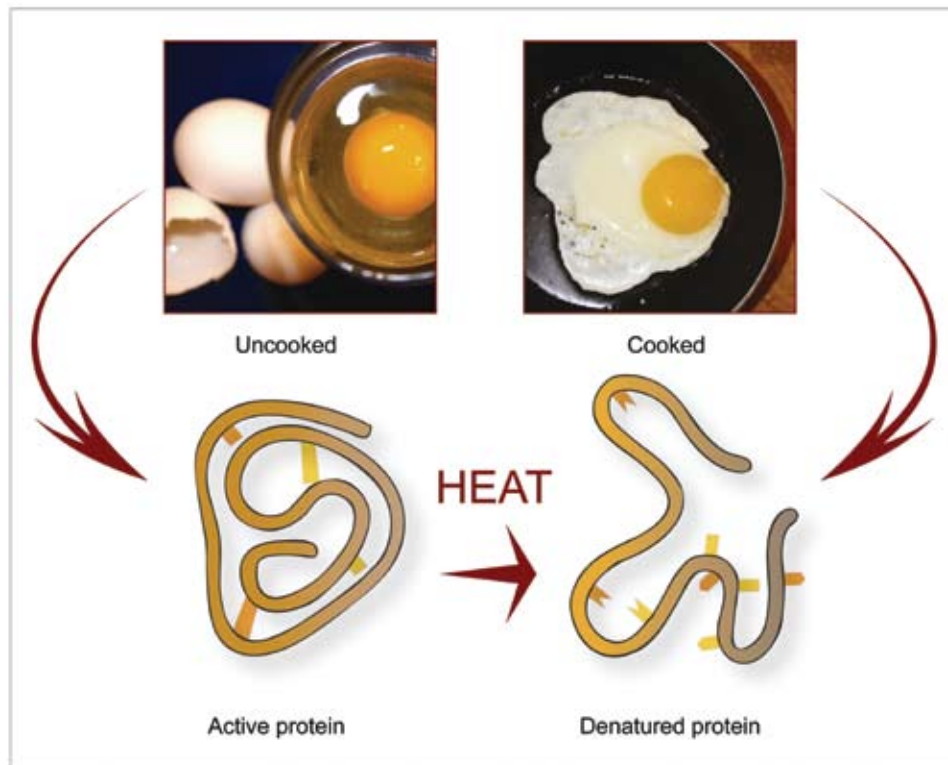
Figure 5. The changes a protein-rich food like an egg undergoes (left) when individual proteins are denatured or unfolded by heat (right).
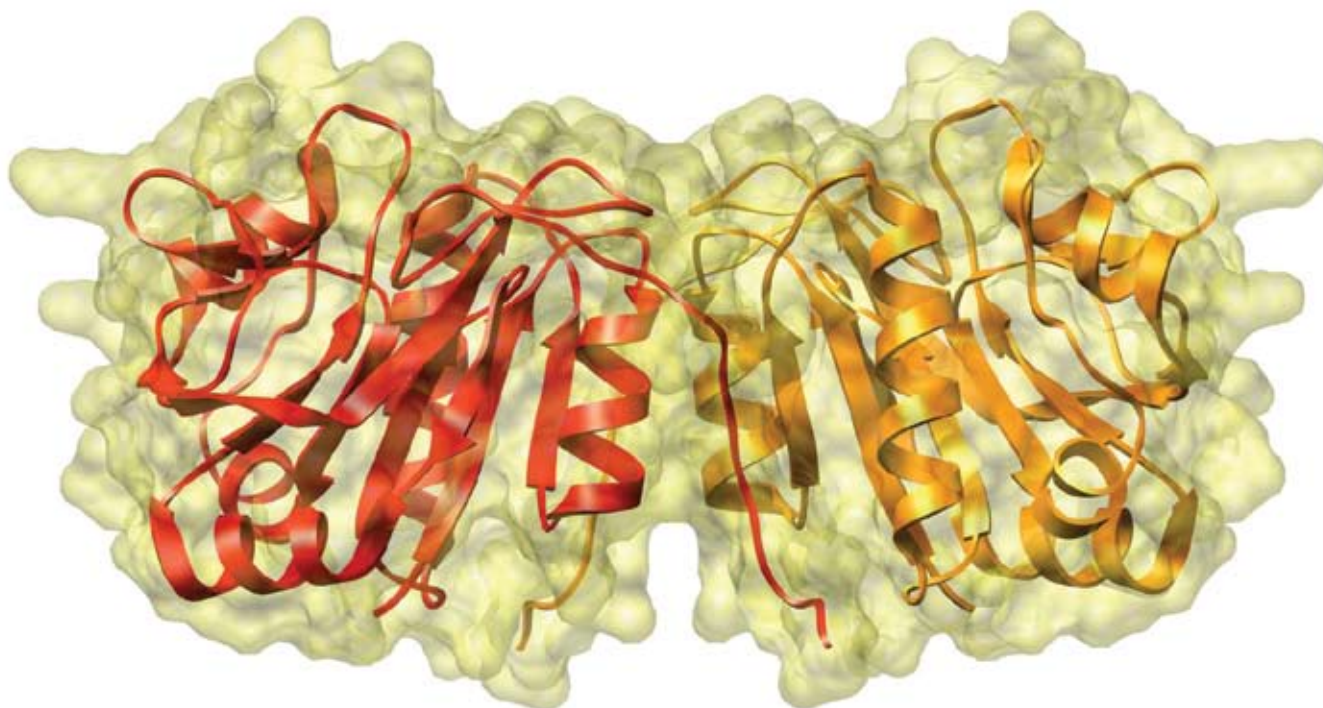
Figure 6. A structural model of an enzyme involved with the transfer of sugars. This glycosyl-transferase was isolated from a thermophilic virus found in the Rabbit Creek area of Yellowstone. The three-dimensional surface of the enzyme has a butterfly shape and is shown in transparency. The protein backbone is represented with red and yellow ribbons. Typical of proteins that have evolved to function at high temperatures, it has a compact structure with short loops connecting the amino acid strands (arrows) and helices (spirals). The model is based on X-ray crystallography data from the lab of TBI researcher Martin Lawrence published in the *Journal of Virology* 80(15), August 2006.

energy is capable of breaking the interactions that control and stabilize the protein fold, thus breaking down the specific structure that is required for function. In the case of enzymes that are not adapted to high temperatures, it is the catalytic function that is inactivated. The heat-induced unfolding or *denaturation* of a protein is often an irreversible process. A classic example of this phenomenon occurs in the cooking of an egg. As the egg cooks, you actually observe the proteins present in the clear liquid egg white denature, forming a white solid (Figure 5). The protein structure is irreversibly changed into another form incapable of carrying out the life-supporting functions of a cell.

What Gives a Protein Thermostability?

Fundamentally, there are few differences between the enzymes that catalyze reactions under moderate conditions, such as those in our bodies, and those found in thermophiles. The mechanisms used to catalyze the chemical reactions are the same, but certain differences at the molecular level allow heat-tolerant proteins to maintain their structures at temperatures that approach the boiling point of water. Intramolecular interactions are the foundation of protein fold and function, and studies of the mechanism of thermal adaptation have revealed that thermally tolerant proteins have a more numerous and extensive set of internal interactions. These proteins are also more compact and generally lack regions such as exposed loops or extended ends. Such loops and ends are typically floppy and represent regions where the stabilizing interactions are vulnerable (Figure 6). Thermophilic proteins don't have as many of these floppy regions and thus resist unfolding. The interior of thermostable proteins is denser than that of proteins adapted for ambient temperatures. In fact, a thermostable protein may be essentially impenetrable to water, thus preventing water from competing for interaction sites that stabilize the fold.

So why aren't all proteins or enzymes built to be stable? The key to this very important question lies in the overall adaptability of a protein that is ultimately stable. Proteins and especially those that function as enzymes are involved in mediating and facilitating dynamic processes and thus are most effective if they can function dynamically. Enzymes that are thermostable are often less effective at accelerating their specific reactions, a necessary tradeoff in high-temperature environments. At moderate temperatures, however, the flexibility and catalytic efficiency of less stable enzymes are of the highest priority.

Can We Put Thermostable Proteins to Use?

The ability of enzymes to accelerate chemical reaction rates up to a million times while maintaining high substrate specificity far exceeds the capabilities of manmade catalysts. However, the conditions under which industrial chemical reactions occur are generally far more harsh than those inside a cell.

Yellowstone thermal areas are teeming with life. Thermal Biology Institute scientists and others who investigate the mystery of thermal stability have a variety of thermal environments to choose from. Hot features near Porcelain Basin (above) are home to acid-loving microbes that obtain energy by oxidizing the minerals sulfur and iron.

Microbial streamers—millions of microorganisms connected in long chains—flow in the thermal runoff along Rabbit Creek.

Mud pots are acid hot springs with a limited water supply. While mud pot activity varies with precipitation and the seasons, they are excellent places to look for thermophiles and their proteins.

If enzymes are to be used to improve the efficiency of industrial reactions they must be able to tolerate such harsh conditions. Naturally, scientists have turned to thermostable enzymes for use in industrial applications.

Thermostable enzymes are a mainstay for the starch industry, which yields products for baking, brewing, detergents, and other applications from starches such as maltose and glucose syrups. The breakdown of starch requires multiple enzymes called amylases. Since starch is soluble in water only at high temperatures, thermostable amylases are essential to this huge industry, which accounts for 30% of the world's industrial use of enzymes.

During the processing and bleaching of wood for paper, xylan and lignin (complex carbohydrates abundant in plant cell walls and wood) must be broken down without degrading the cellulose fibers that go into paper. Enzymes with specific activity for xylan and lignin are advantageous in pulp and paper manufacture; however, they must be able to withstand the high temperature and high (basic) pH conditions used to process wood. Thermostable xylanases are now used in the Kraft process, the most common method of producing paper pulp. Their bleaching properties reduce the need for chlorine, offering a significant environmental benefit. As worldwide demand for paper increases, the use of enzymes reduces the release of halogenated organic compounds, a serious environmental pollutant.

Cellulose, the major component of plant cell walls, is the most abundant natural material on earth. It can be used to produce fuel, fiber, animal feed, and chemicals. However, cellulose is difficult to digest and requires a group of enzymes for complete degradation of the carbohydrate chains. In fact, the most costly step in the production of ethanol from plant material is generating the enzymes that are used. Cellulases are used in color-brightening detergents, cotton production, extracting color from juices, and improving animal feed. All of these processes could benefit from increased thermostability of the

Chemotrophic microorganisms—organisms that make a living on chemical energy—can be invisible to the naked eye, but are found in deep, clear pools like these along White Creek.

enzymes, since the cellulases currently used operate optimally at 50–55ºC (122–131ºF), while many of the processes require higher temperatures.

Chitin, one of the most abundant polymers in nature, can be processed to yield chitosan, a biological resource with many industrial, agricultural, dietary, and medical uses. Chitin is found in the exoskeletons of all crustaceans (shellfish) and insects, as well as in fungi. Thermostable chitinases are valuable in catalyzing the reactions that break down chitin into chitosan. In developing countries along the Pacific Rim,

Applications from Yellowstone organisms are found in the following processes:

- Heat-stable enzymes are used in laundry detergents to break down protein and fat stains on clothing.
- Heat-tolerant microorganisms are being studied to identify enzymes that can degrade alkaline materials including pesticides and explosives like TNT.
- An enzyme from a Yellowstone bacterium is being used to clean up industrial wastewater from hydrogen peroxide bleaching processes used to whiten and disinfect products.
- New thermal-tolerant enzymes from a Yellowstone organism can reproduce DNA more accurately and without the equipment necessary in the current DNA fingerprinting process.
- Metal-containing proteins are being studied for the potential production of hydrogen fuel.
- Enzymes that help speed up the fermentation process and convert plant material like corn into ethanol are being studied for potential biofuel applications.
- Enzymes have been discovered that break down starch at high temperature into the sweeteners trehalose and saccharide, used in processed foods, cosmetics, and pharmaceutical products.

thermostable enzymes could be used to reduce some of the millions of tons of crab and shrimp waste produced each year and return a value-added material in the form of chitosan.

The class of enzymes with the greatest market share in industry are the proteases, which cleave the peptide bond between amino acids and thus break down proteins. One of the most familiar uses of proteases is in enzyme-based stain removers, which use proteases to break down protein-based stains such as blood as well as many other compounds. Industrial uses include leather processing and food and pharmaceutical production.

Our examination of the commercial uses for thermostable enzymes is by no means complete, and many other industries are benefiting from the use of such enzymes in chemical processes. There are a few basic requirements that determine the feasibility of using an enzyme instead of a synthetic catalyst. For use in large-scale industry, an enzyme must be available in large quantities for a reasonable price and should be stable for the duration of the process. Because thermostable enzymes have increased stability at moderate temperatures, they can be more cost effective than other enzymes, even when the maximum temperature of a process does not require use of a thermostable form. Enzymes can also substantially reduce waste products from industrial processes, particularly for reactions in which a heavy-metal catalyst can be replaced with a protein.

Yellowstone's Most Famous Enzyme

Thermus aquaticus is an aerobic bacterium first discovered in 1966 in Mushroom Pool in Yellowstone National Park, and now known to be found in thermal areas worldwide. At that time, *T. aquaticus* was a remarkable discovery because its optimum temperature for growth lay between 70°C and 75°C (158°F to 167°F), near the upper limits for life. This otherwise unremarkable organism

Thermal Biology Institute Research Associate Sarah Korf tests the thermal waters in the Broad Creek area in an effort to better understand how thermophilic bacteria and archaea make a living.

A thermophile is a heat-loving microorganism that not only survives, but thrives, at temperatures above 45°C (113°F). Hyperthermophiles prefer temperatures even hotter; above 80°C (176°F).

was added to a microbial culture collection by microbiologist and early park researcher Thomas Brock.

In the early 1980s Kary Mullis, a scientist with Cetus Corporation, found a use for a heat-tolerant enzyme from *Thermus aquaticus* in the *polymerase chain reaction* or PCR method, a procedure Mullis developed to replicate DNA sequences. The PCR process requires heating the DNA to be copied. However, the DNA polymerase first used in this procedure—the enzyme responsible for copying a DNA molecule—denatured at temperatures greater than 60°C (140°F), making the PCR method slow and arduous because more enzyme had to be added each time the DNA was copied. The DNA polymerase from *Thermus aquaticus* (Taq DNA polymerase) is heat stable, allowing multiple rounds of DNA replication to be performed in a test tube without addition of more enzyme. The use of Taq DNA polymerase led to the automation of PCR and the development of a multimillion-dollar industry devoted to the amplification of tiny amounts of DNA, with applications for basic research, drug discovery, and forensics. For his brilliant idea, Dr. Mullis received the 1993 Nobel Prize in Medicine.

Today, PCR is an important technique used by a wide array of researchers in the park, whether they are studying microbial communities or lake trout populations. PCR is used to amplify tiny bits of DNA found in hair or other biological samples, allowing species such as grizzly bears, wolves, and bison to be identified, thereby providing valuable information about population numbers, distribution, and behavior to park managers and researchers. The discovery

of a heat-stable enzyme from a Yellowstone microorganism brings the idea of resource protection full circle. Protection of Yellowstone resources allowed for the discovery of an organism that is now helping with the management and further protection of this unique ecosystem. Ongoing research in Yellowstone is exploring the use of Yellowstone thermal proteins for a variety of everyday applications.

Yellowstone's Future

Yellowstone truly has something for everyone, first-time visitor and world-famous researcher alike. It is often noted that Yellowstone was established in 1872 for its unusual geology—its geysers, hot springs, and mud pots. Yet we continue to discover and redefine the exact nature and significance of this grand act of preservation. Predecessors would never have suspected the park's significance today as a Biosphere Reserve and World Heritage site. Early park promoters and enthusiasts could not have foreseen the significance of preserving clean air, natural quiet, or nighttime darkness, let alone microbial life and the thermal-stable proteins within. For the scientist most significantly, the park contains vast pristine microbial habitats not duplicated anywhere else on Earth. Preserving these crucial environments is of utmost priority. For this reason, scientists of the Montana State University Thermal Biology Institute work closely with the National Park Service to conduct their research using "leave no trace" practices so that basic and applied research can be conducted without compromising other values—known and unknown. We can only wonder at the vast potential for future discovery.

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John Peters (left) is the Director of the Thermal Biology Institute (TBI) at Montana State University–Bozeman, where he studies the protein structure and function of thermal enzymes. His research is focused on metal-containing enzymes from Yellowstone microorganisms involved with nitrogen metabolism, hydrogen metabolism, and metal reduction, with potential applications tied to bioremediation of metals and clean energy production of hydrogen fuel. **Brian Bothner** (right), Assistant Professor of Biochemistry, is a new member of TBI. His research interests include proteomics and virology. A particular focus of his research is characterizing viruses isolated from Yellowstone thermal features and elucidating the biology of viral infection in Archaeal species such as *Sulfolobus solfataricus*. **Susan Kelly** (center) is the Coordinator of Outreach and Education for TBI. The Institute's outreach activities target the scientific community, the general public, and K–12 audiences.

Colorful microbial mats form in some Yellowstone thermal features. Microbial communities are defined by temperature and chemical composition of thermal waters; thermally adapted proteins allow organisms to thrive in seemingly inhospitable environments.

(More information about TBI, its activities, and applications of Yellowstone thermophiles is posted at www.tbi.montana.edu.)

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