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Hello From the North Country,

Just before the holidays I had a conversation with Matt Kane at NSF regarding the Research Coordination Network proposal that we submitted in late June 2003. The good news is that the proposed RCN is recommended for funding and the proposal reviewed very well. The review panel and Matt are asking us to clarify a few minor concerns, however, and this is to be done with a formal fast lane submission by 1/16/04 as a proposal addendum. Basically, Matt requested a revised Management and Coordination Plan which was one section of the proposal. I have attached a draft of this addendum that responds to the queries raised at NSF. The first page of this file lists verbatim the concerns I received from NSF and then the remainder of the file is the revised Management and Coordination Plan.

If you have time and can have a look at this, please provide any feedback via track changes and I will be happy to incorporate ideas or edits. Matt Kane would like to look at a draft by 1/9/04 before we submit the final on 1/16/04. I have also attached a revised budget (excel file) that will be transferred to the normal NSF budget forms when we submit via Fast Lane on 1/16/04. Basically, the budget is modified only slightly to 1. pay for a full-time rather than 80% time staff to help coordinate the RCN activities. We will initiate a search here very shortly and will include you for input to this process, and 2. NSF recommended fewer workshops and we have obliged by reducing the number of proposed workshops to three over five years. I believe this will be up to us to prioritize as we go along, but for the purpose of budgeting, we have identified three priority workshops as you will see. Otherwise, the proposed budget is the same as submitted earlier.

Let me know what you think and feel free to call at 406-994-5077 if you want to talk about details that are too difficult to do with email,

Thanks again for your participation,

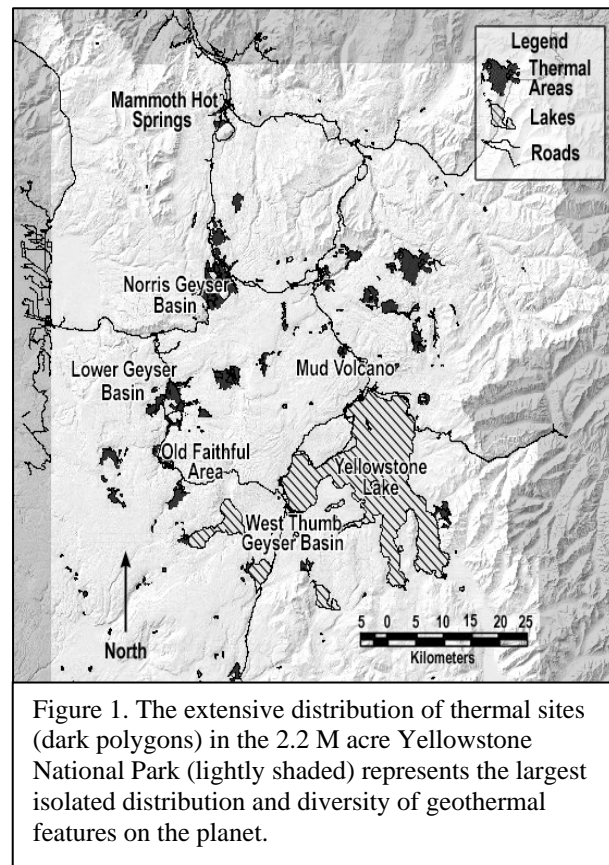
Bill Inskeep

## D. PROJECT DESCRIPTION: Research Coordination Network: Geothermal Biology and Geochemistry in Yellowstone National Park

### 1.0 Introduction

It is estimated that over 10,000 thermal sites exist in Yellowstone National Park (YNP) representing the largest and most varied geothermal basin on the planet. Within these different thermal habitats, a wide range of geochemical and environmental parameters creates an astounding number of microenvironmental niches that host a diverse array of prokaryotic and eukaryotic life, much of which has not been characterized. A large number of the 16S rDNA sequences that have been retrieved from geothermal sites and analyzed using molecular methods are not closely related to known cultured organisms. Consequently, there is often little phylogenetic inference available on which to speculate how these organisms may function in a particular environment or whether aspects of their metabolisms are unique to all of the currently known archaeal, bacterial and eukaryotic diversity. Although 16S rDNA sequence analysis is beginning to provide patterns of sequence distribution in relation to geochemical or physical conditions, more progress is necessary linking metabolic processes important to the evolution of these extremophiles with associated environmental and geochemical signatures unique to geothermal habitats.

The diversity and potential uniqueness of thermophilic organisms in YNP has drawn significant interest from research scientists during the last two decades, and several of the Group Members on the proposed *Research Coordination Network* have their own *Microbial Observatory* or *Biotic Inventory* projects funded by NSF to characterize the microbial populations important in different geothermal habitats in YNP. Furthermore, many other university or federal research scientists (many of which are listed as participants in an upcoming Fall 2003 workshop) hold permits to conduct microbial and or geochemical research on geothermal features of YNP. The diversity and number of current data collections on YNP geothermal biology and geochemistry is impressive, and during the next 2 to 3 years, an even greater number of 16S rDNA sequences will be available from a broad range of geothermal sites. Moreover, U.S. Geological Survey (USGS) and National Park Service (NPS) personnel are collecting data on thermal features necessary for understanding and inventorying a large number of geothermal sites. Coordination and networking activities proposed in this project will improve the quality and coverage of current and future research and encourage interaction among both scientific colleagues and NPS personnel responsible for resource inventory and management.



**Table 1.** Summary of *Group Member* activities regarding geothermal biology and geochemistry in Yellowstone National Park.

<b>Group Member</b>	<b>Title/Affiliation</b>	<b>Expertise</b>	<b>YNP Activity/Data Collections</b>	<b>Databases</b>
<b>Sarah Boomer</b>	Asst. Professor/ Western Oregon State College	Microbiology	Microbial diversity of “red layer” geothermal mats/ Aqueous chemistry, 16S rDNA sequences	NSF MO
<b>Joan Henson</b>	Professor Thermal Biology Institute	Mycology, microbial ecology	Eukaryotic diversity of acidic thermal springs/aqueous chemistry and sequence data	NSF MO/TBI
<b>Bill Inskip</b>	Professor Thermal Biology Institute	Geochemistry, geomicrobiology	Microbial controls on redox cycling of Fe, As and S in acid sulfate springs/ aqueous and solid phase geochemistry and sequence data	NSF MO/TBI
<b>Tim McDermott</b>	Assoc. Professor Thermal Biology Institute	Microbiology, microbial ecology	Molecular analysis of thermal soils and acidic springs/ soil chemical attributes and sequence data	NSF MO/TBI
<b>Dave Mogk</b>	Professor Thermal Biology Institute	Geology Virtual Libraries	Aqueous geochemistry and mineralogy of <i>Sulfolobus</i> habitats/ database development and virtual libraries	NSF MO/TBI
<b>Kirk Nordstrom</b>	Research Geochemist US Geological Survey	Geochemistry, stable isotopes	Geomicrobiology and geochemical processes/ geochemical data on numerous thermal springs	USGS Open File Reports
<b>Anna-Louise Reysenbach</b>	Professor Portland State Univ.	Microbiology	Global diversity and geomicrobiology of the Aquificales / YNP Biotic Survey	NSF Biotic Survey
<b>Frank Roberto</b>	Scientific Fellow and Group Leader/ INEEL <sup>1</sup>	Microbiology, Molecular biology	Isolation and characterization of novel thermoacidophilic bacteria, archaea, and viruses/sequence data	INEEL and NSF MO
<b>John Spear</b>	Research Associate U. Colorado, Boulder	Geomicrobiology Microbial Ecology	H <sub>2</sub> metabolism in YNP geothermal springs /16S rDNA sequences, aqueous chemistry	Pending
<b>Cristina Takacs-Vesbach</b>	Assistant Professor University of New Mexico	Microbial ecology	Biotic Survey of 100 diverse geothermal springs/ aqueous chemistry and sequence data	NSF Biotic Survey
<b>Ann Rodman</b>	GIS Specialist National Park Service	Resource inventory Database systems	NPS Thermal Inventory and NPS Thermophile Inventory/ pH, temperature, location, and description of YNP Thermal features	YNP-NPS
<b>Mark Young</b>	Professor Thermal Biology Institute	Virology, molecular biology	Novel viruses in <i>Sulfolobus</i> habitats/ viral, bacterial and archaeal sequence data	NSF MO

<sup>1</sup> Idaho National Engineering and Environmental Laboratory, Idaho Falls, ID

The *Group Members* assembled for this proposal represent several governmental agencies and university programs all focused on the unique geochemistry and associated biology of geothermal features in Yellowstone National Park (YNP) (Table 1). Although the boundaries of YNP provide a convenient geographic focus, *the primary goal of the proposed coordination network is to develop a more unified effort to characterize, describe, understand and inventory the diverse biota associated with geothermal habitats.* The secondary focus on YNP geothermal systems provides an excellent opportunity for collaboration among research scientists interested in describing geochemical and biotic processes, and agency personnel responsible for resource inventory and management of a constrained geographic unit. Specific objectives outlined in this proposal include coordination and networking activities that contribute directly to advancing research inquiry and productivity, resource inventory and management, and education across many scientific disciplines. *Improved coordination of research and management efforts within YNP will result in greater synergy among research programs and agency personnel as well as provide a platform for comprehensive data sharing, data access, and education across multiple audiences.*

## 2.0 Objectives

1. Develop a coordinated network of research scientists focused on geothermal biology and geochemistry in Yellowstone National Park.
2. Facilitate greater coordination and collaboration on current and future scientific research, data collection, and resource inventory of geothermal features in YNP.
3. Initiate and develop mechanisms to increase information-sharing among group members, and to utilize data collections and educational resources more effectively to reach multiple audiences.
4. Facilitate research and educational activities among group members that capitalize on synergistic overlap and information sharing.
5. Utilize the productivity and progress of a *Research Coordination Network* as a platform to solicit funding for multi-institutional and multi-agency initiatives directed towards future research and education on geothermal biology in YNP.

## 3.0 Rationale

The number and diversity of geothermal features in Yellowstone National Park has captured the interest of scientists and tourists ever since their discovery by western settlers in the early 1800's. Native Americans held the geothermal features of YNP in special regard for several reasons, although much of this history is oral and unknown in our culture. Today, two to three million people from all over the globe visit YNP annually; while they may not always observe *Ursus arctos*, *Alces alces*, or *Canis lupus*, it is a virtual guarantee that they will observe *Solfolubus*, *Cyanidium* or *Synechococcus* from boardwalks and self-guided tours in the many geothermal basins of the park. The opportunity to educate diverse audiences and contribute to literacy in the biological and chemical sciences is tremendous, and numerous programs have already made contributions to education in this arena. The number of scientists holding research permits in YNP has increased during the last decade, and interest has expanded to include studies on novel viruses, bacteria, archaea, fungi, algae and plants that are unique to geothermal habitats. The impacts of geothermal discharge and the subsequent distribution of trace elements such as fluoride and arsenic has further implications for the biology of eukaryotes

inhabiting the geothermal watersheds of YNP, including plants, invertebrates and vertebrates (Garrott et al., 2002; Kocar et al., 2003).

Given the significant interest in the biota and associated geochemical processes important in extreme high temperature environments, and the natural focus of these interests within YNP, we believe that a *Research Coordination Network* will improve future research and educational programs centered on YNP geothermal biology and promote more efficient collaboration among research scientists and agency personnel. In the current proposal we commit to building stronger linkages among scientists and agency personnel in hopes that research findings in YNP are utilized to their maximum benefit across multiple audiences including those interested in:

- discovery of novel prokaryotic and eukaryotic organisms and metabolisms,
- high temperature geothermal environments as potential models for life on other planets,
- geothermal habitats as models for ecosystem dynamics, evolution, and biological speciation,
- description and inventory of geothermal features and associated biota, and
- dissemination and delivery of educational materials to multiple audiences.

#### **4.0 Justification for a Research Coordination Network Focused On YNP Geothermal Biology and Geochemistry**

Currently, there are nearly 70 active research permits to work on YNP geothermal sites, representing numerous types of thermal features, geochemical characteristics and associated prokaryotic and eukaryotic diversity. In addition to high temperatures, many geothermal sites exhibit extreme chemical conditions including pH ranges from 1.5 to 10, and high concentrations of trace elements such as arsenic (As), antimony (Sb), mercury (Hg), and boron (B) (Fournier, 1989; Phelps and Buseck, 1980; Ball et al., 1998, 2001, 2002; Stauffer and Thompson, 1984). Geothermal discharge waters often contain elevated levels of  $H_2(aq)$ ,  $H_2S(aq)$ ,  $CO_2(aq)$ , and other reduced species such as Fe(II) and Mn(II) (Langner et al., 2001). Many of the alkaline-chloride springs such as *Octopus* sustain vibrant phototrophic (cyanobacterial) and associated heterotrophic mat communities (Ward et al., 1998; Ward and Castenholz, 2000). Many of the acid-sulfate chloride springs support a distribution of chemolithoautotrophic populations capable of utilizing electron donors such as  $H_2(aq)$ , S(-II), S(0), S(IV), Fe(II) and perhaps As(III) for energy sources (Langner et al., 2001; Jackson et al., 2001). These microbial populations represent primary colonizers in extreme low pH, high temperature environments, followed by eukaryotic phototrophs such as the acid-tolerant algal species *Cyanidium caldarium* commonly observed up to temperatures of  $\sim 50^\circ C$  (Seckbach, 1994). Biomineralization is a common occurrence in YNP geothermal systems where microbial processes are responsible for deposition of Si, As, S, Fe, Mn, and carbonate solid phases with unique morphological and or chemical signatures. These modern-day stromatolites are excellent models for understanding and recognizing microbial life forms that may be preserved in the geologic or planetary record (Ferris et al., 1986; Fouke, 2001). Interest in life in extreme geothermal environments is especially relevant to NASA programs focused on the potential existence of extremophiles in extraterrestrial environments or evidence of past life on other planets. Moreover, given the diverse range and combination of potential electron donors, electron acceptors, and C sources in geothermal habitats, there are likely a number of novel metabolisms yet to be discovered in YNP (Amend and Shock, 2001).

In addition to promising scientific advancements in understanding microbial diversity and microbial ecology, the NPS has obligations to inventory, describe and understand the nature of biological resources existing in YNP. The USGS also has directives that include inventory and description of water and geological resources. In most cases, the specific objectives and short-term goals of different academic and agency programs are not in direct conflict or competition, despite the obvious overlap in

focus on geothermal habitats. In some cases, different parties may have data collections from the same thermal site, perhaps for different but complimentary objectives. However, there are many examples where we currently do not have effective means of communicating and updating information among one another to capitalize on the synergistic opportunities that exist when several individuals may have complimentary data on an identical or similar type of geothermal feature. It is essentially guaranteed that numerous university and governmental scientists, and NPS managers will continue to collect data and make observations of thermal features in YNP, irregardless of whether we coordinate. The challenge is to understand the diversity of ongoing research and data collection along with the needs of agency personnel to develop a network that enhances data sharing and communication in a manner that assists individuals in meeting their specific objectives, as well as extends their efforts to contribute to education, management or research audiences. In most cases, individuals distributed across university departments, governmental laboratories or federal agencies do not have the time or mandate to fully network with all possible audiences. *Coordination activities must be structured with outcomes that enhance individual as well as collective productivity.*

#### **4.1 Summary of Directives Focused on YNP Geothermal Biology and Geochemistry**

**4.1.1. Academic Research Interests.** Academic scientists have contributed a significant amount of numerical and descriptive data on the surficial characteristics and biota associated with geothermal features in YNP. Certainly, Brock (1978, 1987) led a pioneering effort to describe the immense prokaryotic and eukaryotic diversity located in the many thermal habitats of YNP. Over the last two decades several research groups have made excellent progress characterizing microbial populations important in specific thermal features such as the cyanobacterial and green nonsulfur populations of *Octopus Spring* (Ward et al., 1998) and green sulfur bacteria of New Zealand thermal springs (Wahlund et al., 1991). The difficulty in accurately describing the important microbial populations utilizing cultivation approaches has limited progress in understanding the diversity of biota associated with thermal features. However, with the rapid pace of molecular techniques (i.e. routine amplification of 16S rDNA genes; Pace et al., 1986; Amann et al., 1995), progress towards a better understanding of the diversity and distribution of prokaryotic and eukaryotic organisms in geothermal habitats has improved considerably (Barns et al., 1994, 1996; Pace, 1997; Hugenholtz et al., 1998). Although the diversity of thermal springs under direct study by research permit holders is impressive, the scope and scale of investigation varies widely, yielding data sets that are by nature of the specific questions asked, independent and variable in content. Research objectives focused on YNP thermal features can range from focused efforts to cultivate metabolically unique isolates to studies focused on biomineralization of solid phases to surveys of microbial diversity. Collectively, the results generated by active research scientists in YNP represent an impressive portfolio of information on geothermal biology that could be utilized more effectively to assist in resource inventory and in education. Moreover, the probability of discovery of novel microorganisms (both prokaryotic and eukaryotic) in geothermal habitats is extremely high, considering that only a small percentage of the total thermal features in YNP have actually been studied.

Past research on the microbiology and microbial ecology of thermophilic organisms in YNP has contributed significantly to our understanding of life forms and novel metabolisms in high temperature and unique geochemical environments. Only recently, with the advent of routine molecular methods (e.g. primarily 16S rDNA sequence analysis), have we begun to understand the extent of microbiological diversity represented by the numerous geothermal habitats (Ward et al., 1998; Pace, 1997; Reysenbach et al., 1994; 2000). Continued research will undoubtedly make further contributions to identifying novel organisms that have no cultured relatives to date, and in most cases, we have little to no information regarding the physiological or metabolic capabilities of these organisms. Past research in YNP has also contributed significantly to our understanding of microbial speciation across

chemical and physical gradients and how these environmental factors may control the evolution of *phylotypes*, closely related microorganisms exhibiting adaptive radiation in response to important environmental gradients, and the importance of these gradients in defining microbial species (Mayr, 1982; Donoghue, 1985; Davis and Nixon, 1992; Fox et al., 1992; Goodfellow et al., 1997; Ward, 1998). Such adaptations, which may not be easily ascertained from 16S rDNA sequence and phylogenetic analysis, are likely critical links in understanding the coevolution of microbial populations which define a geothermal microbial community and the degree to which environmental attributes are correlated with the distribution and diversity of genetic code (Reysenbach and Shock, 2002).

In essence, a critical mass of scientists has already assembled around the geothermal habitats of YNP. In just the past five years, four NSF Microbial Observatories and a NSF Biotic Survey project have been awarded to *Group Members* represented here. In addition, YNP research scientists are funded heavily by other NSF programs, NASA, USGS, and NPS. All Microbial Observatory projects are required to develop accessible web content, reflecting data collections and other results. One of the primary objectives of the NSF Biotic Survey and Inventories project (Group Members: Takacs-Vesbach, Reysenbach, Rodman) is to develop a comprehensive web-accessible database containing detailed geochemical data and microbial inventories of 100 diverse thermal springs. Other investigators, some with long track-records focusing on the microbial ecology of thermal features in YNP (e.g. Ward, Reysenbach, Pace, Castenholz) have excellent data collections on specific thermal sites. Much of this is published in the refereed literature and 16S rDNA sequences are in many cases available via Accession Numbers in GenBank (Altschul et al., 1997).

More recently, the formation of the *Thermal Biology Institute* at Montana State University (funded as a satellite node to NASA's exobiology programs emphasizing life in extreme environments) has resulted in additional focus on YNP geothermal features ranging from studies on thermo-tolerant grasses (Stout et al., 1997; Al-Niemi and Stout, 2002), novel viruses in geothermal environments (Rice et al., 2001; Snyder et al., 2003), plant-fungal symbionts important in geothermal soils (Redman et al., 2002), pathogenic amoeba in acid-sulfate springs (Sheehan et al., 2003) and chemolithoautotrophs in acid-sulfate springs (Jackson et al., 2001; Inskeep et al., 2003) to identification of novel viral cages that may be important in nano-material biosynthesis (Douglas et al., 2002). Currently, 10 faculty members at Montana State University are supported by the *Institute* on projects focused on YNP thermal environments. The projects supported by TBI cross a substantial breadth of disciplinary expertise, which reflects the primary mission of the *Institute*: **to conduct and promote research and education focused on the biology and interrelated physical and chemical processes of geothermal environments in the Greater Yellowstone Ecosystem**. The *Thermal Biology Institute* will also be expanding its website to include links to individual programs, highlighting data collections and current research. More specifically, TBI has longer-term goals to lead and or participate in development of a searchable virtual library where links to other research and educational resources would be easily accessible. Given that numerous academic and government scientists will continue to collect worthwhile geochemical and biological data and publish results in refereed journals, it is our position that a *Research Coordination Network* will enhance the productivity of the YNP research community and the quality and scope of individual data collections.

**4.1.2. U.S. Geological Survey Programs.** The U.S. Geological Survey (USGS) also has a long track-record of characterizing the geothermal waters and geology of YNP (dating back to Gooch and Whitfield, 1888). Much of the early effort within the USGS was focused on chemical characterization (older citations) of numerous springs, geysers or mud pots as well as an intensive effort to utilize chemical signatures as a mechanism to map sources of hydrothermal discharge and understand relative contributions of meteoric water and hydrothermal water to surficial discharge (Fournier, 1989). Past

studies supported by the USGS have resulted in comprehensive data sets on the distribution of arsenic (As) and antimony (Sb) (Stauffer et al., 1984) in geothermal springs. More recently, USGS scientists (led by Group Member Nordstrom) have inventoried numerous springs in YNP, collecting thorough water chemical data and summarizing these data sets in USGS Open File Reports (Ball et al., 1998, 2001, 2002). Research scientists at the USGS have also made important contributions to understanding processes important in the speciation of sulfur in numerous springs including *Cinder Pool* in Norris Basin, YNP (Xu et al., 1998; 2000), and more recently, in the discovery of numerous thermal vents in Yellowstone Lake (L. Morgan, USGS).

The focus of USGS inventory and data collection has been primarily chemical composition and hydrogeological dynamics important for understanding the source and spatio-temporal distribution of hydrothermal features in YNP. Surficial chemical signatures are often extremely valuable in tracking sources of hydrothermal fluids and in establishing mixing ratios with meteoric water. USGS scientists have been interested in ratios of oxidized and reduced species (e.g. Fe[III]/Fe[II]; As[V]/As[III]) as an additional indicator of hydrothermal-meteoric water mixing ratios. Consequently, the role of microorganisms in mediating redox reactions important in geothermal environments is essential for understanding oxidation rates of chemically reduced species such as H<sub>2</sub>, H<sub>2</sub>S, Fe(II), As(III), and NH<sub>4</sub>. Furthermore, USGS scientists are keen on understanding linkages among aqueous and solid phase geochemical characteristics to microbial processes. Currently, USGS scientists are interacting with university scientists on different NSF funded *Microbial Observatories* and *Biotic Surveys* (Group Members: Nordstrom, Inskeep, Henson, Takacs-Vesbach, Rodman). The *Research Coordination Network* proposed here would further cement this type of collaboration where individual parties contribute specific expertise in accomplishing their own goals, but improve the end result by collecting more comprehensive analyses on similar or identical thermal sites.

**4.1.3. NPS Resource Inventory and Protection.** The geothermal features of Yellowstone National Park contain microbiological diversity that has the potential for remarkable scientific, social, and economic impact. However, information on the biological and chemical diversity of thermal areas is scattered and the correlation between sequence distribution and the physio-chemical parameters of thermal habitats poorly understood. The Yellowstone Research Permit Office maintains a database of all researchers who have worked in the park since 1990, but there is no simple way for YNP staff to access the information generated by that research. The NPS has been populating three linked databases that could form an important framework for linking multiple data collections. These databases include a spatial inventory of each thermal feature, a literature search of microorganisms, and a field survey of microbial diversity (Group Members: Takacs-Vesbach, Rodman, Nordstrom).

In 1998, a team of NPS personnel began a spatial inventory of significant thermal features within YNP. Every year, accurate (within two meters) locations of more than 1,300 thermal features are collected with GPS units and added to a spatial database which includes digital photographs, physical descriptions, and measurements of pH, electrical conductivity, and temperature. The 6,700 thermal features documented in this database represent approximately 60% of the known thermal areas in the park. This inventory will continue until all the known thermal areas are represented in the database. In addition, there are plans to add chemical information from previous inventories, including thousands of records from USGS reports on the chemical analysis of thermal waters.

A preliminary inventory of 35 thermophilic microorganisms and their associated thermal habitats was completed in 1996. Those organisms were only linked to the pool where they were first discovered, not to all the other pools where they have subsequently been found. Also, if an organism was originally discovered in a hot spring outside of the park, and was later found in Yellowstone, it was not included in the inventory. A project which began in 2000 examined published literature and

updated the original inventory to include all known microbes and all thermal features where the microbes have been found. This literature review found 406 different organisms that were discovered from 105 different thermal features. The database documents nearly 700 unique organism/pool combinations. This information has been linked with the chemical and physical inventory work described above. Unfortunately, funding has not been available to continue this work and the literature review is only current through 2001. It would be a tremendous benefit to the park to continue updating this database with new information from recent journals.

Although a significant amount of existing information has been collected and included in this database, it is apparent that very little of Yellowstone's microbial biodiversity has been discovered. Of the 700 organism/pool combinations included in the database over half are from only two features, *Octopus Spring* and *Obsidian Pool*. Most microbiology research projects in Yellowstone have focused on a specific organism. This means that we know the feature that the specific organism was collected from but we know nothing about the other members of that microbial community. In 2002, NPS staff in cooperation with university researchers, began a field survey of microbial diversity. This database is linked directly to the thermal inventory database already described.

The process of inventorying the physical, chemical, and biological characteristics of Yellowstone geothermal areas is crucial to developing a thorough understanding of these areas and how they change over time. Protection of the thermal areas and recognition of their uniqueness was a driving force behind the creation of our enabling legislation. This protection requires an integrated management approach that depends heavily on scientific research. Our success in preserving the microbial diversity in thermal pools depends largely on the acquisition and application of scientific knowledge to the management of these resources.

**4.1.4. Idaho National Environmental and Engineering Laboratory.** The U.S. Dept. of Energy (DOE) has primary missions of energy generation and national security. The Biotechnology Dept. at the INEEL has historically focused on microbial processing of large volume, low value materials, including wastes, contaminants, fossil fuels, and commodities such as mineral ores. Bioprocessing using microorganisms has addressed DOE missions in the *Offices of Science* (environmental and biological research, including bioremediation of hydrocarbons, halocarbons, heavy metals and radionuclides, natural attenuation, climate change, and carbon sequestration), *Fossil Energy* (coal biobenefication, enhanced oil recovery, methane hydrates), *Conservation and Renewable Energy* (biomass utilization, chemical feedstocks from waste biomass, biomining, bioprocessing for fuels and chemicals), and the *National Nuclear Security Administration* (biotechnology solutions for the intelligence community, detection of priority pathogens). Yellowstone National Park has been a frequent source of novel microorganisms with potential to solve technological challenges facing the nation.

INEEL staff are engaged in independent research projects utilizing the unique natural resources of Yellowstone, including projects seeking microorganisms with enhanced capacity to dissolve metal sulfide ores at low pH and high temperature, microbes expressing thermophilic enzymes to improve the processing of commodity chemicals, paper, pulp, biomass, and wastes, and basic studies considering the mechanisms of survival and growth under extreme environmental conditions (Johnson et al., 2001). Collaborative research projects with academic and industrial partners are common, and currently include a Montana State University-led *Microbial Observatories* project (Group Members: Young, Roberto, Mogk) focused on viral diversity in YNP, as well as projects with Idaho State University, Utah State University, and University of Wales faculty. INEEL staff has also developed a prototype GIS database to overlay microbial inventories on available geographic, environmental,

chemical, and geological data that may be helpful in development of a more comprehensive virtual library (Stoner et al., 2001).

#### 4.2 The Case for Coordination

The brief review of university and agency programmatic efforts focused on geothermal features in YNP suggests that individual scientists and agency personnel have common interests which serve as a strong nucleus for coordination. Many individual participants will benefit directly from coordination initiatives, including improved collaboration among university scientists, increased accessibility and data sharing among research groups and agency personnel, and expanded education and outreach programs that represent network participant priorities.

#### 5.0 Research Coordination Activities

##### 5.1 Formation of the Research Coordination Network.

The formation of a more formal *Research Coordination Network* focused on YNP geothermal biology and geochemistry will represent in part a coalescence of several research and management clusters that are forming naturally as a result of both scientific and agency collaboration. In addition, the commitment of the *Thermal Biology Institute* to facilitate greater coordination and provide networking opportunities is a positive catalyst for forming a sustainable research focus group with aspirations to improve discovery of novel organisms and metabolisms in geothermal habitats, and to contribute to data sharing and accessibility. The *Thermal Biology Institute* is hosting the First Biannual Workshop on *Geothermal Biology and Geochemistry in Yellowstone National Park* on October 9-11, 2003. One of the primary goals of this workshop is to bring together a critical mass of scientists and agency personnel working in YNP to share current research thrusts and to initiate a working group that will improve networking among scientists focused on thermal biology in YNP. The *Thermal Biology Institute* is funding participant travel and accommodations necessary to attend the Fall 2003 workshop, and will be publishing a professional proceeding to represent the diversity and scope of work focused on YNP geothermal biology. To date, we have received an excellent response from individuals invited to attend (see Table 2 for a complete list of participants who have agreed to attend, present findings and participate in several break-out planning sessions).

**Table 2.** Additional individuals (not listed in the current proposal as *Group Members*) who have at the time of proposal submission agreed to participate in the First Biannual Workshop (October 2003) on *Geothermal Biology and Geochemistry in YNP*.

<b>Workshop Participants, Affiliation</b>		
Carmen Aguilar, U. Wisconsin	Eric Mathur, Diversa Corp.	Pat Shanks, USGS
Steve Bell, U. of Cambridge	Michael Madigan, S. Ill. U.	David Stahl, U. of Washington
Fred Cohan, Wesleyan Univ.	Michael Meyer, NASA	Karl Stetter, U. of Regensburg
Sherry Cady, Portland State	Lisa Morgan, USGS	Rich Stout, MSU/TBI
Ken Cullings, NASA Ames	Tracy Norris, Univ. of Oregon	Vicki Thompson, INEEL
David DesMarais, NASA Ames	Gary Olsen, Univ. of Illinois	John Van Der Oost,
Trevor Douglas, MSU/TBI	John Peters, MSU/TBI	Wageningen John Varley, YNP
Christie Hendrix, YNP	Frank Robb, U. of Maryland	Dave Ward, MSU/TBI
Martin Lawrence, MSU/TBI	Lynn Rothchild, NASA	Jon Wraith, MSU/TBI
Donald Lowe, Stanford Univ.	Everett Schock, ASU/Tempe	Brent Ybarrondo, Adams St. U.

It is important to add that these meetings are not intended to be exclusive of any individual or group conducting scientific studies or inventory of YNP geothermal features. Undoubtedly, we have missed important individuals during the planning stages for this first workshop; *however, it is our goal that this workshop represent the first in a series of focused goal-oriented workshops intended to improve coordination and productivity, and the list of invitees will be expanded as visibility of the Coordination Network grows.*

The diversity represented by *Group Members* assembled in the current proposal (Table 1) reflects an interest to bring together disparate programs with different but overlapping objectives. For example, we have purposely included several different disciplines and different agency personnel in hopes of capturing the breadth of programmatic effort focused on geothermal biology and geochemistry. Group members represent a range of expertise including geology, geochemistry, microbial ecology, biology, microbiology, and database development as well as a range in focus on both undergraduate and graduate training. The Fall 2003 workshop provides an excellent opportunity to formalize a *Research Coordination Network* involving many of the active research and agency programs focused on geothermal biology and geochemistry in YNP. As will be discussed in the Budget Justification section, the *Thermal Biology Institute* is willing to provide continuing support for a *Research Coordination Network* by providing funds for participants to attend future biannual workshops.

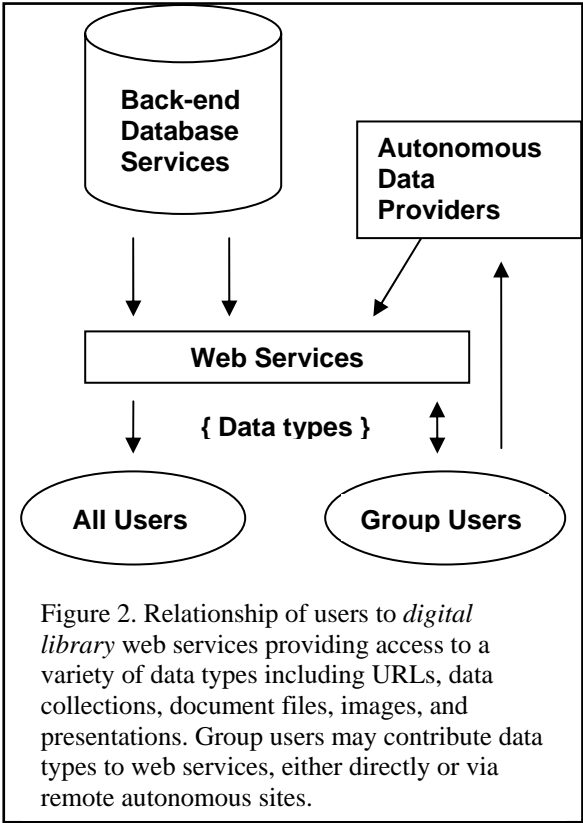
In addition to presenting research results at the Fall 2003 workshop, individuals will participate in planning sessions intended to initiate more formal coordination among research groups and agency personnel. Participants will discuss prioritization and strategies for implementing several coordination efforts including (i) networking and data accessibility, (ii) enhanced education and outreach, (iii) targeted focus group workshops, (iv) cross-training of graduate students or postdoctoral associates, and (v) equipment sharing. The priorities of this *Research Coordination Network* include programs to (i) enhance collaboration among research faculty focused on interrelationships among chemical and physical factors and the distribution of biota in thermal landscapes, (ii) promote greater synergy among academic faculty and NPS personnel, (iii) improve educational opportunities for graduate students, postdoctoral associates, science educators, YNP interpretive staff, undergraduate students and other audiences interested in thermal features.

## **5.2 Networking, Information Sharing and Digital Libraries**

The amount of information available on thermal features of YNP is actually quite impressive despite the fact that only a small percent of the geothermal sites have been characterized. As mentioned, this information ranges from detailed microbial and geochemical analyses of a specific geothermal site to cursory surveys of many springs. Significant amounts of geochemical data are available for different spring types, collected either by individual scientists or USGS personnel, and numerous 16S rDNA sequencing efforts have been completed and many more are in progress. Consequently, there is much to be gained by establishing a formal *Coordination Network* that will focus on initiating digital networks that will allow information sharing across RCN participants and external users. As we have explored methods and tools available to build digital networks, one of the most convenient and user-friendly formats is the concept of digital libraries. A digital library is essentially a web-based service that provides users the opportunity to contribute, store, organize, maintain, or utilize network-accessible documents that are distributed among many owners. Several excellent examples of digital-library style formats have been established including the Science Education Resource Center for education in the Earth Sciences ([serc.carleton.edu](http://serc.carleton.edu)), the University of Alaska Museum containing a large library of biological collections and specimens ([uaf.edu/museum/](http://uaf.edu/museum/)), and the Digital Library Project at UC, Berkeley ([elib.cs.berkeley.edu](http://elib.cs.berkeley.edu)). *One of the goals of the current proposal is to begin a focused effort to create a digital library providing access to information collections on the geobiology*

and geochemistry of thermal features in YNP. A digital library will be initiated after discussion and agreement among Group Members and RCN participants, but will follow the conceptual structure outlined in Figure 2. The technology infrastructure at MSU-Bozeman is able to support our efforts to create a digital library. Enterprise-class hardware and database software are supported for TBI faculty in the Office of Information Technology Systems (Montana Agricultural Experiment Station, MAES). Furthermore, our unit employs dedicated information technology specialists focused on database management systems. As part of the proposed work we will contract with Mr. Matt Rognlie and Mr. Ric Roche (MAES) to work closely with a *Communications Director* (supported by the proposed RCN) developing the digital library. Mr. Rognlie will also participate in our database system workshop so that construction of a digital library takes place iteratively with input from primary contributors and RCN participants (see supplemental documentation). Although the physical location of the server is really unimportant, the *Thermal Biology Institute* will accept responsibility for initiating this effort, preserving autonomous data providers whenever preferred by Group Members and RCN participants (Figure 2).

Advantages of making data, documents, images, URLs and educational resources available through a digital library format are numerous; *the primary goal is to provide a mechanism to manage and make available distributed information collections to multiple users.* A database management system allows users to populate, maintain and search collections. The information collections may continue to reside on appropriate network-accessible sites or be stored directly on a back-end data server. Group members and RCN participants will play an important role in providing library content and the *Communications Director* for the proposed RCN will spend considerable effort targeting specific datasets and information collections currently available. In addition, we will be educating users on mechanisms for creating new collections or modifying existing collections, and in doing so begin an iterative process where specific database tools are in part built based on research and educational user contributions. One of the key issues for discussion among RCN participants is creating standards for data formatting and defining appropriate metadata that provide maximum interoperability among users. In part, this is an exercise in defining controlled vocabulary that provides mechanisms for maximum data access and searchability. The RCN will initiate this process with a goal that by the end of the second year of the project, a first generation digital library will be ready for testing among RCN participants and contain prototype data collections and or links to appropriate network-accessible sites. Further, by the end of the proposed project, the digital library will be adequately populated for open use by multiple audiences. We believe that creation of a digital library will improve coordination among scientists and agencies involved in basic research and in management of geothermal sites in YNP.



### 5.3 Workshops.

Several types of workshops will be offered as part of the proposed *Research Coordination* activities to encourage both broad information sharing and more focused efforts targeting specific research and agency needs. The Fall 2003 Workshop is focused on the breadth of current and past research on YNP geothermal features and will serve to both educate one another and to identify common interests and priorities. We propose a flexible and rotating workshop schedule, where biannual meetings will be intended to provide thorough coverage of research and agency activities and alternating workshops will be focused on specific questions important for progress on high priority initiatives. The proposed workshop schedule is intended to address several high priority initiatives, including (i) virtual libraries and data format standards, (ii) standardization of geochemical and field analytical protocols, and (iii) the use of molecular tools for determining the distribution of microbial species as a function of geochemical and thermal parameters.

**1. Fall 2003.** *First Biannual Workshop.* Focus on thorough coverage of YNP research and agency activities. The outcome of the workshop will include a professional workshop proceeding for distribution to research scientists, agency personnel and environmental science students. This workshop is already planned and will be funded by the *Thermal Biology Institute*.

**2. Spring/Fall 2004.** *Database Workshop.* Focus on creation of virtual libraries, database accessibility, and identification of common metadata for employing database searchability and interoperability among users and contributors. The intended audience will include all research scientists and agency personnel working in YNP that have an interest in database development and accessibility. This workshop would be the first one funded as part of the proposed *Research Coordination*, and will represent an important step for encouraging progress towards a virtual library style format for research and educational resources.

Given the computing and technological capabilities for networking, it is fairly simple and perhaps unoriginal to articulate as a major goal that we desire to improve networking and data accessibility. However, progress in this arena will require well-focused objectives regarding desired content, intended priority audiences, searchability, and interoperability. A focused workshop on virtual libraries, information networking and database cross-talk will be an important step in bringing participants to a point of common understanding. Data resources are not static, and cannot be fully populated overnight, especially without a coordinated effort among individual participants to contribute important data or relevant sources of information. *Mechanisms should be established where RCN participants are encouraged and desire to become both library users and contributors.* For the proposed work, a short-term goal will be to begin a virtual library web service with links to currently available research data and educational resources, and where Group Members and RCN participants assist in identifying and or contributing appropriate *library* content. Several of the Group Members have specific interests and past experience in database development and these individuals will assist in outlining methods of data standardization and creation of common metadata. Specifically, NPS personnel have been working on *Thermal Inventory* databases (Group Member Rodman), INEEL and YNP personnel have established prototype database tools for YNP (Stoner et al., 2001), and a *Biotic Survey and Inventory* project will develop a microbial and geochemical database of 100 thermal springs (Group Members Takacs-Vesbach and Reysenbach). Finally, Group Member Mogk has extensive experience in digital library formats, having just worked on a major project in Earth Sciences education (serc.carleton.edu). Consequently, these individuals will be important contributors to the proposed database workshop.

**3. Fall 2005.** *Second Biannual Workshop.* The focus would alternate back to a thorough coverage of YNP research and agency activities, along with breakout sessions focused on virtual library implementation and population. The outcome of this workshop will include a professional workshop proceeding as well as subcommittee reports on breakout sessions.

**4. Spring/Fall 2006.** *Workshop on Aqueous and Solid Phase Geochemical and Field Analytical Protocols.* This workshop will focus on aqueous and solid phase geochemistry important in geothermal systems, including thermodynamic and kinetic approaches, chemical speciation using aqueous models, and analytical tools for chemical analysis of geothermal waters, soils, sediments and or microbial mats from YNP geothermal sites. The outcome of this workshop will include publication of a document entitled *Chemical Characterization of Geothermal Sites in YNP: Guidelines and Suggested Methodologies.*

**5. Fall 2007.** The *Third Biannual Workshop* will alternate back to a thorough coverage of YNP geothermal biology research, continuing an open invitation to include new research scientists and agency personnel involved in YNP geothermal biology. This workshop will continue to use breakout sessions to establish smaller working groups for targeted initiatives.

**6. Spring/Fall 2008.** *Workshop on Microbial and Molecular Methods.* This workshop will cover two themes related to the identification of relevant and dominant microorganisms in geothermal systems and their functional relationship to the surrounding geochemical or physical environment. Methods of 'routine' 16S rDNA sequence and phylogenetic analysis will be reviewed, along with FISH and real-time PCR applications; however, considering more advanced molecular methods, emphasis will include functional gene approaches, full genome projects and functional inferences from gene annotation, microarrays, and community genomics. A second theme in this workshop will include cultivation using novel strategies designed from measured environmental constraints, and the important role of physiologic and metabolic characterization in confirming functional attributes of phylogenetically defined microbial populations. This is particularly important considering that in many cases the high percentage of novel 16S rDNA sequences in geothermal environments limits our ability to infer functional attributes of dominant organisms. If a subset of these organisms can be successfully cultivated, we expand our appreciation of prokaryotic and eukaryotic diversity, and importantly, their potential role in associated geochemical environments.

**7. Fall 2009.** A *Fourth Biannual Workshop* will alternate back to the full participant list and provide thorough coverage of research on geochemistry and geothermal biology. This would represent the last workshop funded by the proposed Research Coordination Network; consequently, breakout sessions will focus on project continuation, networking prioritization and participant or group member assignments necessary for securing funds for a second five year period. As mentioned, the *Thermal Biology Institute* is committed to assist in this coordination network and will be working to identify mechanisms by which the project could be continued for another five year time period.

#### **5.4 Student/Staff/Faculty Exchange.**

Research coordination often requires personnel exchange among participant laboratories. The *Thermal Biology Institute* has been supporting such activities in several ways during the last 3 years, and will continue to provide support for exchange programs that will benefit the proposed Research Coordination. For example, TBI has funded several visiting scientists for periods ranging from 1 week to 6 months, and in all cases these exchanges have led to increased collaboration and research coordination (examples include R. Castenholz, W. Zillig, F. Cohan and D. Or). TBI has also supported small travel grants for students and other staff to visit external laboratories for collaborative purposes,

and will continue to support scientific exchanges or coordinated sampling trips to YNP field sites. Finally, a successful weekly seminar program focused on thermal biology and comprised of approximately 70% outside speakers has been an excellent mechanism for enhancing collaboration among other research scientists focused on geothermal chemistry and or biology.

## 5.5 Management and Coordination Plan

The proposed RCN will be managed and institutionalized in the general operating framework of the *Thermal Biology Institute* at Montana State University. The potential synergistic relationship between an institute that is focused on thermophilic and thermo-tolerant organisms in YNP geothermal habitats, and a *Research Coordination Network* focused on improved collaboration and data-sharing is a positive and significant catalyst for coordination activities. Although centered at Montana State University, the *Institute* is committed to assisting and or facilitating the activities of other research scientists, agency personnel or educators; *the TBI exists to serve programs that advance the many facets of biology, ecology, evolution, geochemistry and geothermal hydrology important in describing thermal habitats*. There are several important advantages of utilizing the operating management structure of TBI to oversee and guide the proposed work. PIs-Inskeep and Young are currently serving as co-directors of TBI with terms that run for 2 more years, and will be responsible for guiding the proposed work. Three additional TBI faculty (currently McDermott, Stout and Wraith) serve on an Executive Board. A weekly board meeting with the five voting members is used to conduct and approve TBI business, and this type of structure ensures that TBI initiatives move forward in a timely manner. The added dimension of the proposed work will include communication with a much larger network of what initially may be as many as 40 to 50 individuals. The role of core participating scientists will include attendance at workshops, contributing access to *library* content, collaborating when possible on focused research or management initiatives, and evaluation of RCN activities on a yearly basis. A democratic process will be established on major decisions of the proposed RCN where email communication and postings on the current *Thermal Biology Institute* website will be used to keep RCN participants and group members apprised of all announcements, status reports, workshop plans, and self-evaluation forms. The *Thermal Biology Institute* is not interested in dictating the outcomes of the proposed RCN activities, but rather offers a mechanism to capitalize on the modest but important critical mass of individuals and programs already established and located directly in the Greater Yellowstone Ecosystem (GYE).

Other advantages of hosting a *Research Coordination Network* within the overall framework of the *Thermal Biology Institute* include:

- Leadership and democratic governance is already established,
- Physical facilities and infrastructure available at MSU can be used as logistical support for external scientists and educators,
- TBI actively encourages discussion and networking with all scientists focused on geothermal habitats, including younger scientists and international colleagues,
- NPS personnel are within 90 miles of MSU allowing for frequent coordination visits,
- TBI programs and progress are reviewed annually by a Scientific Advisory Board for guidance and critical comments, and by an independent AAAS review team,
- Other TBI staff including a *Program Manager* and an *Outreach Coordinator* will contribute directly to the proposed work through facilitation of workshops and publication of *library* content, and
- TBI has committed funds to assist in supporting participant travel stipends to attend workshops.

We also recognize that the proposed *Research Coordination Network* will link several individuals funded on NSF Microbial Observatory and Biotic Survey projects and it is not expected that the RCN activities will supplant or compete with objectives of and or individual responsibilities to those programs. Conversely, it is our hope that the RCN efforts proposed here will not only assist these individuals in meeting data sharing objectives but also provide a mechanism by which individual data sets or educational resources can be used to reach a larger number of audiences, including fellow scientists and agency personnel. We are also aware that principal investigators on *Microbial Observatory* projects may also participate in efforts to coordinate among the many different *Microbial Observatories*. Certainly, activities to improve visibility and coordination of the entire *Microbial Observatory* network will not overlap significantly with the proposed focus on geothermal biology and geochemistry in YNP. If a global MO network is developed it would be our goal to ensure cross-talk and linkage between that effort and activities of the four YNP Microbial Observatories represented here (Boomer, Henson, McDermott and Young).

## **5.6 Information and Material Sharing**

Participants will be encouraged to make data, images, reports, educational resources or other information available to the digital library network. In many cases, it will be advantageous for participants to contribute information because of the increased exposure of important individual contributions. In some cases, the *Communications Director* supported on the proposed project may identify existing data sets or information resources and assist participants in contributing this to a digital library, or establishing the necessary links to access the information on a remote server (Figure 2). Individual participants will retain ownership of all information contributed, and this fact will be clearly identified in the digital library. When appropriate, required copyright permission will be the responsibility of the contributor. If documents placed in a collection are encumbered, it will be the contributors' responsibility to ensure that intellectual property rights are not violated. Certain contributions might be password protected where smaller workgroups or audiences are the only intended information users.

All RCN group members and participants that are directly involved with focused initiatives and that result in publications, shared databases, or joint projects on educational resources will be credited with authorship. The products and outcomes of the *Research Coordination Network* will reflect the efforts of individual participants; consequently, these individuals will be credited with the work.

## **5.7 Increasing Diversity**

Many young scientists and new investigators are involved in research activities in YNP and they will be encouraged to participate in hopes that a vibrant research community will assist their efforts to establish a productive research and or outreach program important to their individual position descriptions and job performance. We have purposely included a full range of younger scientists and well established research scientists all with varying and diverse backgrounds to represent the initial Group Members (Table 1) and to represent the diversity reflected in the larger participant list. Our plan for incorporating new members and young scientists is reasonably simple: identify promising post-doctoral associates and Ph.D. students using participant contacts, encourage them to attend workshops by providing travel stipends and accommodations, and solicit their direct participation (independent of their current mentor) as users and contributors to the virtual library network. The philosophy of the proposed RCN will be to encourage participant diversity and this is reflected in the breadth of Group Members represented in the current proposal. The diverse backgrounds and physical locations of these mentors will assist in increasing participation by under-represented groups.