**REPORT**

**Draft 03/13/05**

# "DARK ENERGY" WORKSHOP

**Clark Laboratory, Quissett Campus, WHOI**
***October 3 - 5, 2004***

**Sponsors:** Deep Ocean Exploration Institute and Ocean Life Institute, Woods Hole Oceanographic Institution

**Organizers:** Wolfgang Bach (WHOI), Katrina Edwards (WHOI), John Hayes (WHOI), Stefan Sievert (WHOI), and Mitch Sogin (MBL)

## Overview:

Energy flow in biological and geochemical systems is governed by the same thermodynamic rules. Electrochemical gradients in the deep ocean and below the seafloor exist at scales from micrometers to kilometers. How mass and energy cycles of geological and biological systems in these environments are related, however, is basically unknown.

Of critical importance for developing an improved understanding of the linkages between geochemistry and microbiology is the development of methods that allow us to measure in situ electrochemical gradients and gain information on the composition and activity of microbial communities thriving in these gradients.

A goal of the Dark Energy Workshop was bringing together experts in (1) theoretical geochemistry and biochemistry, (2) molecular microbiology and geochemistry, and (3) field/experimental/sensor specialists. Questions to be explored include: What controls interactions between cells and the environment in the deep sea and subseafloor? What is the minimum energy required for growth and maintenance of cells? How does the geochemical environment control microbial activity and community structure?

This workshop report provides a summary of lecture notes and breakout group discussions with focus on the primary intellectual questions, promising techniques and research strategies, and possible implementations.

**Table of content**

**List of Participants:**

Robert Alberty, MIT
Jan Amend, Washington Unv.
Wolfgang Bach, WHOI
John Baross, U Washington
Joan Bernhard, WHOI
Alexander Bradley, MIT
James Cowen, U Hawaii
Jan de Leeuw, NIOZ
Mark Dennett, WHOI
Ashita Dhillon, MBL
Steve D'Hondt, U Rhode Island
Katrina Edwards, WHOI
Tim Ferdelman, MPI Bremen
John Hayes WHOI
Kai-Uwe Hinrichs, U Bremen
Tori Hoehler, NASA Ames Research Center
Julie Huber, U Washington
Susan Humphris, WHOI
Julius Lipp, U Bremen
George Luther, U Delaware
Betty Jean Luther, U Delaware
Tom McCollom, U Colorado
Michael McInerney, U Oklahoma
Ann Pearson, Harvard
Olivier Rouxel, WHOI
Jeff Seewald, WHOI
Jessica Seiber, U Oklahoma
Stefan Sievert, WHOI
Mitch Sogin, MBL
Helen Sturt, WHOI
Roger Summons, MIT
Craig Taylor, WHOI
Meg Tivey, WHOI
Joe Vallino, MBL
Ben van Mooy, WHOI
Frank Wenzhoefer, MPI Bremen
Jean Whelan, WHOI
Linda Amaral Zettler, MBL

## Workshop program

# *Dark Energy: The Deep Oceanic Biosphere Workshop*

**(Co-sponsored by WHOI Deep Ocean Exploration Institute and Ocean Life Institute)**

**October 3 – 5, 2004**

**Woods Hole Oceanographic Institution**

**Woods Hole, Massachusetts 02543**

| | |
|---|---|
| **Sunday, October 3, Clark Lab, Room 507, Quissett Campus, WHOI** | |
| 5:00 p.m. | Icebreaker reception and buffet dinner |
| | |
| **Monday, October 4, Clark Lab, Room 507, Quissett Campus** | |
| 8:00 a.m. | Shuttle from Hotel |
| 8:15 - 8:40 | Continental Breakfast, Clark Foyer |
| 8:45 - 9:00 | Welcome (Susan Humphris, WHOI) |
| 9:00 - 9:20 | Introduction –"The role of dark energy in the earth system" (John Hayes, WHOI ) |
| **Lecture Presentations (35 min. + 10 min. for discussion)** | |
| 9:30 - 10:15 | Jan Amend, Washington University<br>"Energy-yields and energy-demands in microbial geochemistry" |
| 10:15 - 10:30 | Coffee Break |
| 10:35 - 11:20 | John Baross, University of Washington<br>"Phylogenetic and physiological diversity of subseafloor microbial communities" |
| 11:25 - 12:10 | George Luther, University of Delaware<br>"Chemical sensors to understand biogeochemical processes - total element analyses versus chemical speciation analyses" |
| 12:15 - 1:15 | Lunch (catered in Clark Foyer) |
| 1:20 - 3:20 | 1st Breakout Session; disciplinary groups; charge: "What can we measure?" |
| 3:25 - 3:35 | Coffee break |
| 3:40 - 4:50 | Presentation of Breakout Group Results and Discussion |
| 4:50 - 6:00 | Poster Session |
| 6:30 - 8:30 | Dinner (Captain Kidd Restaurant, Woods Hole Village) |
| | |
| **Tuesday, October 5, Clark 507** | |
| 8:00 a.m. | Shuttle from Hotels |
| 8:15 - 8:40 | Continental Breakfast, Clark 507 Foyer |
| 8:45 - 10:45 | 2nd Breakout Group; cross disciplinary groups; charge: "What can we do together?" |
| 10:40 - 10:50 | Coffee Break |
| 10:55 - 12:05 | Presentation of breakout group results and discussion |
| 12:10 - 1:10 | Lunch, Clark 507 Foyer |
| 1:15 - 3:15 | 3rd Breakout Session; cross-disciplinary groups; charge: "How can we implement it?" |
| 3:20 - 3:40 | Coffee Break |
| 3:45 - 5:15 | Presentation of breakout group results and discussion |
| 5:30 p.m. | Shuttle to hotels; dinner on your own |

# 1. State of the art: Summary of Plenary Lectures

## *1.1. Introduction: The Role of Dark Energy in the Earth System*

**John Hayes**, Department of Geology and Geophysics, Woods Hole Oceanographic Institution

Most of the volume of the ocean lies below the photic zone. These dark environments include (1) the oxygen-minimum zone with anaerobic respirers and important nitrogen-cycling microorganisms; (2) mid and deep waters with planktonic archaea and bacteria; (3) the benthos with aerobic heterotrophs and, near vent sites, diverse autotrophs; (4) anaerobic sediments with fermenters, sulfate- and metal-reducing bacteria, and methane-cycling archaea; (5) deep sediments in which cell numbers decrease systematically with depth and in which life appears to be sustained by thermal alteration of organic matter; and (6) igneous basement rocks from which oxidizable substances are released and used by microbial chemoautotrophs that depend on electron acceptors and nutrients supplied by the circulation of seawater., and (7) cold seeps and methane hydrates where energy sources for chemosynthetic bacteria are methane and hydrogen sulfide.

Recent work has indicated that deep-sea crenarchaeota, which comprise a third of the microbial biomass in the oceans, fix carbon autotrophically. This discovery challenges the concept that life in the deep sea depends on sinking organic matter produced in the euphotic zone. Autotrophic primary production in mid waters, the deep sea, and the subseafloor is unrepresented in models of the carbon cycle. Similarly, isotopically based mass balances for the carbon cycle have neglected inputs of mantle carbon and most processes in subduction zones. These simplifications have probably hidden some important redox imbalances. Recognition and investigation of these processes is central to understanding the energy budget for the ocean's dark zones. Solving this rich and compelling scientific problem requires an integrated approach. How are the mass and energy cycles of geological and biological systems in the deep ocean and subseafloor related? By what processes and at what rates are carbon and electrons exchanged between the crust and the mantle? What factors control the redox state of earth's surface?

Answers to these questions – and considerable scientific excitement – can result if we combine our interests and abilities. The team needs players at every position: chemists, molecular biologists, microbial physiologists and ecologists, thermodynamicists, and reaction-transport modelers.

## *1.2. Energy-Yields and Energy-Demands in Microbial Geochemistry*

**Jan Amend**, Washington University, St. Louis

Chemical reactions in the environment may have abiotic or biotic pathways, depending

on the relative rates of abiotic reaction versus enzymatic catalysis. Dissimilation, a microbially induced geochemical reaction, releases energy that can be used for assimilation of microbial biomass. Both types of metabolisms are governed by the same thermodynamic processes. Environments where geochemical energy is released include terrestrial and marine (shallow and deep water) hydrothermal systems. The energy-yields of redox reactions within these systems can be calculated based on thermodynamic principles if the activities of chemical species can be calculated from concentration measurements or, preferred, measured *in situ* with chemical sensors. The Gibbs Free Energies of all possible redox reactions can be ranked in terms of the relative energy yields (per electron or per mole electron acceptor) and that sequence that can be used to predict which dissimilatory metabolic reaction may be most likely in a specific environment. Some energy is lost during conversion of geochemical to biochemical energy (in form of ATP) during dissimilation. The microbial energy quantum, the minimum energy yield required for chemosynthetic microorganisms to survive, is dependent on the specifics of the ADP->ATP conversion. This minimum energy appears to be significantly lower than the commonly cited 20 kJ/mol; field, laboratory, and modeling studies suggest that it may be less than 10 kJ/mol. In addition, the energetics for synthesizing the building blocks (*e.g.,* amino acids, carbohydrates, nucleotides) of cells can now be calculated. It has been shown that these energetics in autotrophs are more than an order of magnitude lower under anaerobic conditions than under aerobic conditions, suggesting that anaerobic chemosynthesizers have a significant anabolic advantage. Because of the strong ties between geochemical energy yields and biological energy demands, there are likely connections between geochemical, energetic, metabolic, and phylogenetic diversities that we can reveal by continued sampling and culturing of subsurface environments and innovative field and experimental studies involving measurements on micro-scales.

### *1.3. Dark for All Seasons – Deep-Sea and Subseafloor Ecosystems*

**John Baross**, University of Washington, Seattle

Questions and issues revolving around the dark energy and deep biosphere debate are: What is the phylogenetic and physiological diversity? What are the physiological adaptations to dark habitats? What are the sources, kinds and transformations: energy, electron acceptors and donors, C, N and P? How diverse and productive are primary producers? What is the fate of carbon produced by microbes? What are the spatial dimensions of dark environments and their interactions with the subseafloor? Is the subseafloor a source of deep sea microbes?

Two first-order questions that we have started to address facilitated by recent developments in ocean drilling and other sampling techniques are: (1) does the subseafloor support a unique microbial community?; and (2) can we predict the physiological diversity of subseafloor microbial communities from fluid chemistry and

temperature? Subseafloor systems include sediments, oceanic spreading centers (magmatic and amagmatic), ridge flanks, gas hydrate deposits and cold seeps, and island arcs and forearcs. All these settings appear to have specific chemical and microbiological characteristics that distinguish them from the deep sea environment.

In deeply-buried sediments, the cell numbers decrease with depth, there are separate zones of sulfate reduction and methanogenesis, and growth rates are likely extremely slow. Ridge flanks constitute the Earth's largest fractured aquifer and may support abundant microbial life supported by trace organic matter and chemical energy released from the rock. Evidence for ridge flank microbial communities is based on (1) molecular evidence for thermophilic microorganisms in 3.5 Ma basement fluids from the Juan de Fuca flank, (2) laboratory and in situ incubation experiments suggesting that microbial growth is supported by the dissolution of basaltic glass, and (3) rock textures, DNA staining results, and carbon isotope compositions that are consistent with the presence of an endolithic (rock-hosted) microbial community.

Axial hydrothermal fluids sampled shortly after volcanic eruptions eject microorganisms from the subseafloor that show a remarkable community diversity, which is different from seawater communities. Isolates from diffuse vent fluids and event plumes show specific physiological adaptations to the mid-ocean ridge environment: They (1) exploit nutrients from rocks and tolerate metals, (2) form biofilms on mineral surfaces, (3) use Fe (III) and S° as electron acceptors, (4) fix $CO_2$ and oxidize hydrogen, (5) grow at temperatures from 2 to 110ºC under aerobic (low-T) to anaerobic (high-T) conditions. The inferred physiologies of mesophilic to hyperthermophilic subseafloor communities include sulfide, iron and methane oxidizers, and heterotrophs (aerobic) as well as sulfate reducers, methanogens, iron reducers, autotrophic and heterotrophic S° reducers (anaerobic).

In borehole and metal dart experiments designed to sample the subsurface microbiology in 3.5 Ma crust at the eastern Juan de Fuca ridge flank, molecular data indicate the presence of diverse Bacteria and Archaea, including gene clones related to known nitrate reducers and thermophilic sulfate reducers and fermenters but lacking methanogens. While Bacteria appear to dominate clone libraries from ridge flank settings, Archaea dominate in mantle-rock hosted hydrothermal systems off slow spreading mid-ocean ridges and in intra-oceanic forearcs. There, interaction between peridotite and seawater leads to the development of alkaline solutions that are enriched in $H_2$ and $CH_4$. Sulfate reduction and methanogenesis appear to be the dominant metabolic reactions in these settings.

These examples illustrate that microorganisms in different geological environments are well adapted physiologically to geochemical conditions (temperature, pressure, pH, metals) in the environment. The production of biofilms by subseafloor microorganisms appears of critical importance to their ability to colonize harsh environments, control electrochemical microgradients and harness geochemical energy.

### *1.4. Chemical sensors to understand biogeochemical processes - total element analyses versus chemical speciation analyses*

**George Luther** College of Marine Studies, University of Delaware, Lewes

Microorganisms in deep sea and subseafloor environments can discern and respond to the energetic state of the system (chemical composition, speciation, temperature, pH). Most techniques make a measurement that does not permit detailed chemical speciation and determination of several species simultaneously at the same region in space to monitor reaction pathways. Detecting several chemical species at once can indicate what biogeochemical processes occur; what organisms might be present and what energy sources are used.

Currently employed sensor techniques include single analyte potentiometric, ion selective electrode, amperometric, membrane, UV fluorescence systems, and enzyme/bacterial sensors as well as multiple analyte FIA methods, OSMO analyzers, UV-VIS spectroscopy, Raman spectroscopy, voltammetry, mass spectrometry, and LIBS. In general, for multiple analyte techniques – what you can measure but do not detect is as important as what you can detect.

A number of recent studies demonstrate that multiple analyte techniques can successfully determine chemical speciation (e.g., Fe and S speciation by voltammetry) in both low and high temperature settings. The examples provide proof that, as theory predicts, chemistry drives biology in sediments, waters and hydrothermal vents. Organisms occupy ecological niches based on chemistry and the energy derived from chemical reactions. Chemical measurements do correlate with molecular biology analyses so *in situ* methods can be used to prospect for life forms that depend on “dark energy”. There is a need for more techniques that are reliable at high temperatures and over long time periods (monitoring).

# 2. Summaries of breakout group discussions

## *2.1. Disciplinary break session (Monday afternoon)*

**Charge: What can our discipline contribute? / What can we measure?**

### *2.1.1. Sensors:*

Muli-analyte sensors enable the detection of several chemical species at a time. Sensors are hence valuable tools in biogeochemical research as they can indicate what geochemical energy sources are used. This is a crucial step toward establishing what biogeochemical processes occur and what organisms might be present in specific dark energy environments.

State-of-the-art multiple analyte techniques are:

FIA methods –
SCANNER (Johnson)
ALCHEMIST (Le Bris) ($H_2S$, Si, $NO_3^-$, Mn, Fe, pH)
SUAVE (Massoth)

OSMO analyzers – Jannasch / Wheat ($NO_3^-$, Fe)

UV-VIS spectroscopy (ISUS) – Johnson ($NO_3^-$, $HS^-$, $Br^-$, DOC)

Raman spectroscopy (DORISS) – Brewer ($CO_2$, $CH_4$, $SO_4^{2-}$)

Voltammetry (ISEA) – Luther ($H_2S$, $S_x^{2-}$, FeS, $Fe^{2+}$, $Mn^{2+}$, $O_2$, $S_2O_3^{2-}$)

Mass spectrometry – Short / Camilli (methane, other small molecules)

Laser-induced breakdown spectrometry (LIBS) – Angel / Miziolek (elemental composition)

The focus should be on the in situ activities of redox-active species. These are critical for making thermochemical predictions and calculations.

Measurements of concentrations of conservative species (e.g., Cl, Br) are also important for constraining physical processes of fluid flow and physicochemical conditions in hydrothermal systems. Very promising are Raman-based detectors for measuring organic compounds. These should be used in concert with inorganic measurements by other methods (e.g., LIBS)

The following table is a wish list for vent and seep sensors from Gallager and Whelan (WHOI-DOEI Sensors Workshop Report, 2004)

| Parameter/Species | Optimal Range | Sensitivity |
|---|---|---|
| Temperature (°C) | 0-400(°C) | ±0.5 units |
| | 0-3(°C) | ±0.001 units |
| pH (general) | 2-10 | ±0.1 units |
| pH ($CO_2$) | 5-8 | ±0.001 units |
| $H_2S$, $H_2$, $CH_4$ | 1-10 nM / 10-100 mM | 2-5% |
| $CO_2$ | 2-100 mM | 2-5% |
| $Cl^-$ | 30-1000 mM | 2-5% |
| $Fe^{2+}$, $Fe^{3+}$, Mn | 1 nM – 25 Mm | 2-5% |
| $O_2$ | 0-250 μM | 2-5% |
| $NO_3^-$ | 0-40 μM | 2-5% |
| $NH_4^+$ | 10 nM – 10 Mm | 2-5% |
| $NO_2^-$, $N_2O$ | 0-4 μM | 2-5% |
| $SO_4^{2-}$ | 1 nM – 1 mM | 2-5% |

The scale of the measurement is less important than the number of measurements made. The scale that measurements should be made at varies significantly between different environments and is dependent on the scale of the redox gradients - i.e., for fine-scale redox measurements at the scale of microorganisms are critical for compressed redox environment, but not necessarily for all systems. Basic surveying is needed prior to the investment of long-term measurement unless other impetuous serves as driver (i.e. biologically, theoretically driven).

In situ measurements in subsurface environments are very challenging. Currently, most direct measurements are made on recovered cores for sedimentary sequences (pore-squeezing). In-situ measurements require technological developments for combined coring and probe deployment directly in the borehole walls.

The currently most pressing limitation is our insufficient ability to measure hydrogen concentrations in-situ. Subsurface environments are often characterized by very low activities or very slow rates, both of which are notoriously difficult to measure with sensors. Another limitation is the often poorly define fluid flow regime / pathways. Only complete knowledge of the physical properties of the environment will enable the interpretation of measurements that we can currently make. The future belongs to Biosensors that use enzymatic-based approaches for increased sensitivity. Long-term monitoring of selected environments should have a high priority.

Measurements of $H_2$, sulfate, and methane need to be improved. Better detection limits for organic compounds are required. Raman spectroscopy is the most promising technique for measuring small organic compounds directly.

The most critical gaps are:

- The need for more powerful multiple-analyte systems.
- Poorly constrained hydrology of the subsurface
- The need for more surveys and time-series (funding and personnel to do this work are insufficient)

*2.1.2. Molecular biomarkers:*

The specific strengths of the molecular biomarker approach are that it provides a tool to answer questions like WHO is there? HOW MANY are there? WHAT are they doing? It also allows us to link modern ecosystems with the geologic record – an immensely powerful tool in paleoecology.

The process we study is often defined by the molecules we study. Advantages of lipids versus nucleic acids are:

- We can look at longer times scales (lipids are fairly stable in the environment)
- We can use compound-specific isotope (C, H, N) analyses.
- Lipid biomarker geochemistry is not selective, there is less of a chance of missing major players in microbial communities.
- The relative abundance of lipids is high.
- We may offer expertise at more complex mixtures (proteins/carbohydrates)

Recent examples of the successful implementation of the molecular geochemical approach are the correct predictions of Annamox (nitrate+ammonium=nitrogen+water) reaction and anaerobic methane oxidation in sedimentary and/or seep environments. Furthermore, biomarkers for the direct identification of planktonic crenarcheota have been developed.

The important processes and parameters that be traced with molecular biomarkers include microbial metabolisms, and element flow through an ecosystem. If there is a C, N, or H isotope signal, then there is potential. The longevity of lipids in sedimentary environments make them particularly useful in integrating over various timescales. It is important to realize that lipids change with environmental parameters, but gene sequences do not. Herein lies a potential key to functional information. Bulk measurements (elemental compositions, stable isotope compositions, defining the substrate) will continue to be important.

The biggest obstacle is sample size. Sample size for subseafloor research is limited by the diameter of the core or volume of fluid. Methods like polymerase chain reaction (PCR) in molecular biology are not available for lipid biomarker research. Further, in situ biomarker work is difficult.

One of the most critical gaps is the lack of a compilation of biomarker properties of relevantly-categorized genes. A genetic basis for lipid synthesis is also missing so that we often do not know what a biomarker means physiologically and phylogenetically. What is

the most severe practical limitation, however, is insufficient personnel support and/or instrument automation.

In the future, lipid-based taxonomy, the analysis of intact (non-degraded) molecules, and the calibration of labile organic components as molecular clocks will be critical. Isotopic studies of environmental carbon pathways and biochemical pathways in general will continue to be very important. We should refine analytical methods to be able to analyse more complex (i.e., larger) molecules (e.g., proteins, carbohydrates). The use of DNA and RNA as functional biomarkers (and their isotopic compositions) will provide an important link between molecular chemistry and biology. We also need to increase our ability to analyze metabolites (both low and high molecular) and their isotopic compositions. Better models of how molecular structure is relevant to the elemental requirements of the environment are needed. Sample throughput and data quality need to be increased by improved standardization, automation, and better uniformity of sample preservation techniques.

### *2.1.3. Molecular Biology:*

It is now well established that cultivation-based techniques have to be supplemented with cultivation-independent methods, i.e. molecular biology based approaches, to describe the full extent of diversity in the natural environment. The application of molecular tools has truly revolutionized the field of microbial ecology. The use of small subunit ribosomal RNA (SSU rRNA) genes as marker molecules has revealed the presence of as-yet-uncultivated microorganisms showing substantial phylogenetic diversity in various habitats. Currently, we are seeing the introduction of genomic approaches into microbial ecology, which hold great promises in answering some of the outstanding questions.

The tool box that microbial ecologist currently have at their disposal is quite impressive, making it possible to address questions that were unthinkable of being tractable even 10 years ago, and allowing us to make important headway in our understanding of microbial ecosystems. Figure 1 depicts a flowchart of a typical molecular biology approach used in microbial ecology.

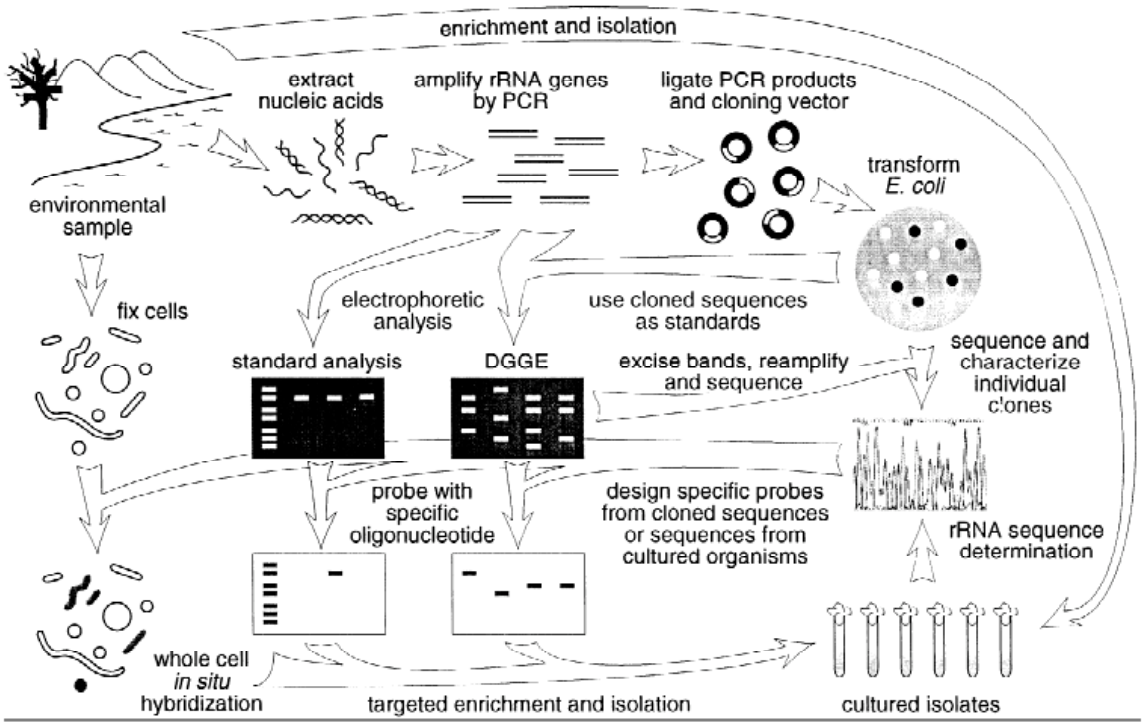


Fig. 1. Commonly used methods in microbial ecology. From Head et al., 1998 (Microbial Ecology 35:1-21)

In this context it is important to realize that all of these tools have advantages and disadvantages and that their application depends on the question being asked. There are methods to provide a snapshot of diversity and that are useful for comparing differences and similarities between different communities, e.g., DGGE, T-RFLP. There are also methods available to assess the abundance of target organisms (whole cell fluorescent in situ hybridization (FISH), quantitative real-time PCR), as well as genomic information and physiology by using functional gene markers and cloning of large genomic fragments. Recently, even shot-gun sequencing has been introduced into microbial ecology. Because genomic approaches tend to be costly and labor-intensive, emphasis should be given to the development of accelerating methodology, such as the serial analysis of V6 ribosomal sequence tags (SARST-V6) developed in Mitch Sogin's lab. This method provides a fast, efficient, and cost-effective assessment of microbial diversity in a particular environment. In general, it was felt strongly that it is beneficial to use a set of complementary tools rather than to depend just on one method to account for pitfalls in each method and to provide multiple lines of evidence.

However, despite the ever-increasing tool box, the field of microbial ecology is in many ways still in an exploratory stage, asking questions like

- Who is there?
- What are they doing?
- How many are there?

- Where are they living?

This is especially the case for the kind of environments relevant for this workshop, which are in general characterized by low biomass and low activity, making the application of many molecular tools challenging. A consequence of this exploration driven research is that it is often incompatible with hypothesis testing, making funding an issue. Until we have not adequately answered these 1st order questions it will be difficult to move to the next phase asking questions like:

- How do microbes interact with/modify their geochemical environment?
- What is their spatial distribution?
- How do communities change with time?
- What are microbial rates of activity?
- How did they all get there?

Answering these questions will require not only the refinement of currently available molecular tools, but will involve their combination with biogeochemical methods (e.g., diagnostic lipid biomarkers, isotope studies), the measurements of process rates (e.g., sulfate reduction), and the detailed characterization of relevant geochemical parameters (e.g., use of microsensors). Methods of great potential in this regard are stable isotope labeling, and combined microautoradiography-FISH which allow us to link the phylogenetic identity of microorganisms with their function. However, with all the buzz about molecular and genomic tools, it is also important to remind ourselves that the study and cultivation of microorganisms remains an invaluable part of microbial ecology. Without isolating organisms and studying their physiology and adaptation to environmental parameters in the laboratory we will not be able to completely understand processes in the environment. In addition, only by including cultivation based studies will it be possible to elucidate the function of the unknown genes and hypothetical proteins identified with genomic tools. The combination of both approaches provides great opportunities, since the molecular tools can help us to identify the key players and to refine our cultivation efforts. At the same time, it is also clear that the concept of a pure culture might not always be the gold standard, as there is increasing evidence that some environmentally important processes are mediated by consortia rather than single organisms, making it questionable that all organisms can be grown in isolation.

It is likely that dark energy environments harbor a huge amount of novel diversity, and we spent considerable time discussing approaches to assess this diversity. In our opinion the newly developed SARST-V6 approach will be a valuable tool in this regard. It is a high-throughput technique that provides a fast and cost-effective way to characterize microbial community composition. Recently, shot gun sequencing has been used to characterize complex microbial communities. However, while this method avoids biases introduced by PCR and is more likely to reflect the true diversity and the relative abundance of organisms, its use is so far limited because of its high costs and the bioinformatic challenge to reconstruct the gene fragments into distinct genomes.

Potentially, the latter could be overcome and with sequencing costs going down this might be an option in the not too distant future. A problem with the currently used PCR based approaches is the dependence on primers that were designed using the current database. This makes it unlikely to discover truly novel organisms, as recently exemplified by the discovery of the Nanoarchaea, a new archaeal kingdom. In this regard, the use of degenerate primers might be appropriate. In addition, large genome fragment cloning is a promising tool in discovering novel diversity and also new functions. However, a limitation so far has been the dependence on 16S gene as a marker for screening. New ways to screen these clone libraries by using functional gene assays are being developed, e.g., the screening for novel iron-oxidizers used by the Edwards lab. Also, the overexpression of unknown genes in organisms like *E. coli* has been used successfully to identify functions of novel genes. A limitation in this regard is the lack of a high temperature expression system to screen for novel genes in high temperature environments like the subseafloor.

Presently, the study of dark energy environments is hampered by several obstacles. One issue is the source of funding. Because of the scope of the projects and the associated logistics, proposals tend to be expensive. The high costs and the fact that research is often exploration-driven rather than hypothesis-driven make it a challenge to obtain funding from traditional funding agencies, e.g. NSF. Thus it will be necessary to identify other potential funding sources such as research foundations. It was also realized that while many methods are available and have been used successfully in microbial ecology, the application of these methods to the low biomass, low activity environments to be studied in the context of this meeting represents a challenge. In many cases, molecular methods require relatively high amounts of nucleic acids, which are not readily available from geological samples (e.g., drill cores of basalt). Thus, specific efforts have to be made to make these highly powerful methods more sensitive and applicable to low biomass, low activity environments. Single cell genome libraries may provide the solution to this problem. Other critical issues are the sample retrieval and tests for potential contamination, the proper fixation of samples (e.g. for RNA based work), the mismatches between the scales of sampling for biology and chemistry/in situ measurements, and the inability to differentiate between active and inactive organisms.

### *2.1.4. Energy/Theory/Experiment:*

Theoreticians explore the utility of purely thermodynamic/energetic models in describing biological systems, using isotopic/mineralogical/geochemical indicators of energy liberation/utilization. The goal is examining the possible links between chemical diversity and microbial diversity. The method is (1) defining the fundamental chemical organizing principles for biological systems and (2) applying the physical chemistry knowledge base to make theoretical predictions for environmentally and biologically relevant conditions. Another application of theoretical studies is suggesting experimental directions that will improve our thermodynamic knowledge base.

The current limitations are incomplete thermodynamic and kinetic data bases for important biomolecules/reactions and environmentally important species. Further, we need better constraints on chemistry and energetics on mineral and biofilm surfaces as well as better constraints on the overall mass and energy transport in different systems.

We need a better coordination of theory, experimentation, and observation. Specifically, lab experiments should be focused on geobiologically relevant systems. Bioenergy education outreach will likely lead to improvements in this regard. Empirical determination of biomass/activity relations of specific metabolic types will make theoretical predictions of biomass production more reliable. Hydrogen measurements are critical, and hydrogen microsensors are absolutely required as are lower detection limits for organic metabolites.

In the future, we need to flesh out a concept of "energetic habitability" in dark, chemosynthesis-driven biogeochemical systems. Our ultimate goal is a convergence between phylogenetic and energy trees.

### *2.2. First cross-disciplinary break session (Tuesday morning)*

**Charge: What can we do together?**

#### *2.2.1. Interdisciplinary benefits/synergistic effects*

Understanding complex biogeochemical systems and their ecology requires a bio-geo-physio-chemo-engineering approach. An example is mass and energy transport within crust, sediments, oceans and across interfaces, impacts on biology and feedbacks between biological, chemical, and physical components of the system.

Different methodologies, approaches, and perspectives may be applied by representatives of different disciplines to tackle the "same" problems/questions. A benefit of this is the availability of independent measurements often at different scales (spatial and temporal). When researchers with different backgrounds work on the same problem, new ideas spawn and new means of investigation are created. Successful cross-disciplinary collaborations also allow the exploitation of interdisciplinary funding program

A successful interdisciplinary approach must begin at the planning stage of a research project. For instance, thermodynamic theory helps frame the question. Basic measurements of physicochemical parameters can be planned accordingly (temperature, pH, etc.). Advanced geochemical and microbiological techniques can then used to determine elemental flow and community structure within a given system.

Together this kind of advance planning creates hypotheses about processes.

Examples for the successful application of this principle are:

Amend and Shock (Yellowstone, etc.):

- geochemical data for natural waters
- design media with similar properties
- cultivated novel organisms

Acid mine drainage studies of Jill Banfield and co-workers

- geochemical data for drainage fluids
- characterization of populations
- metagenomic studies/interactions of limited populations

Anaerobic methane oxidation

- theoretical prediction of metabolism
- targeted search for microbes (consortia)
- geochemical and isotopic confirmation of the process

Following is a list of how the different core disciplines can contribute to the "dark energy" research theme:

- Chemistry:
  - focus experimental and theoretical efforts on biologically relevant aqueous reactions
  - improve measurements of concentrations/activities of species/phase changes.
  - study the reaction rates (abiotic and biotic kinetics)
  - improve thermodynamic and kinetic modeling of microbe-mineral-fluid systems and make models more transparent/user-friendly.
- Biology:
  - focus on who is there in terms of diversity/complexity (consortia, symbiosis, parasitism)
  - what they are doing (activity, tolerance ranges, assimilatory/dissimilatory reactions)?
  - what are the linkages between diversity / actitivity and geochemical energy flow/gradients?
- Physics:
  - provide relevant data on fluid flow (currents, sediments, basement, sulfide chimneys)
  - -physical properties (porosity, permeability, tortuosity, thermal conductivity, temperature, salinity, density, pressure, radiation, magnetism)
- Geology:
  - develop increased understanding of crustal hydrology, sedimentation and diagenesis, bioturbation,
  - interpretation of paleo-record

- water-rock reactions, volcanism, and hydrothermal fluxes

An example for synergistic /interdisciplinary linkages is the effect of biofilms on physical (flow rates, transport, boundary layers) and chemical processes (mineral dissolution, chemical exchange) and feedbacks, e.g., the effect of mineral surface properties on biofilm development.

### *2.2.2. Limitations / Solutions*

Some of the benefits of interdisciplinary research are also pitfalls. If, for example, biological and chemical measurements in the same environment are made over different temporal and spatial scales, it will be difficult to explore the results for biogeochemical linkages. The basic limitations are insufficiencies in education, communication, and planning/documentation. We will also need to understand each other's fields at more than a superficial level. At the same time we should limit the jargon inherent to one's field when discussing projects and objectives with people from other fields. Interdisciplinary themes need to become a more important component in the training of students. (Moore's microbial oceanography course, Agouron's geobiology course, and MBL's microbial diversity course are examples). Field programs provide a unique opportunity for easy interactions with students in an interdisciplinary framework. Multi-laboratory or theoretical collaborations are not as easy. This represents a challenge of the university structure. Training of students in interdisciplinary work and between multiple laboratories is required. Continuing education for faculty is important but often difficult to realize (short workshops at best).

Projects should be integrated from the onset. There also needs to be strong commitment to constant communication and coordination. Understanding strength and weaknesses of methods is more important than to understand exactly how they work or exact terminology. Different fields are more or less tolerant with respect to variation/ uncertainty. Interpretations are more/less plastic in different fields. We all need to increase our awareness of these issues and continue to educate our peers and program managers.

We should look for funding that helps us better coordinate at the grass roots level, (i.e., Ridge Theoretical Institutes, RCN's etc). At the same time we need to find ways to improve communications and efficiency of interdisciplinary research within top-down efforts (e.g., NAI, biocomplexity program).

Specifically we need to strive for:

- more interdisciplinary focus in graduate programs
- rigorous requirement to release data (RIDGE 2000 model)
- rigorous requirement for having pre- and post-cruise meetings
- designing experiments well in advance

- melting together different projects to have more gain
- improved data bases (standardization of formats, accessibility)

### *2.2.3. What is needed*

Improved thermodynamic data bases will be critical for model predictions required at the planning stage and quantification of biomass production within different environments. The latter will also require improved measurements and model descriptions of mass and energy fluxes with focus on mass and energy balances and chemical/thermal gradients. Molecular techniques for more quantitative assessments of biological activity will be increasingly important. Better characterization of electrochemical gradients (more components, better spatial resolution, and longer time-scales) in different environments are of paramount importance. We need to pursue laboratory-based and culture studies, while improving sampling techniques for environmental samples and refining in situ incubation techniques. Better cross-calibration of analytical methods, in particular between field and lab-based systems) is required. For hypotheses of chemoautotrophy in the origin of life, we need better estimates for the levels of oxidants ($CO_2$, $Fe^{3+}$, $S^0$; Limited photocatalytic $NO_x$, HOOH, etc.) that were potentially relevant.

Specifically, we need:

- Process-oriented sensor packages for mm-scale measurements. Ideally these would be built and maintained by a facility (with technical support) and provide CTD, current meters, chemical and biological sensors, in-situ microscopy, etc.
- Sustained long-term observatories (moorings, cables)
- Small scale samplers and probes to characterize surface chemistry (see Table 1 for components and detection limits)
- Combined biomarker (isotope signatures / labeling) studies, flux/ activities/transformation studies, and genetic-base diversity/functional studies
- Matching time/space scales of biological, physical, and chemical measurements and data interpretation and evaluation of sample/measurement reliability
- Affordable metagenomic techniques to link process, function, activity, and substrate (see figure below)
- Improved databases available to and usable by a multi-disciplinary community (NCBI, genetic databases are a good examples)

In general, measurements of rates and activities are not as common as they should be. More focus should be on experiments, both laboratory-based and in situ. New experimental techniques and tracers applications (both radioactive and stable isotopes) will support the relevance of experiments and our ability to use experiments to assess processes and rates in natural environments.

The research problems and strategies are different for different environments. Three break-out groups were formed to discuss implementation of chemoautotrophy-focused research in water column, sediments, and hard rock environments.

### *2.3. Second cross-disciplinary break session (Tuesday afternoon)*

**Charge: How can we implement it?**

*2.3.1. Mesopelagic and bathypelagic water column:*

The mesopelagial represents one of the largest continuous habitats on Earth, yet we know almost nothing about the kind of microbes living in this zone and the global impact of their activities. Traditionally, the role of microbes in this zone has been seen as degraders of organic matter that either rains down from the photic zone of the ocean in the form of marine snow or is advected in the form of dissolved organic carbon. However, recently this perception has been called into question by the finding that planktonic *Crenarchaeota*, which dominate the prokaryotic cell numbers in the mesopelagic zone, could actually be autotrophs, meaning that they are fixing rather than releasing $CO_2$ potentially leading to a decreased rate of $CO_2$-production in this zone. However, up to this point, no one has succeeded in cultivating a representative of this group and their physiology and ecological role remains elusive. This example illustrates our poor understanding of this vast ecosystem and the need for detailed studies to illuminate the secrets hidden in its darkness. We are just beginning to realize that this habitat might contain much more structure than previously thought, providing potential niches for different kinds of microorganisms.

The following questions need to be answered:

- How much overall phenotypic diversity and how many metabolic pathways are present?
- How are they segregated into zones?
- How does this affect microbial diversity?
- What is the role of microheterogeneity (particles, guts, falls)?
- Is the mid- & deep ocean a net sink or source of organic carbon?
- What is the importance of energy (electrons) coming from below, e.g. hydrothermal input?
- What are the prevailing energy sources and metabolic pathways?
- What role does chemoautotrophy in the mesopelagic zone play role in biogeochemical budgets?
- What are the residence times (fluxes) of important reduced species ($NH_4^+$, $S_2^-$, $H_2$) that could potentially support an autotrophic lifestyle?

To address these question will require a multitude of approaches bridging disciplines. An important aspect will be to make a concerted effort to increase our ability

to cultivate microorganisms from this habitat. We need to study environmental communities in the laboratory by setting up experimental systems as well as in situ based approaches. For this, *in-situ* systems or suitable lab-based mimics of natural systems will be required to account for physical (pressure, temperature) and chemical (natural compounds, unknown cofactors) conditions in the environments. Simultaneously we need to increase the amount of genomic sequence information, which will help to identify potential metabolisms and aid in successful culturing work. We also need to improve our ability to chemically characterize deep ocean environments. Chemical speciation, especially for trace metals and organic compounds, is often poorly known.

Elucidating the diversity, ecological role and physiology of the microorganisms inhabiting the mesopelagic zone will require a combination of approaches, including modern molecular biology tools, targeted cultivation, and techniques relating identity with function. This will include the application of high-throughput sequencing (SARST, large genome fragments, shot-gun sequencing), as well as the study of other biomarkers, such as lipids, carbohydrates, and proteins. It will be with the combination of genomic and functional studies that we will be able to discern the physiology of the microbes and discover underexplored, novel mechanisms for harvesting energy (e.g., reduced organic compounds, non-photosynthetic based autotrophy) likely to present in this habitat. In addition, theoretical calculations as well as thermodynamic and kinetic modeling will prove essential to better define the potential energy sources with presently poorly understood budgets (examples: $NH_4^+$, $N_2O$, $NO_2^-$, $CH_4$, CO, $I^-$, $S_2^-$). The conduction of hydrographic surveys and activity measurements of these species will be of paramount importance.

There are many "Dark Energy" opportunities in microbiological oceanography

- A promising study site to look for autotrophic Crenarchaeota and to initiate cultivation attempts would be oxygen minimum zones (OMZ) that can be found in vast areas of the Ocean. Chemolithoautotrophic carbon fixation is confined to redox interfaces and biogeochemical examination of OMZ's from difference areas (e.g., Peru, Namibia) and seasonal variability within these systems would be of great interest

- Hydrothermal plumes are known to carry substantial amounts of reduced chemical species that could be used as electron donors by microbes living in the mesopelagic. Understanding the role of hydrothermal plumes in deep ocean microbial ecology requires a monitoring / time series approach and measurements of the physical dispersal and microbial consumption of reduced hydrothermal components in the water column.

- Freshwater input in the form of rivers and submarine discharge in areas of high sediment permeability is an important contributor of energy sources for chemosynthetic microorganisms in the ocean. Yet, the impact of fluxes of

dissolved reduced and organic substrates on meso and bathypelagic bioproduction in the oceans is unknown.

- A comparison of oligotrophic open ocean and eutrophic coastal ocean sites to constrain the relative maximum/minimum contributions of "Dark Energy"

The new initiative for observatory science at NSF will provide opportunities to install process-specific instrument packages (in-situ sensors for measurements of rates, turnovers, and fluxes). In this regard, the Gulf of Mexico seems of particular interest due to the large input of methane and other energy sources from gas and oil seeps and its confinement. Multiple regional observatories would also allow comparative process studies to assess the role of climate zones, depth zones, passive and active margins, etc. Only observatories will be able to capture the "patchy" & dynamic phenomena (blooms, volcanic events, eddies, storms, earthquakes, etc.) that are likely to play a large role in controlling the distribution of microorganisms in the subphotic ocean.

*2.3.2. Marine sediments*

The first-order questions in sediment microbial ecology and biogeochemistry are similar to those asked for other environments:

- What organisms are down there?
- How abundant are they?
- What are they doing?
- What are their sources of energy?
- How do they interact with their environment?

Systematic questions for oceanic sediments are:

- How does life in subseafloor sediments affect global biogeochemical cycles?
- To what extent are the deep ocean and the subseafloor ocean coupled?
- What are the biogeographies of organisms and genes in the subseafloor ocean?
- How is this biogeography related to that of the deep ocean?

Tackling these questions requires a multi-disciplinary approach. Of critical importance will be an improved determination of the activity of microorganisms in sediments. More sensitive radiotracer techniques for measurements of very slow rates are required. Improved measurements of in situ activities of inorganic energy sources, organic metabolites, and hydrogen re required to assess processes and rates of metabolism in sediments. These measurements will have to include enzyme concentrations. To better describe community composition, combined organic biomarker analyses (compounds and isotopes) and molecular analyses (DNA and RNA) with improved sensitivity at low biomass is needed. Techniques should include CARD-FISH,

mRNA for gene expression, etc. Cultivations will remain important to link individual microbial potentials to geochemical signatures)

A concerted effort is needed to study microbial activity in sediments with focus on representative environments that cover the variability in:

- oceanographic regimes (water depth, seasurface properties)
- temperature regimes
- sediment depth
- sediment age
- subseafloor chemistry (organic flux, redox state, sediment composition)

Particularly interesting are sediments in oligotrophic oceans, where we may be able to find minimum metabolic activities and turnover rates. Accretionary wedge systems in convergent margins and sedimented hydrothermal ridges are also of interest because of the potential feedbacks between hydrology, geochemistry, and microbiology.

The Integrated Ocean Drilling Program (IODP) provides the technology needed to access deeply buried marine sediments. Borehole and core logging will set the thermal, physical, and compositional framework. Microbiological methods continue to be adapted to ocean drilling. Triplicate coring of sediment sites will have to become standard in order to obtain appropriate sample sizes for activity and rate measurements and various chemical and molecular biological methods. The availability of drill core samples for laboratory-based studies is also important.

Observatory science will be facilitated through IODP's CORK (circulation obviation retrofit kits) experiments that now enable in situ geochemical fluid sampling by OsmoSamplers and are amendable to microbial incubation experiments.

*2.3.3. Hard rocks environments:*

Overarching questions in subbasement microbiology are:

- How are fluid-rock reactions harnessed by biology, and how does biology modulate chemical speciation, reaction pathways and rates of chemical exchange?
- What limits growth and productivity (nutrients, electron donors, or electron acceptors)?
- What is the sulfur and Fe geochemistry in hydrothermal systems?
- Is there abiotic synthesis and to what degree does it play into the carbon budget of rock-hosted microbial ecosystems?
- What is the contribution of chemosynthesis to rock-hosted ecosystems?
- What is the genetic potential and physiological/phylogenetic diversity of these systems

The crystalline basement of the ocean floor is the most challenging environment for microbiological and biogeochemiscal studies. In situ analysis (fluid chemistry, physical conditions, microbial growth) at vent sites and in boreholes are desperately needed to overcome numerous sampling and contamination problems. These analyses and rate measurements should be supported by theoretical calculations of energy budgets and biomass production in order to examine how geochemical and microbiological systems may affect each other. Laboratory analyses will provide mineralogy, biological inventory, and isotopic analyses. It will become increasingly important to consider effects of mineral surface chemistry and biofilm development.

The following hard rock environments may harbor microbial life that is probably adapted to the specific temperature/pressure and geochemical regimes.

Ridge Axis:

Rocks: young crust, usually basaltic, less common peridotitic or felsic
Depths: 2500-4500 meters below sea level
Temperatures: 5 to 400 ºC
pH: 3-7
Fluid chemistry: high sulfide and metals
Good target sites are the Endeavour segment, East Pacific Rise 9ºN, Axial seamount (basalt-hosted), and Rainbow and Logatchev sites (peridotite-hosted)

Ridge Flanks:

Rocks: older crust, up to ~50 Ma, sedimented or unsedimented, basalt or peridotite-hosted, hydrology controlled by topography (seamounts)
Depths: 750-4500 meters below sea level
Temperatures: <100ºC
pH: 6-11
Fluid chemistry: seawater-like, high methane and hydrogen in peridotite-hosted systems
Good target sites are the Juan de Fuca Ridge flank (continuous sediment cover, basaltic, warm), the Lost City Site (unsedimented, peridotitic), and North Atlantic ridge flank (discontinuous sediment cover, basaltic, cold)

Island arcs/backarcs:

Rocks: young crust, basaltic to rhyolitic, volatile-rich magmas
Depths: 0-3000 meters below sea level
Temperatures: 5 to 350ºC
pH: 1-7
Fluid chemistry: high sulfur and metals, can have high metalloids, high $CO_2$
Good target sites are the Mariana Arc and the Lau Basin in the West Pacific.

Forearcs:

Rocks: Serpentine mud volcanoes
Depth: 500-2000 meters below sea level

Temperatures 5-20ºC
pH: 8-12.5
Fluid chemistry: high methane and hydrogen, low metals
Good target sites: Marina forearc (South Chamorro Seamount)

Land-based sites can provide opportunities for ground-truthing and developing methodologies that can be applied in subseafloor research.

On the practical side, what is needed is an integration of subbasement microbiology issues with research and observatory plans of big programs such as IODP, RIDGE2000, InterRidge, etc. We need improved analytical abilities for microbiology and geochemistry in hard-rock settings. This will have to include sensor packages that can be adapted to the use in vent site and borehole environments. A metagenomics approach will likely demand private funding sources but needs to be linked into existing programs. Finally, the community must strive for a better coordination of measurements and experiments

## 3. Outlook and recommendations: What are the big issues and how can we work together to solve them?

We need to critically re-evaluate our concept that life in the deep sea is predominantly driven by sinking organic matter produced in the euphotic zone. We do not presently know how important autotrophic primary production in mid water, the deep sea, and the subseafloor is for global carbon cycling. First order questions are still who is there and what are they doing. We need to focus our effort on both the extreme and the common environments. However, currently very little attention is being given to the average deep ocean and ocean crust environments. Careful identification of the players and processes in diverse habitats will allow us to more carefully re-address the balance of the carbon cycle.

In the deep ocean: What is the primary productivity in the deep sea and how does it relate to communication between the pelagic, the benthic, and ocean crust? Is there a biogeography of microbes or a biogeography of genes? To what extent is life on earth dependent on or independent of photosynthesis?

In the subseafloor: What is metabolically active? What are the reaction rates? What are the reaction products? How is growth related to metabolism on a quantitative basis? Is growth in the subsurface only occurring on geological time scales? In anaerobic environments 50% of the C brought in is extruded as metabolites -- how and why? Can this be studied in the field via isotopic labeling? Why do so many of the autotrophs produce energetically expensive extracellular organic matter?

The most important issue is to determine what is there, how many are there, and to constrain this information in the context of the geochemical environment. Principal targets are carbon fixers – base of the food chain and pivotal to the carbon cycle and carbon flow in these systems. Improved abilities to measure microbial activity and the activities of energy-providing chemical species and metabolites will be of paramount importance. Monitoring metabolite levels in the environment is a particularly important measure for microbial activity. At first order monitoring secondary metabolites is a chemical problem. The composition and quantity of by products can be worked out in the laboratory and the results taken to the field to address carbon flow. It will be important to make all chemical and biological measurements (in situ and lab-based) on the same spatial and temporal scales.

The lines of positive evidence that can be used to constrain environmental processes are: isotopic data, cultures, molecular biological data, inorganic geochemical data, lipid data. It is important to play one technique off another in low biomass or otherwise difficult environments in order to maximize detection. Genomic data and biomarker studies could help identify key markers that could help increase our detection of players distinct/unique to the subsurface. Larger data sets of cultured representatives will increase the probability of honing in on the important players.

We are getting first glimpses of microbial communities in various geological settings that indicate a strong coupling between the physiological diversity and the geochemical framework set by volcanic degassing and hydrothermal water-rock reactions. Many of the observed microorganisms possess the ability to produce biofilms, which allows them to control the microchemical environment while attached to a mineral surface. So it is likely that, while the relation between fluid chemistry and microbiology is certainly discernable, it is not always a direct one. In situ measurements of mineral surface properties and chemical activities at, within, and below biofilms will reveal a more detailed understanding of interactions between microorganisms and their chemical environment.

In the future, it will be important to move beyond a pure description of microbial diversity to unravel the governing forces behind the pattern of microbial species distribution. At present, we know almost nothing about the ecology and physiology of the uncultured, albeit environmentally significant microorganisms living in dark-energy environments. We need to develop and refine methods that will allow us to link the diversity of microbial communities with the function and the physiology of the populations making up these communities. This will allow us, then, to move on to address ecological questions that are currently unresolved, yet critical for our understanding of these environments. In this regard it seems of particular interest to decipher the correlation between the observed geochemistry and biological diversity and to address whether natural selection operates at the level of functional genes or of microbial species. This leads to the question whether there exists a biogeography of genes or of microbial species and how lateral gene transfer might play into this. At present we do not understand the evolutionary underpinnings of biogeochemical cycles in different

environments, e.g., to what extent do they share the same functional groups or guilds of microbes or have the same functional gene representation? Molecular and in particular genomic tools hold great promises to address these questions, but they need to be applied in concert with other lines of inquiries including microbiology, geochemistry and geology in order to advance our understanding of these fascinating ecosystems.