



Mesoscale Chemistry: A Workshop Report

DETAILS

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MESOSCALE CHEMISTRY

A WORKSHOP SUMMARY

Kathryn Hughes, Camly Tran, and Joe Alper, *Rapporteurs*

Chemical Sciences Roundtable
Board on Chemical Sciences and Technology
Division on Earth and Life Sciences

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Acknowledgment of Reviewers

This workshop summary has been reviewed in draft form by persons chosen for their diverse perspectives and technical expertise in accordance with procedures approved by the National Research Council's Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will assist the institution in making the published report as sound as possible and to ensure that it meets institutional standards of objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberative process. We wish to thank the following individuals for their review of this report:

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Acronyms and Abbreviations

ATP	adenosine triphosphate
BCST	Board on Chemical Sciences and Technology
BESAC	Basic Energy Sciences Advisory Committee
CSR	Chemical Sciences Roundtable
DOE	U.S. Department of Energy
DOPC	1,2-dioleoyl-sn-glycero-3-phosphocholine
GDP	guanosine diphosphate
GTP	guanosine triphosphate
HIV	human immunodeficiency virus
mV	millivolt
NIH	National Institutes of Health
NRC	National Research Council
NSF	National Science Foundation
PNNL	Pacific Northwest National Laboratory
STM	scanning tunneling microscopy
TEM	transmission electron microscopy
TOF-SIMS	time-of-flight secondary ion mass spectrometry

1

Introduction and Overview¹

In the past few decades great strides have been made in chemistry at the nanoscale, where the atomic granularity of matter and the exact positions of individual atoms are key determinants of structure and dynamics. Less attention, however, has been paid to the mesoscale—it is at this scale, in the range extending from large molecules (10 nm) through viruses to eukaryotic cells (10 microns), where interesting ensemble effects and the functionality that is critical to macroscopic phenomena begins to manifest itself and cannot be described by laws on the scale of atoms and molecules alone. Thus, mesoscale systems bridge the molecular and the macroscopic. The progress made in nanoscience can serve us well as we explore the mesoscale, as noted in a recent report for the Basic Energy Sciences Advisory Committee (BESAC) at the Department of Energy (DOE) (BESAC Subcommittee on Mesoscale Science 2012): “With our recently acquired knowledge of the rules of nature that govern the atomic and nanoscales, we are now well positioned to unravel and control the complexity that determines functionality at the mesoscale.”

Understanding phenomena at the mesoscale presents opportunities for developing new functionality of materials and understanding of biological and interfacial systems, as well as challenges for analysis and description. As Whitesides et al. noted, “the distinctive properties of

mesoscale systems arise when the characteristic length of a process of interest, such as ballistic movement of an electron, excitation of a collective resonance by light, diffusion of a redox active molecule close to an electrode, or attachment and spreading of a eukaryotic cell is similar to a dimension of the structure in (or on) which it occurs. These processes involve interactions with many atoms or molecules rather than interactions with small, localized ensembles of atoms or molecules” (Kumar et al. 1995).

The complexity of mesoscale systems is due to their collective and often nonlinear behavior, and their study requires new approaches to synthesis, fabrication, and analysis beyond those used to study the molecular and macroscopic realms. Some mesoscale structures, such as micelles and liquid crystals, are transient yet thermodynamically stable, while others, particularly those from the biological world such as folded proteins, the capsids of viruses, and the calcium carbonate in mollusk shells, are longer lived.

Jennifer Curtis, Distinguished Professor of Chemical Engineering and Associate Dean for Research in the College of Engineering at the University of Florida, co-chair of the Chemical Sciences Roundtable (CSR), and a member of the workshop organizing committee, explained that the DOE BESAC report and other ad hoc workshops on the mesoscale have had more of a materials science focus rather than a chemistry focus. To explore how knowledge about mesoscale phenomena can affect chemical research and development activities and vice versa, the CSR decided to organize and convene a day-and-a-half workshop on mesoscale chemistry with the following statement of task:

¹The planning committee’s role was limited to planning the workshop, and the workshop summary has been prepared by the workshop rapporteur as a factual summary of what occurred at the workshop. Statements, recommendations, and opinions expressed are those of individual presenters and participants and are not necessarily endorsed or verified by the National Research Council, and they should not be construed as reflecting any group consensus.

An ad hoc committee will plan and conduct a public workshop in November 2014 in Washington, DC. Advances in theoretical, computational, synthetic, and analytical techniques have supported the extension of research into the study and development of mesoscale structures and processes. In the nanoscale to microscale size range, interesting ensemble effects exist that present opportunities for developing new functionality of materials, and understanding of biological systems and interfacial systems, as well as challenges for analysis and description.

This 1.5-day symposium will focus on the research on chemical phenomena at the mesoscale, and participants will be invited to actively participate in discussions responding to panels of speakers to identify opportunities and challenges in chemical and chemical engineering research at the mesoscale with a particular focus on collective and emergent behaviors at this scale. Questions considered during the workshop will include the following:

- *What is the current state of the art of research at the mesoscale in chemistry and chemical engineering?*
- *What is the particular value to chemists and chemical engineers of studying and exploiting individual and collective behaviors at this scale?*
- *What opportunities and challenges exist for research in this area?*

The workshop held November 6-7, 2014, in Washington, DC, focused on exploring the collective and emergent behaviors at the mesoscale. Participants examined the opportunities that utilizing those behaviors can have for developing new catalysts, adding new functionality to materials, and increasing our understanding of biological and interfacial systems. The workshop also highlighted some of the challenges for analysis and description of mesoscale structures. It is the hope of the CSR that, as the interest in mesoscale chemistry continues to grow, this workshop will provide a snapshot of the current research on chemical phenomena at the mesoscale and will provide inspiration for ideas for research and research programs. Over the course of

the workshop, a few important points were raised repeatedly by various participants. These points are as follows:

- There exists a lack of broadly applicable theoretical and conceptual frameworks for understanding and predicting phenomena at the mesoscale.
- Studying molecular-level interactions using both theoretical and empirical tools is useful to understand and improve our ability to adequately describe mesoscale phenomena.
- A distinguishing aspect of mesoscale chemistry research is the need to understand and investigate a system as a whole as opposed to breaking down the system into discrete parts.
- Metastable and nonequilibrium phenomena have a larger effect at the mesoscale than at other length scales.
- Gaps remain in the understanding of the emergence of structure and/or function through collective interactions and motion.
- Entropy plays an increasingly important role in describing behavior at the mesoscale.
- Having fine control over these states in the laboratory is a continuing challenge.
- Modeling and measurement challenges are presented by the stable or metastable states that exist at the mesoscale.

ORGANIZATION OF THE SUMMARY

This publication summarizes the presentations and discussions that occurred throughout the workshop (see Appendix A for the agenda), highlighting the key lessons presented and the resulting discussions among the workshop participants (see Appendix D for a list of attendees). Chapter 2 presents an overview of mesoscale phenomena and how they bridge the behavior of atomic, molecular, and nanoscale structures and those of the macroscopic world. Chapter 3 discusses the role that mesoscale phenomena play in catalysis and how those phenomena can inform strategies for synthesizing and stabilizing catalysts. Chapter 4 examines membrane behavior as a function of mesoscale interactions and how the ability to manipulate ensemble-scale phenomena may lead to the development of novel microchemical systems. Chapter 5 recounts the presentations and discussions

on the mesoscale aspects of biomineralization and geochemical systems, as well as the development of microanalytical tools to probe geochemical heterogeneity and interpret the geochemical record on, within, and beyond Earth. Chapter 6 describes some of the computational approaches that are being used to better understand mesoscale phenomena and design novel materials and protein complexes, improve solar energy conversion, and represent thermodynamic properties of molecular ensembles.

In accordance with the policies of the National Research Council, the workshop did not attempt to establish any conclusions or recommendations about

needs and future directions and focused instead on issues identified by the speakers and workshop participants. The beginning of each chapter contains a list of key points. These key points reflect statements made by speakers and participants at the event and do not reflect a consensus view of the Roundtable or the National Research Council. The organizing committee's role was limited to planning the workshop. The workshop summary has been prepared by workshop rapporteurs Kathryn Hughes, Camly Tran, and Joe Alper as a factual summary of what occurred at the workshop.

2

Growing (Up) from the Nanoscale to the Mesoscale

Key Points

- Mesoscale studies benefit from the experimental, theoretical, and simulation tools developed to explore nanoscale phenomena and build the field of nanotechnology. These studies also benefit from the expertise and knowledge base acquired over decades of semiconductor manufacturing, from a century or more of advances in synthetic chemistry, and from the biotechnology and molecular biology revolution.
- Following the dynamics of these systems and understanding how they evolve at the mesoscale is important, not at the level of each and every atom or molecule, but at a level that will provide useful insights.
- It is also important—and challenging—to understand the role of heterogeneity at the mesoscale. The ability to control chemical functionality is a key to mesoscale studies because that will in turn control and tune the interactions and interfaces at the mesoscale in ways that can be used to couple materials together to develop new capabilities.
- Chemists have used their tools to design building blocks that can be put together to create larger structures in ways that provide specific control over molecular interactions and interfaces. Thus they can study and investigate these phenomena at the mesoscale and develop a theoretical understanding of how mesoscale properties emerge.

In a plenary session to start the workshop, Paul Weiss, Distinguished Professor of Chemistry and Biochemistry and of Materials Science and Engineering, and the Fred Kavli Chair in NanoSystems Sciences at the University of California, Los Angeles, described the defining chemical, physical, optical, mechanical, and electronic properties of surfaces and supramolecular assemblies and discussed the cooperative behavior observed between functional molecules. He explained that there are two ways to approach the mesoscale, just as there is the nanoscale—from the bottom up or from the top down—though in the mesoscale, the requirements for precision in measurement and synthesis are more relaxed than they are when operating at the nanoscale. For example, when looking at the statistical and ensemble properties at the mesoscale or at cooperative effects across different regions or

materials in a mesoscale structure, there are some details that can be ignored or minimized without sacrificing precision in describing the phenomena that are occurring in a given system. The two approaches are complementary, however, and Weiss noted that mesoscale studies are benefiting from the experimental, theoretical, and simulation tools developed to explore nanoscale phenomena and build the field of nanotechnology, as well as from the expertise and knowledge base acquired over decades of semiconductor manufacturing, from a century or more of advances in synthetic chemistry, and from the biotechnology and molecular biology revolution.

Weiss explored many key components of mesoscale phenomena: granularity, defects, energy quantization, collective behavior, fluctuations and variations, interacting degrees of freedom, and structural and dynamical heterogeneity. At the

mesoscale, these components play important roles in determining the band structure of conductors and semiconductors and are important to understand for creating photonic, plasmonic, spintronic, and metamaterials. Collective effects, such as superconductivity, ferroelectricity, and piezoelectricity, also result from interactions occurring at the mesoscale.

One important challenge in studying mesoscale collective effects is accounting for heterogeneity and fluctuations. “We want to understand these, and we do not want to say that there’s an ensemble distribution,” said Weiss, because in studying these systems, “heterogeneity turns out to be critically important.” Using the human immunodeficiency virus as an example of a structure that fits between the nanoscale and microscale, he explained that there are many repeated patterns in the structure of a virus, but there is also a great complexity of extreme importance found in the details of the encoded genome of the virus, in the variations and fluctuations in that genome, and in their effects on billions of people worldwide.

When working at the nanoscale, said Weiss, it is possible to exercise exquisite control over the position of every atom, but working at the mesoscale requires giving up that kind of control and instead embracing the granularity of structures at this scale. He noted that this is where techniques developed in the 1990s by the semiconductor industry could prove valuable for developing and studying mesoscale structures and that there are many unused semiconductor foundries built to produce earlier-generation computer chips that could be turned to this work. At a time when research in chip design and development was pushing the boundaries of Moore’s Law to ever smaller scales, there were advantages, Weiss explained, in backing away from those boundaries and working on a broader range of materials. As he put it, nobody will complain about the exact placement of impurities in a semiconductor when working at the mesoscale. Techniques such as soft lithography, microcontact printing (Dameron et al. 2005, Love et al. 2002), dip-pen lithography (Hong, Zhu, and Mirkin 1999), and chemical lift-off lithography (Liao et al. 2012), which work well at the 100-nanometer scale but can also work at the centimeter scale, are proving useful in creating structures at the mesoscale. However, they are not applicable for creating the latest semiconductor structures. He stressed that the ability to control

chemical functionality is the key to this work because that will in turn control and tune the interactions and interfaces at the mesoscale in ways that can be used to couple materials together to develop new capabilities.

Working at the mesoscale and the averaging that comes with that does mean giving up some details. For example, x-ray data from crystals of DNA led to deducing the double-helix structure of DNA, but it would not provide any information on the sequence of bases. One of the surprises from the Human Genome Project, Weiss noted, was that though there are a million different proteins that are present in human beings, there are only about 21,000 genes to provide that coding. Sequencing alone does not reveal the mechanisms by which this complexity is achieved, and there are lessons to be learned by studying this system about how “mix-and-match” systems can create such a variety of results. As an aside, he reminded the workshop that the U.S. government’s investment in the Human Genome Project has generated outsized returns in terms of economic activity and gains in personal income, and that mesoscale science has the same potential for return on investment.

Returning to the subject at hand, Weiss also reminded the workshop participants that it is important when making materials at the mesoscale to keep in mind the desired function of those materials and the phenomena that need to be measured. “There’s this beautiful interplay taking place now in nanoscience and nanotechnology where we build devices in order to understand properties. I think the same is going to be extremely true of the mesoscale,” he said. “What can we build that will give us feedback about the key components in terms of structure, function, spectra, and so forth?”

Working from a chemistry-based perspective in both the nano- and mesoscale provides useful insights, especially when coupled with tools and knowledge drawn from other fields. In nanotechnology, researchers have already learned to bridge the communication gaps among chemists, physicists, material scientists, biologists, clinicians, mathematicians, computer scientists, and others to exchange ideas and benefit from each other’s expertise. Chemists, meanwhile, have used their tools to design building blocks that can be put together to create larger structures in ways that provide specific control over molecular interactions and interfaces to study and investigate these

phenomena. The construction of colloidal fullerene crystals with precise structures (Claridge et al. 2009) supports that idea, said Weiss, as does the work that his group did using various inorganic building blocks and linkers to create zero-, one-, two-, and three-dimensional materials with well-defined band gaps. Using tools and understanding from other fields, his group could predict the values of these band gaps from an understanding of the properties of the linker's electronic structure. "From very simple rules of thumb, we could predict the properties of the materials," said Weiss. The reason for that predictability, he explained, is that the interactions between the different components in these systems are relatively weak because of the way the linkers hold the different components apart from one another. Using chemistry, it is possible to create additional clusters and materials that can in turn inform the theoretical understanding of how mesoscale properties emerge and to test those theories. Weiss built on this example of the benefit of chemistry as part of an interdisciplinary approach to research at the mesoscale with a few more examples.

Turning to the subject of defects, Weiss explained that they can be exploited for patterning but that they can also lead to pattern dissolution. In early studies of mesoscale defects, he and his colleagues showed that mesoscale systems using self-assembled monolayers are never at equilibrium but that the defects can be guided to a specific location and with a specific density and then frozen at the place (Weiss 2008). "We could annihilate things such as substrate vacancy islands and voids and step edges by adding more molecules to the matrix, by allowing flow to get rid of those defects, and by filling in the kinds of defects we didn't want," he explained. The defects that remained were ones that could be used to insert functional components into the material, and these functional components could be observed and measured using either scanning tunneling microscopy or spectroscopic methods (Zheng et al. 2013).

Studies of carboranes—molecules in which the carbon atoms are hexacoordinate rather than tetracoordinate and are therefore electron deficient—demonstrate one aspect of the kind of control that can be achieved over mesoscale structures. Placement of the carbon atoms in these materials determines the direction of the dipole in the carborane molecule. When these cage molecules

self-assemble in monolayers, they can have the dipole oriented parallel or perpendicular to the material surface (Figure 2-1), which creates either polar or nonpolar structures (Hohman et al. 2009) that can in turn be mixed to create organoelectronics with controlled interfacial energy (Kim, J., et al. 2014). Weiss and his colleagues have learned how to control the average surface charge of these self-assembled monolayers to create "essentially perfect band alignment while preserving an important property of the polymer—how it wets." In contrast, the typical approach to modify surface charge is to use partial fluorination, but this changes the wetting property of the polymer and the morphology of the active layer.

Weiss noted that it is possible to measure buried dipoles in these systems. In one set of experiments, his group wanted to know if aligned dipoles are responsible for the competitive advantage of the nonpolar surface and, in fact, aligned dipoles do create a defect-tolerant system. Spectroscopic measurements and mathematical analysis showed that the dipole interaction occurs over a long enough range to create a two-dimensional ferroelectric system. "If we only had neighbor-neighbor interactions, that would be classically forbidden," explained Weiss. Instead, these mesoscale interactions allow the molecules to cross step edges that are a couple of angstroms high and cross domain boundaries that are offset by a couple of angstroms. The result is that these self-assembled monolayers have large regions where the dipoles are aligned even when there are defects that would normally prevent such alignment from occurring.

In his final remarks, Weiss drew some lessons from nanotechnology to comment on the type of tools that need to be developed and applied to understand mesoscale phenomena. In nanotechnology, he said, there were structural tools that let researchers observe and control the placement of individual atoms and molecules to develop some predictive rules regarding structure and function at the nanoscale. "What we learned, and what we are going to need to learn in the mesoscale, is structural control at multiple scales all at the same time. We're going to need to associate those and use those as input for theory and simulations, and that's going to ultimately give us control over chemical, physical, electronic, optical, and biological properties," he said.

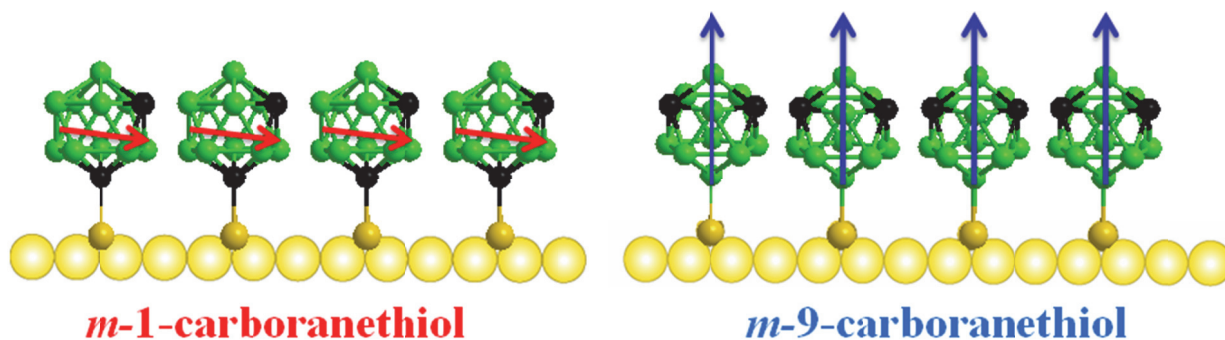


Figure 2-1 The alignment of dipoles on the surface of a self-assembled monolayer has an important impact on the chemical properties of the resulting surface. SOURCE: Hohman et al. (2009). Reprinted with permission from *ACS Nano*.

He also noted that while he did not talk about dynamics, it is going to be important to follow dynamics and understand how these systems evolve at the mesoscale, not at the level of each and every atom or molecule but at a level that will provide useful insights. He predicted that mathematical tools will be useful in this regard. The ability to measure and detect rare events in mesoscale structures will also be important, particularly for developing novel catalysts, where rare structures are often the ones that have the largest catalytic activity. “One of the keys there is going to be figuring out what those are and guiding the system into those areas,” said Weiss. He concluded his talk with the prediction that the tools needed to understand the mesoscale are going to be complicated and it will be necessary to piece together information in a way that does not require knowing the position and function of every atom in these structures.

DISCUSSION

In response to a question about the field’s ability to create well-defined mesoscale systems at a commercial scale, Weiss said roll printing of organic electronics is an example of mesoscale structures being produced at scale. However, he noted that much of the work with organic electronics has been empirical rather than driven by predictions based on well-formulated theory, and this is an area where mesoscale research could produce significant advances in terms of understanding how to intentionally control chemical and polymer properties to produce specific properties.

Weiss then addressed the issue of metastability at the mesoscale. He stated that when researchers

were first performing chemical conversions on self-assembled monolayers, they would use the most vigorous chemical reactions they could find so that these conversions would go to completion. That, however, turned out to be a bad idea because the monolayers would anneal. What was needed, he explained, was to develop chemistry that preserves metastable structures and yet goes to completion or to develop methods of only carrying out chemistry at defects. These systems, he said, are metastable systems, not equilibrium systems, and it is necessary to preserve that metastability to produce materials with novel and useful properties.

3

Catalysis

Key Points

- Confinement and control of local environments are essential features of development of functional catalysts, and chemists can provide this control by using both chemical and biological approaches to modify mesoscale parameters.
- Controlling the environment surrounding the active sites of catalysts within constrained environments has proved to be difficult, as is the understanding of the effects that the reaction products have on a constrained environment inside a pore.
- For native enzymes and synthesized catalytic mimics, the outer coordination sphere plays a critical role in determining reactivity. Large, dynamic changes in the ligands around an active site can result in changes in the reactivity of functional groups in other parts of the protein.
- There are many opportunities within modeling to address mesoscale phenomena and to develop new theoretical paradigms for examining mesoscale regimes within different types of catalysts. Such work would be particularly valuable for understanding mesoscale heterogeneity, fluctuations, and transport effects.
- Heterogeneity and long-range interactions, which had not been factored into modeling studies until recently, are important for generating catalytic activity.
- The role of entropy in driving the reactions at the mesoscale is recognized as important but not yet well understood, raising a challenge that needs to be addressed.
- The tools available in mechanical engineering, fluid control, and synthesis provide a greater ability to design structures with varying pore diameters and shapes to create catalysts reliably for specific functions, but key synthetic challenges remain.
- Processes developed for the semiconductor industry, such as atomic layer deposition, are being employed to build mesoscale catalyst structures via a bottom-up strategy.

The workshop's first panel session featured five presentations on various aspects of how mesoscale chemistry is important for catalysis. Andrew Borovik, Professor of Chemistry at the University of California, Irvine, described the architectural features within local environments that are instrumental in regulating function at a catalytic center. Wendy Shaw, Scientist in the Physical Sciences Division at the Pacific Northwest National Laboratory (PNNL), discussed how an understanding of the interactions between an enzyme's protein scaffold and its catalytic active site can inform efforts to improve molecular catalysts. Yi

Lu, the Jay and Ann Schenck Professor of Chemistry at the University of Illinois, Urbana-Champaign, presented an example of how to elucidate and employ key features of mesoscale biocatalysis for designing artificial enzymes with catalytic efficiencies similar to that of native enzymes. Cynthia Jenks, Assistant Director for Scientific Planning and Division Director of Chemical and Biological Sciences at the Ames Laboratory, focused on hybrid multifunctional systems and their use in modulating catalytic activity and selectively controlling accessibility of an active site to reactants. She also discussed design concepts based on

mesoscale phenomena for tandem catalysts. Peter Stair, the John G. Searle Professor of Chemistry and Chair of the Chemistry Department at Northwestern University, concluded the presentations by reviewing two basic approaches to synthesizing mesoscale catalysts and then focusing on strategies for molecular and atomic assembly on solid platforms to create new catalysts. A panel discussion, moderated by session chair Bruce Garrett, Director of the Physical Sciences Division at the PNNL and a member of the workshop organizing committee, followed the five presentations.

ARCHITECTURAL COMPLEXITY AT THE MOLECULAR LEVEL

To start his presentation, Andrew Borovik showed images of four enzymes containing metal cofactors that are essential for their enzymatic activity and noted two key features shared by these enzymes. The first is that if the metal cofactors were removed from the active site, the chemistry these enzymes would perform would be pedestrian at best and of almost no use to chemists. “But when we insert these cofactors into the active site, they perform remarkable chemistry, efficient, robust, selective chemistry that as a synthetic chemist who tries to make catalysts, I have an amazingly difficult time to achieve,” said Borovik. The second important feature is that key forces or interactions that control the local environments around the metal atom cofactors are noncovalent.

These two features are not found solely in biology, however, and Borovik showed an example of a porous organic host that his group has synthesized (Sharma and Borovik 2000). It is able to bind and release oxygen using a confined cobalt atom within a suitable molecular complex. He also described a relatively simple copper complex that, when embedded within a zeolite, had very high activity as an oxidation catalyst for converting methane to methanol. He characterized this reaction as one of the most difficult chemical transformations to accomplish (Woertink et al. 2009). The lesson from these two examples, he said, is that confinement and control of local environments are essential properties for developing functional catalysts, and that the main challenge for chemists aiming to manipulate these two properties is to control noncovalent interactions. The key question,

he said, is “How can you design something to promote these intramolecular processes in order to promote overall function?”

To illustrate this challenge, Borovik focused on a simple reaction—the oxidation of water to form oxygen—and the biological construct that promotes this reaction. The molecular complex that accomplishes this reaction in nature is called Photosystem II, and it orchestrates the movement of eight different particles with specific timing to catalyze this reaction, which lies at the heart of photosynthesis. Photosystem II is a large membrane-bound protein complex whose active site structure was solved in 2011 (Umena et al. 2011), revealing the arrangement of metal atoms within the active site and providing important information about how water is oxidized to molecular oxygen. There are four manganese centers and one calcium center that are surrounded by a rich network of hydrogen bonds which extends beyond the active site (Figure 3-1). While many of the steps of the water oxidation reaction are known, the details of the key step of generating the oxygen–oxygen bond are still largely a mystery. Identifying those details will likely depend on computational methods to provide some mechanistic insights.

One thought regarding potential mechanisms for the reaction, said Borovik, is that one of the manganese centers becomes highly oxidized, making it “amazingly electron deficient” and capable of forming a high-energy oxygen intermediate that can react with one of the water molecules in the active site to create molecular oxygen. That mechanism, however, would require chemistry that has not ever been observed in the laboratory. To explore possible mechanisms, Borovik and his colleagues have been creating small organic constructs that can bind metal ions and promote intermolecular hydrogen bonding networks similar to those in the active site of Photosystem II. These can then be used to study fundamental properties such as proton and electron flow, and findings of these studies can then be applied to the creation of synthetic catalysts.

One such organic construct contains a metal binding pocket and several nearby amine groups to promote intermolecular hydrogen bonding. Borovik’s research group has created a number of versions of this molecule with different functionality and obtained both structural data using x-ray diffraction and spectroscopic data to follow electron

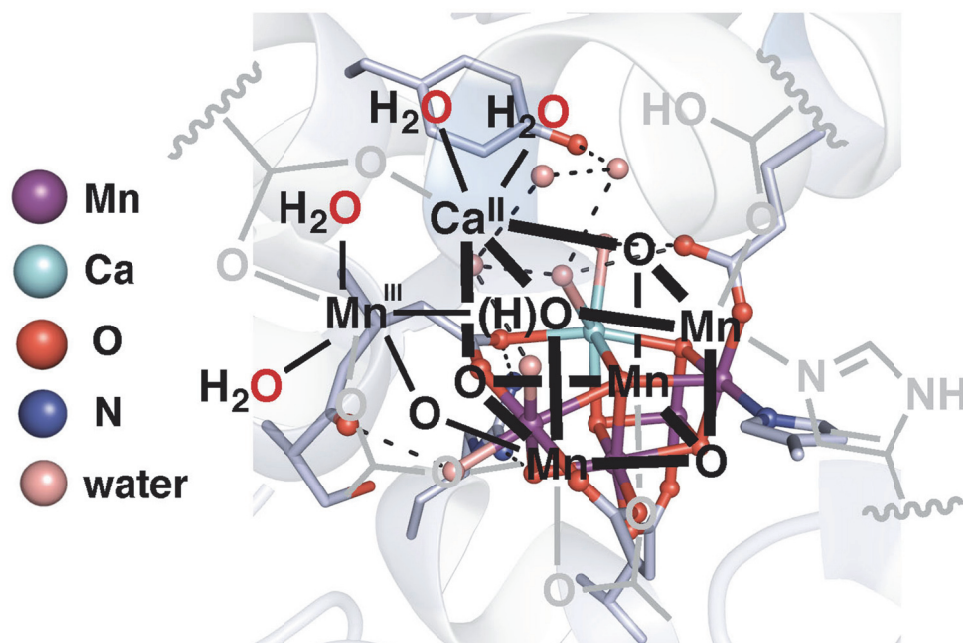


Figure 3-1 The oxygen-evolving complex of Photosystem II. SOURCE: Cook and Borovik (2013). Reprinted with permission from *Nature Chemistry*.

transfer and proton movement. These data are supporting the development of a better understanding of the thermodynamics and kinetics of the oxidation reaction and how the key reactive intermediate could form in a biological active site.

Another key question his group is exploring is the role the calcium atoms play in active site function. To examine this question, they redesigned the active site in a construct to promote hydrogen bond formation and to include both manganese and calcium in the active site, which would resemble the active site of Photosystem II (Figure 3-2). The next step, he explained, is first to introduce more manganese atoms into this complex followed by water molecules to see if the resulting complex will oxidize water to molecular oxygen.

Borovik and his colleagues are also exploring ways of modifying their small molecule complexes to move protons out of the active site. One of the molecules they created was able to catalyze a related reaction that reduces molecular oxygen to water at room temperature. “This just shows you that by changing the local environment and managing the protons that we can actually install new catalytic function in our systems,” Borovik said. Taking this work to the next scale, his team is now inserting these metal–organic complexes into larger structures such as the Streptavidin protein complex. This work

takes advantage of the interface between two of the Streptavidin subunits, known as the vestibule, and is attempting to tune the local environment around the vestibule to alter the mesoscale interactions in order to observe the effect of those environmental changes on inserted metal–organic complexes. Recently, his team created a structure that connects two spatially separate copper complexes—one bound to each Streptavidin subunit—via a water channel. “We can now combine chemistry and biology and in some cases genetics to create modifications within the vestibule to control the local environment,” said Borovik in closing. “The ultimate goal is to understand how these weak interactions control function and to make catalysts that can do the same kind of transformations that occur in biology.”

BRINGING MESOSCALE PROPERTIES TO MOLECULAR CATALYSTS BY ATTACHING AN OUTER COORDINATION SPHERE

Like Borovik, Wendy Shaw is attempting to gain insights into catalytic function by controlling the environment around the active site, but her approach is to use amino acids and peptides. She is also studying a different system—the enzyme known as hydrogenase, which converts protons to

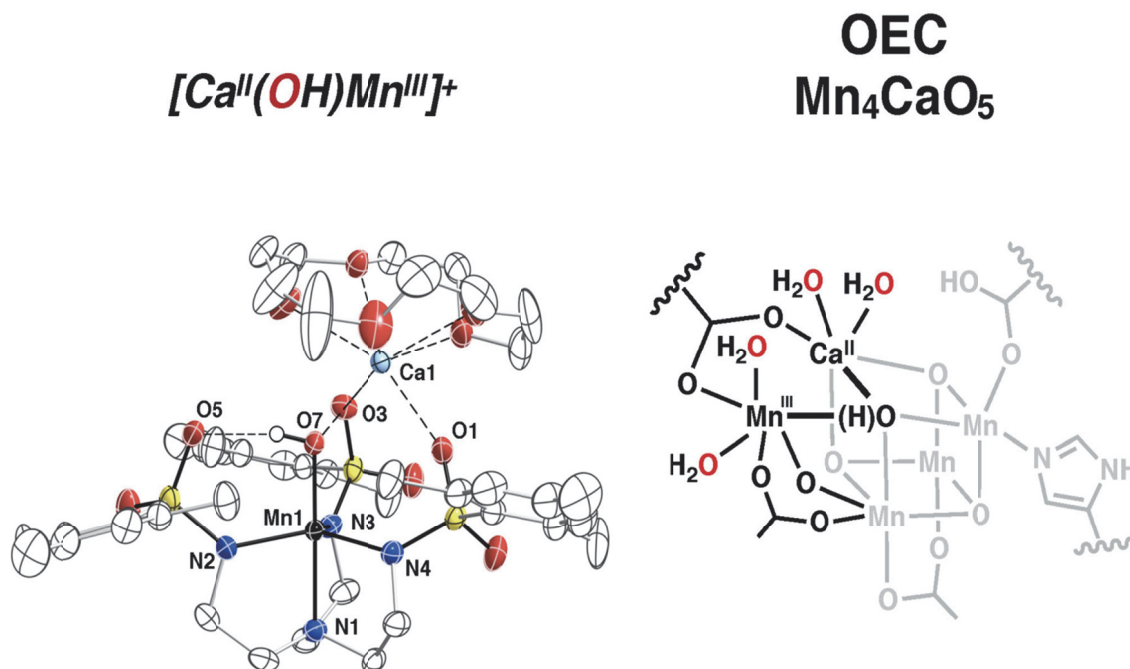


Figure 3-2 A molecular structural comparison between a synthetic active site and that of the oxygen-evolving complex. SOURCE: Umena et al. (2011). Reprinted with permission from *Nature Chemistry*.

molecular hydrogen and back again. Hydrogenase is extremely efficient at catalyzing this reaction, operating at a rate of 10,000 conversions per second at less than 100 millivolts (mV) overpotential. She explained that overpotential is a measure of the extra energy required to initiate the reaction, and the lower the overpotential, the more energetically efficient the reaction.

There are multiple hydrogenases in nature, and the one Shaw studies has two iron atoms in the active site along with pendant amine groups that are positioned such that they can bring hydrogen into the active site for cleavage or bring protons together to be released as molecular hydrogen. These pendant amines are critical for the enzyme's efficient function, and Shaw noted there are no known active catalysts that purely mimic the active site of the enzyme. She added that a colleague of hers has created active site mimics that reproduce the function of the pendant amines (Figure 3-3), producing some of the fastest hydrogen oxidation catalysts known; they operate at 50 conversions per second and with a 400-mV overpotential (DuBois 2014). Nevertheless, the underperformance of this synthetic catalyst relative to the native enzyme shows that the complexity of the outer coordination

sphere is essential to function. It is for this reason that she focuses her work on introducing the outer coordination sphere to see if she can recapture some of that complexity and its function.

The outer coordination sphere of hydrogenase, explained Shaw, controls mesoscale functionality by creating channels that allow protons, hydrogen gas, and electrons to reach and leave the active site in a precise way, in terms of both location and timing. "It ends up being a very orchestrated process to get protons and electrons at the active site at the right time to react," said Shaw. She also noted that each of the ligands in the hydrogenase active site is bound to metals or hydrogen-bonded to another group around it, which allows for the precise control at an atomic level around the active site. This hydrogen bonding controls redox potentials and the structure and dielectric potentials of the active site. Shaw said that there are many things about the environment of the active site that are not yet understood, but that it is clear that this environment is controlled by the large number of functional groups within the complex of 20 amino acids that surround the active site.

Ligand dynamics also plays an important role in the active site's catalytic activity. Large, dynamic changes in the ligands around an active site can

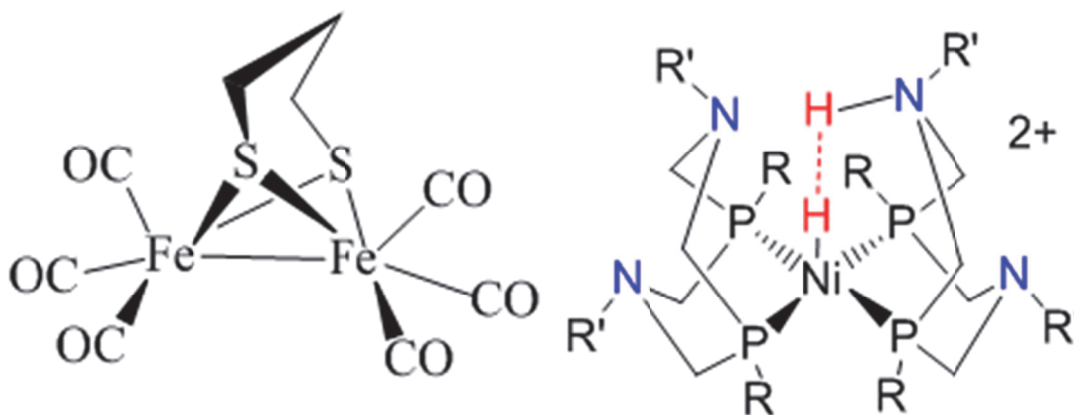


Figure 3-3 Two mimics of the iron–iron hydrogenase: one that reproduces the active site of the enzyme but is inactive (left) and one that introduces pendant amine functionality and that is active (right). SOURCE: DuBois (2014). Reprinted with permission from *Inorganic Chemistry*.

result in changes in the reactivity of functional groups in other parts of the protein, a phenomenon known as allostery. At the same time, there are many smaller, slower structural changes occurring in the vicinity of the active site that can control the local dielectric and structure and cause the enzyme to catalyze the reverse reaction. “That’s something that we don’t have good control of in molecular catalysts,” said Shaw, who said that the important lesson when considering all of the dynamics described so far is that the interaction and cooperativity of the active site with the protein scaffold is necessary to generate function.

There are many mesoscale features in hydrogenase and Shaw discussed how the study of one of these features provides evidence of concerted proton and electron transfer. Data from a molecular dynamics study looking at motion as a function of protonation state show that when the protein is in a protonation state that is preferential for hydrogen oxidation, there is motion at the mouth of the channel that ferried protons out of the active site, as well as at the redox partner binding site (Ginovska-Pangovska et al. 2014, McCullagh and Voth 2013). However, when the protonation state changes to one that favors hydrogen production, motion at the proton channel stops while motion at the redox binding partner increases. “While this is by no means conclusive, it indicates that the redox partner binding region is sensitive to what is happening in the proton channel and is suggestive of proton-coupled electron transfer,” said Shaw.

One challenge is to understand how motion in one part of the enzyme is driving protonation at the active site or vice versa, she said. Another challenge is to incorporate these kinds of allosteric interactions into molecular catalysts, both to create novel catalysts and to better understand how enzymes work. There are two general methods for approaching the work, either from the “top down” or the “bottom up.” A top-down approach to control mesoscale complexity takes a molecular catalyst and inserts it into an already formed protein structure, such as the work that Borovik discussed using Streptavidin. The problem with this approach is that it produces a very large molecule that may provide challenges for studying and modeling the system.

Shaw has taken a bottom-up approach that begins with a molecular catalyst and uses peptides to build a scaffold that adds functional groups to the outer coordination sphere. She started with a nickel-phosphorous-nitrogen hydrogen oxidation catalyst and added various peptide-based outer coordination spheres that resemble the enzyme but are much smaller. The first construct she created to oxidize hydrogen operated at three reactions per second with an overpotential of 900 mV. Adding a proton channel to move protons more quickly out of the active site reduced the overpotential by 300 mV—a 30 percent improvement in efficiency—but it also slowed the reaction rate slightly (Lense et al. 2014). “I think this result captures the idea of the mesoscale in that you can introduce a feature that you think is going to do one thing and it does something completely different,” said Shaw.

The next step was to insert a glycine into the core catalyst and the result was “pretty interesting,” said Shaw. The resulting catalyst has a reaction rate that is competitive with the catalyst described previously but with a low overpotential that is competitive with what is seen in the enzyme. She credits a strategically positioned carboxylic acid group that can move a proton quickly between a nitrogen atom and an oxygen atom in the active site and allows for rapid proton transfer from the pendant amine (Dutta et al. 2013). Adding another proton relay using arginine instead of glycine, which adds guanidinium functionality in the outer coordination sphere, raises the overpotential slightly but increases the reaction rate by about an order of magnitude, she said (Dutta, Roberts, and Shaw 2014).

While these results indicate that guanidiniums are enhancing catalysis, it is not clear how they are doing so. To answer that question, Shaw and her colleagues looked at the catalytic cycle and began to test possible steps in the cycle, including deprotonation followed by hydrogen addition. Beginning with the idea that deprotonation was the initial step—a step that would be independent of hydrogen concentration—she and her colleagues increased the hydrogen pressure and expected to see no change in rate as deprotonation would be the rate-limiting step. In fact, they identified a linear dependence on hydrogen concentration, suggesting that the guanidiniums in the outer shell are in some way affecting hydrogen addition but not deprotonation. Subsequent experiments showed that there appears to be an arginine–arginine interaction within the protein that aids in catalysis. Shaw believes that the interaction between the two arginines helps stabilize the nickel-to-nitrogen distance slightly and that in turn is helping to facilitate hydrogen addition.

Reversibility is a hallmark of enzymes and mesoscale interactions, and though Shaw and her colleagues had managed to create a fairly efficient molecular catalyst, this catalyst is not reversible at room temperature. Shaw credits a decrease in mesoscale interactions between the active site and the protein scaffold for the reversibility displayed by the native enzyme. However, when the molecularly catalyzed reaction was run at 40°C to 50°C instead of room temperature, the molecular catalyst was completely reversible just as it is in the enzymes. In light of those results, she and her colleagues hypothesized that if arginine stabilizes the

guanidinium–guanidinium interaction, then pi–pi interactions, such as those introduced by phenylalanine or tyrosine, could provide similar stabilization. In fact, when Shaw and her team replaced the arginine with tyrosine, the result was a catalyst that operates reversibly at room temperature. “What’s exciting here is that we’ve made a pretty small change really far from the active site, and we’ve completely changed the reversibility of these complexes as well as the temperature at which it operates,” said Shaw.

Going forward, Shaw and her colleagues plan to add more complexity by using peptides instead of amino acids in the outer shell and to employ computational methods to inform future designs. Computation, said Shaw, has the potential to predict structural stability and functional group positioning and to limit the experimental guessing that comes with trying to perform protein structure prediction. She noted that David Baker, a colleague at the University of Washington, made a structure prediction game that anyone can play on the Internet, and that work has identified two different solutions that look to be promising structures. She and her coworkers are doing some optimization work now, and she noted that they are at the point of introducing multiple functional groups to the system and seeing how those can control catalytic activity. Future work will also investigate the role of water within these catalytic structures. She stated that the ultimate question to answer will be how to implement various features so that they work together. “We’ve been able to implement two different functional groups, but when we add a third will it be complementary, or will we have to do something to make them talk to each other?” she asked in closing her remarks.

ELUCIDATING KEY FEATURES OF MESOSCALE BIOCATALYTIC SYSTEMS AND EMPLOYING THEM IN DESIGNING FUNCTIONAL ARTIFICIAL ENZYMES WITH HIGH ACTIVITY

Continuing the theme of exploring the role that mesoscale phenomena play in enabling the catalytic activity of enzymes, Yi Lu described his group’s work on the enzyme cytochrome c oxidase, which catalyzes the reduction of oxygen to water and in doing so creates the proton gradient that drives the

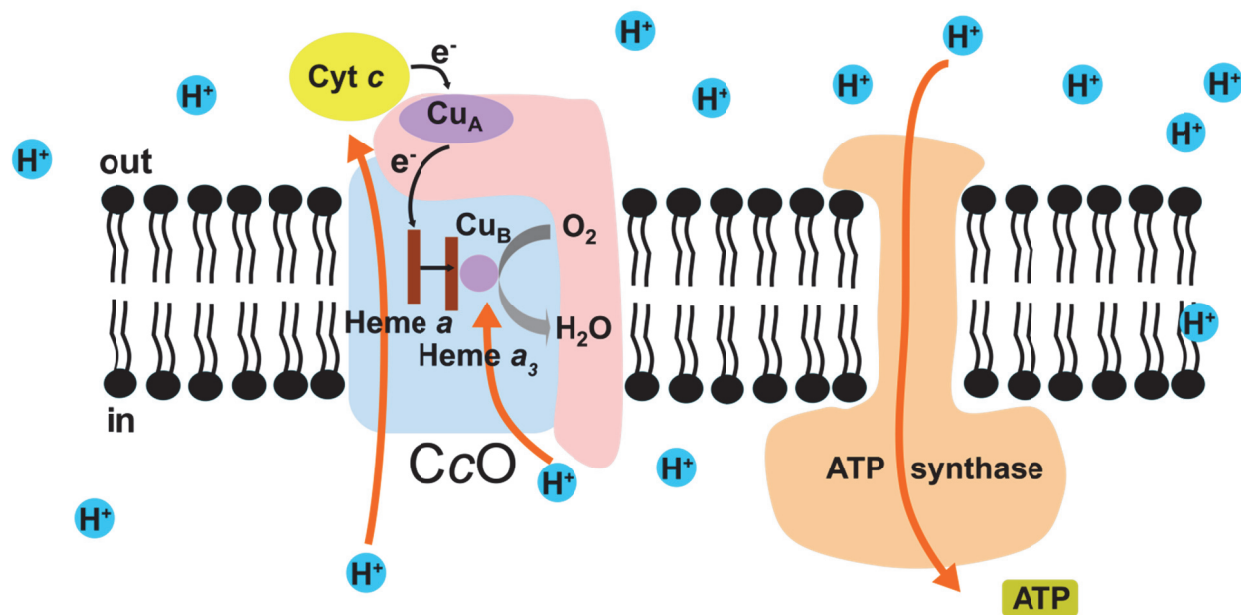


Figure 3-4 Cytochrome c oxidase catalyzes the reduction of oxygen to water, producing four protons and four electrons that are used to drive adenosine triphosphate (ATP) synthesis. SOURCE: Hwang and Lu (2004). Reprinted with permission from *Proceedings of the National Academy of Sciences of the United States of America*.

synthesis of adenosine triphosphate (ATP). Moreover, this enzyme is capable of catalyzing this reaction without releasing toxic reactive oxygen species (Figure 3-4). Lu noted that one of his interests in studying this enzyme is its potential importance as a model for creating new catalysts for the oxygen reduction reaction in fuel cells because of its fine control over the movement of electrons with high efficiency. While there is a relatively efficient platinum-based catalyst that is used in today's fuel cells, this catalyst is not only expensive because of its platinum content, but it operates at a higher overpotential than does cytochrome c oxidase (Kjaergaard, Rossmeisl, and Nørskov 2010). Besides wanting to identify the mesoscale features that make cytochrome c oxidase such an efficient catalyst, Lu said that he also wants to understand why chemists have been unable to create a synthetic biocatalyst with high activity despite 30 to 40 years of biomolecular modeling aimed at creating such a catalyst.

One answer to this second question is that most biomimetic modeling work has focused on the enzyme's active site and has treated the environment surrounding the active site as being largely hydrophobic. In fact, said Lu, there are many

hydrophilic, positively charged, and negatively charged residues around and near the active site and many water molecules that are strategically positioned around the active site. "So if you look at the true picture of the active site, it is no longer just a hydrophobic environment. It's actually quite hydrophilic, particularly at the mesoscale," said Lu, who added that the role of these mesoscale heterogeneous environments surrounding the active site had not been factored into synthetic modeling studies until recently.

A number of groups have created synthetic model systems for this enzyme, and James Collman's group at Stanford has produced several different synthetic iron and copper biomimetic analogs of the copper-containing active site of cytochrome c oxidase that faithfully reproduce not only key physical features of the active site, but also its ability to catalyze the reduction of oxygen into water (Collman et al. 2007). Daniel Nocera's group at the Massachusetts Institute of Technology has created a copper-containing complex that is also able to catalyze the reduction of oxygen to water (Dogutan et al. 2010). Both of these model systems use small organic molecules to mimic the enzyme's active site.

Lu's approach is to use the protein myoglobin as the scaffold for creating a catalytically conducive environment around a synthetic active site. Myoglobin is much smaller than cytochrome c oxidase, making it easier to work with, and its rigid protein scaffold allows for the facile incorporation of mesoscale interactions into the design of the biosynthetic catalyst. In addition, a large body of work by many research groups has created the means to produce modified myoglobin molecules that can be used to explore how well-characterized changes in the protein's structure affect those mesoscale interactions and catalytic activity (Lu et al. 2009).

Lu's initial work aimed at incorporating a heme-coordinated copper into myoglobin that had catalytic activity, but it only reduced oxygen to the peroxide species and not all the way to water (Sigman et al. 2003). Further modeling activity identified three key histidine residues and a tyrosine residue within cytochrome c oxidase, as well as an elaborate hydrogen-bonding network involving two water molecules, that are critical in creating catalytic activity (Blomberg, Siegbahn, and Wikstrom 2003). The challenge then became one of introducing these residues and this hydrogen bonding network into the structure of a modified myoglobin. The group's first step was to use a different heme to coordinate copper—one that added a particular hydroxyl group to the active site. The second was to introduce a tyrosine residue into the active site next to one of the key histidine residues. The resulting synthetic enzyme was able to reduce oxygen to water at a rate of thousands of reactions per second (Miner et al. 2012). Lu summarized these findings by emphasizing the importance of the heterogeneous environment and long-range interactions for generating catalytic activity.

The next challenge his group addressed was to try to understand and control the cascade of electron and proton transfers that occur in cytochrome c oxidase and similar enzymes, such as the family of blue copper proteins in which the copper atom is coordinated to two nitrogen atoms and two sulfurs in the active site. One interesting feature of these blue copper proteins is that they have nearly identical structures yet have a wide range of overpotentials ranging from 130 to 618 mV. Lu's group decided to explore the role that mesoscale structure plays in producing this wide range of catalytic activity by changing the hydrophobicity and the hydrogen

bonding network near the active site using various combinations of four mutations to alter amino acid residues in the protein. Using these mutations, either singly or in combination, Lu's team was able to tune the overpotential over a 700-mV range, surpassing the highest and lowest potentials reported for any member of this enzyme family without altering the metal binding site (Marshall et al. 2009). Lu and his colleagues have since used these proteins to generate the first experimental support for what is known as a Marcus inverted region that has been proposed to be important in tuning the forward and backward electron transfer rates that create the charge separation needed to drive reactions such as photosynthesis (Farver et al. 2013).

Lu's team has since collaborated with a group in India to engineer these artificial enzymes onto the surface of an electrode, a necessary step for creating a commercially useful catalyst. To ensure that the proteins have a specific orientation on the surface of the electrodes, computer models were used. The collaboration has generated a model system that has a pseudo-first-order rate that is better than the native enzyme and with a turnover rate approaching a quarter of a million reactions per second. Lu's group has since improved the catalytic activity of its biomimetic system by incorporating nonnative cofactors and unnatural amino acids into their designed myoglobin oxidase catalysts (Bhagi-Damodaran et al. 2014).

Turning to a different subject in the final few minutes of his presentation, Lu described the use of catalytic DNA to control the assembly and disassembly of nanoparticles at the mesoscale for use in sensing, imaging, and drug delivery applications. Using lessons learned from the error-correcting functions of the ribosome, Lu's team has developed catalytic DNA that can find defects in nanoparticle assembly and remove them (Tan, Xing, and Lu 2014).

In summary, said Lu, it is possible to make functional biomimetic models of complex metalloenzymes such as cytochrome c oxidase with high activity using much simpler native proteins, by taking into account several mesoscale parameters:

- the heterogeneous environment around the active site that includes many noncovalent interactions, such as water and associated hydrogen-bonding networks;
- the control of cascade reactions; and

- the role that the homo- and heterogeneous reactions play in catalytic activity.

MESOSCALE CONCEPTS FOR DESIGN AND BEHAVIOR IN HETEROGENEOUS CATALYSIS

Heterogeneous catalysts differ from their homogeneous counterparts in that they are easier to separate out of a reaction mixture and regenerate. They are typically robust and have higher activities than homogeneous catalysts and usually have good stability at higher temperature, but aside from those features, these two broad classes of catalyst have much in common, said Cynthia Jenks. As is the case with the homogeneous catalytic systems that the first three speakers described, controlling key features of the heterogeneous catalysts that she studies requires consideration of the environment surrounding the active sites. In this presentation, Jenks focused on her work on immobilizing heterogeneous molecular catalysts in mesoporous silica nanoparticles, an environment that is more constrained than when these catalysts are in solution. These nanoparticles have cylindrical pores whose shape, size, and chemical composition and surface functionalization can be controlled during nanoparticle synthesis.

Confinement was the first aspect of these catalysts that Jenks addressed. When creating these catalysts, it is possible to put them onto the convex surface of a solid particle or onto the concave inside of the pore. In one experiment, she and her colleagues ran a catalytic addition reaction in a hexane solution, with the catalyst tethered to the outside of a particle and inside a mesoporous silica nanoparticle. Tethering the catalyst to the nanoparticle surface enhanced the reaction rate slightly, but putting it inside a nanoparticle pore produced a huge jump in reaction rate. Changes in the morphology and size of the pore—both its length and diameter—also can have a significant impact on catalytic activity. In another experiment, Jenks and her colleagues ran an aldol condensation reaction using a catalyst that was embedded in nanoparticles with pores that were either 400 or 800 nm long. Activity—measured by the percent of starting materials converted to the final product—dropped substantially with the longer pore, perhaps because the reaction products could not exit the pore efficiently. On the other hand, said Jenks, increasing pore diameter had a significant positive impact on percent conversion, raising it from less than that of

the homogeneous catalyst when it was in a 2.8-nm-diameter pore to approximately 10-fold higher than the homogeneous catalyst when pore size was enlarged to 3.6 nm in diameter. These results show that modifying the physical, mesoscale environment of the catalysts can significantly affect the reactivity of the systems. Given the tools available in mechanical engineering, fluid control, and synthesis, one can imagine designing structures with varying pore diameters and shapes to reliably create catalysts for specific functions.

Aside from tuning pore size and shape, Jenks and her colleagues have also explored tuning catalytic activity by modifying the chemical composition of the internal pore wall using pentafluorophenyl propyl groups, which creates a hydrophobic environment inside the pores and enhances catalytic activity. It appears that this hydrophobic environment causes water to be extruded from the mesopores, driving the equilibrium of the reaction toward completion. Changing solvents can also have a big effect on reaction and conversion rates, with the effect depending on the nature of the catalyst and the solvent being used (Kandel et al. 2013a). For example, when using an immobilized amine to catalyze an esterification reaction, the percent conversion is high when hexane is the solvent and the catalyst is a secondary amine, but the conversion rate is low when water is the solvent, probably because water stabilized a reaction intermediate, which in turn inhibits the reaction. Understanding the fundamental aspects of the interaction between the solvent and reactive intermediates at the catalytic site represents another area for future investigation.

Jenks has also been exploring the creation of systems in which two catalysts are immobilized, one on the particle surface, the other in the pores, to enable tandem reactions to occur, much as location-coupled enzymes do in nature. These could also provide opportunities to take advantage of a combination of enzymatic specificity in a mild, external environment and also allow for less selective, harsher reactions to occur in a designed, reactor-like environment within a pore. As an example, she described a system in which the enzymes alcohol dehydrogenase and catalase are tethered to the outside of the particle and an alkylamine oxidation catalyst is immobilized inside the pores to perform a sequence of oxidation and aldol coupling that transforms short-chain alcohols

into longer-chain molecules with high selectivity (Kandel et al. 2013b). She also noted that it is possible to do one reaction on the surface of the particle and then cap the pores using gold or silver to carry out a second reaction in a more confined environment or to employ some gatekeeper functionality that only admits certain molecules into the pores. Another way porous materials can enable interesting chemistry is by enabling reactions to be run under different temperature and pressure conditions, she added (Lewis et al. 2013).

There are a number of challenges in designing these types of systems that are related to mesoscale phenomena. Tethering homogeneous catalysts is often complicated, she said, as is being able to distribute reactive sites throughout the particles and controlling the chemistry within the pore. Controlling the environment surrounding active sites in the pore has proven difficult, and so too has been understanding the effect that the reaction products have on the constrained environment inside a pore. Unintended plugging of the pores can also be a problem. Solutions to some or all of these challenges could come from promising work in mesoscale modeling of reactions in these different environments (Nagasaka et al. 2007, Piccinin and Stamatakis 2014) and molecular tethering in pores (Wang et al. 2014).

Jenks concluded her presentation by noting that there are many opportunities within modeling to address mesoscale phenomena. “Being able to develop new paradigms to enable us to look at these mesoscale regimes within these particular types of catalysts would be particularly valuable to enable us to understand at the mesoscale spatial heterogeneity, fluctuations, and transport effects,” said Jenks. Key challenges that she sees include the ability to design catalytic groups where they are desired, to design in gatekeepers and caps, and to bridge length scales in modeling and combine and control chemistry and transport.

STRATEGIES FOR THE SYNTHESIS OF MESOSCALE CATALYST DESIGNS

In the final presentation of this first panel session, Peter Stair discussed methods and strategies for making solid catalytic materials using one of two approaches: the one-step assembly of preformed molecular units into porous scaffolds and the step-

by-step assembly of molecular units on preformed porous platforms. In the former process, pore structures and catalytic sites are determined by the assembling units, and in the latter, pore structures and catalytic sites are adjusted by assembly.

The synthesis of zeolites starting with aluminum or silicon hydroxyl anions is one example of the one-step synthesis approach. Under the right conditions of pH and temperature, these anions will assemble into a gel, form secondary units that ultimately form larger building blocks, and then form a host of zeolite structures at the mesoscale. Stair reminded the audience that one particular zeolite made in this fashion catalyzes the production of gasoline from oil, the largest human-performed chemical transformation in history. One feature of this strategy, he said, is that it runs at equilibrium conditions throughout the reaction process, so even when there is a significant amount of solid material formed, the reaction of the smaller soluble units continues at equilibrium.

Another, more recent example of the one-step approach is one that produces metal organic frameworks. In this case, the building blocks are more complicated than simple anions; they include a large variety of linkers and metal salts. Typically, the linkers determine what the structure of the metal organic framework will be when they combine with the metal salts under the right solvent conditions. This approach is capable of producing structures with pores that are much larger than those found in zeolites, and because the synthesis occurs in near-equilibrium conditions, it tends to produce crystalline materials.

The second approach to solid catalyst synthesis—step-by-step synthesis—uses a solid, porous material, usually ceramic, into which catalytic function is added. The ceramic forms can take a wide variety of macroscopic shapes. A variety of methods such as precipitation, adsorption, and impregnation are used to add catalytic function to the preformed materials. In impregnation, which Stair characterized as the most important of these methods, the porous ceramic material is combined with a solution of a metal salt, for example, where the volume of solution is such that it matches the pore volume of the ceramic material. Once the liquid fills the pores, a simple drying process leaves the metal salt behind as little crystallites of various sizes and shapes located at different locations in the pores. The catalyst is then activated by calcining it in

oxygen or reducing in hydrogen at high temperature. Typically, said Stair, because of the porous structures of the ceramic materials and the variations of density and shape of the crystallites within those pores, the final catalyst is considered inhomogeneous in size, shape, and composition.

There are pros and cons to each of these approaches for making solid catalysts. The advantage of the one-step assembly process, said Stair, is that it produces materials with uniform crystalline structure and a large surface area, and it provides the ability to carefully control pore size and the uniformity of those pores. The disadvantage of this approach is that the resulting catalysts are of limited stability and complexity, particularly with the metal organic frameworks. The two-step or post-platform assembly method produces materials with good stability, flexibility, and complexity. Pore size and morphology are well controlled, but the main disadvantage is that the active sites are determined more by statistics than by molecular control in most cases.

A new third approach that Stair and his collaborators are developing is to create materials using the atomic layer deposition process developed by the semiconductor industry as a bottom-up strategy to build mesoscale catalyst structures. This strategy starts out with atomic and molecular building blocks and assembles catalysts with well-defined composition and structure. As an example of how atomic layer deposition works, Stair described one synthesis in which a metal oxide surface covered with hydroxyl groups was exposed to a reactive organometallic complex such as trimethylaluminum. The resulting vigorous reaction created a new surface in which all of the hydroxyl groups are gone, replaced by a new surface decorated by alkyl groups that could be converted to hydroxyl groups again by treatment with water. The advantage of this approach is that because it is self-limiting it provides control by allowing uniform, layer-by-layer deposition of the coating without concerns about clogging the mouth of the channel, which is a concern for other coating techniques such as vapor deposition (Figure 3-5). Stair noted, too, that “you can make all of the interesting catalytic compositions that you would ever want using atomic layer deposition.”

Atomic layer deposition can also be used to make nanoparticles when using precursors of late transition metals such as palladium and platinum (Lu

and Stair 2010, Lu et al. 2014). The nanoparticles tend to be capped by the ligands that were in the original organometallic precursor, and the deposition process can therefore be repeated to create tiny nanoparticles of well-defined size and composition. It is also possible to deposit multiple metals on the same nanoparticle to explore mesoscale effects related to the proximity of one metal atom to another.

Stair then noted that atomic layer deposition can be used to create apertures, pores, and cavities, as well as structures with or without facets. He highlighted one surprising example from his group in which he and his collaborators took a bare supported palladium nanoparticle and tried to use it to catalyze the dehydrogenation of ethane to make ethylene. The result was that the reaction produced carbon, methane, and hydrogen but little ethylene. Wondering what would happen if they covered the palladium nanoparticle, they added a layer of aluminum oxide that eventually buried the particle, eliminating all catalytic activity since reactants could not reach the nanoparticle. However, this coating has a high water and hydroxyl content and heating the material generated pores in the deposited layers that reached down to the nanoparticle. Now, this construct is “beautifully selective for producing ethylene and hydrogen,” said Stair, characterizing it as an example of how changing the environment around the nanoparticle totally changes the particle’s catalytic chemistry.

Elaborating on this idea, Stair said it is possible to bury the nanoparticles in layers that themselves have catalytic activity such that when the channels are formed, the result is a metal-based catalyst at the bottom of the channel and a different function, such as an acid catalyst, higher up in the channel to carry a second chemical transformation within the same pore. It is also possible to envision creating layers that generate a gradient of composition along the pore that would allow a particular chemical species to diffuse along the surface of the pore in one direction. “You might be able to channel reagents in and out of the catalytic site by using such a gradient,” Stair explained. He noted that these are just possibilities as his group has yet to generate such structures.

Atomic layer deposition can also be used to make cavities by starting with a catalytic oxide and adding a template molecule that will not be covered during the deposition process. The cavities can be

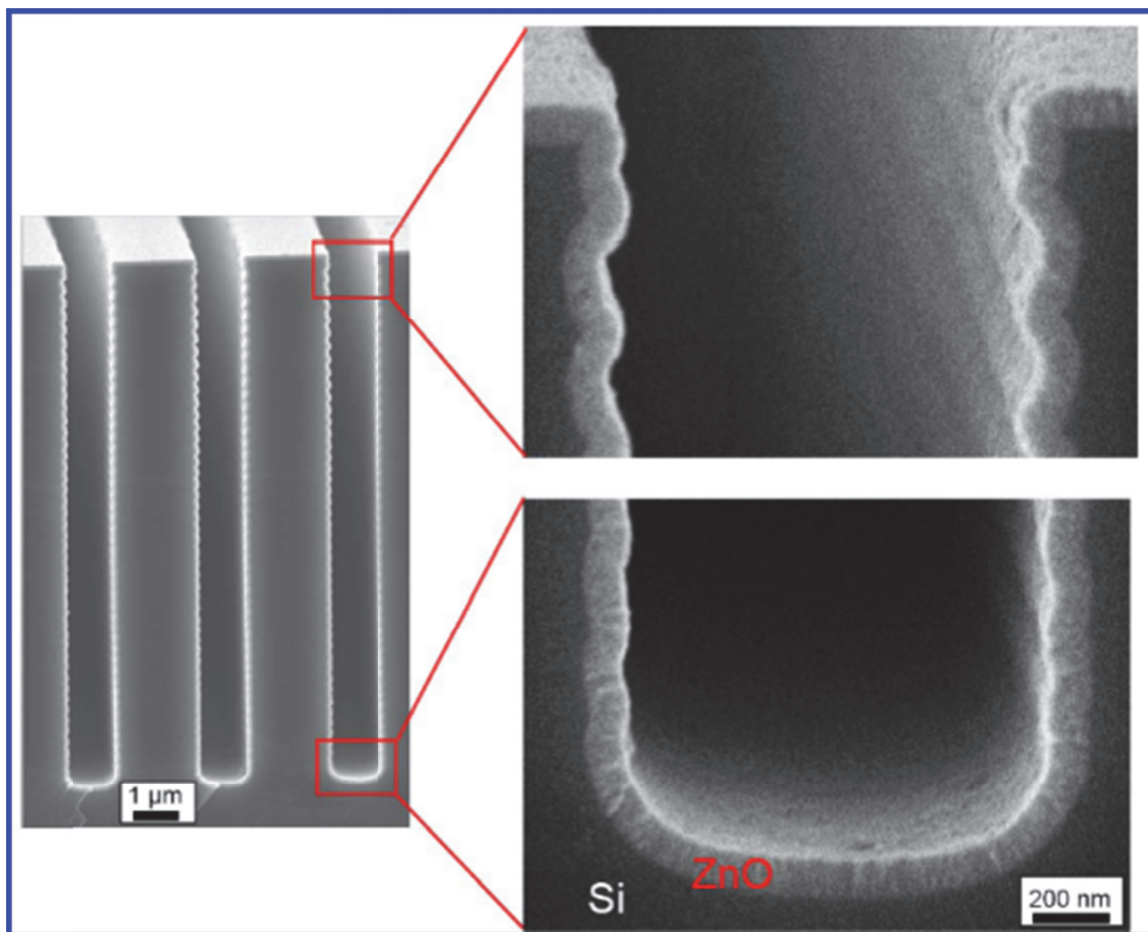


Figure 3-5 Conformal zinc oxide coating of a silicon trench without buildup at the pore entrance. SOURCE: Stair (2014). Reproduced with permission from Jeffrey W. Elam.

constructed so that they only let certain molecules reach the catalyst sitting at the bottom of the pore to create selectivity. His group has demonstrated this approach is capable of discriminating between closely related chemical species in a competitive photoreduction reaction (Figure 3-6) (Canlas et al. 2012). Stair added that it should be possible to create sterically defined cavities that can perform chiral catalysis, though again his group has yet to make such catalysts. In response to a question about these chiral cavities from Donna Blackmond of the Scripps Research Institute, Stair explained that the premise here is that creating a spiral of functional groups in the cavity could create binding sites capable of discriminating between two enantiomers.

Stair concluded his presentation by noting that with the essentially limitless possibilities of atomic layer deposition it would be good to develop design rules to help guide future work. He added that he

would also like chemists to develop more selective synthesis chemistries based on the atomic layer deposition reaction and analytical techniques that can provide more information about the structures and chemistries of the resulting cavities.

DISCUSSION

Neil Henson from the Los Alamos National Laboratory started the discussion by asking the panel for their thoughts on the statement in the Department of Energy report that, when thinking about mesoscale phenomena, the density of atoms is more important than the specific positions of those atoms (BESAC Subcommittee on Mesoscale Science 2012). He added that it was his opinion based on what he heard from the four speakers that this statement is wrong. Stair said that structure does matter and that maybe it would be

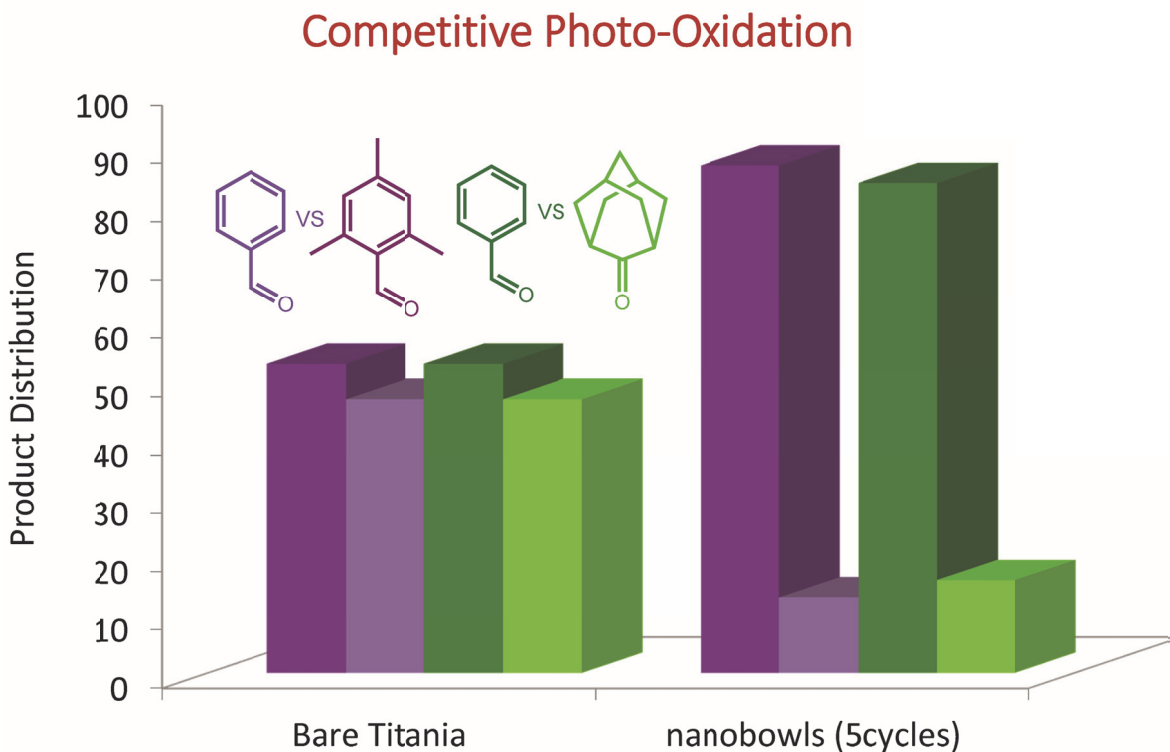


Figure 3-6 Catalysis by nanocavities over titanium dioxide with structural selectivity. SOURCE: Stair (2014). Reproduced with permission from Justin Notestein.

important to consider structure over a longer length scale. Shaw agreed completely that the report was wrong, particularly with regard to enzyme function where the position of specific atoms makes a difference in activity. Session Chair Bruce Garrett said that the Energy Department's report was oriented more toward materials rather than chemistry and chemists worry about atomic position more. Jenks added that the density of sites does matter.

Garrett asked the panel to comment on the need to couple transport to reactivity, which several of the panelists mentioned in their talks. Stair said that moving reagents where you want them to go is important and is something that could be improved in heterogeneous catalysis. Borovik added that he and others working in homogeneous catalysis have not done a good job with this either. He also noted that understanding transport issues includes understanding the timing and reagents coordinated so that the molecules arrive at the active site in the right sequence to facilitate the reaction. Jenks said that her work has shown that transport dynamics is tremendously important and that one challenge is

controlling transport as pores get deeper and reactions get more complex. Lu said that structural biology can provide clues on how nature accomplishes transport with high precision.

Jim de Yoreo of PNNL asked about solvent effects for catalytic reactions done in solution as opposed to the gas phase. Shaw said that solvent plays an important role in determining catalytic activity. Enzymes, for example, have pockets of water in them that have been demonstrated to be important, though the details are not yet well understood. Borovik said there is not yet much evidence that solvent effects can alter the catalytic process, but anecdotal evidence does support that idea. "But I don't think we have much experimental evidence to know exactly how the solvent is manipulating the process," Borovik said. Blackmond noted that the work Jenks described with the aldol condensation supports the idea that solvent effects are important.

Garrett then asked if there is a difference between mesoscale environmental effects around active sites and confinement. Shaw said that there is

a difference: “The environment around the active site is controlling what the chemistry around that active site looks like. Confinement could at its simplest be just making sure the molecules are close enough to react.” Garrett also wondered about the example that was discussed where confinement decreased the reaction rate when he would have expected it to increase because reagents are kept in closer proximity to one another. Jenks explained that confinement can mean that product molecules could also end up being confined in the active site which could block further reactions from occurring.

Yong Wang from PNNL asked Stair to elaborate on the kind of design rules he would like to see developed to help guide the use of atomic layer deposition. Stair replied that one place to start would be to have a better idea of what the structures created using this method actually looked like, such as when particles are being partially covered with some parts left exposed. It would be helpful, he added, to have some notion about what structures would be interesting to make and to have reagents that will react selectively with the edges of a nanoparticle versus its facets or vice versa. Garrett, in a follow-up question, asked if there was any real idea of whether edges or facets are left uncovered when creating these structures and Stair said that infrared spectroscopy does provide a fairly good picture of whether facets or edges are exposed and that the results agree with calculations based on theory.

Turning to the subject of measurement, Garrett asked the panel to comment on the challenges of characterizing active sites in these constructs. Lu said that the characterization of systems that can form crystals is well developed, but the interface between homogeneous and heterogeneous catalysis is where more work is needed. Also, characterizing structures on surfaces in three dimensions is extremely challenging, Lu added. “I think the biggest challenge is doing in situ characterization of heterogeneous catalysis on surfaces,” he said. Shaw added that a major challenge is developing methods for determining if minor species present in catalysts are actually doing most of the catalysis.

De Yoreo commented that with regard to measurement, it is easy to address the problem of what is happening at the atomic scale using computational methods, while from the measurement standpoint it is easy to address what is happening at the ensemble scale. Linking the two scales is where the difficulty lies. It would be useful,

though, to resolve motions at the mesoscale and new measurement techniques are needed to do this. Some optical techniques have sufficient time resolution, he noted, but they do not have the necessary spatial resolution. Pulsed imaging techniques, such as those using free electron lasers, might be able to produce snapshots but only while destroying the system. De Yoreo suggested that low-dose dynamic transmission electron microscopy (TEM) might be able to get both the necessary time and spatial resolution, but that has yet to be demonstrated. Stair added that TEM is good at seeing something solid but poor at seeing voids in structures.

Lu said that his group is exploring the possibility of using the optical and magnetic properties of the metal atoms in their catalysts to see if they can get useful microscopic images of single proteins and observe their dynamics. So far, his collaborator has obtained single protein images and can measure spectroscopic changes that provide information on dynamics. “It’s quite challenging and we cannot see the complete catalytic process,” said Lu. Borovik asked if this method can see the movement of the protein 10 to 20 angstroms from the active site. Lu said when the microscopy and spectroscopy data are combined with calculations and information from crystal structures there is the potential to produce a series of snapshots of protein dynamics.

Gregory Schenter from PNNL stated that it is his opinion that the issue of a system being confined is closely coupled to the nature of the fluctuations that influence the reactions from the mesoscale, be it density fluctuations, vibrational fluctuations, electronic structure fluctuations, or electric field fluctuations, and he asked the panel for their comments. Borovik agreed with Schenter and that all of those properties are governed by the local environment. He noted that changes in the hydrogen-bonding network his group creates change the redox potential of the system and the acid–base properties, all of which are connected to function.

Miguel Garcia-Garibay, of the University of California, Los Angeles and a member of the organizing committee and the Chemical Sciences Roundtable, said that one way of looking at compartmentalization and reduction of dimensionality is to reduce the statistical entropy of the system. Given that nobody on the panel talked about entropy, he asked if the panel had any thoughts on this matter. Borovik said that he thinks entropy is important and has examined how the local

confinement provided by the cavity around a catalyst, particularly the hydrogen-binding cavity in one of the systems he studies, can influence the reaction process and impacts the energy of activation. His group found that there is a tremendous effect that can change the entire mechanism of the reaction and provide unexpected rate enhancements. He acknowledged, though, that he would like to have a better understanding of how to take advantage of the local confinement, which is an entropic effect to make systems function better. Although entropy is well defined, the entropic effect of this local confinement might be more difficult to quantify.

In response to a comment from William Noid of the Pennsylvania State University about the relative

importance of entropy and atomic structure in high-energy versus low-energy reactions, Shaw said that she believes that, even at the mesoscale, atomic structure matters. “Maybe it’s possible that we don’t need to understand atomic structure in detail, but I think it’s critical to have different materials or different catalyst functions regardless of the length scale that you’re looking at,” she said. Jenks added that there just is not enough knowledge yet to know how understanding the atomic structure is impacting the macroscale behavior of a system. As a final comment, Borovik reiterated that structure does matter at the mesoscale and that the reason entropy is not discussed much is because the field does not know how to think about it yet, raising a challenge that needs to be addressed.

4

Membrane Behavior and Microchemical Systems

Key Points

- While a great deal is known about membrane function and overall structure, little is known about the organization of lipids in the membrane that form mesoscale structures and how they modulate the function of membrane-bound proteins.
- Given the ability to create asymmetric vesicles—those with different lipid mixtures in the inner and outer layer—opportunities and challenges exist for research on these systems to gain a better understanding of biomembrane structure and function.
- Digital microfluidics is a member of a family of techniques that have interesting mesoscale characteristics and challenges associated with them but that can also serve as tools to understand questions related to molecular transport.
- Microchemical systems present several opportunities for mesoscale chemical engineering by manipulating local mass and heat transport and chemical phenomena at micrometer to millimeter scales.
- There are many challenges confronting those who are attempting to turn the promise of mesoscale chemical engineering into practical devices.

The workshop's second panel featured three speakers. Erwin London, Professor of Biochemistry and Cell Biology and Professor of Chemistry at Stony Brook University, spoke about the collective mesoscale behavior of the lipid bilayer in biological membranes as studied using a new experimental model that more closely represents the asymmetric composition of the cell membrane. Aaron Wheeler, the Canada Research Chair of Bioanalytical Chemistry at the University of Toronto, described his group's work developing digital microfluidic systems that can manipulate fluid droplets in, around, and through solid materials. Benjamin Wilhite, Associate Professor of Chemical Engineering at Texas A&M University, reviewed recent advances in both microreactor development and membrane research, with an emphasis on energy-related applications. A panel discussion moderated by session chair Vernon Anderson, Program Director in the Division of Pharmacology, Physiology, and Biological

Chemistry at the National Institute of General Medical Sciences and a member of the workshop organizing committee, followed the three presentations.

ASYMMETRIC LIPID VESICLES FORMATION AND PROPERTIES

The lipid bilayer that forms the membrane that surrounds all cells is a mesoscale structure comprising individual lipid molecules, each with a polar and nonpolar region, that self-assemble and self-organize with the polar portions in contact with water, explained Erwin London. Proteins, he added, are embedded within the lipid bilayer. While a great deal is known about membrane function and overall structure, little is known about the organization of lipids in the membrane that form mesoscale structures and how they modulate the function of membrane-bound proteins. It is also still a mystery

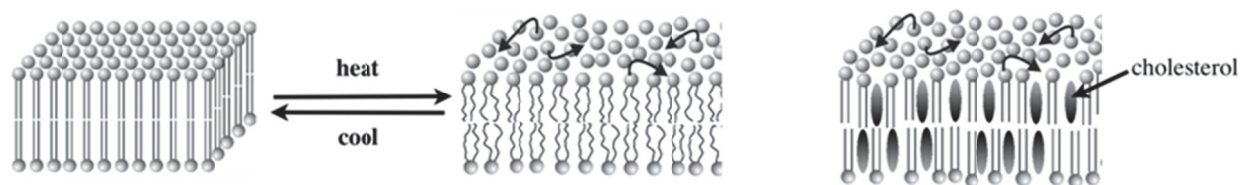


Figure 4-1 Lipid bilayers can exist in different physical states. SOURCE: London (2014). Reproduced with permission from Erwin London.

as to why natural lipids have so many different chemical structures, he added.

Lipid bilayers can exist in different physical states, London explained (Figure 4-1). In the solid-like “gel” state, the acyl chains are packed tightly in a highly ordered, almost crystalline, arrangement. Lateral diffusion within the membrane is slow in this state. At higher temperatures, a transition occurs, characterized by a melting temperature that depends on the type of lipids in the bilayer. This transition leads to a liquid-like disordered state, called the Ld state, where lateral motions are fast and in which the acyl chains have kinks in them. A third physical state, which occurs only when cholesterol is present in the membrane and is known as the Lo state, is both liquid and ordered. London characterized this as an interesting state of matter that shares properties with both the solid-like gel state and the liquid-like disordered state. In this third physical state lateral motions are fast but the acyl chains are extended, straight, and tightly packed against themselves and the cholesterol molecules.

Saturated lipids—those without double bonds—can pack together more easily and bilayers formed from saturated lipids have high melting temperatures and tend to form the solid, gel-like state at physiological temperatures. Unsaturated lipids have *cis* double bonds that act as permanent kinks that tend to inhibit lipid–lipid interactions. As a result, they pack together more loosely and have low melting temperatures. London said that in the 1960s and 1970s, when researchers came to appreciate the ways different lipids pack together, the question arose as to how the mixtures of lipids in a real cell membrane would behave, if phase separation occurred within membranes, and if the interesting biology that occurs in a membrane would take place in regions where coexisting domains occur.

It turns out that in model membrane systems coexisting domains are observed and studied (Figure

4-2), but in real cell membranes these domains are vanishingly small. These different domains in model and real systems are important because each membrane protein has a particular affinity for these different domains, with the result that mesoscale lipid structure has a direct effect on protein–protein interactions. For example, proteins with similar affinities may interact in the same domain or be segregated from each other if their affinities are different. A protein’s conformation, and therefore its activity, can also be affected by the domain state. London noted that as these domains form or break up, or when smaller domains coalesce into larger domains, it can turn on or off biological processes driven by membrane-bound proteins. Ordered lipid domains, also known as lipid rafts, have been proposed to play a role in signal transduction, sorting of proteins to different cell membranes, infection by bacteria and viruses, and other membrane processes. “So the question we’re faced with is how can we better understand the formation of these domains, what are the principles that are behind their formation, and what are the principles that control their size,” said London. He added that this last question is a contentious subject among researchers who are trying to understand why they are small in many systems and larger in many of the model systems.

One of the issues that the field faces is that many of the model membranes, which are made by mixing lipids in water, are symmetric, while real cell membranes are asymmetric—they have a different lipid composition in the inner and outer layers (Figure 4-3). In mammalian membranes, the outer layer, or leaflet, is rich in sphingolipids and the inner leaflet is rich in aminolipids such as phosphatidylethanolamine and phosphatidylserine. Being able to reproduce this type of distribution is important because the sphingolipids and cholesterol

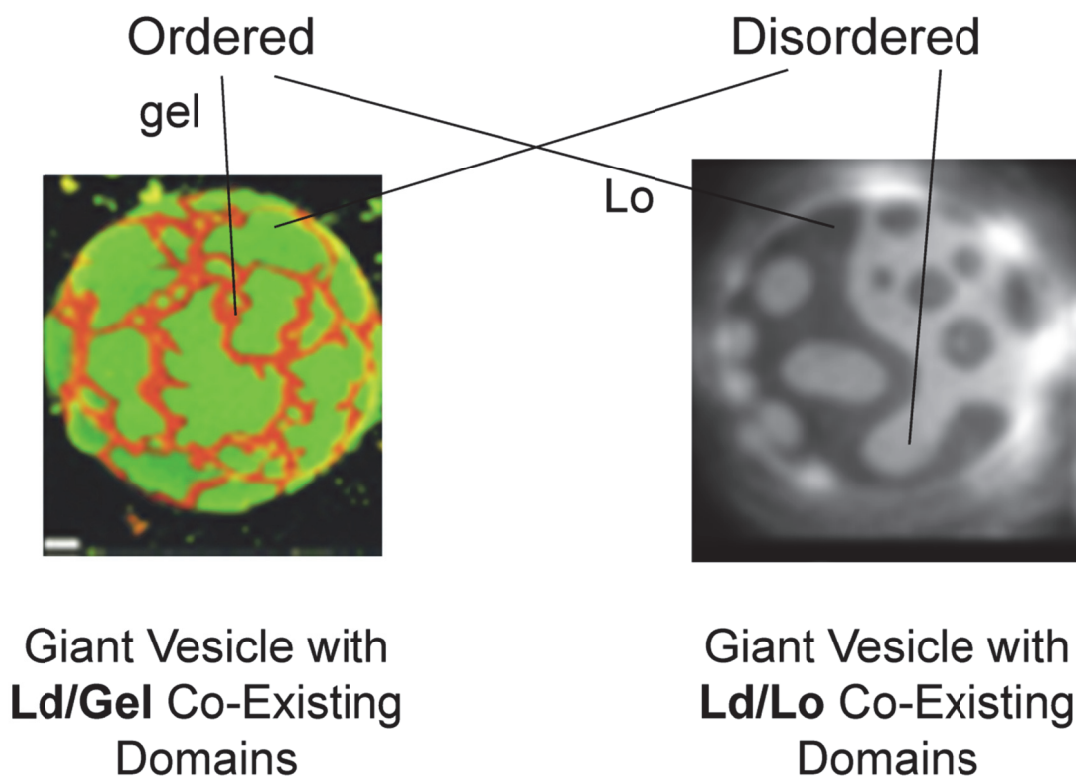


Figure 4-2 Membranes with mixtures of lipids can have coexisting domains with different compositions and different states. SOURCE: Feigenson and Buboltz (2001, right) and Veatch and Keller (2003, left). Reprinted with permission from *Biophysical Journal*.

create ordered domains and the sphingolipids are restricted largely to the outer leaflet. At the same time, membrane reorganization in the outer leaflet is somehow transmitted to the inner leaflet and the proteins in the inner leaflet respond to clustering in the outer leaflet, London explained.

To study the effects of asymmetry, London and his collaborators create model asymmetric membranes using a family of molecules known as cyclodextrins, which are cyclic glucose polymers containing six to eight glucose units. Cyclodextrins are hydrophilic but with a hydrophobic cavity that can be used to bind lipids from a donor vesicle and deposit them in the outer leaflet of an acceptor vesicle of different lipid composition. The result is an asymmetric vesicle that can be separated by centrifugation. London's group recently improved this method so that they can create asymmetric vesicles with controlled amounts of cholesterol in the inner and outer leaflets that come close to mimicking the plasma membrane from cells.

Using this method, London's group has been

studying coupling between the outer and inner leaflets. They have created vesicles with one of two different sphingolipids in the outer leaflet: milk sphingomyelin, which has a long hydrocarbon chain that extends into the inner leaflet, and egg sphingomyelin, which has shorter hydrocarbon chains and does not extend into the inner leaflet. The outer leaflet of these vesicles also contains cholesterol and an unsaturated lipid—1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC)—that forms disordered domains, while the inner leaflet only contains DOPC. The outer leaflet has a mixture of ordered and disordered domains, while the inner leaflet by itself would only form the disordered state.

To probe the two leaflets, London's group used fluorescent dyes that locate either in Ld or Lo domains. Images of the asymmetric vesicles show that the inner leaflet, which has no sphingolipid, has responded to the outer leaflet and formed ordered domains that correspond to where the outer leaflet has formed ordered domains, demonstrating that there is strong interleaflet coupling. In vesicles with

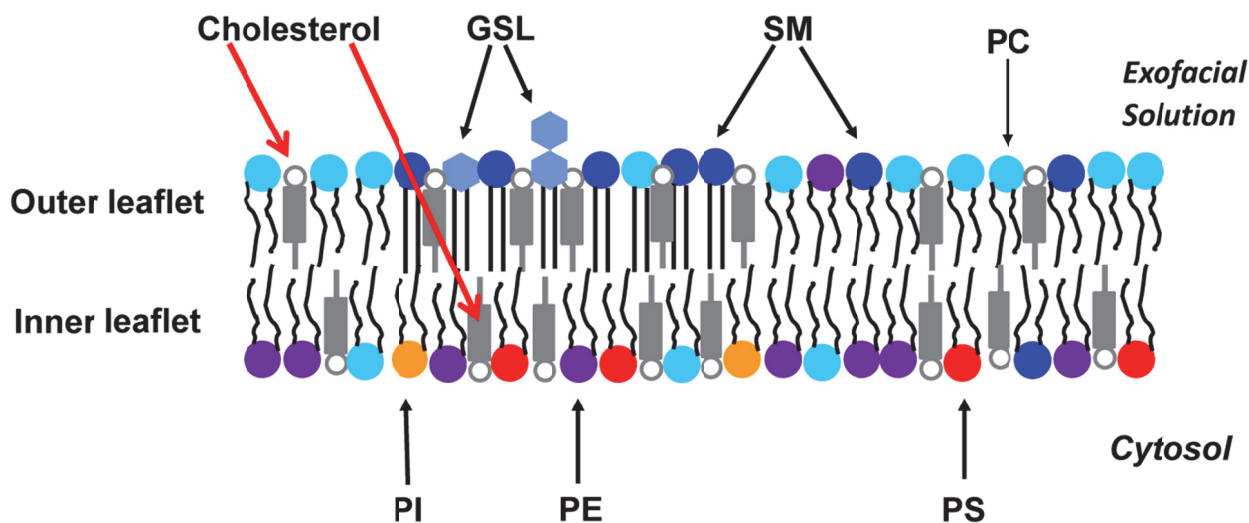


Figure 4-3 Lipid bilayers in cells are asymmetric. In mammalian cells, the major lipids in the outer leaflet are sphingomyelin (SM), glycosphingolipid (GSL), phosphatidylcholine (PC), cholesterol, and some phosphatidylethanolamine (PE). The inner leaflet's major lipids are PE, phosphatidylserine (PS), phosphatidylinositol (PI), cholesterol, and some PC. SOURCE: London (2014). Reproduced with permission from Erwin London.

milk sphingomyelin, the inner leaflet properties match those of the outer leaflet, which London said suggests that the ordered domains forming in the inner leaflet are more similar to those in the outer leaflet. In the vesicles with egg sphingomyelin, the properties of ordered domains in the inner leaflet are different than those in the outer leaflet, suggesting that with longer hydrocarbon chains that can interdigitate with the inner leaflet, there is much stronger coupling. London noted that the results of these experiments also suggest that the ordered domains of the inner leaflet are enriched in cholesterol.

London concluded his presentation by listing some of the opportunities and challenges that exist for research in this area given the availability of asymmetric vesicles that can be used to gain a better understanding of biomembrane structure and function:

- What lipids reduce or resist this interlipid coupling *in vivo*?
- What controls where membrane proteins go and how do they partition between these domains in realistic asymmetric membranes?
- What controls the affinity of membrane proteins for different membrane domains?

- How does domain structure impact membrane protein interactions, conformation, and function?
- Can the principles identified in studying asymmetric membranes be extended to enable the preparation of vesicles or materials other than lipids in an asymmetric layer-by-layer fashion?
- What are the principles that control domain size, which can vary from large domains to nanoscale domains?
- Can we create domain patterned materials with controlled mesoscale heterogeneity in terms of domain size and chemical composition?
- Can we use asymmetric vesicles for improved encapsulation applications such as drug delivery?

DIGITAL MICROFLUIDICS: NOT JUST FOR LIQUIDS ANYMORE

As introduced by Aaron Wheeler, digital microfluidics is a member of a family of techniques that have interesting mesoscale characteristics and challenges associated with them but that can also serve as tools to understand some of the questions that this workshop is addressing. This is particularly true for questions related to molecular transport. He

noted that though the focus is on one set of techniques in this talk, there are quite a few different tools to study transport phenomena that use enclosed microchannels of a wide variety of interesting geometries.

As an example of a digital microfluidic device, Wheeler showed a schematic of a system consisting of a droplet trapped between an insulator sitting above an array of electrodes and a counter electrode. The insulator is a critical design element that enables fine control over the electrodes. With this system Wheeler's group can charge a given electrode and "drag" the droplet over to the charged region of the device. He noted that his group has developed computational models to describe this type of system, and they understand that the forces being applied to the droplet are on the order of tens of micronewtons. Devices such as these can be used to carry out chemical reactions in a precise and controlled manner thanks to the ability to deliver droplets to specific places on the microfluidic device. In the example he showed, the droplets are on the order of tens of nanoliters in volume and the reaction space was a few hundred microns. Wheeler explained that this type of device enables programmed chemistry to take place with controlled, small quantities of reagent delivered with exquisite precision in terms of sequence and location.

Wheeler said that while much of the work in digital microfluidics has been done using liquids or even cells, he began asking himself a number of years ago what would happen to this type of system if he used it with solids and for making three-dimensional heterogeneous systems. To do that, his group had to answer a number of questions, such as what happens to the driving and resistive forces in a three-dimensional heterogeneous system and how can they be used and under what conditions to move solids through such a device. In working to answer these questions, his group has been using digital microfluidics to move hydrogels, polymer plugs, and magnetic particles. "Each of these systems has its own unique challenges and questions associated with it," Wheeler explained as he described the device that his group created to work with hydrogels.

The first question his group explored was what would happen to the hydrogel in a digital microfluidic device as reagents were moved through the device. In one set of experiments, members of his research group showed that they could move

water droplets through a hydrogel without disturbing the hydrogel. "We weren't sure what to expect, and in fact we thought that perhaps the presence of the hydrogel would present some significant impediments to moving the droplet, but that turns out not to be the case at all," he explained. Given that result, his group then followed the diffusion of a tracer either into or out of the gel in a passive state, where the droplet is sitting in place, to calculate a diffusion coefficient, and it matched what they expected from the literature. However, moving the droplet repeatedly through the hydrogel triggers an active transport process that is three times faster than passive diffusion. Moreover, this active transport rate changes depending on the velocity of droplet movement, the size of the gel, and the size of the droplet. Wheeler noted that this type of device could prove useful for studying transport under varying conditions.

One application of digital microfluidics with solids that his group has been exploring involves the notion of confining catalytic systems to take advantage of different rates or types of chemistry. One such project involves confining proteolytic enzymes to hydrogels to create a device for use in proteomic analysis, and it started with developing an epoxide-based chemical reaction to tether these enzymes throughout the hydrogel. Confocal microscopy showed that this immobilization process did distribute enzyme throughout the three-dimensional structure of the hydrogel. With this chemistry in hand, Wheeler's group then created microreactors with different enzymes connected to microfluidic channels through which they could deliver samples for proteomic analysis and then collect the enzymatic digest for analysis. The eventual goal of this work is to create a device containing a large number of different proteases to produce multiple protein digests that will provide a high level of amino acid sequence coverage to enable high-throughput proteomics. Currently, this project is at the stage where Wheeler's group has started collaborating with a mass spectroscopy company that wants to use these devices in conjunction with automated mass spectroscopic analysis of the protein digests.

Another project from his laboratory aims to develop hydrogel-containing microfluidic devices for growing mammalian cells. Before describing this device, he reminded the workshop that mammalian cells grown in a three-dimensional conventional gel

develop with a different phenotype compared to when they are grown in two dimensions. Though the three-dimensional growth results in a phenotype that is closer to *in vivo* phenotypes, many researchers choose not to grow three-dimensional structures because the fragility of the conventional gels makes the process arduous. Building on the knowledge within the group, they first refined the process for creating gels for this device, for example, group developing gel crosslinking methods and ways of controlling surface energies that enable them to form a wide variety of gel shapes. With these techniques in hand, his team has built microfluidic devices with arrays of gels that are each addressable individually.

When cells are placed in and evenly distributed throughout these devices, they grow in a three-dimensional arrangement with the same phenotype expected from the literature for cells grown using conventional gel systems. However, when comparing the robustness of the conventionally grown three-dimensional cellular spheroids with those grown in the hydrogels, a significant and surprising difference was identified. The resulting cell spheroids grown within the microgels are far more robust than those grown in conventional gels. This makes it possible to deliver reagents to these cells in ways that were not possible with those grown in conventional gels. One potential application for these devices that his group is exploring with collaborators is to produce an array-based three-dimensional tissue culture system for medical screening applications. He noted that “this is an interesting system where there may be some opportunity for mesoscale science . . . and again I’ll point out that the surface has just been scratched.” He concluded his talk by noting that “these types of tools may end up being useful for studying some of the things that this workshop is interested in exploring.” He also noted in answering a question from a workshop participant that these microfluidic devices work in air, oil, and other environments and that once the device is set up, operations are carried out electronically.

ENGINEERING AT THE MESOSCALE: MICROREACTORS AND MEMBRANES

Benjamin Wilhite began the last presentation in this session with the comment that, from his perspective as a chemical engineer, he has a different concept of mesoscale. “To the chemical

engineer, microchemical systems present several opportunities for mesoscale chemical engineering by manipulating local mass and heat transport and chemical phenomena at micrometer to millimeter scales,” he said. From the perspective of a materials scientist, he added, membrane materials present several opportunities for mesoscale chemistry by manipulating the chemical structure of membranes at the mesoscale in a way that enables new kinds of separations to occur. He also noted that chemical engineers usually work at such large scales of reactors, reactants, and catalysts that the local manipulation of chemistry at the mesoscale is not something that has been considered very often. That awareness did start to change about 20 years ago, he added, when chemical microreactors were first developed. The ability to build miniaturized chemical reactors created a situation where chemical engineers had to start paying attention to various physical processes such as fluid dynamics, laminar flow, and conductive heat transfer that become dominant in these smaller systems. “What that allowed us to do to solve those problems was to manipulate, by way of the structure of the device, the local transport rates in such ways as to manipulate indirectly the chemistry of the reaction,” Wilhite explained. “Finally, we can live up to what all engineers aspire to be, which are master manipulators,” he added.

The first decade of microreactors saw a wealth of firsts, and all could be summed up with catchphrase, “on a chip”: the first gas–solid catalytic reactor on a chip (Srinivasan et al. 1997), the first gas–liquid mixer on a chip, the first gas–liquid disengaging unit on a chip, the first gas separation unit on a chip, the first fully integrated trickle-bed microreactor on a chip (Wilhite et al. 2008), and so on. This decade established the basic chemical engineering operations that were possible on a chip and how to perform them, as well as what did and did not work. Since the 2000s, the emphasis has shifted from the novel to the practical, and research has proceeded down one of two paths: either creating lab-on-a-chip devices for chemical analysis, such as the work that Wheeler described, or building devices that perform chemical processing and replace the large-scale reactors and processing equipment that are traditionally in the realm of chemical engineering.

Researchers going down this second path, including Wilhite, soon realized that chemical

engineering on individual chips was never going to replace chemical refineries because of the scale of industrial chemical production per month. Research then began to look at ways of coupling different devices, much like Lego bricks, into modular reforming processes that operate at the mesoscale rather than the microscale and that can produce enough of a chemical to be useful on an industrial scale. Wilhite described a few of these modular chemical plants. One such device can perform laboratory-scale organic systems with modules that can be assembled and disassembled quickly to make different products as needed. “This has great potential in the chemical industry where nowadays the market is volatile in terms of what chemicals need to be made for society,” said Wilhite. “The idea of having a chemical plant that you design and build and make money off of for 20 years without the feedstock or the desired product changing, those days are gone. Now we need modular chemical plants that can be quickly field stripped, reassembled, and retrofitted.”

Another promising area of research and development activity aims at creating a personal “turnkey” chemical plant capable of converting natural gas to hydrogen as needed at home or at a local fueling station, which could enable an immediate transition to hydrogen fuels for automobile use by leveraging the existing natural gas infrastructure. Similarly, researchers are working on a modular system that would convert the natural gas now being flared at the wellhead of what are known as stranded oil wells—those too far from a natural gas pipeline for it to be economical to run additional line to capture the gas associated with shale oil—into synthesis gas using Fischer-Tropsch chemistry. The resulting liquid fuel could then be more readily and economically captured and transported. Wilhite noted that Qatar is already doing this on a large scale to turn natural gas into diesel fuel.

There are many challenges confronting those who are attempting to turn the promise of mesoscale chemical engineering into practical devices. One of the first challenges researchers addressed was the difficulty in getting liquids to mix in these mesoscale devices. Mixing at the bulk scale is dominated by turbulent motion of free liquid when agitated, and mixing is controlled either by stirring or not stirring a mixture of liquids. At the mesoscale, mixing is dominated by the diffusion of momentum

to such an extent that when two fluid streams are brought together in microchannels they do not mix at all on any kind of useful time frame. However, forming channels with submillimeter ridges and fins on the walls disrupts laminar flow and exquisite control of mixing (Liu et al. 2013, Stroock and Whitesides 2003).

Another challenge that Wilhite and his group addressed was to develop the means of manipulating gas-phase chemistry at membrane surfaces. One of the first examples of accomplishing this was when Wilhite’s group created a device that used a membrane to remove contaminants from a natural gas stream that would rapidly corrode a second, more expensive catalytic palladium-containing membrane (Figure 4-4) (Kim et al. 2010, Moreno, Damodharan, and Wilhite 2010). Not only did this system eliminate corrosion of the catalytic membrane, but it also increased the rate of permeation through that second membrane by about 30 percent, Wilhite explained.

Heat transfer in microreactors has also proven to be a challenge, particularly with regard to preventing heat loss in a device with hundreds of tiny channels passing through a block of material. The key to addressing this problem was to develop manufacturing techniques that would isolate each microchannel and remove the common solid substrate (Besser 2011), Wilhite said. “What that has allowed us to do is to focus the patterning of a catalyst within a heat-exchanging microreactor so that we can control the local mesoscale rates of heat transfer, rates of heat production, and rates of heat utilization by a chemical reaction, in order to get significant improvement in the amount of reaction we can push through one small volume,” said Wilhite.

In the final portion of his presentation, Wilhite discussed some of the challenges of using membranes in gas separations. Membrane-based gas separation has become the standard industrial process since the first gas-separation membranes were commercialized in the 1980s as a less-expensive and lower-energy-use alternative to distillation. The main challenge is overcoming the inherent limit to the efficiency of these membranes, a property revealed by what is known as a Robeson map that plots purity of the separation versus the permeability of the membrane to that gas. To overcome the tradeoff between purity and rate of separation, researchers have been adding structured

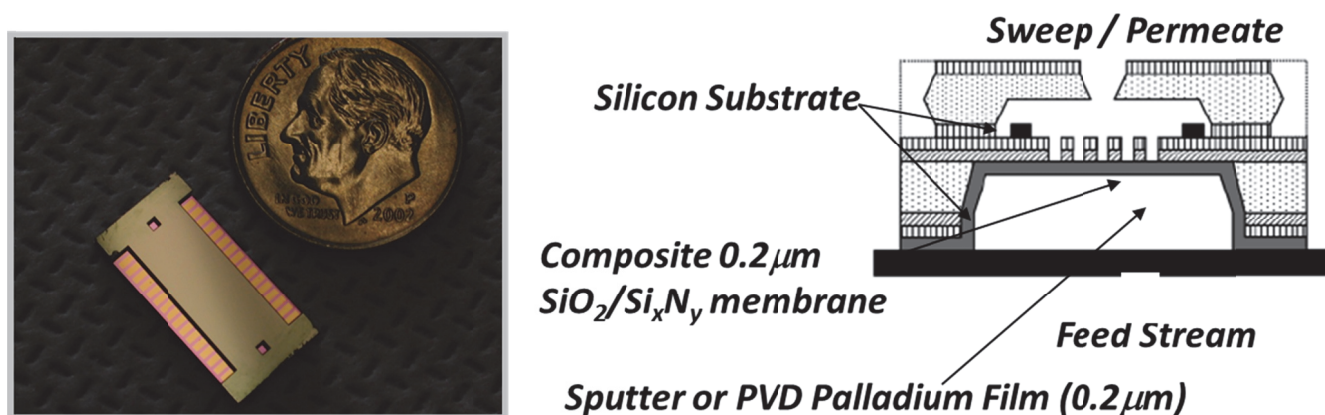


Figure 4-4 Manipulation of gas-phase chemistry at membrane surfaces enabled the first demonstration of portable high-purity hydrogen generation from methanol. SOURCE: Wilhite, Schmidt, and Jensen (2004). Reprinted with permission from *Industrial & Engineering Chemistry*.

inorganic materials such as zeolites and metal oxide frameworks to the polymer membranes (Kim, D., et al. 2014).

Wilhite and his collaborators have developed a layer-by-layer deposition technique that allows them to control the chemistry of deposition by building a film one layer at a time, alternating positively charged and negatively charged polymers. He noted that this process is not as precise as atomic layer deposition, but that it works well for creating membranes. The example he described used polyethyleneimine and polyacrylic acid and the membrane grows by allowing the polymers to adhere electrostatically to the surface. By adjusting the pH of the dip solutions, it is possible to tune the level of charge in the resulting membrane film as well as the crosslinking between the polymer layers. The result is a thick, robust membrane with well-defined pores that will separate hydrogen gas from carbon monoxide, carbon dioxide, oxygen, and nitrogen with performance characteristics that exceed the upper bounds predicted by the Robeson map by an order of magnitude. “We are able to have a higher separation than anything else in the literature because of using mesoscale chemistry to manipulate the deposition conditions used to make the membrane,” Wilhite said in closing.

DISCUSSION

In response to a question from Yi Lu about how to incorporate different enzymes into a membrane

with known spatial definition, London replied that this is a difficult task in an asymmetric lipid bilayer. “Controlling protein orientation is still a mystery,” London explained, adding that there is no solution to this problem at present. One possibility would be to manipulate the shape of the proteins given that orientation in the lipid bilayer is controlled to some degree by protein shape. Wheeler added that Petra Dittrich and her colleagues at ETH Zurich are exploring some innovative approaches to achieve protein orientation.

Vernon Anderson from the National Institutes of Health asked about the lateral stability of proteins in the artificial bilayers that London creates. London replied that proteins move laterally quickly, on the order of many times per second, but that the rate of flipping from one side of the membrane to the other is very slow and reaches hours or days.

Jim De Yoreo asked Wilhite about work that might have been done to make synthetic analogs of sequence-defined polymers that can operate in drug environments. Wilhite replied that he did not know of any work in this area but that the membranes he and his collaborators make will separate gases under drug conditions and that the membranes have good stability when used in dry conditions. Even in the absence of water, these membranes maintain their structure and their separation properties.

London then fielded a question from Wendy Shaw, who asked if he had any details on what was driving the phase separation seen with different membranes and if microfluidics could help gain

insights into that process. He replied that the driving force of the phase separation is simply the tendency of one lipid to prefer a solid phase and the other to prefer a liquid phase at some intermediate temperature at which these two states will exist. Van der Waals forces drive the lipids to pack tightly in the solid state, and this is amplified by the presence of cholesterol, which also likes to pack tightly in the presence of water.

Andrew Borovik then asked Wheeler if his devices could be used to deliver electrons as well as reagents in a spatially and temporally defined manner. Wheeler's answer was yes, and that his group has in fact done that kind of chemistry for electroanalysis and that it should be possible for electrocatalysis, too. Borovik commented that this capability could be useful not just for doing chemistry but also to better understand the mechanisms of electrocatalytic reactions.

Bruce Garrett asked Wilhite if the problem of heat transfer was now solved given the results he obtained, and Wilhite said that it was not. Heat, he said, is still lost to such an extent that it cannot yet be recaptured to drive other reactions on the chip. Researchers are working on this problem but it has yet to be solved. Wilhite also responded to a question from Yong Wang of the Pacific Northwest National Laboratory about whether industry is working to scale these devices to reach the necessary capacity for its purposes by noting that industry is still concerned about how to monitor and control

these devices and about the robustness of the systems. Because the channels are packed tightly into these devices, it is difficult presently to also fit in the amount of instrumentation needed to monitor chemical processes to the extent that they are monitored now in large petrochemical and fine chemical manufacturing facilities.

Lu then asked Wilhite if the devices can operate under pressure, and Wilhite said that this, too, is a challenge because the devices as they are currently made are welded laminates that can fail under pressure. Going to a tube-within-a-tube design for creating channels reduces the number of welds needed to hold the device together. London asked if three-dimensional printing can overcome this problem by eliminating the need to weld layers together, and Wilhite said this was an interesting possibility, one that he wants to explore. Cristian Contescu from Oak Ridge National Laboratory invited Wilhite to use the three-dimensional printers in the manufacturing demonstration facility there and then asked Wilhite about the cost of these devices and whether it would ever be practical to use these devices on a large scale. Wilhite acknowledged that cost was a big issue and that there are groups working to reduce the cost of manufacturing the devices by using stamping and rolling processes instead of micromachining in order to scale and automate production of the devices. He noted, too, that tube-in-tube systems should also be less expensive to make.

5

Biom mineralization and Geochemical Processes

Key Points

- An important research challenge is understanding the interaction between organic molecules and nanoparticle reactivity and how those interactions differ in the laboratory versus in the natural world.
- Increased understanding of the role of proteins in nucleation and biom mineralization processes could aid in the development of tools to direct the assembly of nanoparticles.
- The growth of biom minerals provides a rich area for research at the mesoscale, and new analytical tools are changing fundamental understanding about the growth behavior of even common, well-studied materials. Additional theoretical, computational, and in situ experimental tools that can visualize molecular events could be useful in furthering this work.
- A comprehensive model that quantitatively describes the diverse pathways of mineralization is lacking, and one essential challenge for developing that model is to describe the ensemble behavior of coupled solvent–solid systems.
- Models for crystal growth that work well for describing the growth of bulk soluble crystals at low supersaturation do not work as well when applied to crystals that grow via particle-based processes.
- Bulk analysis of paleo proxies—geochemical signatures that are recorded in an archive that has been preserved and that can be measured—have proven important for understanding large-scale changes in the Earth’s geochemistry, but these proxies display unusual and unpredicted properties when examined at the mesoscale, in part as a result of large compositional heterogeneity in the materials.

The third session of the workshop explored mesoscale phenomena involved in biom mineralization and geochemical processes. Pupa Gilbert, Professor of Physics at the University of Wisconsin-Madison, described two kinds of biom mineral structures and discussed how knowledge of those structures proved indispensable for understanding the mesoscale biom mineral formation pathways. John Spencer Evans, Professor at the Center for Skeletal Sciences at New York University, talked about the families of extracellular matrix proteins, how they guide biom mineralization processes, and how that leads to interesting material features. Jim De Yoreo, Chief Scientist for Materials Synthesis and Simulation across Scales at the Pacific

Northwest National Laboratory (PNNL), spoke about the ongoing advances in the temporal and spatial resolution of in situ imaging methods and how those methods are being applied to understand the emergence of order in protein matrices and the controlling factors in matrix-directed mineral formation. Alex Gagnon, Assistant Professor in the School of Oceanography at the University of Washington, discussed work from his laboratory that aims to explain small-scale heterogeneity in the geologic record and to understand the implications regarding efforts to explore how the carbon cycle has changed over the course of the Earth’s history. In the final talk, Andrew Madden, Associate Professor in the School of Geology and Geophysics

at the University of Oklahoma, presented a number of important directions for future research for mesoscale chemistry in the Earth and planetary sciences. An open discussion followed, moderated by Patricia Thiel, the John D. Corbett Professor Chemistry and Distinguished Professor of Chemistry and of Materials Science & Engineering at Iowa State University, co-chair of the workshop organizing committee, and a member of the Chemical Sciences Roundtable (CSR).

FORMATION PATHWAYS IN BIOMINERALS REVEALED BY THEIR STRUCTURE

Pupa Gilbert started her presentation by showing a micrograph of single crystals of calcite that form the spines of sea urchins. These crystals have rounded structures that defy the usual understanding of crystals as structures with flat faces and sharp edges. She pointed out that these crystals co-orient with each other within 0.025 degrees. Using spectroscopic methods to explore the electronic structure of the crystals reveals that they have a sequence of mineral faces that develop as the crystals grow from amorphous precursors (Politi et al. 2008, Radha et al. 2010). During the animal's lifetime, a sea urchin's spines can grow as large as 30 cm long. Each spine comprises a single crystal that has holes on the order of 10 microns, and this crystal grows to length in about 10 months, whereas with current techniques in the laboratory it would take about 82 years if it could be grown that large at all, said Gilbert. Understanding the mechanisms that support such growth and reproducing it in the laboratory could have implications for the electronics and solar panel industries and for carbon sequestration.

The crystal growth mechanism that is most familiar is one where crystals grow from ions attaching one at a time to a nucleus, which grows into a bulk crystal with facets and edges. Urchin calcite crystals take a different path, one of particle-by-particle growth. The process starts with amorphous nanoparticles that can be as large as 50 to 100 nm. These nanoparticles aggregate into a three-dimensional but amorphous solid that then undergoes crystallization. This random-walk process spreads in three dimensions as the crystal grows. A question that emerges from this is whether the two methods produce similar crystals, and if not, what are the differences in the crystals that result from the

different growth patterns? Those are questions that Gilbert and her collaborators have been exploring.

No differences are apparent using x-ray diffraction techniques, but other methods do highlight some variations. Gilbert showed a calcium distribution map of the triradiate sea urchin spicules imaged using x-ray absorption near-edge spectroscopy and photoelectron emission spectromicroscopy (Figure 5-1). Photoelectron emission spectromicroscopy reveals the different states of calcium carbonate present in the spicule, that is, whether it is crystalline calcite, anhydrous amorphous calcium carbonate, or hydrated amorphous calcium carbonate. These images reveal that freshly deposited material at the outer edge of the growing spicule comprises a mixture of amorphous and hydrous amorphous calcium carbonate, while the center is mostly crystalline calcite. Crystallinity propagates from the center to the outer rim of the spicule (Gong et al. 2012) in a series of phase transitions that occur as the urchin grows from about 30 hours after fertilization occurred to create the urchin embryo. By day three, the spicules are completely crystalline, which means the phase transitions in the animal are different when this process occurs in the laboratory. "So question number one is how do biological systems control these phase transitions?" Gilbert said. Energetically, this phase transition is exothermic, with calcite being the lowest energy state.

These amorphous precursors, said Gilbert, explain how the resulting calcite crystals can have rounded shapes and grow much larger and 100 times faster than in the laboratory. "The morphology is determined before crystallization, at the time of aggregation," she explained.

Gilbert then discussed the production of nacre as a second example of biomineralization at the mesoscale. Nacre, the lamellar material out of which many mollusk shells are made, is iridescent because it is layered, and the thickness of the layers is about 0.5 microns. Nacre, she said, is among the most well-studied biominerals, with over 1,000 papers published on its structure and properties, yet when Gilbert and her team used polarization-dependent imaging contrast to examine the aragonite crystals in nacre, they found that the published structures were wrong (Olson et al. 2013). Instead of the C axis of the aragonite crystals in nacre being oriented parallel to one another and perpendicular to the nacre layers, their orientation differs depending on the species

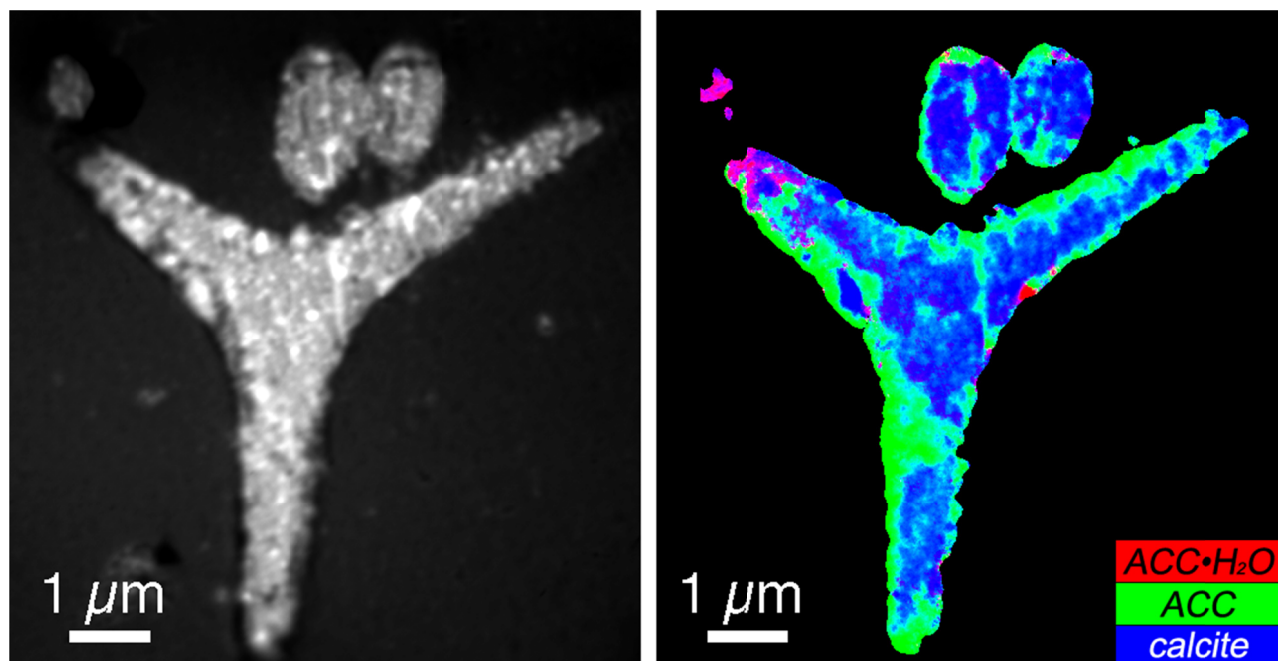


Figure 5-1 Sea urchin spicules imaged using x-ray absorption near-edge spectroscopy (left) and photoelectron emission spectromicroscopy (right). SOURCE: Gong et al. (2012). Reprinted with permission from *Proceedings of the National Academy of Sciences of the United States of America*.

(Figure 5-2). In one sample, for example, the crystals were staggered diagonally, in another they formed sheets, and in a third they stacked in columns.

Of the many features of nacre that Gilbert's lab has since elucidated, one is that the aragonite crystals grow epitaxially and a second is that these crystals order gradually by a physical, not biological, process. During epitaxial growth, the crystals are almost always connected to one another, and the ones that are not oriented correctly to the other crystals appear to stop growing and die out, while oriented crystals grow faster and fill space faster. "This is a perfectly abiotic mechanism," said Gilbert. "It's a competition for space model and there's beautiful math that goes along with that. [It] is precisely the same math that describes the evolution of biological organisms in computation for space, food, and other resources."

Gilbert's team also found that the initial layers of nacre are more disordered than in the final state, and that these initial crystals die out as the co-orientation process takes over. The speed with which co-orientation occurs is species dependent, she added. In some species, co-orientation begins within 10 to 20 microns of the initial growth, while in

others it can take 100 to 200 microns for order to develop. She concluded her remarks by noting that there are 3 different polymorphs of calcium carbonate, with the ones she discussed being just two of them. "Each and every one of them can teach us something very interesting technologically," she said.

NUCLEATION EVENTS, MINERAL ASSEMBLY, AND CRYSTAL MODIFICATIONS PERFORMED BY DISORDERED PROTEINS

In biological systems, cells are the architects of the mesoscale, said John Evans. Cells have the genomic blueprints that dictate how the process is going to go from initiation to its conclusion. He also noted that there are more than 60 different biominerals on Earth produced by 55 phyla in all five kingdoms. Of these, 80 percent are crystalline materials and 20 percent are amorphous, and they are made from 22 different elements. It is likely, he added, that there are common mechanisms for forming these biominerals (Veis 1990), though this hypothesis has yet to be proven.

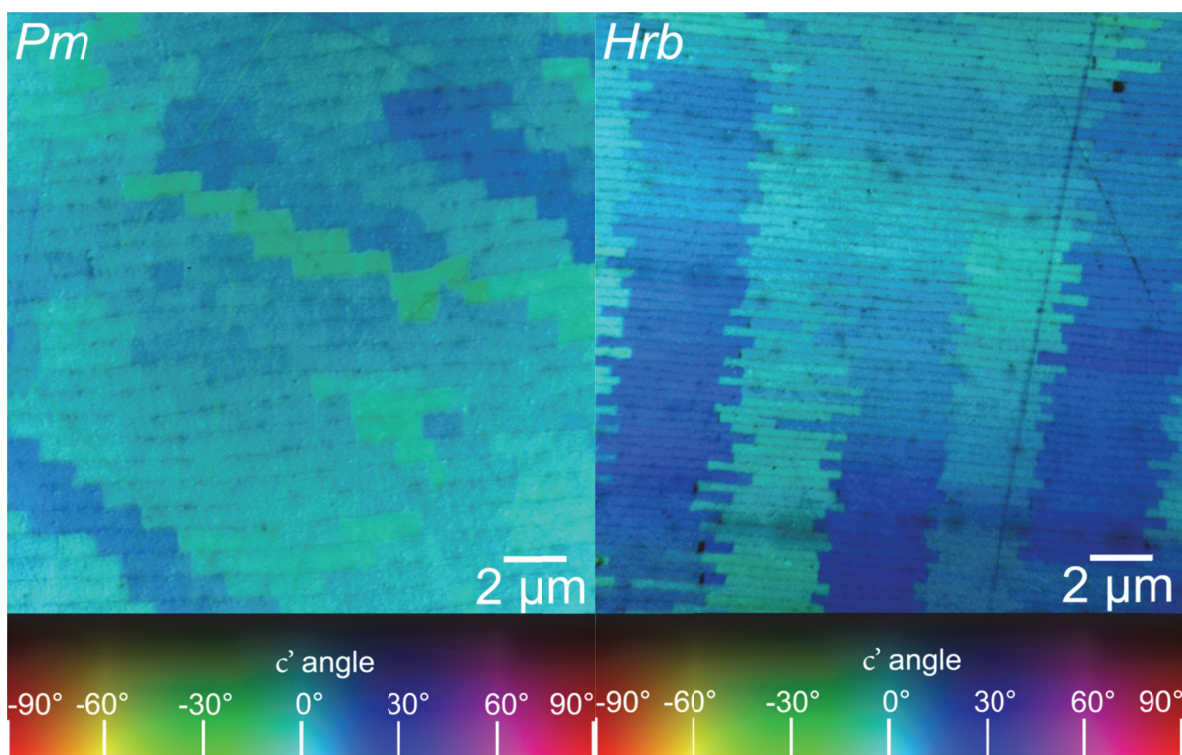


Figure 5-2 Side view of fractured nacre from two different species of mollusk shows the different crystal arrangements when imaged using polarization-dependent imaging contrast. SOURCE: Olson et al. (2013). Reprinted with permission from the *Journal of Structural Biology*.

If cells are the architects of biominerals, then biomolecules are the construction workers of the mesoscale, and biomineralization can be considered a “hard–soft” assembly process in which “soft” nanocomponents, particularly proteins, assemble to form “hard” mesoscale and larger biomineral structures. Cells use proteins to generate the nanoscale components that assemble into larger-scale solids with either short- or long-range ordering, a process that involves protein-based mediation of nucleation and disorder-to-order transformation. Using nacre from mollusk shells as a model system, each nacre tablet comprises a coherent aggregation of calcium carbonate nanograins that range in size from 3 to 10 nm, and nanoscale bridges and asperites—rough, nanosized bluffs—on the tablet surfaces help interlock the tablets and keep them from being displaced during crystal growth.

Proteins play a key role in creating the tablets. Each table comprises a polysaccharide layer of beta-chitin onto which is deposited a silk protein gel matrix. Inside this matrix is a family of proteins called framework proteins that are responsible for

nucleation that occurs in the gel phase. Inside the tablets is another set of proteins known as intercrystalline proteins and they exist as inclusions or voids inside the table crystals. Each of these proteins contains regions of amino acids that do not fold and do not form stable structures; these regions are labile, dynamic, and they destabilize aggregation. These proteins also contain regions of amino acids that are prone to aggregate similar to the way that beta-amyloid plaques aggregate in the brains of patients with Alzheimer’s disease. Together, these different regions simultaneously promote and destabilize aggregation, which is what is seen in atomic force micrographs (Figure 5-3). “We see evidence of aggregation but no real ordering of the structures,” said Evans, who added that these proteins can actually scuttle along the surface of the developing crystals.

As far as what these protein phases, as he called the aggregates, do in the nucleation process, Evans said that a nonclassic nucleation model postulates that nucleation starts from supersaturated conditions when prenucleation clusters form. These

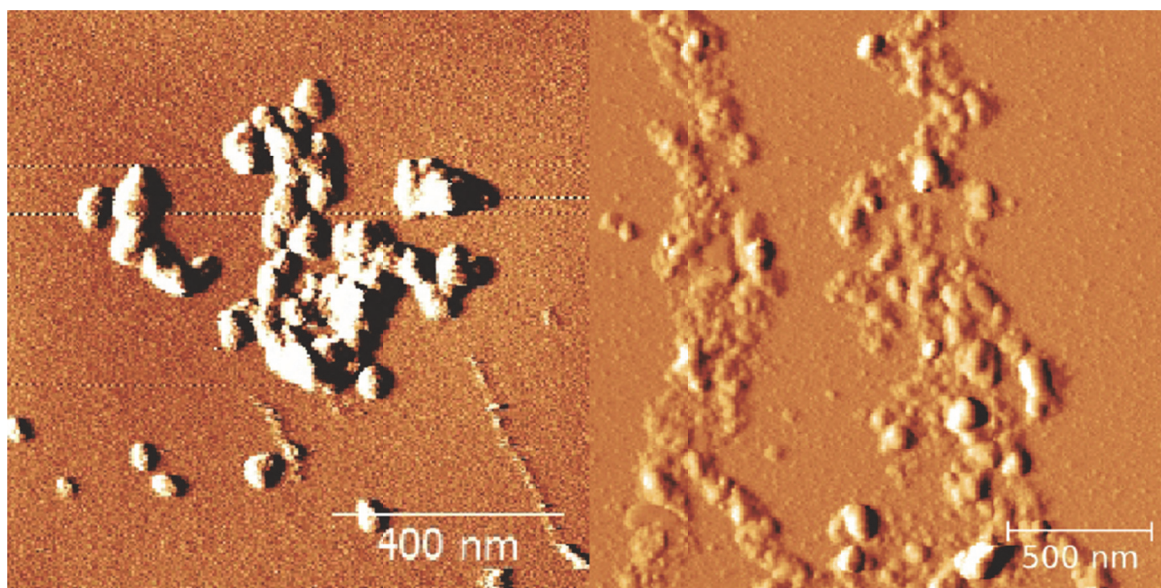


Figure 5-3 Atomic force micrographs of framework protein N16.3 (left) and the intracrystalline protein AP7 (right) showing regions of aggregation but no ordering. SOURCE: Evans (2014). Reprinted with permission from John S. Evans.

prenucleation clusters are small ion chains that are no more than 1 to 3 nm across and that can co-assemble into an amorphous aggregate of about 100 nm in size. This amorphous precursor has one of two fates: it can dissolve and precipitate again to form a crystalline phase or it can persist in an amorphous phase if there is something present to stabilize that state. Evans explained that some interesting mesoscale effects are seen during this process, and the role of some proteins in the nucleation process is beginning to be understood. Focusing on a pair of proteins, Evans noted that the intercrystalline protein AP7 (Figure 5-3, right) slows down but supports the formation of the prenucleation clusters by a factor of 2, while the N16.3 (Figure 5-3, left) framework protein affects a different part of the process and destabilizes those prenuclear clusters.

Using flow cell technology with electron microscopy to observe the nucleation process, Evans and his collaborators have shown that with AP7 intercrystalline protein present in a saturated solution of calcium carbonate, the mineralization reaction forms clusters of ring-like or donut-like structures that eventually produce branching mineralized structures (Figure 5-4), whereas when AP7 is absent, amorphous calcium carbonate forms rapidly in a chaotic manner (Chang et al. 2014b). As the AP7-mediated process continues over a period of minutes, calcite crystals grow and become coated with

protein, which directs nanoparticles of calcite to attach to the growing crystal to create a three-dimensional, orthogonally arranged, ordered structure that again has protein on its surface (Figure 5-5). “There is a continual deposition of a protein phase on top of an existing crystal, causing nucleation and the assembly of nanoparticles on the surface,” said Evans. The framework protein N16.3, on the other hand, appears to be directing the emergence of new crystal growth directions and creating nanotexturing along the sides where emerging crystals are growing (Figure 5-6) (Chang et al. 2014a). Slicing through crystals grown in the presence of AP7 reveals that they are porous and that there are micro-, meso-, and macrosized pores all within the same crystal. “So not only do we modify the surface, we modify the interior as the crystal grows,” said Evans.

Evans and his colleagues have also studied what happens when there is more than one intercrystalline protein present and found that the kinetics of crystal formation can change significantly depending on the ratios of the different proteins. In one set of experiments, they paired AP7 with PFMG-1, a protein found in Japanese oyster pearl nacre and that by itself forms clusters of crystals that resemble pine cones. When the two proteins are mixed in equal mole amounts, the result is a pine cone structure with nanoparticles growing on top of it.

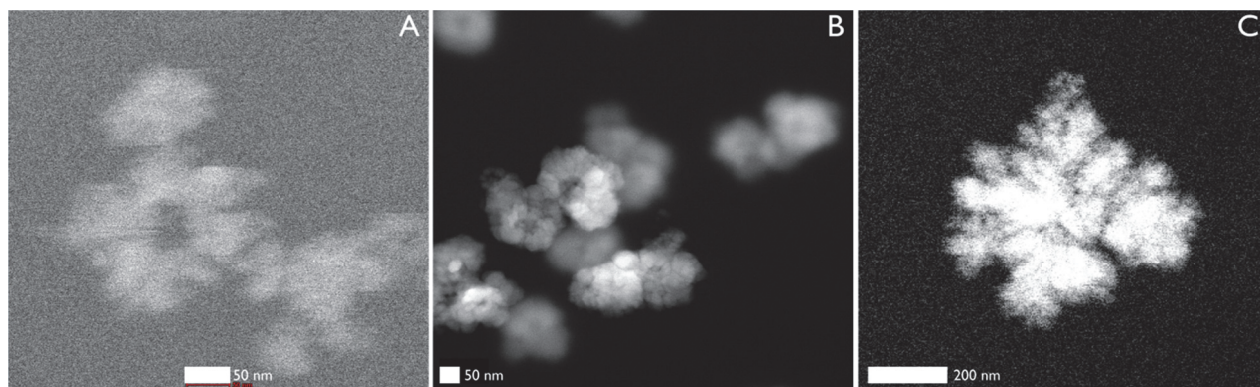


Figure 5-4 AP7 protein phases assemble and organize mineral nanoparticles, starting with ring-like structures in a premineralization step (A) and proceeding through early (B) and late (C) mineralization steps. SOURCE: Perovic et al. (2014b). Reprinted with permission from *Biochemistry*.

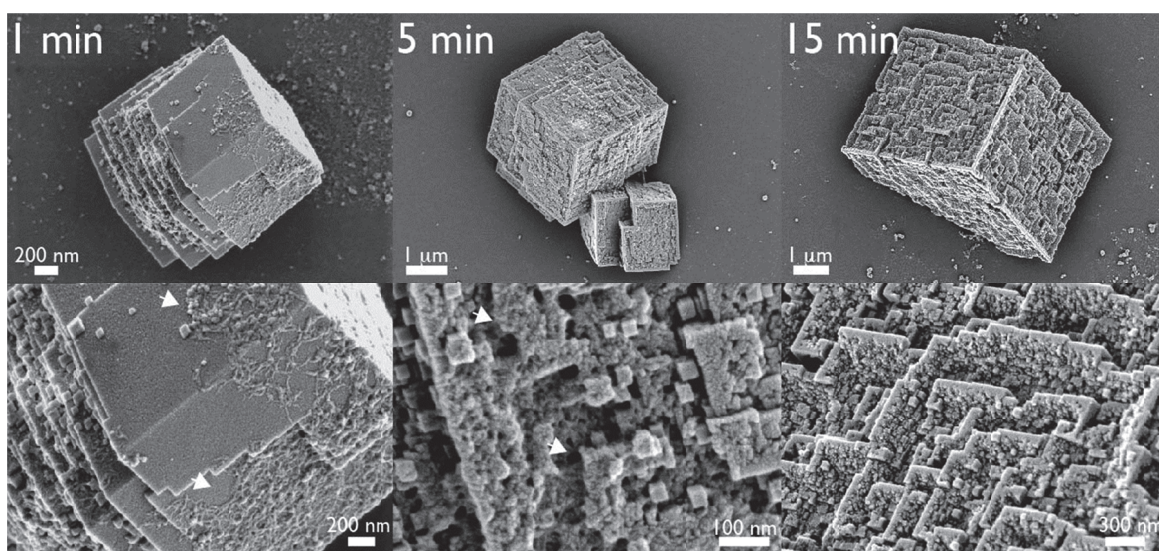


Figure 5-5 Intercrystalline protein AP7 induces the growth of ordered single-crystal nanocalcite coatings. SOURCE: Chang et al. (2014b). Reprinted with permission from *Biochemistry*.

Measurements of amorphous calcium carbonate solubility as a function of time showed that nucleation events are slightly destabilized when the AP7:PFMG-1 ratio was 10:1, a little more destabilized at a 1:10 ratio, and strongly destabilized at a 1:1 ratio, though on a longer time frame. At equal molar amounts, explained Evans, “it takes longer for amorphous calcium carbonate to form and it is highly unstable relative to what either protein does on its own.” In addition, there is a second, smaller nucleation event that occurs later when the two proteins are present in equal amounts. Further experiments showed that nucleation can even stop with the result that a stable phase forms in which nucleation is essentially suspended. This is called a

polymer-induced liquid-phase precursor, something that was observed a decade ago with polymer systems that form lipid phases with amorphous precursors. He noted that this interesting phenomenon needs further study. He also predicted, in closing, that these biomineral assembly proteins could be used to direct the assembly of nanoparticles made of other minerals.

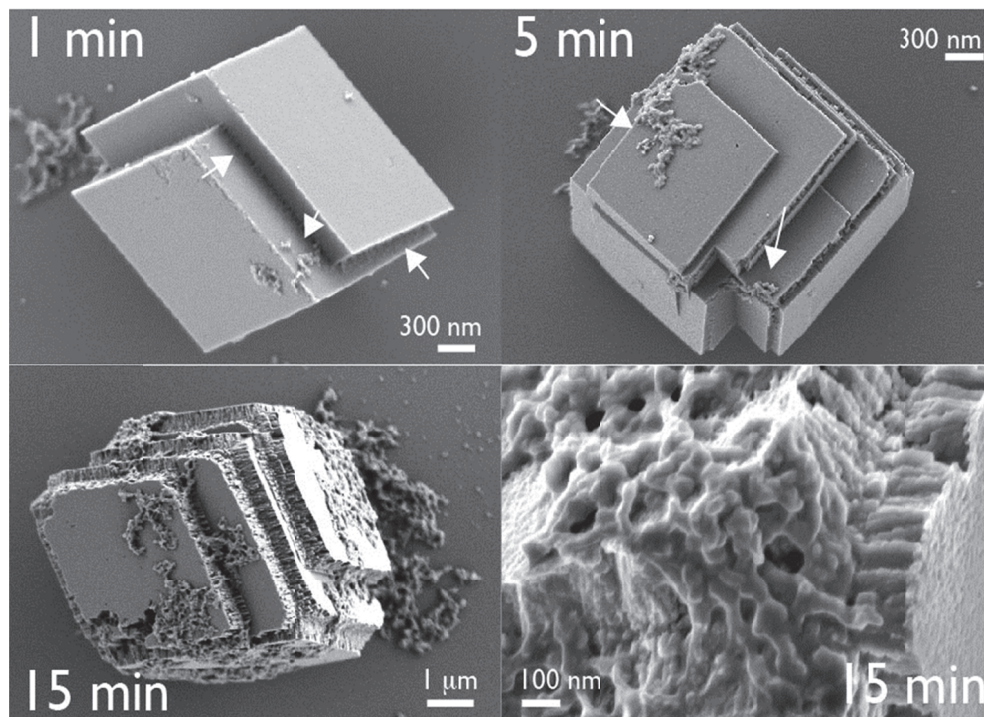


Figure 5-6 Framework protein N16.3 directs crystal growth and nanotexturing. SOURCE: Chang et al. (2014b). Reproduced with permission from *CrystEngComm*.

THE DYNAMICS OF MACROMOLECULAR ASSEMBLY AND SUBSEQUENT MINERAL FORMATION: UNDERSTANDING THE EMERGENCE OF ORDER

“Mineral formation is a manifestation of the intimate link that exists between biology and the environment,” said Jim De Yoreo. The process of biomineralization, he added, occurs on such a vast scale that it affects the planet’s chemistry and geology. The White Cliffs of Dover, for example, are made up almost exclusively of the deposits of a single organism and they record the interaction between the planet and its biota stretching back through the Cambrian Period. He also noted that the nucleation of ice in clouds is driven by mineral aerosols that originate in soils, often the result of biological activity. “The point is, you cannot separate the near-surface mineralogy of the Earth from the action of biology,” said De Yoreo.

Nucleation, he said, occurs through unstable density fluctuations that overcome a free energy barrier, a theory put forth by Josiah Williard Gibbs in two papers published in 1876 and 1878 as a means of explaining how water droplets form but

that was subsequently applied to crystals. De Yoreo explained that the rate of nucleation can be written in terms of two exponential Boltzmann factors, one of which describes the atomistic processes involved in crystallization, including the desolvation of ions, and the other describing the free energy barrier that Gibbs postulated. He also explained that the free energy expressions have a dependence on volume that is stabilizing and a dependence on area that is destabilizing. The volume effect arises because precipitation into the bulk drops the free energy of the system to zero, while the surface effect comes with a free energy penalty. Together, these competing energy terms create the free energy barrier.

As Evans noted in his presentation, recent data and simulations argue for nonclassical mechanisms of nucleation in which multi-ion clusters first aggregate to form amorphous particles that then transform to crystals. In this model, the classical nucleation barrier is eliminated by the aggregates of these clusters, said De Yoreo. However, there are also data regarding the distribution of smaller clusters of calcium carbonate that are seen in cryoelectron micrographs that he said argue for

classical mechanisms of nucleation. “If you look at the distribution, they look essentially classical,” said De Yoreo, who added that this debate needs further study to resolve, he said.

Another possible mechanism posits that these systems crystallize by first phase-separating into two liquids, one of which is a dense liquid phase that then dehydrates and turns into a crystalline phase. Molecular dynamics simulations suggest that the free energy of clustering is a downhill drop with no barrier, and that the diffusivity of the ions within these clusters is essentially that of liquids and orders of magnitude larger than what would be expected for solids, he explained (Bewernitz et al. 2012, Wallace et al. 2013).

There has also been a change in thinking in recent years about what takes place during crystal growth after nucleation. Classical crystal growth happens by the addition of monomers to step edges that can be generated by two-dimensional nucleation or via a dislocation. This classical view works well for bulk soluble crystals growing at low supersaturation. Many crystals, however, grow instead through particle-based processes, both of biogenic and nonbiogenic origin, such as those that Gilbert and Evans discussed and that other investigators have described. What is needed, said De Yoreo, is a comprehensive model that quantitatively describes the diverse pathways of mineralization (Figure 5-7). The essential challenge, he said, is to describe the ensemble behavior of a system that represents a coupled solvent–solid system. “That coupling happens across scales, and I don’t just mean length scales, I mean time scales and energy scales. And we want to describe the ensemble process, but we want it to be predictive, and that means we have to maintain molecular fidelity where it’s needed,” said De Yoreo.

The challenge, then, is to create theoretic frameworks and computational tools to describe ensemble phenomena with molecular fidelity and in situ experimental tools that can visualize molecular events. In terms of key mesoscale science challenges, De Yoreo listed the need to understand solution structure and fluctuations at solution–solid interfaces and confined interparticle regions; the evolution of fields and forces during assembly and translation into motion; the size dependence of free energy of formation, solvation energy, and phase stability; and the existence of prenucleation clusters and dense liquid states in electrolyte solutions.

His group is tackling some of these challenges by using in situ methods to watch how nucleation and growth occur. In one set of experiments, his group is using in situ atomic force microscopy to examine how microbial membranes form two-dimensional crystals with a well-ordered lattice structure. These crystals form with the aid of proteins that start out in a disordered form but over time become crystalline. Once they reach the crystalline stage, tetramers form along the growing crystals’ edges. De Yoreo explained that because these tetramers never form before crystallization starts, it is “apparently not possible for the protein to find its way to the folded tetrameric state without first passing through this liquid-like state.” Once they do form, however, they never leave and the clusters never disappear, so this is not a nucleation process because the solid does not communicate reversibly with the reservoir of proteins in solution. Taken together, these observations suggest that structural fluctuations occur on a longer timescale than density fluctuations and that conformational entropy drives a two-step pathway via an irreversible formation of liquid-like precursors. The conformational entropy drives the system through this two-step pathway to irreversibly form liquid-like droplets in which the proteins are in close enough contact for a long enough time to find the specific bonds they need to form an ordered structure. In this system, De Yoreo said, the emergence of order catalyzes assembly (Chung et al. 2010).

Mineral systems are no less complex, said De Yoreo. Studies on calcium carbonate nucleation demonstrated that multiple processes can occur simultaneously. In one process, amorphous calcium carbonate grows in size until a new nucleation event, which occurs on the surface of the particle, transforms amorphous calcium carbonate into crystalline aragonite. What these experiments showed was that all of the phases of the calcium carbonate system are stable relative to the solution and metastable relative to calcite. Moreover, the level of supersaturation needed to stabilize amorphous calcium carbonate makes all of the pathways available and which one dominates in the transition to calcite is unpredictable.

Living systems, however, cannot tolerate this type of flexible system, and they use proteins and

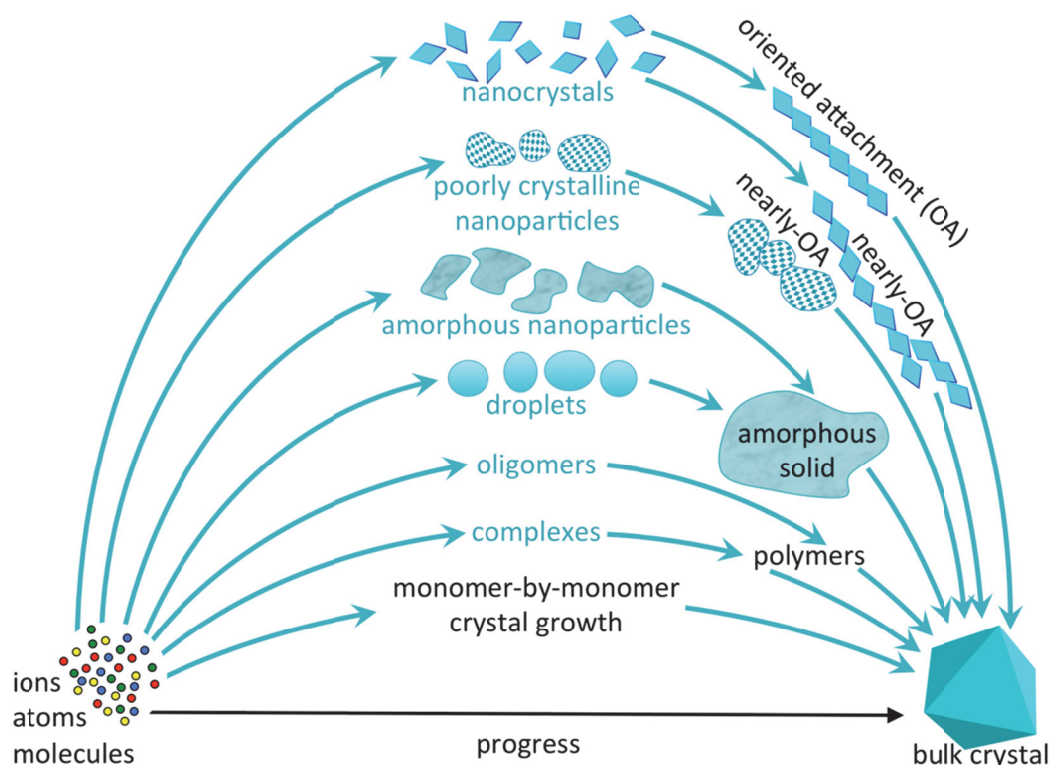


Figure 5-7 The many diverse pathways of mineralization. SOURCE: De Yoreo (2014). Reproduced with permission from Jim De Yoreo and Pupa Gilbert.

other biological polymers to direct transformation to specific crystalline forms. In nature, surfaces are covered in proteins and other organics, which impacts both nucleation rate and the mineral formation pathway. De Yoreo explained that when a nucleus forms on a surface, this creates an interfacial energy between the crystal and the substrate, but it also eliminates an interface between the substrate and liquid that had existed. The result is that the new interfacial energy is a composite term that is equal to the old one times a correction factor.

It turns out, said De Yoreo, that adding organic polymers to a supersaturated solution of calcium carbonate can redirect nucleation pathways. For example, polystyrene sulfonate, a polyelectrolyte polymer, binds large percentages of the calcium ions in solutions and produces globules that are 20 to 80 nm in size. Adding carbonate to the solution results in nucleation only occurring in these globules and the nuclei that form are amorphous. In another set of experiments with iron oxide, De Yoreo and his colleagues observed that ferrihydrate forms

dumbbell-shaped particles that engage in long-range interactions over 5 to 10 nm with other particles that causes them to jump to each other over a 5- to 10-angstrom distance (Nielsen et al. 2014). Plots of translational and angular velocity versus distance show a sharp increase occurring when two particles come within about 1 nm of each other (Li et al. 2012). De Yoreo said that calculations suggest that van der Waals and ion correlation forces can act at long range to bring the particles together, but that electrostatic forces dominate in this case.

He concluded his presentation with his view of some of the key mesoscale science challenges regarding understanding nucleation and growth. “We don’t know what the solution structure and its fluctuations are at solution–solid interfaces, and we know even less about it in the region between particles. We don’t know how the fields and forces evolve during assembly, and how those fields and forces translate into motion. We have to understand what the size dependence of free energies, solvation energies, and phase stabilities are and those impact

whether or not prenucleation clusters really exist and take part in the formation process or whether there are dense liquid states within the system,” said De Yoreo. Above all, he added, there is a need for theoretical frameworks and computational tools that can describe the ensemble phenomenon in these systems while keeping molecular fidelity where it is needed, and experimental tools that can visualize molecular-scale events in real time.

SMALL COMPOSITIONAL SIGNALS WITH A BIG IMPACT: FROM SUBMICRON GEOCHEMICAL HETEROGENEITY TO CLIMATE RECORDS

Paleo proxies are one of the fundamental tools used to understand Earth’s history and climate, explained Alex Gagnon. The chemical composition, or geochemical signature, of that archive reflects how mass and energy has moved around the planet. While measurements of these paleo proxies have been incredibly important for conducting bulk analysis, they display unusual and unpredictable properties when used at the mesoscale. “If you look at a really small scale, what we typically see, especially in biominerals but in a large number of systems, is small-scale but large-magnitude compositional heterogeneity that presents both a challenge in how we interpret how we think proxies work and an opportunity in that there is rich information encoded in this heterogeneity that could potentially help us understand and reconstruct climate signals in new and improved ways,” said Gagnon.

One biomineral of particular interest in this regard is produced by a free-living, single-cell organism called foraminifera that produces a calcium carbonate shell. When this organism is building its calcium carbonate shell, it is incorporating the O^{16}/O^{18} ratio that exists in the ocean at that particular time. This ratio is influenced by the amount of water that is deposited in ice—the process of evaporating water and making snow fractionates stable isotopes, and when done over a long enough period of time, such as during an ice age, the composition of seawater will change and that is reflected in this O^{16}/O^{18} ratio. As these organisms die, they fall to the bottom of the ocean and build up large deposits of carbonates that can be sampled by taking cores. Analyzing those cores for

their O^{16}/O^{18} ratio provided the first insights into the fact that there had been regular ice ages in the past and that analysis, conducted in the 1960s, showed both the time and pace of these ice ages.

Another important paleo proxy, one used widely to reconstruct sea surface temperatures, also uses foraminifera and it is based on the empirical observation that its skeleton is not pure calcium carbonate but includes a number of impurities, particularly magnesium. The empirical observation is that if these organisms are cultured at different temperatures, the magnesium/calcium ratio of a collected group of these organisms changes as a function of temperature, and that an analysis of sediment cores for that ratio provides a proxy for past sea surface temperatures. At the bulk scale, the correlation between temperature and magnesium/calcium ratio is robust within a particular species of foraminifera. Again, the signals from this paleo proxy show alternating periods of warm conditions and ice age conditions, and they are reproduced in sediments from around the world. One interesting feature of this paleo proxy is that it provides tropical and high-latitude temperatures and the differences between them are what drive heat and power weather systems around the planet.

The problem with these proxies arises when looking at individual foraminifera, and the reason is that temperature is not the only factor involved in influencing the magnesium/calcium ratio, and a number of different anomalies hinted at the problem, including the fact that simple precipitation experiments produced much different results than what is seen in biominerals. The most conclusive experiments were done on individual foraminifera that had been grown in the laboratory at constant temperature. Accurate spot analysis of individual shells showed that the magnesium/calcium ratio could vary by as much as a factor of 2 to 4 in a single organism cultured at constant temperature. Measurements using a variety of microanalytical techniques—including nanoscale time-of-flight secondary ion mass spectrometry (TOF-SIMS), which provides compositional isotopic maps at the submicron scale on spots the size of a few hundred nanometers—revealed regular banding of magnesium and calcium levels even with the shells of individual foraminifera. This finding raises the question, then, of what modulates this banding beyond temperature.

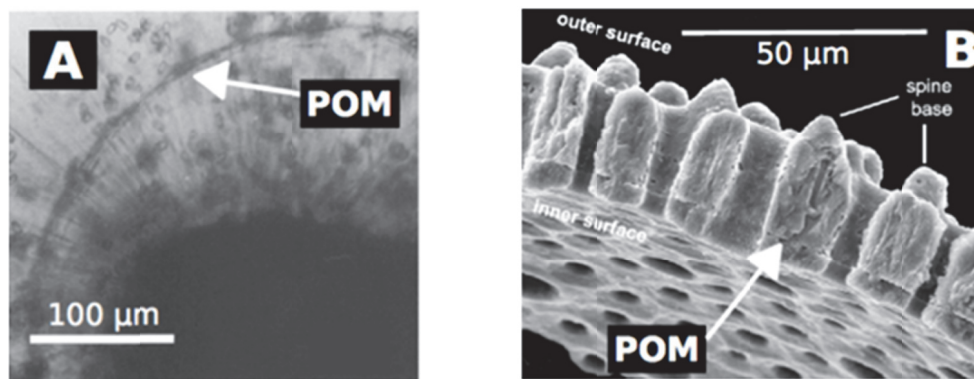


Figure 5-8 Micrographs showing the primary organic membrane (POM) on which foraminifera and corals grow their mineralized structures. SOURCES: (A) Spero (1988). Reprinted with permission from *Marine Biology*. (B) Eggins, Sadekov, and De Deckker (2004). Reprinted with permission from *Earth and Planetary Science Letters*.

One possibility is that pH could be playing a role, and indeed, when Gagnon and his collaborators grew foraminifera in the lab in a range of pHs, they saw the submicron scale of magnesium/calcium banding changed as a function of pH. Foraminifera cultured at low pH showed high-amplitude banding, while those grown at high pH showed much lower-amplitude banding. Additional analyses done on impurities other than magnesium, such as sodium, identified similar patterns of variation at the mesoscale. His group is now collecting data for a number of different ions using nano-TOF-SIMS to construct detailed compositional maps of foraminifera grown under different conditions. He noted that in every foraminifera examined so far there is a high-sodium region associated with a known organic membrane that is laminated to the calcite skeleton. He also said that his team has found these variations at the mesoscale in corals and other biominerals.

Taken together, these data raise questions about how these mesoscale processes of biomineralization affect the interpretation of records of Earth's climate history. What is needed, said Gagnon, is a set of tools to help map this heterogeneity and to explain this heterogeneity at a chemical scale. He said that he believes this heterogeneity can provide important clues about the past climate history of the Earth that are presently missing. In addition, a better understanding of these processes could provide inspiration for biomimetic approaches for controlling the composition of minerals and materials at very small scales.

Turning to the issue of identifying mechanisms that might be producing these banding patterns,

Gagnon first reviewed some possible mechanistic forces that could be operating, including diurnal forcing of the foraminifera microenvironment, the dynamics and chemistry at the site of calcification as influenced by organismal ion-specific pumping, and the mineral growth kinetics and thermodynamics as affected by organic mineral interactions. Foraminifera, it turns out, are a good model for studying how organic–mineral interactions might affect composition, in part because they have a compositional variability that is structured and because they lay down a very clear organic layer, known as the primary organic membrane, upon which they grow their skeletons (Figure 5-8). Examining the detailed composition of the minerals extending out from this membrane could provide information on how the organic mineral interface influences composition.

Gagnon and his team have used atom probe tomography, a method for reconstructing the atom-by-atom composition of a sample, to explore this interface in detail. This field emission technique is typically used with conductive materials because it requires applying a large charge to the sample in order to ionize individual atoms from a prepared section of the sample, but with some creative and brute-force approaches and by taking advantage of the fact that carbonates are insulators, his team was able to analyze the atomic composition at the organic mineral interface from a single foraminifera skeleton. One observation from these experiments was that the sodium concentration is increased dramatically on the organic side of the interface, a finding that he said can help distinguish how

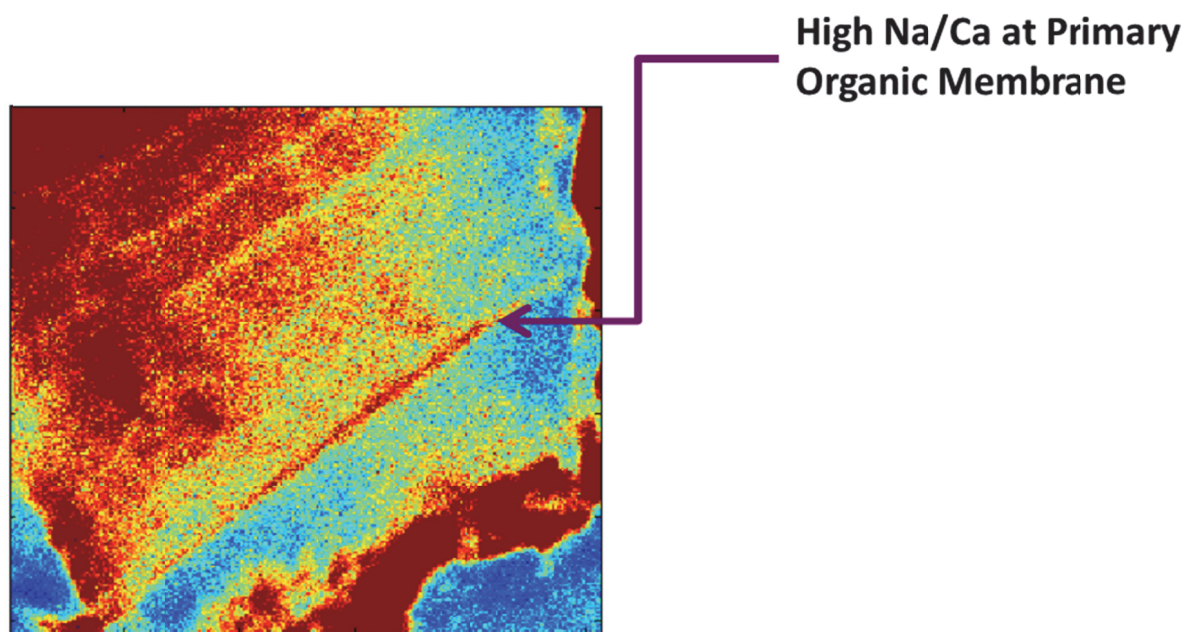


Figure 5-9 Sodium intensity image obtained using nanoscale TOF-SIMS. SOURCE: Gagnon (2014). Reproduced with permission from E. Bonnin and Andrew Gagnon.

organics and the organic–mineral interface influences composition.

“There is a compositional signature, and the location can tell us about mechanism,” he said, adding that these data are consistent with the signature of the large sodium signature detected with other methods, such as time-of-flight secondary ion mass spectrometry (Figure 5-9), that are used to make measurements at larger scales. “What we’ve now shown is that we can scale up from nanometer-scale measurement that tells us about the chemistry of these interactions to microscale maps of composition and then move from these micron-scale maps of composition to be able to tell us about ensemble behavior and global climate,” said Gagnon.

MESOSCALE GEOCHEMISTRY RESEARCH OPPORTUNITIES ON, WITHIN, AND BEYOND EARTH

In the final presentation of this session, Andrew Madden illustrated the wide applicability of mesoscale chemistry to the study of Earth and other planetary systems. To do so, he presented three unrelated vignettes. The first vignette explored how to investigate size-dependent reactivity in the natural world. One approach would be to do laboratory

experiments and study processes that might be important for the environment, make observations of the natural world, and see if the two sets of data correlate with each other. “But what we actually find in the natural environment is that nanoparticles aren’t really isolated,” said Madden, suggesting that it might be possible to use observations from the natural environment to inform new laboratory experiments. “In that way, we could say whether or not size-dependent reactivity and nanoscale size effects were important for the natural world.”

In fact, what Madden and his colleagues did was to do experiments in both the lab and nature simultaneously by taking three different sizes of magnetite nanoparticles and putting them in groundwater in nature and in synthetic groundwater in the laboratory. What they found from comparing the results from these two environments is that the nanoparticles were far more reactive in the laboratory than in the field. Moreover, in the lab the smallest particles were the most reactive, but in the field it was the largest particles that displayed any reactivity at all (Swindle et al. 2014). What was happening, explained Madden, was that large organic molecules accumulate on the nanoparticles in natural groundwater, which has the effect of passivating the ability of the nanoparticles to react (Figure 5-10).

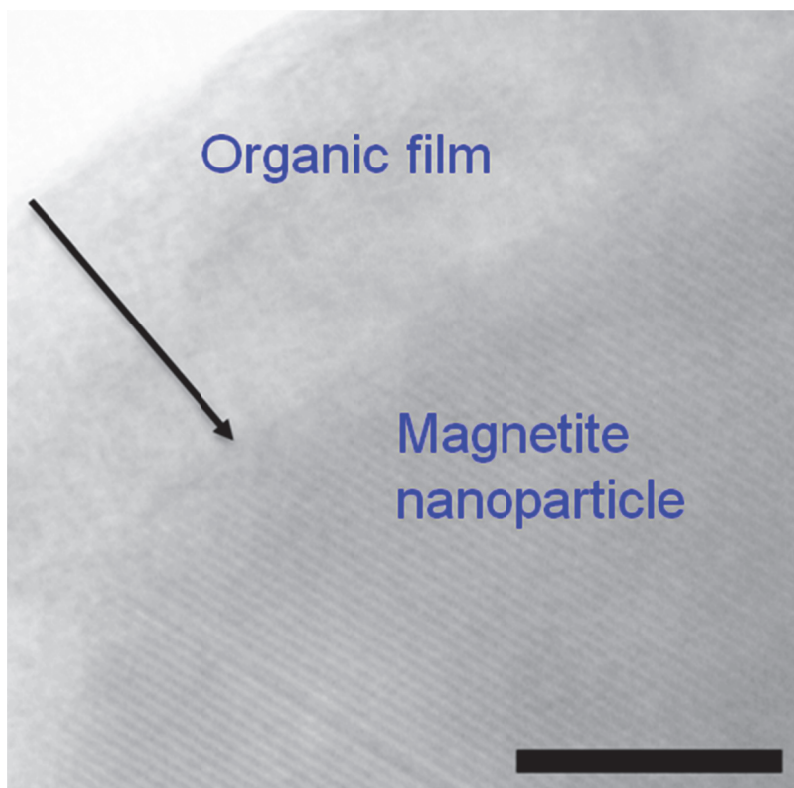


Figure 5-10 An organic film on a magnetite nanoparticle suppresses field reactivity. The black bar represents 10 nm. SOURCE: Swindle et al. (2014). Reprinted with permission from *Environmental Science & Technology*.

Another project, this one involving a bacterium that oxidized magnesium, found that organics were required for optimal manganese oxidation and crystal formation (Learman et al. 2011). In these experiments, manganese oxidation under abiotic conditions produced disordered, poorly crystalline manganese oxide as the end product, but when conducted with cell-free extracts prepared from a bacterium that oxidized manganese, the result was initially the same, but over time much larger, and ordered manganese oxide crystals formed. “So one of the research challenges we could think about in applying nanoscale size effects to the natural world is how can we sort out when organics passivate nanoparticle reactivity and when they actually promote nanoparticle reactivity, and what’s the balance between those in different environments,” said Madden.

The second vignette examined whether nanomaterials and mesomaterials could be involved in the processes that trigger earthquakes. Following the 1999 Chi-Chi earthquake in Taiwan, researchers collected core rock material from the fault zone that

was active during that earthquake. While this might seem to be a stretch, a careful examination of the material collected showed that it was made out of nanoscale to mesoscale particles (Ma et al. 2006). Using an instrument that can simulate the type of processes occurring in fault zones, Madden and University of Oklahoma colleague and collaborator Ze’ev Reches found the same type of nanoparticulate materials in the gouged rocks. It turns out that these nanoscale and mesoscale particles that are gouged out of the rock as a fault begins to slip may be coming together to create what are essentially roller bearings. These bearings then reduce the friction at the fault line and allow the fault to continue to slip, producing an earthquake (Figure 5-11) (Reches and Lockner 2010).

Madden explained that the drop in friction between the two plates in Reches’s instrument that simulate a moving fault correlates with the number and diameter of the rollers present between the moving plates. He noted, too, that there is a fundamental aspect of how the inner particle forces in the gouge particles are creating strength enough to hold those

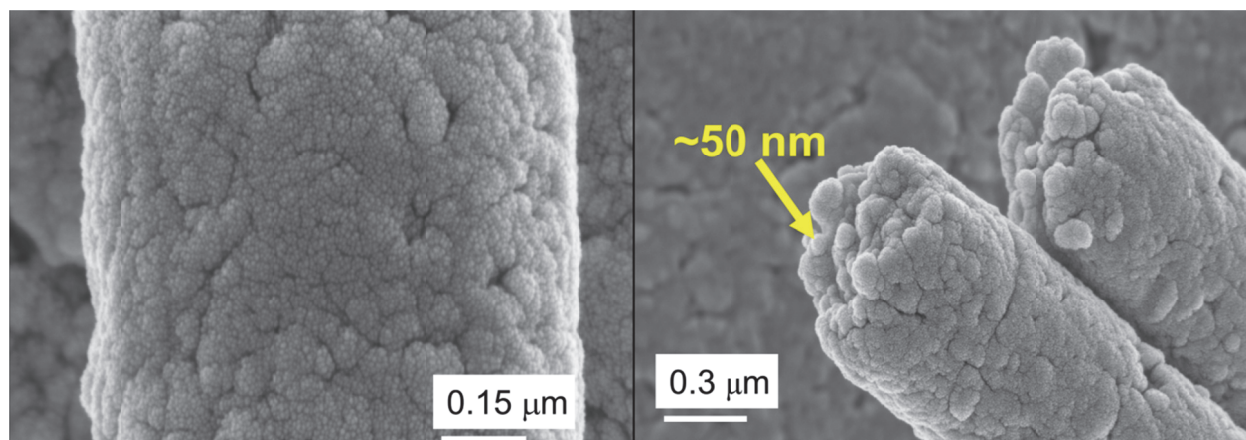


Figure 5-11 Rollers composed of nanoscale mineral grains with composition similar to bulk rock are formed under conditions re-created to mimic those that occur during a fault-slip earthquake. SOURCE: Madden (2014). Reproduced with permission from Xiaofeng Chen, Andrew Elwood Madden, and Ze'ev Reches.

rollers together until they reach a critical size, at which point they fall apart. Understanding the forces involved will require further study, he said. Vignette number three dealt with efforts to trace the history of water on Mars and to understand how long rocks on Mars were in contact with water and how that contact produced the geological signatures seen today. The key mineral of interest in this study is called jarosite, a hydrous sulfate of potassium and iron that only forms a very narrow set of chemical conditions. Outside of those conditions, jarosite breaks down with continued contact with water to produce simpler iron oxides. “The fact that jarosite is found on Mars tells us that the duration of water–rock interaction was not that long, and we can actually try to time it,” explained Madden.

Madden, working with the principal investigator for this project, Megan Elwood Madden, measured the dissolution kinetics of jarosite under various conditions and saw what thousands of previous geochemical experiments have seen—solute release into an aqueous solution rises quickly and then plateaus. However, further experiments using saturated brines found that when jarosite is in contact with these saturated brines, the dissolution reaction suddenly accelerates at some point as a result of a feedback process involving the dissolution products. Further experiments found that when jarosite dissolves, it releases iron, which precipitates, and potassium sulfate, which does not. The sulfate ions combine with calcium to form gypsum, which now disrupts the charge balance in the system and the residual chlorine then attacks the

jarosite, hastening its dissolution. These results are of interest because mesoscale structures known as shales are now being tapped as sources of natural gas and oil. The shales contain pores that can be filled with the briny solutions being injected into shale deposits. “I think that once we start interrogating these rocks in different ways, we’re going to find some unique mesoscale effects that will change the types of mineral–water interactions that might be important from what we thought otherwise,” said Madden in concluding his presentation.

DISCUSSION

Session moderator Patricia Thiel started the discussion by asking if the panelists saw any connections between their work and the work on catalysis that was discussed in the workshop’s first panel presentation. Gilbert responded first by noting that there is one big difference between catalysis and biomineralization processes: catalysis is about accelerating reactions, whereas biomineralization retards both the nucleation and crystal growth process that proceed rapidly under abiotic conditions. Evans elaborated on Gilbert’s remarks by noting that in biomineralization there is a “pause button” where biomolecules come together and regulate nucleation and subsequent steps in a way that allows many other components to come into play at different times in the nucleation and growth phases to further regulate and control the process to

produce biominerals with the necessary physical properties.

Gagnon said that there are analogies between the two fields because both are trying to understand how different components of a complex system interact at the mesoscale to produce a desired function. Just as model systems of enzymes that only recapitulate the inorganic metal centers are informative but do not capture the full behavior of the native enzyme, abiotic models of biomineralization provide useful insights but do not capture the full complexity of the system in nature. De Yoreo added that one similarity both fields have is that they are trying to understand the energy landscape that drives or inhibits reactions, and in both catalysis and biomineralization there are proteins that are manipulating energy landscapes, in the former to accelerate a chemical reaction and in the latter to control the kinetics of crystal formation.

Anne Chaka, from PNNL, asked the panel what role water positioning and transport plays in biomineralization processes. Gilbert replied that one of the keys to biomineralization reactions in general is to exclude water and that biomineralization always occurs behind closed membranes from which water is excluded. She added that going through an amorphous precursor, which she described in her presentation as playing an important role in the formation of calcite, is a “smart trick because if you calculate the volume of a supersaturated solution that would be needed otherwise, it would be orders of magnitude larger than the entire organism.” In short, she said, there is no water involved in biomineralization. De Yoreo tempered Gilbert’s comments by saying that the biomineral world is vast. “The number of cases that have been studied enough to understand whether or not particular aspects such as the level of hydration or the utility of water in transformations in general is small, and we really can’t say,” said De Yoreo. “We will just have to see over time how that develops.” Gagnon added, “There’s quite a wide range of strategies that different organisms appear to use about what the water-to-mineral ratio is during biomineralization.”

Wendy Shaw, from PNNL, asked De Yoreo and Evans if they had any insights on what drives iron oxide particles to make a 5- to 10-angstrom jump from one crystal to another during biomineralization or why some minerals when they’re forming start out having a donut shape with a void. De Yoreo’s short answer was that the mechanism is unknown, and that there are many potential forces at work.

“The only thing we can say for certain is we know what the accelerations are and calculate the force, and we know what the change in potential energy is during that process,” said De Yoreo. Calculation using Coulomb’s Law can provide a reasonable answer that suggests that electrostatics plays a role, which would be consistent with the length of the jump, but interfaces are complex and there are many potential interactions that could be responsible. “This is one of the open questions about particle-based growth processes and oriented attachment in particular,” said De Yoreo. Evans added his conjecture, which was that prior to becoming visible, there are actually clusters already forming that are not visible yet simply because they haven’t reached a critical density that enables them to be visualized. De Yoreo said, however, that there are data that argue against that view of assembly. Evans said that another possible model is that there are oligomer gels that are not visible that are attracting ions and creating localized supersaturation that triggers nucleation on the gel filaments.

Donna Blackmond asked the panel if there are chiral biominerals, and both De Yoreo and Evans said that they were not aware of any chiral biomineral crystals. Andrew Stack, from Oak Ridge National Laboratory, then asked if the field was advanced enough to be able to predict how a given biomineral will grow into a given shape, such as a mollusk shell. The unanimous answer from the panelists was no, though Gagnon noted that it is starting to be possible to modify existing processes to change morphology or composition, but he characterized that as a baby step toward total control and prediction. De Yoreo categorized the state of the field as being at the stage of having intuitive phenomenological approaches but not able to make predictions. Gilbert summarized the state of the field by saying, “We don’t know the laws of nature at the mesoscale and because we don’t know the fundamental laws, we cannot make predictions.”

Benjamin Wilhite asked Evans about the generalizability of some of the processes he described, that is whether instead of making carbonates these processes could be adapted to work with nitrate precursors, or if instead of working with calcium they could be applied to work with other transition metals. In particular, he was wondering if it would be possible to adapt the biological systems to make columnar structures and oriented crystals for use in energy applications, for example. Evans

replied that the “devil is in the details,” and that the idea of borrowing from biology is “tantalizing.” One question that needs answering with regard to this question, said Evans, is how these proteins would behave under solution conditions, such as pH or ion concentrations, that differ from the conditions they see in nature.

Workshop organizing committee and CSR member Miguel Garcia-Garibay, from the University of California, Los Angeles, asked if the products of biomineralization processes are in a stable state, and De Yoreo said that in most cases the answer is yes. The aragonite crystals in nacre that Gilbert talked about are an exception because aragonite is not the stable state of calcium carbonate at room

temperature. “That system is caught in a metastable state,” said De Yoreo. Garcia-Garibay noted that vertebrate bone, a biomineral, is in a homeostatic state, not a stable state. Peter Stair followed that comment by asking the panelists if they had any ideas on what factors are involved in creating stable structures in the mesoscale realm. Gilbert replied that the answer is no, but that proteins are likely involved. She noted that aragonite only forms when there is an excess of magnesium present along with calcium carbonate even though there is no magnesium found in the aragonite crystals. It may be that magnesium is influencing the behavior of proteins that are involved in regulating aragonite production.

6

Computational/Chemical Processes in Self-Assembly

Key Points

- There is a need to develop theory and simulation that can connect models across scales and incorporate emergent phenomena to realize functionality by design capabilities. In spite of great advances in modeling at all levels, particularly with coarse-grained models, there is still a critical lack of understanding about how long-range order on the mesoscale emerges from very short-range interactions and what one could treat less precisely.
- There are many tools in the computational chemistry toolbox that can be brought to bear on the problem of bridging the microscopic and macroscopic world, and among them are the coarse-grained models and atom-by-atom simulation of whole systems. These models can provide complementary information about mechanism and mesoscale behavior.
- Entropy is usually thought of as counteracting the enthalpic driving force for self-assembly, but in many mesoscale systems, entropy is actually helping the assembly process, though the degree to which that occurs is still unclear.
- The shape of particles can have a profound effect on self-assembly even in the absence of interparticle interactions.
- Designing proteins with specific three-dimensional structures by changing amino acid sequences so that they would then self-assemble into interesting mesoscale structures has been a long-standing engineering challenge that has still yet to be solved.

The workshop's final session featured five presentations on how computation and chemistry can be brought to bear on the challenge of understanding self-assembly of mesoscale structures. Todd Yeates, Professor of Chemistry and Biochemistry at the University of California, Los Angeles, provided some examples of the role that symmetry plays in self-assembly and its use in creating a broad range of materials with diverse applications. William Noid, Associate Professor of Chemistry at Penn State University, described various approaches for multiscale modeling, including the use of bottom-up models for representing thermodynamic properties. Klaus Schulten, Professor of Physics and Director of the National Institutes of Health Center for

Macromolecular Modeling & Bioinformatics at the University of Illinois, Urbana-Champaign, discussed how intelligent mesoscale biological systems can be resolved entirely in chemical detail and how that information can be used to mimic the robust and adaptive mesoscale molecular strategies that evolved in nature. Sharon Glotzer, the Stuart W. Churchill Collegiate Professor of Chemical Engineering, and Professor of Materials Science and Engineering, Physics, Applied Physics, and Macromolecular Science & Engineering at the University of Michigan, addressed the role that entropy, order, and function play in self-assembly processes. Gregory Voth, the Haig P. Papazian Distinguished Service Professor of Chemistry at the University of Chicago, discussed how multiscale simulations can help

reveal key features of the structure and self-assembly of large multiprotein and protein-membrane complexes such as those involved in remodeling membranes. An open discussion moderated by workshop organizing committee and Chemical Sciences Roundtable member Miguel Garcia-Garibay, from the University of California, Los Angeles, followed the five presentations.

DESIGNING NOVEL SELF-ASSEMBLING PROTEIN MATERIALS: RECENT SUCCESSES AND FUTURE PROSPECTS

The past few decades have seen spectacular success in using nucleic acids, particularly DNA molecules, to assemble into sophisticated objects, said Todd Yeates, and the reason for that success comes from the fact that the rules by which DNA molecules self-associated are simple, following the basic rules of Watson–Crick base pairing (Han et al. 2013, Ke et al. 2012, Rothmund 2006, Seeman 2010). In contrast, designing proteins with specific three-dimensional structures by changing amino acid sequences so that they would then self-assemble into interesting objects has been a long-standing engineering challenge that has still yet to be solved. However, using the underlying principles of symmetry that exist in nature, research teams such as the one headed by Yeates have recently developed design strategies that are realizing success in designing protein molecules that self-assemble into mesoscale structures.

Some 20 years ago, a graduate student of Yeates was working on the problem of trying to understand why proteins crystallize with a specific preference for different kinds of symmetries. She discovered that a key element for understanding why proteins crystallize in the arrangements that they do is a mathematical property known as the minimum contact requirements for achieving connectivity in a molecular solid (Wukovitz and Yeates 1995). Crystalline solids follow space group symmetries, Yeates explained, but what had never been examined before was the minimum different number of ways molecules had to touch each other in order to form three-dimensional structures. “This turned out to be a math problem, and the question of how many different distinct ways a molecule has to contact itself in order to form a connected network is essentially a problem of group theory,” said Yeates.

What this insight means is that the minimum contact number is a function of symmetry. For example, to create an arrangement with what is called P2 symmetry, the molecule has to touch itself in at least three different ways no matter the shape of the individual molecule. In contrast, P4 symmetry requires just two contacts. What this rather simple but powerful concept translates into is that “an astonishing range of architectures and symmetries can be generated using only two symmetric points of protein–protein interaction,” explained Yeates. That realization, he added, frames the engineering problem of how to take a molecule and engineer it so that it self-assembles.

With part one of the problem solved—coming up with a rule to guide engineering work—Yeates and his team turned to part two, figuring out how to design a protein to have the necessary two points of contact and build in the interfaces needed to create the right geometry. He noted that his group started down this path before the advent of sequence design—the idea of taking a protein molecule and redesigning its amino acid sequence in a specific way so that it would interact with itself. Instead, they used natural protein oligomers that already had natural interfaces built into them. For example, many proteins form dimers or trimers and creating a fusion product of two of these proteins, using genetic fusion to create a gene that codes for both components, is in principle creating a larger protein that has the two natural interfaces needed to create bigger structures.

The one problem with this approach is that it is necessary to control the degrees of freedom in the fusion protein so that the two halves orient themselves correctly relative to one another. “If you just fuse two protein molecules together, you’d probably expect to get some kind of gel or just some kind of glue,” said Yeates, “but since we were trying to design very specific symmetric geometries, the question is how to fuse together two protein molecules in a way that you can control or anticipate what the geometric relationship would be between the parts that are joined.”

A solution to this problem was to work with proteins that had a terminal alpha helix because that afforded the opportunity to use computational methods to predict how the rigid nature of the alpha-helical structures would dictate geometry (Padilla, Colovos, and Yeates 2001). This is not a way to control geometry, said Yeates, but a way of

anticipating it, and it triggered a combinatorial chemistry exercise of taking known proteins with known types of helices, hooking them together, sometimes with short alpha-helical linkers, and modeling how these constructs would self-assemble. As a guide for this design effort, Yeates' team used the angles of intersection in Platonic solids to develop the geometric rules for combining different symmetry elements, and then built one protein that their calculations predicted would assemble into a single 12-unit construct with tetrahedral symmetry. In fact, the protein did self-assemble but into multiple species that had a much broader size distribution than expected.

Yeates commented that the resulting paper drew little attention, even among the members of his own group, and this effort languished for more than a decade until a new bioengineering student joined the lab and decided to revisit this earlier work. By taking advantage of the better graphics technology that had been developed during the intervening years and turning a fresh eye on the structures, this student decided that there was one amino acid in the original design that, because of its location, could interfere with the predicted self-assembly process. When the "corrected" protein was expressed, it folded neatly into four distinct species that were separable using gel electrophoresis. These species, said Yeates, corresponded to the four possible ways of taking twofold and threefold axes of symmetry to make closed symmetries (Lai, King, and Yeates 2012). An additional amino acid change produced a construct that then self-assembled into one species that could be crystallized and whose structure was determined by x-ray crystallography to be a somewhat squashed tetrahedron (Figure 6-1) (Lai, Cascio, and Yeates 2012). This student then repeated this work with another fusion protein that self-assembled into a 230-angstrom cube containing 24 subunits, and in this case the crystal structure varied from the prediction by roughly 1 angstrom (Lai et al. 2014).

A second approach that has realized success has been taken by a former student of Yeates' who is using sequence design rather than combinatorial approaches. The idea here was to take one natural oligomer and use computational methods for sequence design to introduce the second interface at exactly the right geometry (King et al. 2012). Of the 90 different constructs that this computational approach predicted would work, two did and they have since been crystallized. The resulting crystal

structures matched the predicted structures within about an angstrom, said Yeates. Further work showed that this approach could be extended to two-component assembly by defining an intended target symmetry, identifying arrangements where the molecules touch each other, and then using computational methods to introduce the right amino acids to make that happen.

In his final remarks, Yeates said that of the nearly 50 possible combinations of two symmetry point groups to create self-assembling structures, only two have been explored. Going forward, the goal will be to demonstrate that these other possibilities can be made. If successful, these designed mesoscale assemblies could have use in creating multivalent antigen displays, bi- and multispecific reagents for cell recruitment, and layers and three-dimensional materials for high-density enzyme or receptor display.

BOTTOM-UP MULTISCALE APPROACHES FOR MESOSCALE PHENOMENA: PROGRESS AND OPPORTUNITIES

In preparing his presentation for this workshop, William Noid reviewed the Department of Energy report on mesoscale science and came away with two thoughts about mesoscale phenomena. The first is that there is a need to develop theory and simulation that can connect models across scales and incorporate emergent phenomena to realize functionality by design capabilities. The second idea is that there are many tools in the computational chemistry toolbox that can be brought to bear on this problem of bridging the microscopic and macroscopic world, and among them are the coarse-grained models of the sort that he uses in his research. The challenge, he said, is that if the goal is to capture the properties that emerge when moving from the atomic to the mesoscale, it will be important to ensure that a low-resolution, coarse-grained model will incorporate the physics that are governed by atomic interactions.

Coarse-grained models, Noid explained, can simulate longer length scales and time scales than atomic-scale models, and they can reach equilibrium, something that is difficult with fine-grained models because of the computational requirements of those more detailed models. Given that coarse-grained models take so much less

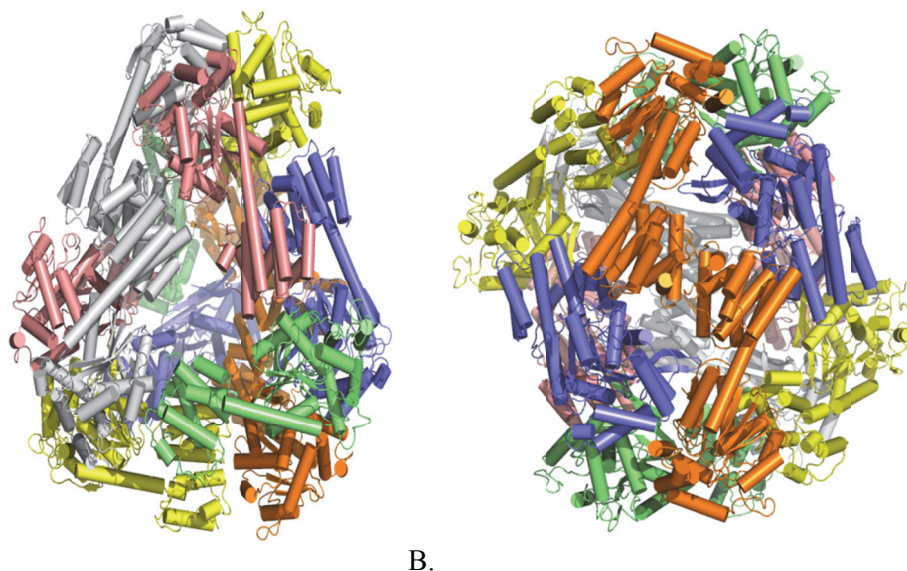


Figure 6-1 (A) The atomic structure of a designed protein cage with 12 subunits in a pseudotetrahedral symmetry. (B) The intended symmetry of the same protein cage with 12 subunits. SOURCE: Lai, Cascio, and Yeates (2012). Reprinted with permission from *Science*.

computational power, it is easy to run a simulation multiple times and determine the statistical confidence of simulated properties, as well as to systematically study parameter space and the impact of changing external conditions. From a conceptual point of view, coarse-grained models have advantages that go beyond their reduced computational burden. For example, it is possible to tailor coarse-grained models for particular systems and to focus efforts on essential features of a system while eliminating unnecessary details. Some coarse-grained models, for example, represent groups of water molecules or some details of some amino acids as a single entity rather than as multiple atoms. Another type might represent a protein not as a collection of atoms but as some kind of elastic network medium. Coarse-grained models provide a great deal of freedom, so it is up to the researcher to decide how best to represent a system and to create an abstract representation of the important pieces of the system and how they interact with one another.

Noid noted that while there are some theoreticians who believe that coarse-grained models are a waste of time—that the goal should be to build as detailed a model as possible and wait for computer technology to advance to the point that these models can be run in their full glory—the fact is that new things happen on different length and

time scales and so describing them with the same microscopic physics is missing the point. “You want to be able to capture the physical forces driving phenomena at particular length scales,” said Noid. “That is real physics and that is what coarse-grained models allow you to do.”

There are two different broad strategies for coarse-graining, top down and bottom up. Top down entails taking an experimental observation to parameterize a coarse-grained model. Alternatively, it is possible to create a coarse-grained model to observe what type of features emerge on particular length and time scales as the properties of the model change, which is what Sharon Glotzer talked about in her presentation. The bottom-up approach to coarse-graining takes a more detailed model of the system that is believed to be an accurate representation of a particular system. Not only would one use the model to capture generic phenomena, but one would also to try to figure out the mesoscopic manifestations of the atomic interactions for that particular system. This is a powerful approach, said Noid, because it enables the development of rigorous theories that connect these different length scales. The bottom-up approach, he added, relies upon a rigorous statistical mechanics framework to go from a well-validated fine-grained model to a coarse-grained model that captures the

phenomena of interest. The challenge in this approach, though, is to ensure that when going from a high-resolution to a lower-resolution model the correct forces and physics are captured.

Noid outlined a few of the computational approaches that people have employed to calculate wave functions and probability distributions. He described a method, known as force matching, that takes the forces generated by an atomistic model and the forces generated by a coarse-grained model and tries to minimize the difference between the two when averaging over structures or particles. This last approach is quite powerful, said Noid, because not only does it provide the desired coarse-grained energy potential, it also provides a variant framework that enables a systematic improvement of the model's approximations. Also, this approach boils down to a direct linear least-squares problem, which means that it can be solved exactly for many different types of systems, and it makes predictions that work in practice for many systems even without force information for a structure. "You can show in practice that you can generate a model of any protein in a databank and recover the underlying potentials quantitatively," said Noid.

He concluded his talk by noting that his group focuses on bottom-up methods and finds that they are very accurate for soft materials, but not always so accurate for modeling more complex biological materials. More work needs to be done there, he added, as there is a need to develop theories for working out the best way to represent systems. "The key idea though is that this many-body potential of mean force in statistical mechanics gives us an exact rigorous framework for any system, for any resolution, to try to make this perfect coarse-grained model, and now we just have to figure out how to make good approximations for that," said Noid.

VIEW OF INTELLIGENT MESOSCALE BIOLOGICAL SYSTEMS AT CHEMICAL RESOLUTION: WHAT CAN BE LEARNED

To Klaus Schulten, computation serves as a microscope to provide views of biomaterials that are otherwise unavailable. He said that one of the great lessons he has learned over 40 years of studying biological materials is that "if you really want to play your cards well in the mesoscale world of materials and devices that you should think not only

about how to build these materials, but that there is a lot of good chemistry that you can do with these materials. That is something that the physicists cannot do and the material scientists cannot do, but you chemists can do."

As an example of that, Schulten discussed the cellular microtubule, which he said not only has interesting material properties, such as mechanical stability, but the ability to control chemistry, particularly the hydrolysis of guanosine triphosphate (GTP). Fresh microtubules, he explained, are loaded with GTP, and when they're spent and fall apart they have guanosine diphosphate, but they have an intermediate state that is slightly disordered and more interesting in terms of its mechanics. More interesting, he said, is the fact that the physical properties of these materials are controlled by the molecules they bind.

He also discussed the cellulosome, which is a finger-like structure on the surface of bacteria that produce cocktails of enzymes needed to break down cellulose and other plant cell wall components. But besides producing the right enzymes with the best distribution to work on the substrates at hand, the cellulosomes themselves have polymer properties that enable them to help pull apart the cellulose in wood so that the enzymes have better access to their substrates.

The virus capsid, another example of a mesoscale biological "device," was long thought to be simply a container of genetic material, but that notion was wrong. "The capsid is actually a communication surface: It is communicating with the cell that is being infected to make the cell help with its cellular effectors, its proteins, to guide the capsid to the right place in the cell," said Schulten.

Returning to the example of the microtubule, Schulten said it has a number of structural properties that enable it to interact with proteins that change its properties, such as its flexibility, in response to chemistry going on at the microtubule surface. Using data from several sources, including electron microscopy and crystallographic studies, Schulten and his team have created a computational model of the microtubule at the atomic level that can reproduce the microtubule's behavior as chemistry is happening on its surface. Similarly, his team has modeled the modular nature of the cellulosome to try to understand the changes it undergoes as it interacts with cellulose to generate strong chemical bonds that help it pull cellulose apart.

His group has also solved the structure of the HIV-1 capsid using cryoelectron microscopy and all-atom molecular dynamics simulations to make sure that the structure they deduced is stable (Zhao et al. 2013). One thing these experiments revealed is that the surface of the capsid is more heterogeneous than the typical icosahedral virus and more closely resembles Ebola or influenza virus. All told, there are some 1,300 protein molecules that pattern the capsid surface and that can move and distribute themselves and create barriers and channels through which ions can move. The capsid can also interact with cyclophilin, a protein that cells use to guide molecules to the nucleus, which the HIV-1 capsid has to do in order to inject its genetic material into the nucleus. He noted that this information should be useful for developing drugs that interfere with HIV-1 function.

In the final minutes of his presentation, Schulten discussed work aimed at understanding the mesoscale structure and function of the organelles that house the many enzymes and cofactors needed for photosynthesis. He briefly described the process by which light energy is harvested by chlorophylls and carotenoids, triggering a flow of excitons that travel to the photosynthetic reaction center, where ATP is generated, and then discussed how modeling and simulation work has enabled his group to detail the movement of molecules within the photosynthetic apparatus to enable photosynthesis to occur and provide a better understanding of the chemistry involved in this process.

EMERGENCE OF ENTROPY, ORDER, AND FUNCTION AT THE MESOSCALE

From Sharon Glotzer's perspective of someone who is interested in entropy and order, there are three emerging trends in mesoscale science. The first is the idea of what she called patchy particles and shape entropy. A decade or so ago, when researchers began synthesizing a wide range of nanoparticles with a variety of shapes and functionalizing them with different ligands, it became clear that much of the physics and chemistry driving the self-assembly of building blocks into higher-ordered nanostructures was very similar despite the fact that these materials and the processes used to make them were quite different. To make sense of this, Glotzer and her colleagues developed a conceptual

framework that was useful for understanding what kind of structures would be possible by tuning different anisotropic characteristics of the building blocks (Glotzer and Solomon 2007). This framework was developed by doing a principle component analysis that broke apart all of these different types of attributes, and it has served as a guide for further experimentation and for deciding what types of particles are likely to self-assemble into a desired structure (Figure 6-2).

Using a coarse-grained approach of the sort that Noid discussed, Glotzer's group has been working with Christopher Murray's group at the University of Pennsylvania to understand why the particles that Murray's group was making were not assembling into the predicted structures. As an example, Glotzer described how lanthanum fluoride plates functionalized with oleic acids produced two very different geometries and how her group's modeling work showed that as the aspect ratio of the plates changed, different facets became expressed more than other facets. Moreover, the model showed that the ligand binding density on the surfaces are sensitive to which facets are expressed. Thus, the aspect ratio controls the expression of facets, which controls the ligand binding density and, ultimately, the strength of the interactions between the various surfaces and the particles and shapes that emerge. This illustrates how interaction patchiness and shape control structure (Ye et al. 2013a).

The Glotzer and Murray groups were able to extend this work to understand what would happen when mixing together particles of completely different shapes, such as spheres and rods (Ye et al. 2013b). "Depending on how you tune the asymmetry of these interactions you can get anything from a fully phase-separated state to a lamellar phase, a crystal of the rods with the spheres in between to a full and proper crystal, just by subtle changes in the patchiness of the interactions around these antisymmetric particles," said Glotzer.

Typically, when looking at this type of self-assembly, enthalpy dominates, with van der Waals interactions and hydrogen bonding coming into play at nanometer and colloidal scales. Glotzer noted, though, that there is an emerging understanding that entropy and entropic bonds also can control self-assembly, and that this appreciation could lead to the ability to make even more complex mesoscale structures through self-assembly processes. As an example, she described studies that her group

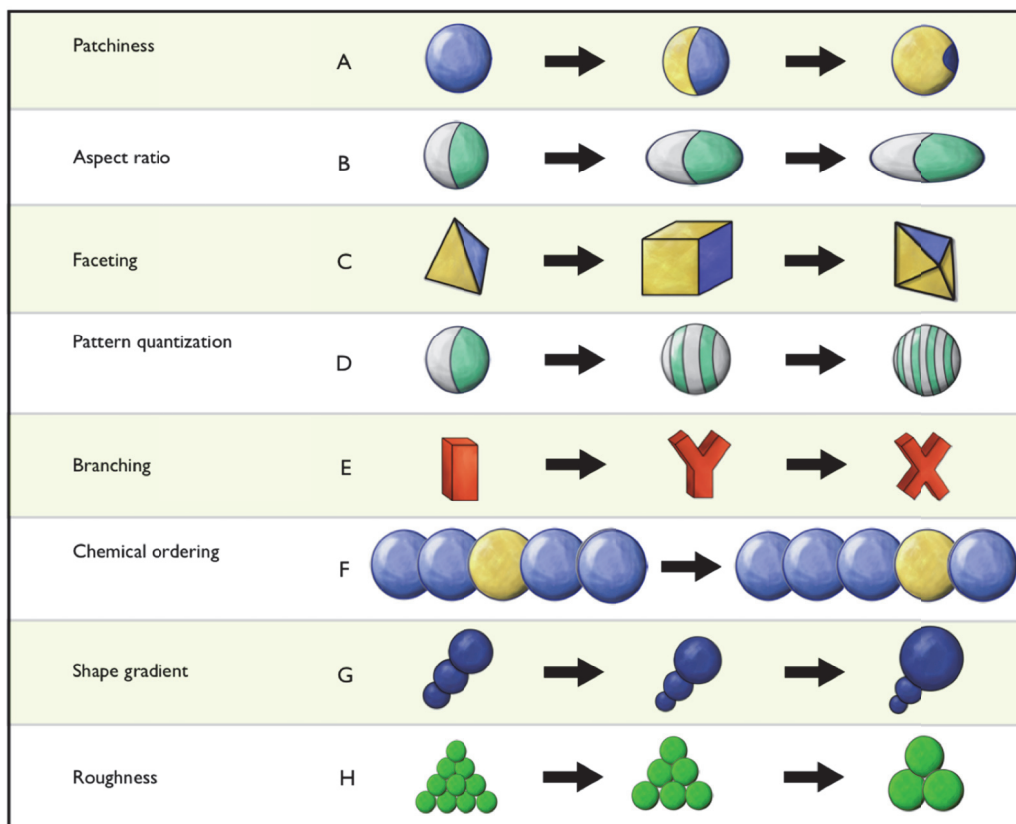


Figure 6-2 Principal component analysis reveals a nearly infinite-dimensional design space for nanoparticles. SOURCE: Glotzer and Solomon (2007). Reprinted with permission from *Nature Materials*.

conducted with cadmium telluride and cadmium selenide nanoparticles. These particles, which are tetrahedral in shape, are coated with ligands, are charged, and have dipole moments. Using a coarse-grained model, Glotzer and her collaborator were able to predict a dozen different types of structures, including sheets, wires, twisted chiral shapes, and superparticles, that they were then able to observe.

It has been known for decades, Glotzer explained, that entropy maximization can cause a disordered system to order, a principle upon which colloid physics depends. To see if entropy alone could drive structure formation, she and her collaborators modeled what would happen if they threw tetrahedrally shaped nanoparticles into a box. What emerged was a complete surprise—a dodecagonal quasicrystal of very complex structure with 82 particles in the unit cell and with no interactions emerging from the surrounding fluid (Haji-Akbari et al. 2009). This discovery, she said, turned out to be the tip of the iceberg in terms of the structures that could be generated simply by tuning

particle shape (Damasceno, Engel, and Glotzer 2012a). Glotzer said she now believes that it is possible to generate every space group observed for crystals just by tuning particle shape and relying solely on entropy (Figure 6-3), and her group is now looking into that possibility. Already, her group has generated upward of 50 different space groups including some very complex structures such as chiral beta manganese with 20 particles in a unit cell and gamma brass, with 52 particles in a unit cell from slight truncated and augmented dodecahedra.

Entropy has usually been thought of as counteracting the enthalpic driving force for self-assembly, but Glotzer's group has used potential mean force and torque modeling to quantify how much entropy is actually helping the assembly process. This method calculates the potential of mean force and torque between two particles in the middle of a sea of particles, and it can quantify how entropy maximization produces emergent effective entropic bonds that help align particles with one another (Damasceno, Engel, and Glotzer 2012b).

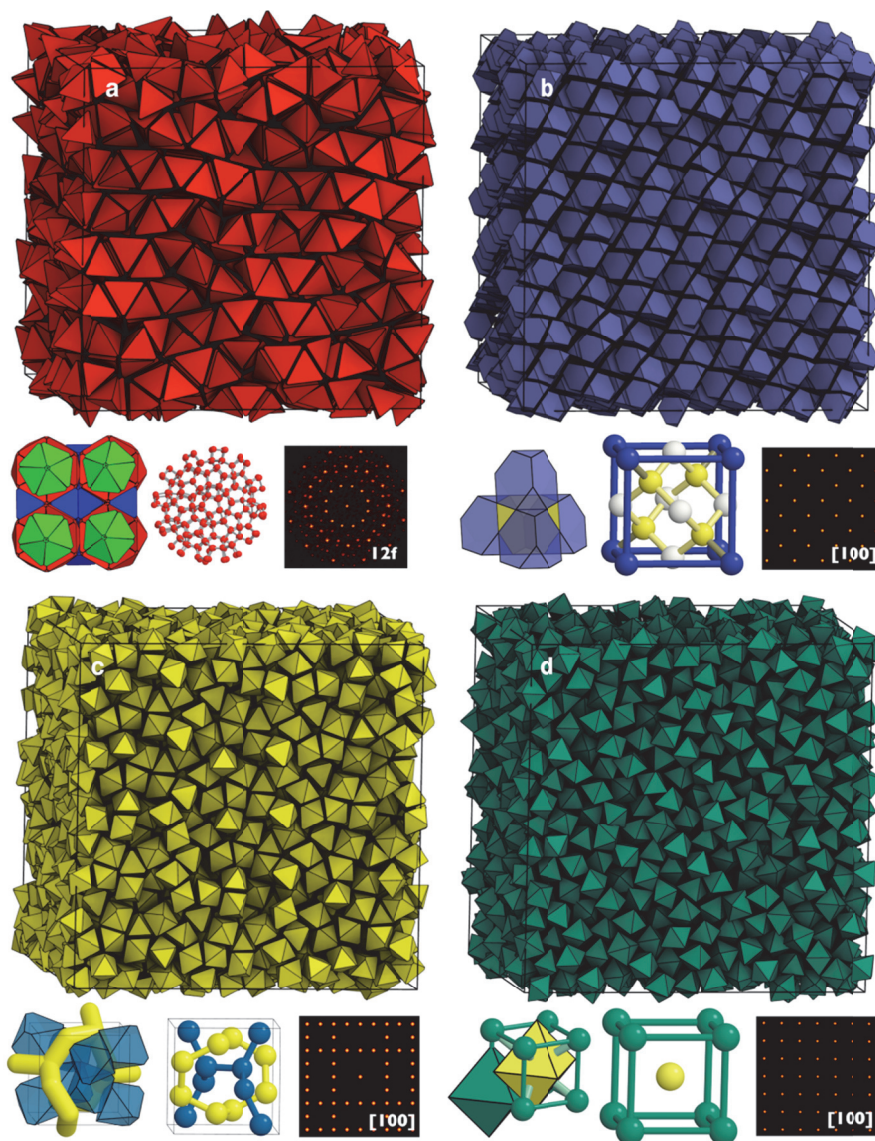


Figure 6-3 Tuning entropy by tuning shape. SOURCE: Damasceno, Engel, and Glotzer (2012a). Reprinted with permission from *ACS Nano*.

“We can now start to put these entropic bonds on the same footing as other kinds of chemical bonds and think about how to incorporate them into design rules,” said Glotzer.

The second major trend in mesoscale science identified by Glotzer is understanding the forces that are involved in self-assembly, meaning how long-range order develops from short-range forces. The goal of this work is to understand how giant, complex unit cells and other structures form with a periodicity and order well beyond the range of interparticle forces and to use that information to design for metastability. Already, Glotzer and her

collaborators have generated the first icosahedral quasicrystal ever self-assembled from a disordered fluid in a computer simulation, and what is particularly noteworthy about this achievement, she said, is that they were able to self-assemble this structure from a one-component system of spherically symmetric particles interacting with a completely isotropic pair potential that is less than three particle diameters (Engel et al. 2015). “We’re seeing for the first time that experimental capabilities and simulation capabilities for these very large systems are starting to overlap so that we can start to understand exactly how these structures are

growing and understand nucleation and growth problems,” said Glotzer.

For example, two different types of particles, rhombic dodecahedra and spheres, both self-assemble from a fluid into a face-centered cubic, but spheres make poor crystals while rhombic dodecahedra make beautiful crystals. It would be good to know why that is so, said Glotzer, and to understand the kinetic pathway that these particles are following as they self-assemble. The goals are to begin to understand why differently shaped particles will self-assemble into the same crystal structure, predict which of those shapes will make the best crystal, and determine if an assembly pathway can be designed to force the system to make that crystal.

The third major trend that Glotzer addressed is the development of functional building blocks that will not only self-assemble but that are reconfigurable. As an example, she described the self-assembly of ellipsoid Janus particles that were gold on one side and poly(methyl methacrylate) on the other side into long braid-like fibers that were predicted by computer simulation. What is interesting about these particles is that turning an electric field on and off polarizes the gold and causes the fibers to stretch, acting as a colloidal actuator that could represent a first step toward creating artificial muscle fibers (Shah et al. 2015). She also described two other examples in which polydispersed distributions of particles and mixtures of particles and proteins that first self-organize into large superparticles then self-assemble into crystals. In one case, using cadmium telluride particles mixed with cytochrome c enzymes, the transmission electron micrographs showed that the individual particles were heterogeneously mixed within the mesoscale crystals (Park et al. 2014). She noted as an aside that these crystals are photoactive and could be used to carry out chemical reactions.

She also discussed work leading to reconfigurable colloidal clusters that she has been doing with David Pine’s group at New York University. These clusters are made by mixing spherical particles with spheres that have a dimple in them, and in a painstaking set of experiments using optical tweezers, they were able to get these colloids to transition back and forth between different configurations. “If we can assemble these into regular structures that can transition from one state to another, you could store information in this system,” said Glotzer, who estimated that it could be

possible to store a terabyte of information in a teaspoon of such a colloid.

She concluded her remarks with a brief description of another project her group has started that looks at active particles and whether they will rotate with the same fixed applied torque in opposite directions when energy is pumped into the system. A constant input of energy rotates the particles and induces effective interactions among the particles that drive emergent, mesoscale phenomena.

MULTISCALE COMPUTER SIMULATION OF MESOSCALE BIOMOLECULAR COOPERATIVITY

In the workshop’s final presentation, Gregory Voth discussed the use of a full multiscale simulation hierarchy as a means of understanding membrane remodeling during endocytosis, a process that he said is not well understood at the mesoscale. There are many different pathways for endocytosis, one of which is the clathrin-mediated pathway in which a complex network of proteins cooperates to bring things into or take things out of the cell through the formation of a so-called “bud” at the membrane’s surface. This bud begins as a dent or pit in the membrane and then proceeds to encapsulate the material entering the cell and allow it to be transported within the cell. One set of proteins involved in this pathway are known as the BAR domain proteins, which resemble tiny bananas and are believed to be curvature sensors and curvature generators. As the membrane bud forms, these proteins form a scaffold upon which other proteins can build to facilitate the clipping of this bud to reform the cell wall and bring the bud into the cell.

When inserted into liposomes, BAR domain proteins such as amphiphysin and endophilin, all of which have a cluster of positively charged amino acids that interact with the negatively charged lipids of the cell membrane, can form some interesting structures that change shape (Figure 6-4) and that mesoscopic simulations can reproduce computationally (Ayton et al. 2009). The two questions of importance regarding this observation, said Voth, are whether it is possible to understand what is happening and whether they are relevant to biology. The answer to the second question, he added, is “not so relevant.”

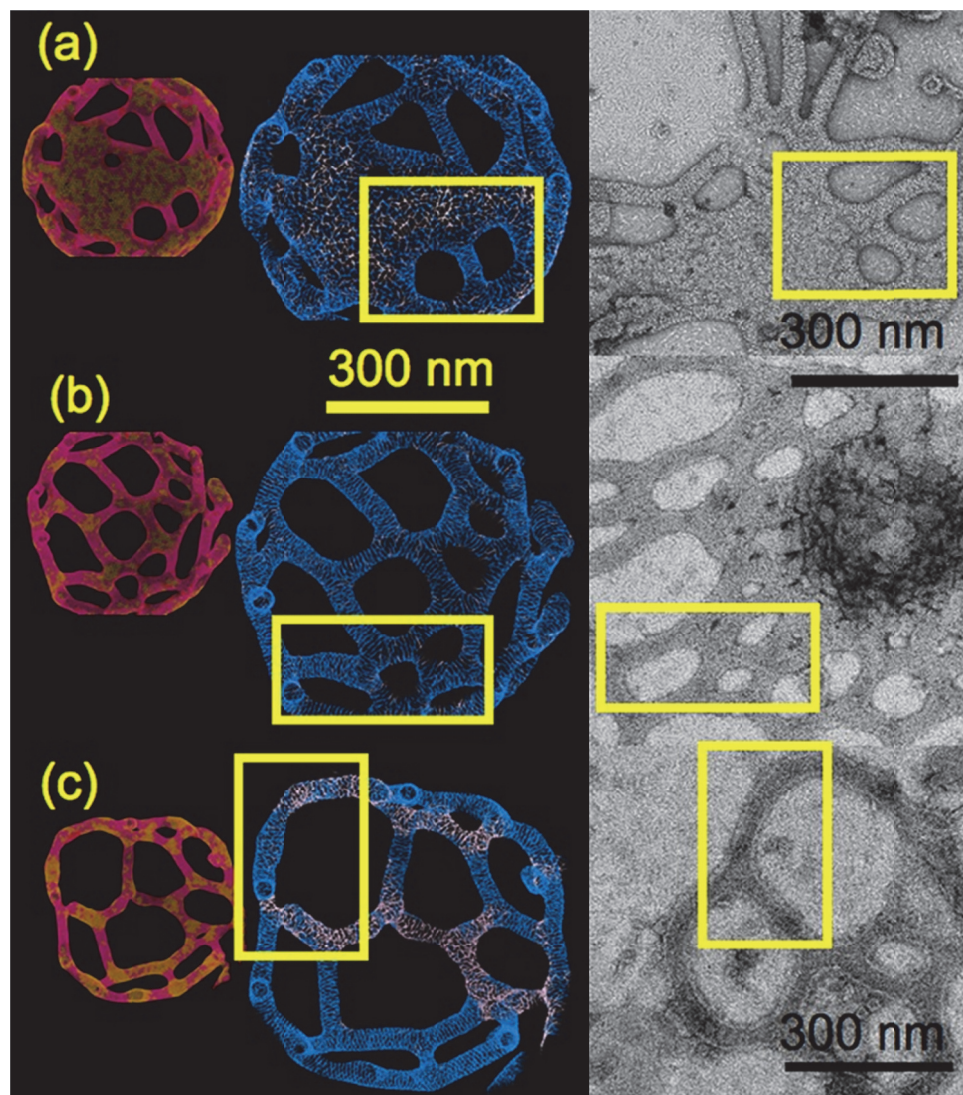


Figure 6-4 Mesoscopic simulations (left) and electron micrographic images (right) of liposomes containing BAR-domain proteins. SOURCE: Ayton et al. (2009). Reprinted with permission from *Biophysical Journal*.

Several years ago, said Voth, research on the binding of endophilin to liposomes of different sizes, and thus curvatures, found that binding affinity increased significantly as the curvature of the liposome increased. The hypothesis proposed to explain this observation was that endophilin was binding to defects that occur in the membrane as it curves, and as the curvature increased, so, too, would the number and size of the defects (Bhatia et al. 2009, Drin et al. 2007, Hatzakis et al. 2009). Voth's group calculated the free energy of binding at the molecular scale and found that there is a 5-kilocalorie-per-mole driving force for endophilin's helical structure to fold into a defect (Cui, Lyman,

and Voth 2011). "That was a critical step in our ability to model these systems, tying into atomistic scale information and building that into coarse-grained models," said Voth. A critical element of this binding, he added, are the helical elements of these proteins.

To further explore the role that these helices play in membrane interactions and remodeling, Voth's group ran simulations comparing BAR-domain proteins with their helices, called N-BAR, or without their helices, called BAR because the N-terminal helices are absent. These simulations showed that the helices are critical to the stability of the membrane-protein complex. With the BAR

proteins, the membranes are very disordered and will fall apart in the simulations. With the N-BAR proteins, the simulations produced structures with zigzag packing of the helices, which can be observed at poor resolution with cryoelectron microscopy. He noted that new techniques using single-electron detectors may help solve this resolution problem.

Additional simulations with flat membranes found that at high density—one protein molecule for every 300 lipid molecules—N-BAR forms a scaffold that induces the membranes to take on a uniform tubular curvature and develop small membrane invaginations. Further simulation work predicted that at high density, N-BAR will cause 200- to 300-nm-diameter liposomes to transform into reticulated tubular structures. A three-dimensional reconstruction of a cryoelectron micrograph tomogram confirmed the existence of a tubular network (Simunovic et al. 2013).

It turns out, Voth explained, that most of what these simulations produced can be described using a mesoscopic field theory that treats membranes as elastic and that allows the membrane to couple to an energy field provided by the proteins. This type of model couples the motion of the lipids with the motion of the proteins, as well as interactions between the proteins, and it produces the same results as the coarse-grained molecular-level model. He noted that “we’ve gotten pretty good at going from the atomistic level to the coarse-grained level, but what’s really missing is a direct map between the two.”

Examining the richness of these remodeled vesicles reveals a very rich phase diagram at high protein densities that predicts that different kinds of structures will form. These structures are modulated by the strength of protein binding to the surface of the liposome and its composition, density of protein at the surface, and other factors. One of Voth’s graduate students noticed that string-like features can form even at the initial phase of remodeling at high protein densities and that the formation of these strings is much less when the helices are missing. This led to simulations at much lower protein densities that produced a remarkably different behavior. Instead of forming lattices or even partial lattices, these long banana-like proteins formed strings. Moreover, these strings go into troughs in the membrane’s curvature in the opposite way from the high-density cases where tubules preform (Simunovic, Srivastava, and Voth 2013). In addition,

the vesicles show large deformations that appear to be responding to string formation. What appears to be happening is that proteins binding to the surface of the membrane form linear aggregates and that two lines of protein form in side-by-side interactions. The proteins then induce local invagination or welling and curvature of the membrane, a high-energy process that is compensated for in part by the binding energy of the protein.

What is most interesting about this arrangement of proteins and lipids is that it has the effect of lowering entropy, which would make the free energy of this process go in the wrong direction, Voth explained. What seems to be happening, he said, is that membrane tension modulates protein–protein interactions in a way that disfavors linear aggregation and instead causes the proteins to form strings. Preliminary experiments using atomic force microscopy have produced images with striking similarity to those predicted by coarse-grained simulations.

In conclusion, Voth said that the low-density regime, rather than high-density conditions, is consistent with experimental observations of endocytosis, with linear aggregation of protein at the initial stages of remodeling. In the high-density region, excess bending energy breaks the membrane and as a result creates tubules.

DISCUSSION

Neil Henson, from Los Alamos National Laboratory, started the discussion and asked if a fair representation of a modeling approach for simulating a mesoscale object that does chemistry would be to “first understand the chemical reaction at the atomic level, and then the barriers, thermodynamics, and kinetics, and then abstract that information into a more coarse-grained model and perhaps to a mesoscopic theory in order to understand the effect of that chemistry on the mesoscale structure.” Voth said that this is not always the case and that many successful modeling efforts start with the coarse-grained model. Glotzer said that both atomic-scale and course-scale models are needed. “If the community had waited to study nanoparticle self-assembly by first studying nucleation and growth of the nanoparticles in solution to see how they grow, and then throwing ligands in to stop the growth and built all that up, we would never have gotten to self-assembly,” said Glotzer. She noted that starting at

the coarse-grained level can work well if there are insights from experimental data or finer-scale simulations that can be used to parameterize the model.

Schulten stated that today's atomic-scale models can simulate a volume of about 1 cubic micron, or about 100 million to 1 billion atoms. Given that capability, the most straightforward approach would be to take the best force field parameters available and use those to simulate at the atomic scale and build from there. "My own principle is that I can only simplify what I understand and that it might be dangerous to go straight to coarse-graining before you really know the chemical details," said Schulten. Voth added that working at the molecular scale is challenging because of limits in sample space and free energy barriers, and Glotzer wondered what the restrictions are with regard to time scale. Schulten replied that his group simulated the entire HIV-1 viral capsid for 1.5 microseconds and that there are methods for extending these simulations for multiple milliseconds, such as replica exchange molecular dynamics and transition pair sampling. "Using smart sampling methods, you can go for many milliseconds, but if you don't know where you are heading, you are limited by a microsecond or so," said Schulten.

Session moderator Garcia-Garibay noted that one of the things about mesoscale phenomena that the field often thinks about is emergent behavior, and he asked the panel if simulation and modeling can address emergent behavior or are there behaviors at the mesoscale that cannot be described in terms of first principles, such as geometry, topology, and long- and short-range interactions. Yeates said that, at the scales at which he works, the models generate more or less exact structures, but as he moves to larger scales he expects that there will be mesoscopic behavior where what will be important is the length scale over which order persists and those will be a challenge given what is known today. He noted, though, that Glotzer's work with rigid materials is dealing with long-range order, but it is not clear whether these methods will work with soft, more flexible materials. "I think we'll find out how length scales relate to flexibility as we go to extended symmetries instead of closed symmetries," said Yeates.

Schulten said that electron microscopy is a natural tool for these longer length scales and that experimental data can inform these modeling efforts.

One of the difficulties in using microscopy, said Voth, is that they require assuming that there is some sort of symmetry present and that they are done at liquid nitrogen temperatures, which limits motion and entropy considerations among other things, and so the translation to room-temperature behavior might not be straightforward.

Chris Mundy from the Pacific Northwest National Laboratory (PNNL) asked the panelists if they were considering the role of solvent in their studies given that solvent effects at the nanometer scale can be significant. Voth said that coarse-grained models of the sort that his and Glotzer's groups run have an implicit solvent represented as hydrophobic associations that have to be estimated. "You have to build in the physics," said Voth. Glotzer added that her approach has been to first do fully atomistic simulations with water present and gain insights from those models to feed into the coarse-grained models, an approach that Schulten agreed is a good one for including solvent effects in larger-scale models.

Patricia Thiel asked if there is a connection between these modeling studies and what is happening in biomineralization. Glotzer said there is an absolute link because these modeling efforts are asking the exact same questions: How do these assemblies grow, how does nucleation occur, and where do these processes lead once crystal growth starts? Jim De Yoreo then asked how these modeling approaches apply to biomineralization given that they are run at very high densities and natural systems operating at low volume fractions. Glotzer acknowledged that her work, for example, will not explain what is happening initially at low density, but once interactions start happening and molecules or smaller structures start aggregating, factors such as entropy can explain phenomena seen in the mesoscale.

Garcia-Garibay then asked the panel to comment on the idea that Paul Weiss put forth in the workshop's plenary address that many things at this scale are metastable, that they are constantly in flux. Glotzer said that a major trend in mesoscale modeling now is to design for metastability, but that in fact, little is known about metastability on the mesoscale and how to guide assembly down a path to metastability instead of the globally favored stable energy state. She added that the active matter work that she mentioned briefly is all about dealing with systems that are never in thermodynamic

equilibrium and that are always driven to some steady state that is not the low-energy stable state. The big question there is whether it is possible to map that state to a thermodynamic equilibrium-like system that has a free energy function. “Is there a function that can describe your active system so that you can treat it as though it’s thermodynamic?” she asked. That is an open question that she predicted will be answered in the next 10 years.

Schulten remarked that living systems are metastable for a good reason, which is that the result is a more robust system. “Life is not stable, life is changing, and you have to adjust to the circumstances. That is why these systems are never in a very rigid state, because they have to be in different states,” said Schulten. During repair, for example, structures are being disassembled and rebuilt, usually starting from some existing surface that can guide reassembly. “Metastability is a principle of adaptability and robustness,” he added.

Bruce Garrett from PNNL asked the panel to comment on the importance of entropy in these systems, and Voth said that entropy is very important. “I think a lot of current thinking in biology is driven too much by structural biology, which is ordered, but biology is driven by free energy, not equilibrium, so why wouldn’t biology utilize entropy?” said Voth. “What we’re increasingly finding is that proteins may be driven to bind because some part of them becomes disordered

and so it’s free energy that’s facilitating that behavior.” Glotzer remarked that entropy is a global concept that cannot be understood locally. By definition, it is an emergent property of a system, she said, and understanding the role of entropy is a terrific opportunity. “We don’t know yet how to design to exploit entropy to give us the structures that we want,” said Glotzer.

Noid commented that, as you change the resolution of the model, what you’re effectively doing is you’re taking configurational entropy out of the high-resolution model and you’re transferring it into the effect of interactions of the low-resolution model. And so, depending upon the level of resolution that you look at and describe your system with, your interactions become increasingly entropic and decreasingly energetic; you can show that very simply and mathematically.” He added that there are cases where there are general design principles that are well understood with regard to how entropy drives those processes, but in terms of predicting the role of entropy in driving most systems, that is still a challenging prospect. Schulten made the final comment, which was that in protein folding there is a very subtle balance between entropy and enthalpy that can be shifted by very subtle factors. As an example, he cited the case of blood clotting, which is triggered by a conformational change in protein structure triggered by small shifts in blood flow.

References

- Ayton, G. S., E. Lyman, V. Krishna, R. D. Swenson, C. Mim, V. M. Unger, and G. A. Voth. 2009. "New insights into BAR domain-induced membrane remodeling." *Biophys. J.* 97(6):1616-1625.
- BESAC Subcommittee on Mesoscale Science. 2012. *From Quanta to the Continuum: Opportunities for Mesoscale Science*. Washington, DC: U.S. Department of Energy.
- Besser, R. S. 2011. "Thermal integration of a cylindrically symmetric methanol fuel processor for portable fuel cell power." *Int. J. Hydrogen Energy* 36(1):276-283.
- Bewernitz, M. A., D. Gebauer, J. Long, H. Colfen, and L. B. Gower. 2012. "A metastable liquid precursor phase of calcium carbonate and its interactions with polyaspartate." *Faraday Discuss. R. Soc. Chem.* 159:291-312.
- Bhagi-Damodaran, A., I. D. Petrik, N. M. Marshall, H. Robinson, and Y. Lu. 2014. "Systematic tuning of heme redox potentials and its effects on O₂ reduction rates in a designed oxidase in myoglobin." *J. Am. Chem. Soc.* 136(34):11882-11885.
- Bhatia, V. K., K. L. Madsen, P. Y. Bolinger, A. Kunding, P. Hedegard, U. Gether, and D. Stamou. 2009. "Amphipathic motifs in BAR domains are essential for membrane curvature sensing." *EMBO J.* 28(21):3303-3314.
- Blomberg, M. R., P. E. Siegbahn, and M. Wikstrom. 2003. "Metal-bridging mechanism for O-O bond cleavage in cytochrome C oxidase." *Inorg. Chem.* 42(17):5231-5243.
- Canlas, C. P., J. Lu, N. A. Ray, N. A. Grosso-Giordano, S. Lee, J. W. Elam, R. E. Winans, R. P. Van Duyne, P. C. Stair, and J. M. Notestein. 2012. "Shape-selective sieving layers on an oxide catalyst surface." *Nat. Chem.* 4(12):1030-1036.
- Chang, E. P., J. A. Russ, A. Verch, R. Kröger, L. A. Estroff, and J. S. Evans. 2014a. "Engineering of crystal surfaces and subsurfaces by framework biomineralization protein phases." *CrystEngComm* 16(32):7406-7409.
- Chang, E. P., J. A. Russ, A. Verch, R. Kröger, L. A. Estroff, and J. S. Evans. 2014b. "The intrinsically disordered C-RING biomineralization protein, AP7, creates protein phases that introduce nanopatterning and nanoporosities into mineral crystals." *Biochemistry* 53(27):4317-4319.
- Chung, S., S.-H. Shin, C. R. Bertozzi, and J. J. De Yoreo. 2010. "Self-catalyzed growth of S layers via an amorphous-to-crystalline transition limited by folding kinetics." *Proc. Natl. Acad. Sci. U. S. A.* 107(38):16536-16541.
- Claridge, S. A., A. W. Castleman, Jr., S. N. Khanna, C. B. Murray, A. Sen, and P. S. Weiss. 2009. "Cluster-assembled materials." *ACS Nano* 3(2):244-255.

- Collman, J. P., N. K. Devaraj, R. A. Decréau, Y. Yang, Y.-L. Yan, W. Ebina, T. A. Eberspacher, and C. E. D. Chidsey. 2007. "A cytochrome C oxidase model catalyzes oxygen to water reduction under rate-limiting electron flux." *Science* 315(5818):1565-1568.
- Cook, S. A., and A. S. Borovik. 2013. "Inorganic chemistry: Deconstructing water oxidation." *Nat. Chem.* 5(4):259-260.
- Cui, H., E. Lyman, and G. A. Voth. 2011. "Mechanism of membrane curvature sensing by amphipathic helix containing proteins." *Biophys. J.* 100(5):1271-1279.
- Damasceno, P. F., M. Engel, and S. C. Glotzer. 2012a. "Crystalline assemblies and densest packings of a family of truncated tetrahedra and the role of directional entropic forces." *ACS Nano* 6(1):609-614.
- Damasceno, P. F., M. Engel, and S. C. Glotzer. 2012b. "Predictive self-assembly of polyhedra into complex structures." *Science* 337(6093):453-457.
- Dameron, A. A., J. R. Hampton, R. K. Smith, T. J. Mullen, S. D. Gillmor, and P. S. Weiss. 2005. "Microdisplacement printing." *Nano Lett.* 5(9):1834-1837.
- De Yoreo, J. 2014. "The Dynamics of Macromolecular Assembly and Subsequent Mineral Formation: Understanding the Emergence of Order." Presented at the Chemical Sciences Roundtable Workshop on Mesoscale Chemistry, Washington, DC, November 6-7, 2014.
- Dogutan, D. K., S. A. Stojan, R. McGuire, M. Schwalbe, T. S. Teets, and D. G. Nocera. 2010. "Hangman corroles: Efficient synthesis and oxygen reaction chemistry." *J. Am. Chem. Soc.* 133(1):131-140.
- Drin, G., J. F. Casella, R. Gautier, T. Boehmer, T. U. Schwartz, and B. Antony. 2007. "A general amphipathic alpha-helical motif for sensing membrane curvature." *Nat. Struct. Mol. Biol.* 14(2):138-146.
- DuBois, D. L. 2014. "Development of molecular electrocatalysts for energy storage." *Inorg. Chem.* 53(8):3935-3960.
- Dutta, A., S. Lense, J. Hou, M. H. Engelhard, J. A. S. Roberts, and W. J. Shaw. 2013. "Minimal proton channel enables H₂ oxidation and production with a water-soluble nickel-based catalyst." *J. Am. Chem. Soc.* 135 (49):18490-18496.
- Dutta, A., J. A. S. Roberts, and W. J. Shaw. 2014. "Arginine-containing ligands enhance H₂ oxidation catalyst performance." *Angew. Chem., Int. Ed.* 53(25):6487-6491.
- Eggins, S. M., A. Sadekov, and P. De Deckker. 2004. "Modulation and daily banding of Mg/Ca in *Orbulina universa* tests by symbiont photosynthesis and respiration: A complication for seawater thermometry?" *Earth Planet. Sci. Lett.* 225(3-4):411-419.
- Engel, M., P. F. Damasceno, C. L. Phillips, and S. C. Glotzer. 2015. "Computational self-assembly of a one-component icosahedral quasicrystal." *Nat. Mater.* 14(1):109-116.
- Evans, J.S. 2014. "Slouching Toward the Mesoscale: Nucleation Events, Mineral Assembly, and Crystal Modifications Performed by Disordered Proteins." Presented at the Chemical Sciences Roundtable Workshop on Mesoscale Chemistry, Washington, DC, November 6-7, 2014.
- Farver, O., N. M. Marshall, S. Wherland, Y. Lu, and I. Pecht. 2013. "Designed azurins show lower reorganization free energies for intraprotein electron transfer." *Proc. Natl. Acad. Sci. U. S. A.* 110(26):10536-10540.
- Feigenson, G. W., and J. T. Buboltz. 2001. "Ternary phase diagram of dipalmitoyl-PC/dilauroyl-PC/cholesterol: Nanoscopic domain formation driven by cholesterol." *Biophys. J.* 80(6):2775-2788.

REFERENCES

- Gagnon, A. 2014. "Small Compositional Signals With a Big Impacts: From Sub-Micron Geochemical Heterogeneity to Climate Records." Presented at the Chemical Sciences Roundtable Workshop on Mesoscale Chemistry, Washington, DC, November 6-7, 2014.
- Ginovska-Pangovska, B., M. H. Ho, J. C. Linehan, Y. Cheng, M. Dupuis, S. Raugei, and W. J. Shaw. 2014. "Molecular dynamics study of the proposed proton transport pathways in [FeFe]-hydrogenase." *Biochim. Biophys. Acta* 1837(1):131-138.
- Glotzer, S. C., and M. J. Solomon. 2007. "Anisotropy of building blocks and their assembly into complex structures." *Nat. Mater.* 6(8):557-562.
- Gong, Y. U. T., C. E. Killian, I. C. Olson, N. P. Appathurai, A. L. Amasino, M. C. Martin, L. J. Holt, F. H. Wilt, and P. U. P. A. Gilbert. 2012. "Phase transitions in biogenic amorphous calcium carbonate." *Proc. Natl. Acad. Sci. U. S. A.* 109(16):6088-6093.
- Haji-Akbari, A., M. Engel, A. S. Keys, X. Zheng, R. G. Petschek, P. Palffy-Muhoray, and S. C. Glotzer. 2009. "Disordered, quasicrystalline and crystalline phases of densely packed tetrahedra." *Nature* 462(7274):773-777.
- Han, D., S. Pal, Y. Yang, S. Jiang, J. Nangreave, Y. Liu, and H. Yan. 2013. "DNA gridiron nanostructures based on four-arm junctions." *Science* 339(6126):1412-1415.
- Hatzakis, N. S., V. K. Bhatia, J. Larsen, K. L. Madsen, P. Y. Bolinger, A. H. Kunding, J. Castillo, U. Gether, P. Hedegard, and D. Stamou. 2009. "How curved membranes recruit amphipathic helices and protein anchoring motifs." *Nat. Chem. Biol.* 5(11):835-841.
- Hohman, J. N., P. Zhang, E. I. Morin, P. Han, M. Kim, A. R. Kurland, P. D. McClanahan, V. P. Balema, and P. S. Weiss. 2009. "Self-assembly of carboranethiol isomers on Au111: Intermolecular interactions determined by molecular dipole orientations." *ACS Nano* 3(3):527-536.
- Hong, S., J. Zhu, and C. A. Mirkin. 1999. "Multiple ink nanolithography: Toward a multiple-Pen nanoplotter." *Science* 286(5439):523-525.
- Hwang, H. J., and Y. Lu. 2004. "pH-dependent transition between delocalized and trapped valence states of a CuA center and its possible role in proton-coupled electron transfer." *Proc. Natl. Acad. Sci. U. S. A.* 101(35):12842-12847.
- Kandel, K., S. M. Althaus, C. Peeraphatdit, T. Kobayashi, B. G. Trewyn, M. Pruski, and I. I. Slowing. 2013a. "Solvent-induced reversal of activities between two closely related heterogeneous catalysts in the aldol reaction." *ACS Catal.* 3(2):265-271.
- Kandel, K., S. M. Althaus, M. Pruski, and I. I. Slowing. 2013b. "Supported hybrid enzyme-organocatalysts for upgrading the carbon content of alcohols." Pp. 261-271 in *Novel Materials for Catalysis and Fuels Processing*. Washington, DC: American Chemical Society.
- Ke, Y., L. L. Ong, W. M. Shih, and P. Yin. 2012. "Three-dimensional structures self-assembled from DNA bricks." *Science* 338(6111):1177-1183.
- Kim, D., D. Donohue, B. Kuncharam, C. Duval, and B. A. Wilhite. 2010. "Toward an integrated ceramic micro-membrane network: Effect of ethanol reformat on palladium membranes." *Ind. Eng. Chem. Res.* 49(21):10254-10261.
- Kim, D., P. Tzeng, K. J. Barnett, Y.-H. Yang, B. A. Wilhite, and J. C. Grunlan. 2014. "Highly size-selective ionically crosslinked multilayer polymer films for light gas separation." *Adv. Mater.* 26(5):746-751.
- Kim, J., Y. S. Rim, Y. Liu, A. C. Serino, J. C. Thomas, H. Chen, Y. Yang, and P. S. Weiss. 2014. "Interface control in organic electronics using mixed monolayers of carboranethiol isomers." *Nano Lett.* 14(5):2946-2951.

- King, N. P., W. Sheffler, M. R. Sawaya, B. S. Vollmar, J. P. Sumida, I. Andre, T. Gonen, T. O. Yeates, and D. Baker. 2012. "Computational design of self-assembling protein nanomaterials with atomic level accuracy." *Science* 336(6085):1171-1174.
- Kjaergaard, C. H., J. Rossmeis, and J. K. Norskov. 2010. "Enzymatic versus inorganic oxygen reduction catalysts: Comparison of the energy levels in a free-energy scheme." *Inorg. Chem.* 49(8):3567-3572.
- Kumar, A., N. L. Abbott, H. A. Biebuyck, E. Kim, and G. M. Whitesides. 1995. "Patterned self-assembled monolayers and meso-scale phenomena." *Acc. Chem. Res.* 28(5):219-226.
- Lai, Y. T., D. Cascio, and T. O. Yeates. 2012. "Structure of a 16-nm cage designed by using protein oligomers." *Science* 336(6085):1129.
- Lai, Y. T., N. P. King, and T. O. Yeates. 2012. "Principles for designing ordered protein assemblies." *Trends Cell Biol.* 22(12):653-661.
- Lai, Y. T., E. Reading, G. L. Hura, K. L. Tsai, A. Laganowsky, F. J. Asturias, J. A. Tainer, C. V. Robinson, and T. O. Yeates. 2014. "Structure of a designed protein cage that self-assembles into a highly porous cube." *Nat. Chem.* 6(12):1065-1071.
- Learman, D. R., S. D. Wankel, S. M. Webb, N. Martinez, A. S. Madden, and C. M. Hansel. 2011. "Coupled biotic–abiotic Mn(II) oxidation pathway mediates the formation and structural evolution of biogenic Mn oxides." *Geochim. Cosmochim. Acta* 75(20):6048-6063.
- Lense, S., A. Dutta, J. A. S. Roberts, and W. J. Shaw. 2014. "A proton channel allows a hydrogen oxidation catalyst to operate at a moderate overpotential with water acting as a base." *Chem. Commun.* 50(7):792-795.
- Lewis, E. A., D. Le, A. D. Jewell, C. J. Murphy, T. S. Rahman, and E. C. Sykes. 2013. "Visualization of compression and spillover in a coadsorbed system: Syngas on cobalt nanoparticles." *ACS Nano* 7(5):4384-92.
- Li, D., M. H. Nielsen, J. R. Lee, C. Frandsen, J. F. Banfield, and J. J. De Yoreo. 2012. "Direction-specific interactions control crystal growth by oriented attachment." *Science* 336(6084):1014-1018.
- Liao, W. S., S. Cheunkar, H. H. Cao, H. R. Bednar, P. S. Weiss, and A. M. Andrews. 2012. "Subtractive patterning via chemical lift-off lithography." *Science* 337(6101):1517-1521.
- Liu, Y., Y. Deng, P. Zhang, Z. Liu, and Y. Wu. 2013. "Experimental investigation of passive micromixers conceptual design using the layout optimization method." *J. Micromech. Microeng.* 23(7):075002.
- London, E. 2014. "Asymmetric Lipid Vesicles Formation and Properties." Presented at the Chemical Sciences Roundtable Workshop on Mesoscale Chemistry, Washington, DC, November 6-7, 2014.
- Love, J. C., D. B. Wolfe, M. L. Chabinyc, K. E. Paul, and G. M. Whitesides. 2002. "Self-assembled monolayers of alkanethiolates on palladium are good etch resists." *J. Am. Chem. Soc.* 124(8):1576-1577.
- Lu, J., and P. C. Stair. 2010. "Low-temperature ABC-type atomic layer deposition: Synthesis of highly uniform ultrafine supported metal nanoparticles." *Angew. Chem. Int. Ed. Engl.* 49(14):2547-2551.
- Lu, J., K. B. Low, Y. Lei, J. A. Libera, A. Nicholls, P. C. Stair, and J. W. Elam. 2014. "Toward atomically-precise synthesis of supported bimetallic nanoparticles using atomic layer deposition." *Nat. Commun.* 5:3264.
- Lu, Y., N. Yeung, N. Sieracki, and N. M. Marshall. 2009. "Design of functional metalloproteins." *Nature* 460(7257):855-862.

REFERENCES

- Ma, K.-F., H. Tanaka, S.-R. Song, C.-Y. Wang, J.-H. Hung, Y.-B. Tsai, J. Mori, Y.-F. Song, E.-C. Yeh, W. Soh, H. Sone, L.-W. Kuo, and H.-Y. Wu. 2006. "Slip zone and energetics of a large earthquake from the Taiwan Chelungpu-fault Drilling Project." *Nature* 444(7118):473-476.
- Madden, A. 2014. "Mesoscale Geochemistry Research Opportunities On, Within, and Beyond Earth." Presented at the Chemical Sciences Roundtable Workshop on Mesoscale Chemistry, Washington, DC, November 6-7, 2014.
- Marshall, N. M., D. K. Garner, T. D. Wilson, Y. G. Gao, H. Robinson, M. J. Nilges, and Y. Lu. 2009. "Rationally tuning the reduction potential of a single cupredoxin beyond the natural range." *Nature* 462(7269):113-116.
- McCullagh, M., and G. A. Voth. 2013. "Unraveling the role of the protein environment for [FeFe]-hydrogenase: A new application of coarse-graining." *J. Phys. Chem. B* 117(15):4062-4071.
- Miner, K. D., A. Mukherjee, Y. G. Gao, E. L. Null, I. D. Petrik, X. Zhao, N. Yeung, H. Robinson, and Y. Lu. 2012. "A designed functional metalloenzyme that reduces O₂ to H₂O with over one thousand turnovers." *Angew. Chem. Int. Ed. Engl.* 51(23):5589-5592.
- Moreno, A. M., S. Damodharan, and B. Wilhite. 2010. "Influence of two-dimensional distribution schemes upon reactor performance in a ceramic microchannel network for autothermal methanol reforming." *Ind. Eng. Chem. Res.* 49(21):10956-10964.
- Nagasaka, M., H. Kondoh, I. Nakai, and T. Ohta. 2007. "CO oxidation reaction on Pt(111) studied by the dynamic Monte Carlo method including lateral interactions of adsorbates." *J. Chem. Phys.* 126(4):044704.
- Nielsen, M. H., D. Li, H. Zhang, S. Aloni, T. Y.-J. Han, C. Frandsen, J. Seto, J. F. Banfield, H. Cölfen, and J. J. De Yoreo. 2014. "Investigating processes of nanocrystal formation and transformation via liquid cell TEM." *Microsc. Microanal.* 20(2):425-436.
- Olson, I. C., A. Z. Blonsky, N. Tamura, M. Kunz, B. Pokroy, C. P. Romao, M. A. White, and P. U. Gilbert. 2013. "Crystal nucleation and near-epitaxial growth in nacre." *J. Struct. Biol.* 184(3):454-463.
- Padilla, J. E., C. Colovos, and T. O. Yeates. 2001. "Nanohedra: Using symmetry to design self assembling protein cages, layers, crystals, and filaments." *Proc. Natl. Acad. Sci. U. S. A.* 98(5):2217-2221.
- Park, J. I., T. D. Nguyen, G. de Queiros Silveira, J. H. Bahng, S. Srivastava, G. Zhao, K. Sun, P. Zhang, S. C. Glotzer, and N. A. Kotov. 2014. "Terminal supraparticle assemblies from similarly charged protein molecules and nanoparticles." *Nat. Commun.* 5:3593.
- Perovic, I., E. P. Chang, M. Lui, A. Rao, H. Cölfen, and J. S. Evans. 2014a. "A nacre protein, n16.3, self-assembles to form protein oligomers that dimensionally limit and organize mineral deposits." *Biochemistry* 53(16):2739-2748.
- Perovic, I., A. Verch, E. P. Chang, A. Rao, H. Cölfen, R. Kröger, and J. S. Evans. 2014b. "An oligomeric C-RING nacre protein influences prenucleation events and organizes mineral nanoparticles." *Biochemistry* 53(46):7259-7268.
- Piccinin, S., and M. Stamatakis. 2014. "CO oxidation on Pd(111): A first-principles-based kinetic Monte Carlo study." *ACS Catal.* 4(7):2143-2152.
- Politi, Y., R. A. Metzler, M. Abrecht, B. Gilbert, F. H. Wilt, I. Sagi, L. Addadi, S. Weiner, and P. U. Gilbert. 2008. "Transformation mechanism of amorphous calcium carbonate into calcite in the sea urchin larval spicule." *Proc. Natl. Acad. Sci. U. S. A.* 105(45):17362-17366.
- Radha, A. V., T. Z. Forbes, C. E. Killian, P. U. Gilbert, and A. Navrotsky. 2010. "Transformation and crystallization energetics of synthetic and biogenic amorphous calcium carbonate." *Proc. Natl. Acad. Sci. U. S. A.* 107(38):16438-16443.

- Reches, Z., and D. A. Lockner. 2010. "Fault weakening and earthquake instability by powder lubrication." *Nature* 467(7314):452-455.
- Rothmund, P. W. 2006. "Folding DNA to create nanoscale shapes and patterns." *Nature* 440(7082):297-302.
- Seeman, N. C. 2010. "Nanomaterials based on DNA." *Annu. Rev. Biochem.* 79:65-87.
- Shah, A. A., B. Schultz, W. Zhang, S. C. Glotzer, and M. J. Solomon. 2015. Actuation of shape-memory colloidal fibres of Janus ellipsoids. *Nat. Mater.* 14(1):117-124.
- Sharma, A. C., and A. S. Borovik. 2000. "Design, synthesis, and characterization of templated metal sites in porous organic hosts: Application to reversible dioxygen binding." *J. Am. Chem. Soc.* 122(37):8946-8955.
- Sigman, J. A., H. K. Kim, X. Zhao, J. R. Carey, and Y. Lu. 2003. "The role of copper and protons in heme-copper oxidases: Kinetic study of an engineered heme-copper center in myoglobin." *Proc. Natl. Acad. Sci. U. S. A.* 100(7):3629-3634.
- Simunovic, M., C. Mim, T. C. Marlovits, G. Resch, V. M. Unger, and G. A. Voth. 2013. "Protein-mediated transformation of lipid vesicles into tubular networks." *Biophys. J.* 105(3):711-719.
- Simunovic, M., A. Srivastava, and G. A. Voth. 2013. "Linear aggregation of proteins on the membrane as a prelude to membrane remodeling." *Proc. Natl. Acad. Sci. U. S. A.* 110(51):20396-20401.
- Spero, H. J. 1988. "Ultrastructural examination of chamber morphogenesis and biomineralization in the planktonic foraminifer *Orbulina universa*." *Mar. Biol.* 99(1):9-20.
- Srinivasan, R., I. M. Hsing, P. E. Berger, K. F. Jensen, S. L. Firebaugh, M. A. Schmidt, M. P. Harold, J. J. Lerou, and J. F. Ryley. 1997. "Micromachined reactors for catalytic partial oxidation reactions." *AIChE J.* 43(11):3059-3069.
- Stair, P. 2014. "Strategies for the Synthesis of Mesoscale Catalyst Designs." Presented at the Chemical Sciences Roundtable Workshop on Mesoscale Chemistry, Washington, DC, November 6-7, 2014.
- Stroock, A. D., and G. M. Whitesides. 2003. "Controlling flows in microchannels with patterned surface charge and topography." *Acc. Chem. Res.* 36(8):597-604.
- Swindle, A. L., A. S. Elwood Madden, I. M. Cozzarelli, and M. Benamara. 2014. "Size-dependent reactivity of magnetite nanoparticles: A field-laboratory comparison." *Environ. Sci. Technol.* 48(19):11413-11420.
- Tan, L. H., H. Xing, and Y. Lu. 2014. "DNA as a powerful tool for morphology control, spatial positioning, and dynamic assembly of nanoparticles." *Acc. Chem. Res.* 47(6):1881-1890.
- Umena, Y., K. Kawakami, J.-R. Shen, and N. Kamiya. 2011. "Crystal structure of oxygen-evolving photosystem II at a resolution of 1.9 Å." *Nature* 473(7345):55-60.
- Veatch, S. L., and S. L. Keller. 2003. "Separation of liquid phases in giant vesicles of ternary mixtures of phospholipids and cholesterol." *Biophys. J.* 85(5):3074-3083.
- Veis, A. 1990. "Biogenic Minerals. Review of *Biomineralization: Cell Biology and Mineral Deposition* by Kenneth Simkiss and Karl M. Wilbur. Academic Press, San Diego, CA, 1989.; and *On Biomineralization*. Heinz A. Lowenstam and Stephen Weiner. Oxford University Press, New York, 1989." *Science* 247(4946):1129-1130.
- Wallace, A. F., L. O. Hedges, A. Fernandez-Martinez, P. Raiteri, J. D. Gale, G. A. Waychunas, S. Whitlam, J. F. Banfield, and J. J. De Yoreo. 2013. "Microscopic evidence for liquid-liquid separation in supersaturated CaCO₃ solutions." *Science* 341(6148):885-889.
- Wang, C.-J., D. M. Ackerman, I. I. Slowing, and J. W. Evans. 2014. "Langevin and Fokker-Planck analyses of inhibited molecular passing processes controlling transport and reactivity in nanoporous materials." *Phys. Rev. Lett.* 113(3):038301.

- Weiss, P. S. 2008. "Functional molecules and assemblies in controlled environments: Formation and measurements." *Acc. Chem. Res.* 41(12):1772-1781.
- Wilhite, B. A., K. F. Jensen, T. F. Hill, L. F. Velásquez-García, A. H. Epstein, and C. Livermore. 2008. "Design of a silicon-based microscale trickle-bed system for singlet-oxygen production." *AIChE J.* 54(9):2441-2455.
- Wilhite, B. A., M. A. Schmidt, and K. F. Jensen. 2004. "Palladium-based micromembranes for hydrogen separation: Device performance and chemical stability." *Ind. Eng. Chem. Res.* 43(22):7083-7091.
- Woertink, J. S., P. J. Smeets, M. H. Groothaert, M. A. Vance, B. F. Sels, R. A. Schoonheydt, and E. I. Solomon. 2009. "A $[\text{Cu}_2\text{O}]^{2+}$ core in Cu-ZSM-5, the active site in the oxidation of methane to methanol." *Proc. Natl. Acad. Sci. U. S. A.* 106(45):18908-18913.
- Wukovitz, S. W., and T. O. Yeates. 1995. "Why protein crystals favour some space-groups over others." *Nat. Struct. Biol.* 2(12):1062-1067.
- Ye, X., J. Chen, M. Engel, J. A. Millan, W. Li, L. Qi, G. Xing, J. E. Collins, C. R. Kagan, J. Li, S. C. Glotzer, and C. B. Murray. 2013a. "Competition of shape and interaction patchiness for self-assembling nanoplates." *Nat. Chem.* 5(6):466-473.
- Ye, X., J. A. Millan, M. Engel, J. Chen, B. T. Diroll, S. C. Glotzer, and C. B. Murray. 2013b. "Shape alloys of nanorods and nanospheres from self-assembly." *Nano Lett.* 13(10):4980-4988. doi: 10.1021/nl403149u.
- Zhao, G., J. R. Perilla, E. L. Yufenyuy, X. Meng, B. Chen, J. Ning, J. Ahn, A. M. Gronenborn, K. Schulten, C. Aiken, and P. Zhang. 2013. "Mature HIV-1 capsid structure by cryo-electron microscopy and all-atom molecular dynamics." *Nature* 497(7451):643-646.
- Zheng, Y. B., B. K. Pathem, J. N. Hohman, J. C. Thomas, M. Kim, and P. S. Weiss. 2013. "Photoresponsive molecules in well-defined nanoscale environments." *Adv. Mater.* 25(3):302-312.

Appendix A

Workshop Agenda

Mesoscale Chemistry

A Workshop by the Chemical Sciences Roundtable
November 6-7, 2014—Washington, DC

November 6, 2014

12:30-12:40 **Introduction to Workshop and Topic**
Speaker: Jennifer Curtis

Session 1: Overview

Chair: Jennifer Curtis

12:40-1:20 **Growing (Up) from the Nanoscale to the Mesoscale**
Speaker: Paul Weiss

Session 2: Catalysis

Chair: Bruce Garrett

1:20-1:40 **Architectural Complexity at the Molecular Level**
Speaker: Andrew Borovik

1:40-2:00 **Bringing Mesoscale Properties to Molecular Catalysts by Attaching an Outer Coordination Sphere**
Speaker: Wendy Shaw

2:00-2:20 **Elucidating Key Features of Mesoscale Biocatalytic Systems and Employing Them in Designing Functional Artificial Enzymes with High Activity**
Speaker: Yi Lu

2:20-2:40 **Mesoscale Concepts for Design and Behavior in Heterogeneous Catalysis**
Speaker: Cynthia Jenks

2:40-3:00 **Strategies for the Synthesis of Mesoscale Catalyst Designs**
Speaker: Peter Stair

3:00-3:30 Panel Discussion

3:30-3:40 Break

Session 3: Membrane Behavior/Microchemical Systems

Chair: Vernon Anderson

- 3:40-4:00 **Asymmetric Lipid Vesicles Formation and Properties**
Speaker: Erwin London
- 4:00-4:20 **Digital Microfluidics: Not Just for Liquids Anymore**
Speaker: Aaron Wheeler
- 4:20-4:40 **Engineering at the Mesoscale: Microreactors and Membranes**
Speaker: Benjamin Wilhite
- 4:40-5:10 Panel Discussion
- 5:10 Workshop Day 1 Adjourns

*November 7, 2014***Session 1: Biomineralization/Geochemical**

Chair: Patricia Thiel

- 8:45-9:05 **Formation Pathways in Biominerals Revealed by Their Structure**
Speaker: Pupa Gilbert
- 9:05-9:25 **Slouching Toward the Mesoscale: Nucleation Events, Mineral Assembly, and Crystal Modifications Performed by Disordered Proteins**
Speaker: John Spencer Evans
- 9:25-9:45 **The Dynamics of Macromolecular Assembly and Subsequent Mineral Formation: Understanding the Emergence of Order**
Speaker: Jim De Yoreo
- 9:45-10:05 **Small Compositional Signals with a Big Impact: From Sub-Micron Geochemical Heterogeneity to Climate Records**
Speaker: Alex Gagnon
- 10:05-10:25 **Mesoscale Geochemistry Research Opportunities On, Within, and Beyond Earth**
Speaker: Andrew Madden
- 10:25-10:55 Panel Discussion
- 10:55-12:20 Lunch on your own

Session 2: Computational/Chemical Processes in Self-Assembly

Chair: Miguel Garcia-Garibay

- 12:20-12:40 **Designing Novel Self-Assembling Protein Materials: Recent Successes and Future Prospects**
Speaker: Todd Yeates
- 12:40-1:00 **Bottom-Up Multiscale Approaches for Mesoscale Phenomena: Progress and Opportunities**
Speaker: William Noid

*Appendix A***Session 2 (cont)**

Chair: Miguel Garcia-Garibay

1:00-1:20 **View of Intelligent Mesoscale Biological Systems at Chemical Resolution—What Can Be Learned**

Speaker: Klaus Schulten

1:20-1:40 **Emergence of Entropy, Order, and Function at the Mesoscale**

Speaker: Sharon Glotzer

1:40-2:00 **Multiscale Computer Simulation of Mesoscale Biomolecular Cooperativity**

Speaker: Gregory Voth

2:00-2:30 Panel Discussion

Closing Discussion

2:30-3:00 Open Discussion

3:00 Workshop Day 2 Adjourns

Appendix B

About the Chemical Sciences Roundtable

The Chemical Sciences Roundtable (CSR) was established in 1996 by the National Research Council and its Board on Chemical Sciences and Technology. The CSR held its first meeting in February 1997, and it now meets three times annually. As a neutral and credible forum for communication among all segments of the chemical enterprise, the CSR provides great potential for enhancing the future well-being of the chemical sciences. The realm of the chemical sciences and technology not only spans the science and engineering disciplines but also encompasses a major segment of U.S. industry.

The CSR is a unique science-oriented, apolitical forum of leaders of the chemical enterprise. Its objectives are to facilitate enhanced understanding of issues in the chemical sciences and technologies that affect government, industry, academic, national laboratory, and nonprofit sectors, and the interactions among them; and to furnish a vehicle for education, exchange of information and discussion of issues and trends that affect the chemical sciences. The Roundtable accomplishes these objectives in two ways:

- First, through exchange of information among its members at three meetings annually of the CSR. The members in turn share the exchanges with others in their own institutions and professional organizations to which they belong.
- Second, by organizing workshops on highly relevant and important topics, for which published proceedings are made broadly available throughout the chemical sciences community. These workshops and the proceedings are designed to enable follow-up discussion or action by others in the chemical sciences community.

The CSR's charter enables government representatives to serve as full members, but consequently precludes it from providing advice and recommendations. Its primary role therefore is to facilitate communication among leaders in the chemical sciences, who can in turn bring important information to the broader chemical sciences community. Typically, CSR members represent the senior chemist or chemical engineer in an organization, providing the basis for strong collaboration and cooperation among federal agencies, nongovernmental organizations, professional and industry associations, universities, the chemical industry, private foundations, federal laboratories, the legislative arena, and the Executive Branch. Through a combination of educational and leadership roles, the CSR will continue its major role in maintaining the health and strength of the chemical sciences in the United States.

Appendix C

Biographical Sketches of Workshop Speakers and Organizing Committee Members

PLENARY SPEAKER BIOGRAPHY

Paul Weiss, Ph.D., University of California-Los Angeles

Paul S. Weiss holds the Fred Kavli Chair in NanoSystems Sciences, and is a distinguished professor of chemistry and biochemistry and of materials science and engineering at the University of California, Los Angeles. He received his S.B. and S.M. degrees in chemistry from MIT in 1980 and his Ph.D. in chemistry from the University of California at Berkeley in 1986. He was a postdoctoral member of technical staff at Bell Laboratories from 1986 to 1988 and a visiting scientist at IBM Almaden Research Center from 1988 to 1989. Before coming to UCLA in 2009, he was a distinguished professor of chemistry and physics at the Pennsylvania State University, where he began his academic career in 1989. His interdisciplinary research group includes chemists, physicists, biologists, materials scientists, mathematicians, electrical and mechanical engineers, and computer scientists. Their work focuses on the ultimate limits of miniaturization, exploring the atomic-scale chemical, physical, optical, mechanical, and electronic properties of surfaces and supramolecular assemblies. He and his students have developed new techniques to expand the applicability and chemical specificity of scanning probe microscopies. They have applied these and other tools to the study of catalysis, self- and directed assembly, and molecular and nanoscale devices. They work to advance nanofabrication down to ever smaller scales and greater chemical specificity in order to operate and to test functional molecular assemblies, and to connect these to the biological and chemical worlds. Two current major themes in his laboratory are cooperativity in

functional molecules and single-molecule biological structural and functional measurements. He has over 300 publications, holds over 20 patents, and has given over 500 invited, plenary, keynote, and named lectures.

Weiss has been awarded a National Science Foundation Presidential Young Investigator Award (1991-1996), the Scanning Microscopy International Presidential Scholarship (1994), the B. F. Goodrich Collegiate Inventors Award (1994), an Alfred P. Sloan Foundation Fellowship (1995-1997), the American Chemical Society Nobel Laureate Signature Award for Graduate Education in Chemistry (1996), a John Simon Guggenheim Memorial Foundation Fellowship (1997), and a National Science Foundation Creativity Award (1997-1999), among others. He was elected a Fellow of the American Association for the Advancement of Science (2000), the American Physical Society (2002), the American Vacuum Society (2007), the American Chemical Society (2010), the American Academy of Arts and Sciences (2014), and an Honorary Fellow of the Chinese Chemical Society (2010). He was also elected a senior member of the IEEE (2009). He received Penn State's University Teaching Award from the Schreyer Honors College (2004), was named one of two Nanofabrication Fellows at Penn State (2005), and won the Alpha Chi Sigma Outstanding Professor Award (2007). He was a visiting professor at the University of Washington, Department of Molecular Biotechnology from 1996 to 1997 and at the Kyoto University, Electronic Science and Engineering Department and Venture Business Laboratory in 1998 and 2000. Weiss was a member of the U.S. National Committee to the International Union of Pure and Applied Chemistry (2000-2005). He has

been the Technical Co-chair of the Foundations of Nanoscience Meetings, Thematic Chair of the Spring 2009 American Chemical Society National Meeting, and the Chair of the 2009 International Meeting on Molecular Electronics. He served as director of the California NanoSystems Institute at UCLA (2009-2014). He was the Senior Editor of *IEEE Electron Device Letters* for molecular and organic electronics (2005-2007), and is the founding Editor-in-Chief of *ACS Nano* (2007-present). At *ACS Nano*, he won the Association of American Publishers, Professional Scholarly Publishing PROSE Award for 2008, Best New Journal in Science, Technology, and Medicine, and ISI's Rising Star Award a record ten times.

SPEAKER BIOGRAPHIES

Andrew Borovik, Ph.D., University of California, Irvine

Andrew Borovik, Ph.D., was born and raised in Chicago, Illinois, and obtained his B.S. degree in chemistry from Humboldt State University, in Arcata, California, in 1981. In 1986, he received his Ph.D. from the University of North Carolina-Chapel Hill, under Professor Tom Sorrell. Dr. Borovik has held postdoctoral positions with Larry Que at the University of Minnesota as an NIH fellow from 1986 to 1988 and Ken Raymond at the University of California, Berkeley, from 1990 to 1992. He has been on the faculty at Ithaca College, Kansas State University, the University of Kansas, and the University of California, Irvine, where he is currently a professor. His research group has been developing new approaches in molecular design to prepare synthetic constructs that emulate the properties of protein active sites. Protein active sites have unique architectural features that control the immediate environment surrounding metal ions (microenvironment). These features, in turn, are instrumental in controlling protein activity, much of which has not yet been achieved in synthetic systems. Noncovalent interactions, particularly hydrogen bonds, have been implicated as key regulators of microenvironmental properties. However, little is known about how hydrogen bonds are able to influence metal-mediated processes. By establishing noncovalent interactions that promote the activation of small molecules, his group can

control the secondary coordination sphere of metal complexes.

Jim De Yoreo, Ph.D., Pacific Northwest National Laboratory (PNNL)

Jim De Yoreo is Chief Scientist for Materials Synthesis and Simulation Across Scales at PNNL and an affiliate professor of materials science and engineering at the University of Washington. He received his Ph.D. in physics from Cornell University in 1985. Following postdoctoral work at Princeton University, he became a member of the technical staff at Lawrence Livermore National Laboratory (LLNL) in 1989, where he held numerous positions including Director of the Biosecurity and Nanosciences Laboratory, and Deputy Director of the Laboratory Science and Technology Office. He joined Lawrence Berkeley National Laboratory in 2007 where he served as deputy and interim director of the Molecular Foundry. De Yoreo is a member of the MRS, APS, ACS, and the AACG. He is an editor for *Bioinspired Materials*, associate editor-in-chief for *Frontiers of Materials Science*, and a member of the Executive Committee of the International Organization for Crystal Growth (IOCG). He has served as president and board member of the Materials Research Society and on committees for the National Academy of Sciences, the Department of Energy, and the U.S. Congress. De Yoreo's research has spanned a wide range of materials-related disciplines, focusing most recently on in situ AFM and TEM investigations of interactions, assembly, and crystallization in biomolecular and biomineral systems. De Yoreo has authored, coauthored, or edited over 195 publications and patents. He is a recipient of the IOCG's Laudise Prize, the AACG Crystal Growth Award, an R&D 100 Award, and the LLNL Science and Technology Award, and is a Fellow of the APS and the MRS.

John Spencer Evans, D.D.S., Ph.D., New York University

Dr. Evans received his B.S. degree at Northwestern University and his D.D.S. degree at the University of Illinois in 1982. After completing residency fellowships at Northwestern University Medical Center and the University of Southern California Medical Center, he obtained his Ph.D. in

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chemistry at California Institute of Technology in 1993. He is currently a professor at the Center for Skeletal Sciences, New York University, and his research emphasis is on protein-mediated biomineralization phenomena.

Alexander C. Gagnon, Ph.D., University of Washington

Alexander C. Gagnon received his B.S. and B.A. degrees from the University of California, Berkeley, in 2002. Remaining on the West Coast, he completed his doctorate studies under Professor Jess Adkins and Douglas Rees at California Institute of Technology with a thesis entitled, “Geochemical Mechanisms of Biomineralization from Analysis of Deep-Sea and Laboratory Cultured Corals” in 2010. From 2010 to 2013 Dr. Gagnon held a postdoctoral fellowship at the Lawrence Berkeley National Laboratory with Donalad De Paolo and James De Yoreo. Gagnon was appointed assistant professor in the School of Oceanography at the University of Washington in 2013, where he is currently.

Pupa Gilbert, Ph.D., University of Wisconsin-Madison

Trained in physics at the First University of Rome “La Sapienza” with Filippo Conti (chemistry, physical chemistry) and Tiziana Parasassi (biology, biochemistry), Pupa Gilbert has 30 years of experience in the fascinating field of biophysics. She has been a staff scientist at the Italian CNR in 1988-1999, and at the Swiss Institute of Technology in 1994-1998, until she joined the physics department at University of Wisconsin-Madison in 1999 as a full professor. She won many awards in Italy, the United States, and internationally. She served as scientific director of the Synchrotron Radiation Center in 2002-2006, on the Scientific Advisory Committee of the Canadian Light Source in 2004-2009, and as chair of the Division of Biological Physics of the American Physical Society (APS) in 2010-2014. She is a fellow of the APS and of the Radcliffe Institute for Advanced Study at Harvard University.

Sharon Glotzer, Ph.D., University of Michigan

Dr. Glotzer is the Stuart W. Churchill Collegiate Professor of Chemical Engineering, and professor of materials science and engineering, physics, applied physics, and macromolecular science and engineering at the University of Michigan in Ann Arbor. She is member of the National Academy of Sciences, and a fellow of the American Academy of Arts and Sciences, the American Physical Society, and the American Association for the Advancement of Science. She received a B.S. degree from the University of California, Los Angeles, and a Ph.D. degree from Boston University, both in physics. Prior to joining the University of Michigan in 2001 she worked for 8 years in the Materials Science & Engineering Laboratory at the National Institute of Standards and Technology (NIST) as co-founder and Director of the NIST Center for Theoretical and Computational Materials Science. Glotzer’s research on computational assembly science and engineering aims toward predictive materials design of colloidal and soft matter, with current emphasis on shape, packing, and assembly pathways. She has nearly 200 refereed publications and has presented over 300 plenary, keynote, and invited talks around the world. Glotzer was the recipient of the Charles M.A. Stine Award in Materials Science and Engineering from the American Institute of Chemical Engineers, holds a National Security Science and Engineering Faculty Fellowship from the Office of the Secretary of Defense, and was named a 2012 Simons Investigator.

Cynthia Jenks, Ph.D., The Ames Laboratory

Dr. Cynthia Jenks is the assistant director for Scientific Planning and the division director of Chemical and Biological Science at the Ames Laboratory. She received her B.S. in chemical engineering in 1986 from the University of California, Los Angeles. She received an M.S. degree in chemical engineering in 1988 and a Ph.D. in chemistry from Columbia University in 1992. She did her postdoctoral work at Iowa State University and the U.S. Department of Energy’s Ames Laboratory. She joined the scientific staff of the Ames Laboratory in 1995. Her research interests are in the areas of surface structure and reactivity, surface structure–property relationships, catalysis, and thin-film growth. She is a Fellow of the

American Association for the Advancement of Science.

Erwin London, Ph.D., Stony Brook University

Dr. London received his B.A. at Queens College of the City University of New York in 1974 and completed his Ph.D. at Cornell University in 1980. After completing his Ph.D., London accepted a postdoctoral fellowship at Massachusetts Institute of Technology from 1980 to 1982. Since then, he has been a part of the faculty at Stony Brook University. He is currently professor in the Department of Biochemistry and Cell Biology and has a joint appointment in the Department of Chemistry. His research group studies biomembrane protein structure and function and biomembrane lipid and protein organization by combining spectroscopic methods, such as fluorescence, with chemical, biochemical, immunochemical, and molecular biological approaches. Recent studies have concentrated upon understanding the organization of biomembranes into domains with distinct lipid and protein compositions. Biomembrane lipid segregation into domains of high structural order (lipid rafts) and lower structural order (liquid disordered domains) has become an important focus of cell membrane studies. Lab studies aim at defining the principles that underlie domain formation and developing methods to detect such domains in cells. To aid in this, the lab has developed a model biomembrane vesicle system that for the first time efficiently reproduces the lipid asymmetry (difference in lipid chemical composition) in the inner (cytoplasmic) and outer (exofacial) monolayers found in the lipid bilayers of many cell membranes.

Yi Lu, Ph.D., University of Illinois, Urbana-Champaign

Dr. Yi Lu received his B.S. degree from Peking University in 1986, and his Ph.D. degree from the University of California, Los Angeles, in 1992 under Professor Joan S. Valentine. After 2 years of postdoctoral research in Professor Harry B. Gray's group at the California Institute of Technology, Dr. Lu started his own independent career in the Department of Chemistry at the University of Illinois at Urbana-Champaign in 1994. He is now

Jay and Ann Schenck Professor of Chemistry in the Departments of Chemistry, Biochemistry, Bioengineering and Materials Science and Engineering. He is also a member of the Center for Biophysics and Computational Biology and Beckman Institute for Advanced Science and Technology. His research interests lie at the interface between chemistry and biology. His group is developing new chemical approaches to provide deeper insight into biological systems. At the same time, they take advantage of recently developed biological tools to advance many areas in chemistry. Specific areas of current interests include (a) design and engineering of functional metalloproteins as environmentally benign catalysis in renewable energy generation and pharmaceuticals; (b) fundamental understanding of DNAzymes and their applications in environmental monitoring, medical diagnostics, and targeted drug delivery; and (c) employing principles from biology for directed assembly of nanomaterials with controlled morphologies and its applications in imaging and medicine.

Andrew Madden, Ph.D., University of Oklahoma

Dr. Andrew Elwood Madden is an associate professor in the School of Geology and Geophysics at the University of Oklahoma where he co-directs the Physical and Environmental Geochemistry Laboratory and directs the powder x-ray diffraction laboratory. His B.S. degree in geology from Michigan State University was followed by a Ph.D. in geochemistry from Virginia Tech while holding an NSF Graduate Research Fellowship and postdoctoral research at Oak Ridge National Laboratory. In recent years, his research on nanoparticulate minerals ranged from fundamental studies comparing the size-dependent reactivity of nanoparticulate minerals in the lab and field, the formation of nanoparticulate phases of radionuclides and metals for groundwater remediation, the frictional properties of nanoparticulate rock gouge that control the behavior of fault zones in earthquakes, to nanodiamonds in natural sediments as possible indicators of an impact event at the Younger Dryas boundary, and analog studies of nanoparticulate iron oxide minerals that constrain the history of water on Mars. He also teaches courses such as physical geology, environmental

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geology, minerals and the environment, and clay mineralogy.

William Noid, Ph.D., Pennsylvania State University

William Noid received his B.S. degree from the University of Tennessee in 2000. He moved to Ithaca to complete his Ph.D. at Cornell University in 2005 for research in quantum-classical theories of nonlinear spectroscopy. He was an NIH NRSA postdoctoral fellow at the University of Utah, where he worked on multiscale modeling theories with Professor G.A. Voth. He started as an assistant professor at Penn State in 2007 and was promoted to associate professor in 2013. His research at Penn State addresses theories for multiscale modeling, glycoprotein biophysics, and intrinsically disordered proteins. This work has been recognized by an ACS Hewlett Packard Outstanding Junior Faculty Award, an Alfred P Sloan Foundation fellowship, a Camille Dreyfus Teacher-Scholar award, and an NSF Career award.

Klaus Schulten, Ph.D., University of Illinois at Urbana-Champaign

Klaus Schulten holds a Diplom degree in physics from the University of Muenster, Germany (1969), and a Ph.D. in chemical physics from Harvard University (1974). He was junior group leader at the Max-Planck-Institut for Biophysical Chemistry from 1974 to 1980, and professor of theoretical physics at the Technical University of Munich from 1980 to 1988. Schulten came to the University of Illinois in 1988, and in 1989 joined the Beckman Institute and founded the Theoretical and Computational Biophysics Group, which operates the NIH Center for Macromolecular Modeling and Bioinformatics. Since 2008 he is co-director of the NSF-funded Center for the Physics of Living Cells. Schulten's awards and honors include a Blue Waters Professorship, National Center for Supercomputing Applications (2014); Professorship, University of Illinois Center for Advanced Study (2013); Distinguished Service Award, Biophysical Society (2013); IEEE Computer Society Sidney Fernbach Award (2012); Fellow of the Biophysical Society (2012); Award in Computational Biology (2008); Humboldt Award of the German Humboldt Foundation (2004); University of Illinois Scholar (1996); Fellow of the American Physical Society

(1993); and Nernst Prize of the Physical Chemistry Society of Germany (1981).

Wendy Shaw, Ph.D., Pacific Northwest National Laboratory (PNNL)

Dr. Wendy Shaw received her B.A. in chemistry from Whitman College in Walla Walla, Washington, and obtained her Ph.D. at the University of Washington in 2000. Soon after, she landed a position at Pacific Northwest National Laboratory in Washington state, where her research interests are in the areas of catalysis and biomineralization. In catalysis, she is interested in understanding the contribution of the outer coordination sphere to organometallic catalysis by incorporating enzymatic features of the protein scaffold into homogeneous catalysts. The features of the outer coordination sphere being investigated include dynamics, the local environment around the active site and the function of proton channels. Dynamics are being studied with novel stimuli controlled ligands for catalyst control, development, and recovery. The role of the environment around the active site and the role of proton channels are being investigated with a combined computational and experimental approach building around nickel-based hydrogen production/oxidation catalysts which mimic hydrogenase enzymes. Her interests in biomineralization processes include developing a fundamental understanding of protein-surface interfaces and biomineralization processes using solid-state NMR dipolar recoupling techniques, neutron reflectivity, and physical chemistry methods, specifically, elucidating the protein structure, protein orientation and nucleation and growth mechanisms of naturally occurring biominerals. Broader implications include understanding nonnatural systems such as protein-polymer interactions, coatings, and tissue engineering. She has received several honorable awards including the Graduate Fellowship, Associated Western Universities from 1996 to 2000, Outstanding Performance Awards, PNNL, the DOE Early Career Program Grant, 2010, and was selected to attend the U.S.-Indonesia Symposium in 2011.

Peter Stair, Ph.D., Northwestern University

Peter C. Stair is the John G. Searle Professor of Chemistry and chair of the Department of Chemistry at Northwestern University. He received a B.S. in chemistry from Stanford University in 1972 and a Ph.D. from the University of California, Berkeley, in 1977 under the supervision of Gabor Somorjai. He has been on the faculty at Northwestern University since 1977. From 1997 to 2012 he was director of the Northwestern University Center for Catalysis and Surface Science. He is director of the institute for catalysis in energy processes, a senior scientist in the Chemical Sciences and Engineering Division at Argonne National Laboratory, and Deputy Director of the Energy Frontier Research Center: Institute for Atom-efficient Chemical Transformations. His research interests are in the synthesis, characterization, and physical properties of heterogeneous catalysts. He has worked in surface science and in situ Raman spectroscopy. His goal is to develop fundamental understanding in catalysis science that leads to advances in industrial chemistry and energy technology. He is a past recipient of the Alexander von Humboldt Senior Scientist Award and recipient of the 2010 ACS George Olah Award in Hydrocarbon or Petroleum Chemistry.

Gregory Voth, Ph.D., University of Chicago

Dr. Voth is the Haig P. Papazian Distinguished Service Professor of Chemistry at the University of Chicago. He is also a professor of the James Franck Institute and the Institute for Biophysical Dynamics, as well as a senior fellow of the Computation Institute. He received a Ph.D. in theoretical chemistry from the California Institute of Technology in 1987 and was an IBM postdoctoral fellow at the University of California, Berkeley, from 1987 to 1989. He is the author or coauthor of approximately 425 peer-reviewed scientific articles, with an h-index of 78 and more than 22,000 citations. Voth is a fellow of the American Chemical Society, American Physical Society, The Biophysical Society, and the American Association for the Advancement of Science. He has received a number of awards and other forms of recognition for his work, including most recently the American Chemical Society Division of Physical Chemistry Award in Theoretical Chemistry and Election to the International Academy of Quantum Molecular

Science, both in 2013. He has proudly mentored more than 160 postdoctoral fellows and graduate students.

Professor Voth is a leader in the development and application of theoretical and computational methods to study problems involving the structure and dynamics of complex condensed phase systems, including proteins, membranes, liquids, and materials. He has pioneered a method known as “multiscale coarse graining” in which the resolution of the molecular-scale entities is reduced into simpler structures, but key information on their interactions is accurately retained (or renormalized) so the resulting computer simulation can accurately and efficiently predict the properties of large assemblies of complex molecules such as lipids and proteins. This method is multiscale, meaning it describes complex condensed phase and biomolecular systems from the molecular scale to the mesoscale and ultimately to the macroscopic scale. Professor Voth’s other research interests include the study of charge transport (protons and electrons) in water and biomolecules—a fundamental process in living organisms and other systems that has been poorly understood because of its complexity. He also studies the exotic behavior of room-temperature ionic liquids and other complex materials such as nanoparticle self-assembly, polymer electrolyte membranes for fuel cells, and electrode–electrolyte interfaces in energy storage devices. In the earlier part of his career, Professor Voth extensively developed and applied new methods to study quantum and electron transfer dynamics in condensed phase systems. Much of this work was based on the Feynman path integral description of quantum mechanics.

Aaron Wheeler, Ph.D., University of Toronto

Dr. Wheeler completed his Ph.D. in chemistry in 2003, working with Dick Zare at Stanford University. After graduating, Aaron spent 2 years as an NIH postdoctoral fellow at UCLA. Since 2005, Aaron has been the Canada Research Chair of Bioanalytical Chemistry at the University of Toronto, with a primary appointment in the Chemistry Department and cross-appointments at the Institute for Biomaterials and Bioengineering and the Banting and Best Department of Medical Research. Wheeler has been recognized internationally with a number of awards, including

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the Merck GmbH H.E.M. Prize and the American Chemical Society Arthur F. Findeis Award, and he is an Associate Editor of *Lab on a Chip*.

Benjamin Wilhite, Ph.D., Texas A&M University

Dr. Wilhite completed his doctoral research at Notre Dame. His focus included emphasizing reactor design and multiphase flow. From there, he moved to Boston, Massachusetts, to complete a postdoctoral fellowship at MIT where he focused upon catalytic microreactors and micromembranes for energy applications. He began his academic career at the University of Connecticut from 2005 to 2010 and is currently a professor at Texas A&M University. His work centers on understanding interplay between transport phenomena, materials science, and catalysis for designing membranes and microreactors for natural gas processing.

Todd Yeates, Ph.D., University of California, Los Angeles

Yeates earned his bachelor's degree at UCLA in 1983. He stayed on at UCLA and earned his Ph.D. in 1988 while doing research under the direction of Professor Douglas Rees. There he helped determine the crystal structure of the bacterial photosynthetic reaction center as part of a team racing to determine the first crystal structures of membrane proteins. He then moved to The Scripps Research Institute to do his postdoctoral research on the structure of poliovirus with Professor James Hogle. Yeates returned to UCLA in 1990 to join the faculty in the Department of Chemistry and Biochemistry. His interdisciplinary research, combining molecular biology with computing and mathematics, has focused on structural, computational, and synthetic biology.

Past research findings in the Yeates lab include an explanation for why proteins crystallize in certain favored arrangements, with implications for the development of racemic macromolecular crystallography; the development of equations for detecting disorder in x-ray diffraction data from protein crystals; the discovery that certain thermophilic microbes are rich in intracellular disulfide bonds; development of comparative genomics methods; and the discovery of novel topological features such as links and slipknots, with implications for protein folding landscapes. Recent

work has focused on giant protein assemblies, both natural and designed. In the area of giant natural protein assemblies, Yeates' group has pioneered the structural biology of the carboxysome and related bacterial microcompartments. These are primitive metabolic organelles in many bacteria, wherein a protein shell (reminiscent of a viral capsid) encloses a series of enzymes in order to sequester a sensitive pathway within the bacterial cell. In the area of designed protein assemblies, Yeates' group has laid out symmetry-based strategies for engineering novel protein molecules to self-assemble into precisely defined symmetric nanocages and materials, and has demonstrated their successful application to creating cubic assemblies on the 15- to 20-nm scale. Yeates is a member of the Molecular Biology Institute, the California Nanosystems Institute, the UCLA-DOE Institute of Genomics and Proteomics, and a Fellow of the American Association for the Advancement of Science.

**ORGANIZING COMMITTEE MEMBER
BIOGRAPHIES**

Miguel Garcia-Garibay* (co-chair) has been a faculty member in the Department of Chemistry and Biochemistry since 1992. He came to UCLA after doing postdoctoral research at Columbia University, which followed his Ph.D. studies at the University of British Columbia, in Canada. The earlier portions of Dr. Garcia-Garibay's education were completed in his native Mexico, at the Universidad Michoacana, where he did research on natural product isolation and characterization. Dr. Garcia-Garibay was promoted to full professor in the year 2000 and he has served as Vice Chair for Education in the Department of Chemistry and Biochemistry since 2005. Dr. Garcia-Garibay is a member of the editorial board of the *Journal of the American Chemical Society* and the *Journal of Organic Chemistry*. He has been a member of the CNSI since 2005. His current research efforts are aimed at the development of artificial molecular machinery in highly organized crystalline media, and to the development of green chemistry by taking advantage of organic reactions in molecular nanocrystals.

Patricia A. Thiel* (co-chair) is the John D. Corbett Professor of Chemistry, and a Distinguished Professor of Chemistry and of Materials Science & Engineering at Iowa State University. She is also a faculty scientist in the Ames Laboratory. She is active in research, teaching, and administration. In research, she is known for her work in three main areas: nanostructure evolution on surfaces, surface properties and structures of quasicrystals (a complex type of metallic alloy), and the chemistry of water adsorbed on metal surfaces. Thiel is an enthusiastic teacher of physical chemistry. She has held several administrative posts, including chair of the Department of Chemistry. Thiel earned her B.A. in chemistry from Macalester College, and her Ph.D. in chemistry from the California Institute of Technology in 1981. After postdoctoral work at the University of Munich as a von Humboldt Fellow, she joined the technical staff at Sandia National Laboratories, Livermore, then moved to Iowa State University in 1983. In her early academic career, Thiel was recognized with awards from the Camille and Henry Dreyfus Foundation and the Alfred P. Sloan Foundation, and by a National Science Foundation Presidential Young Investigator Award. Later, she received the American Chemical Society's Arthur W. Adamson Award, and the American Physical Society's David J. Adler Lectureship. She was also named Fellow of several societies: the American Association for the Advancement of Science, the Materials Research Society, the American Physical Society, and the American Vacuum Society.

Vernon Anderson earned a B.S. in chemical engineering from the University of Missouri-Columbia (1977) and a Ph.D. in biochemistry from the University of Wisconsin-Madison (1981) followed by postdoctoral research at Cornell University. He was an assistant professor and associate professor of organic chemistry at Brown University studying enzymes of fatty acid metabolism. After moving to Case Western Reserve University as professor of biochemistry and chemistry he specialized in using stable isotopes to characterize enzyme reactions, metabolism, and protein modifications along with continued interests in kinetic and spectroscopic analyses of enzyme reactions. In 2009 he moved to the NIH to become a program director in the Division of Pharmacology,

Physiology, and Biological Chemistry where he administers grants in the areas of bioinorganic chemistry, redox enzymology, mitochondrial electron transport, and other aspects of bioenergetics.

Bruce Garrett is responsible for the leadership, management, and operations of the Physical Sciences Division at Pacific Northwest National Laboratory. The focus of research in the division is on developing tools and understanding required to control chemical and physical processes in condensed phases and at complex interfaces. Dr. Garrett has been involved in research in the field of theoretical physical chemistry since the 1970s. His major research accomplishments have been in the area of reaction rate theory, including the development of theoretical methods for predicting rates of chemical reactions. This research contributed to the development of variational transition state theory into a practical, reliable method for including important quantum mechanical effects into calculations of rate constants for gas-phase chemical reactions. Dr. Garrett's research focus in the past several years has been to extend these methods to treat chemical reactions in condensed phase and interfacial systems. His current research interests include developing theoretical methods for including the effects of molecular environment (liquids, solids, and interfaces) on the rates of chemical reaction, advancing molecular theories to describe the kinetics of gas-to-particle nucleation, and understanding the molecular-level structure and energetics of liquid interfaces that will allow a detailed description of the transfer of molecules between phases. Before joining PNNL in 1989, Dr. Garrett cofounded Chemical Dynamics Corporation where he managed and conducted fundamental research. At PNNL, he was Technical Group Leader for Molecular Theory & Modeling from 1989 to 2002, and Associate Director for Molecular Interactions & Transformations from 2002 to 2005, before becoming Director of the Physical Sciences Division.

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Jennifer Sinclair Curtis* is Distinguished Professor of Chemical Engineering and Associate Dean for Research in the College of Engineering at the University of Florida (UF). Professor Curtis received a B.S. in chemical engineering from Purdue University (1983) and a Ph.D. in chemical engineering from Princeton University (1989). She has an internationally recognized research program in the development and validation of numerical models for the prediction of particle flow phenomena. She is the coauthor of over 100 publications and has given over 160 invited lectures at universities, companies, government laboratories, and technical conferences. Professor Curtis is a recipient of a Fulbright Senior Research Scholar Award, an NSF Presidential Young Investigator Award, the American Society of Engineering Education's (ASEE's) Chemical Engineering Lectureship Award, the Eminent Overseas Lectureship Award by the Institution of Engineers in Australia, the ASEE's Sharon Keillor Award for Women in Engineering, and the AIChE Fluidization Lectureship Award. She currently serves on the Governing Board of the Council for Chemical Research and as co-chair of the National Academies' Chemical Sciences Roundtable. She is also associate editor of the *AIChE Journal* and on the editorial advisory board of *Industrial & Engineering Chemistry Research*, *Powder Technology*, and *Chemical Engineering Education*. She has served on the National Academy of Engineering's (NAE's) Committee on Engineering Education and has participated in two NAE Frontiers of Research Symposiums (2003 and 2008). She is a Fellow of AAAS, AIChE, and ASEE.

*Denotes member of the Chemical Sciences Roundtable

Appendix D

Workshop Attendees

Co-chair

Jennifer S. Curtis, University of Florida

Committee Members

Michael R. Berman, Air Force Office of Scientific Research

Steven Bernasek, National Science Foundation

Carole Bewley, National Institute of Diabetes and Digestive and Kidney Diseases

Donna G. Blackmond, Scripps Research Institute

Emilio Bunel, Argonne National Laboratory

A. Welford Castleman, Jr., Pennsylvania State University

Richard R. Cavanagh, National Institute of Standards and Technology

Miguel Garcia-Garibay, University of California, Los Angeles

Sean Jones, Office of Science and Technology Policy

Jack Kaye, National Aeronautics and Space Administration

JoAnn S. Lighty, National Science Foundation

Kenneth G. Moloy, DuPont Company Experimental Station

Robert Peoples, American Carpet Institute

Tanja Pietrass, U.S. Department of Energy

Michael E. Rogers, National Institute of General Medical Sciences

Patricia A. Thiel, Ames Laboratory and Iowa State University

Workshop Co-organizers

Vernon Anderson, National Institutes of Health

Bruce Garrett, Pacific Northwest National Laboratory

Plenary Speaker

Paul Weiss, University of California, Los Angeles

Session Speakers

Andrew Borovik, University of California, Irvine

Jim De Yoreo, Pacific Northwest National Laboratory

John S. Evans, New York University

Alex Gagnon, University of Washington

Pupa Gilbert, University of Wisconsin, Madison

Sharon Glotzer, University of Michigan

Cynthia Jenks, Ames Laboratory

Erwin London, Stonybrook University

Yi Lu, University of Illinois at Urbana-Champaign

Andrew Madden, Oklahoma State University

William Noid, Pennsylvania State University

Doug Ray, Pacific Northwest National Laboratory
Klaus Schulten, University of Illinois at Urbana-Champaign
Wendy Shaw, Pacific Northwest National Laboratory
Peter Stair, Northwestern/Argonne National Laboratory
Aaron Wheeler, University of Toronto
Benjamin Wilhite, Texas A&M University
Gregory Voth, University of Chicago
Todd Yeates, University of California, Los Angeles

Participants

Mouneir Abdel-Hamied

Luke Achenie, Virginia Tech

Lee Bitna, American Association for the Advancement of Science

Todd Brethauer

Phil Britt, Oak Ridge National Laboratory

Anne Chaka, Pacific Northwest National Laboratory

Michael Conroy, Naval Research Laboratory

Cristian Contescu, Oak Ridge National Laboratory

Louise Criscenti, Sandia National Laboratories

Valmor de Almeida, Oak Ridge National Laboratory

Paul DeSario, Naval Research Laboratory

Miles Fabian, NIH/National Institute of General Medical Science

Gregory Fletcher, DOE

Neil Henson, Los Alamos National Laboratory

Aditya Jaishankar, ExxonMobil Research and Engineering

Cory Jensen

Arben Jusufi, ExxonMobil Research and Engineering

Muhammad Kismurtono, Technical Implementation Unit for Development of Chemical Engineering Processes—LIPI

Ahmet Kusoglu, Lawrence Berkeley National Laboratory

Julia Laskin, Pacific Northwest National Laboratory

Eric Lin, National Institute of Standards and Technology

Tu Maobing, Auburn University

Andrew McGill, Naval Research Laboratory

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