To help meet the needs for a new generation of advanced, high-performance composites required for new construction materials, new micro-electronic, optoelectronic and catalytic devices, chemical and biological sensors (Bright, 1994), energy transducers, "smart" medical implants (Newnham, 1997) and faster and higher capacity “biochips” (Kozal et al., 1996; Travis, 1997), materials scientists have turned to the biomimetic production of mineralized nanocomposites modeled on those produced by marine organisms (Eisner, 1991; Mann et al., 1992, 1993; Heuer et al., 1992; Pennisi, 1992; Mann, 1993; Walsh et al, 1994; Hoch et al., 1996; Mann and Ozin, 1996; Yang et al., 1997). These ceramic-like composites of mineral and biopolymers offer unique combinations of strength, anisotropy, biocompatibility, precise structural control at the nanoscale level, and coupling between mineral and organic polymeric phases that make them especially attractive models for commercially valuable new materials (e.g., Bouchon et al., 1995; Hoch et al., 1996).

Our long-term goal is to identify the proteins, genes and molecular mechanisms that control the biological formation of the high-performance mineralized composite materials made by marine organisms, and to learn to utilize these for the practical nanofabrication of commercially useful advanced new materials with potential applications in electronic, optical, and medical devices. Our specific objectives have been to develop a predictive understanding of the molecular mechanisms
governing the structures, self-assembly and functions of the biopolymers that control mineral composite formation, and to apply this understanding to develop economically efficient, environmentally benign, low-temperature routes to the synthesis of useful new mineral-organic composite materials. As reported here, we have made significant steps toward fulfilling that promise with our discovery that the proteins we found responsible for the synthesis and structural control of silica in a California marine sponge can be harnessed to catalyze the synthesis and control the nanostructural alignment of titanium dioxide, gallium oxide, and several other valuable semiconductors. Details are explained below:

We recently discovered that the silica spicules made by a common marine sponge provide a uniquely tractable model system for dissection of the molecular mechanisms controlling silica nanofabrication, and that the integral proteins of this and other marine biomineralized composites can be used to direct the formation of thin films of silica and other minerals with useful optical, electrical and magnetic properties. Our major accomplishment has been a breakthrough revealing the molecular mechanisms Nature uses to make high-performance composite materials from silicon under mild physiological conditions in living organisms, and our discovery that we can harness these mechanisms to make silica and advanced silicone polymer networks with simultaneous control of their structure and shape (Shimizu et al., 1998; Cha et al., 1999, 2000a,b; Zhou et al., 1999; Shimizu and Morse, 2000; Morse, 2000, 2001; Sumerel and Morse, 2003). The present manufacturing of polymers of silicon and oxygen requires extremes of temperature, pressure, acidity or alkalinity to produce a wide range of materials including glasses, ceramics, molecular sieves, catalysts, elastomers, resins, optical coatings, semiconductors, insulators, fiberoptics and photoluminescent polymers. Our discovery of the mechanism of used in the biological synthesis of silicon-based materials offers the prospect of a new and more efficient pathway for the environmentally benign manufacture of many commercially valuable products.
We found that the common marine sponge, Tethya aurantia, produces large quantities of glass-like silica needles (1-2mm length x 30 micron diameter); these are so abundant that they represent 75% of the dry weight of the sponge, making analysis of their constituents and the mechanism of their synthesis possible (Shimizu et al., 1998). The sponge silica needles each contain a central axial filament of protein (1-2mm length x 2 microns diameter). We found these filaments to be composed of three very similar subunits that we named “silicateins” (for silica proteins) (Shimizu et al., 1998).

Characterization of silicatein \( \alpha \) (the subunit comprising nearly 70% of the mass of the filaments) and sequence analysis of the cloned cDNA that codes for this protein revealed that silicatein \( \alpha \) is closely related to members of a well-known family of enzymes (Shimizu et al., 1998). This unexpected discovery suggested the exciting possibility that the role of these proteins in their control of the polymerization of silica might be catalytic, and similar in mechanism to that of their well-known enzyme counterparts.

Consistent with this prediction, we discovered that the purified silicateins, and the silicatein produced from a recombinant DNA that we cloned in bacteria, catalytically accelerate the synthesis of silica and silicone polymers, depending on the substrate we provide (Cha et al., 1999). When the silicateins are assembled into macroscopic protein filaments, they serve as “templates”, guiding the polymerization of the silica or silicone polymers along the molecular contours of the protein filaments. Molecular modeling of the silicatein protein helped us identify the sites on the molecule that function as the catalytically active site. We confirmed this identification, and identified the specific mechanism of catalysis of silicon polymer synthesis, by producing and characterizing genetically engineered proteins with specific modifications (introduced at the level of the cloned, recombinant DNA) that abolished the catalytic activity (Zhou et al., 1999). These findings, in turn, enabled us to make synthetic, catalytically active peptides that incorporate the essential features we found required for the catalytic activity (Cha et
This has allowed us to produce shape-controlled silica and commercially important silicone polymer networks from these wholly synthetic catalysts in vitro. We characterized the structures of the resulting polymer nanocomposites by state-of-the-art 2-dimensional solid-state nuclear magnetic resonance.

We then completed the cloning and sequencing of silicatein $\beta$, the second most abundant protein subunit we found occluded in the silica produced by the sponge. Comparison of the silicatein $\alpha$ and $\beta$ sequences and their computer modeling-predicted 3-dimensional structures reveals that the two proteins are very highly homologous, with the principal differences appearing in those parts of the molecule we believe responsible fit together like a lock and key to direct the assembly of the macroscopic silicatein filament. We have achieved the expression and overproduction of both silicatein alpha and beta from recombinant DNA templates cloned in bacteria, and demonstrated that these proteins work well to catalyze and structurally direct the polymerization of silica and silicones from simple silicon alkoxide precursors at low temperatures and under mild conditions. Based on the structure and function of the silicatein proteins, and our site-directed mutagenesis studies which confirmed our identification of the portions of the molecules that are responsible for the catalysis of silicon-polymer synthesis, we developed both macromolecular (peptide) and small molecule biomimetic catalysts that mimic the activities of the natural proteins. These have been patented, and the results published in a series of frequently cited papers.

In our most significant over the past two years, we discovered that the sponge proteins that catalyze and spatially direct the polymerization of silica and silicones also can be used to catalyze and spatially direct the polymerization of titanium dioxide, gallium oxide, zinc oxide, ruthenium oxide and cobalt oxide, broad-band semiconductors with valuable applications as photovoltaic, electronic, optoelectronic and magnetic materials (Sumerel et al., 2003; Kisailus et al., 2004). Most exciting, we
discovered that the semiconductors formed in this process are partially nanocrystalline, and that the atomic lattices of their nanocrystals are coherently aligned along the silicatein fiber axis! Titanium dioxide is widely used in the development of advanced solar cells, industrial photocatalysts, electronic devices and materials, coatings and health-care products; gallium oxide is a valuable precursor for the synthesis of gallium nitride, a very valuable material used commercially in the growing solid-state lighting industry. Our discovery represents the first instance in which an enzyme has been found that can be used for the environmentally benign biocatalysis of titanium dioxide and other semiconductor synthesis, and the first instance in which a catalyst has been found that can control the nanostructures of these materials. We are working now with our colleagues at DuPont to identify practical applications of this fundamentally significant discovery for improved photovoltaics (converters of sunlight to electrical energy, based on titanium dioxide), and to extend this approach to the structurally controlled synthesis of other electronically useful materials.

In addition to helping explain the biological and molecular mechanisms of nanofabrication of silicon-based materials under mild physiological conditions, our findings have demonstrated that these mechanism can be harnessed for the development of environmentally benign new routes to the synthesis of patterned silicon-based materials, photovoltaic materials (such as titanium dioxide) and semiconductors (Morse, 1999, 2000, 2001: Shimizu and Morse, 2000; Cha et al., 2000a,b, Sumerel and Morse, 2003; Kisailus et al., 2004). We have collaborated with researchers from Dow Corning Corp. (the nation’s largest manufacturer of silicon-based materials) and the Oak Ridge and Los Alamos National Laboratories, developing and testing strategies to immobilize the silicatein molecules in precise arrays to direct the fabrication of oriented titanium dioxide and semiconductor nanocrystallites for potential applications in microelectronics and optoelectronics. We also have established a collaboration with Lucent Corp., a division of Bell Laboratories, Inc., exploring the correlation between the superior
optical performance of the biologically produced glass fibers and their physical structures and properties (Aizenberg et al., 2004; Weaver and Morse, 2003; Weaver et al., 2003).

**Cooperating Organizations:**

Dow Corning Industries (Midland, MI)

Genencor International, Inc. (Palo Alto, CA)

Bell Laboratories Inc. (Murray Hill, NJ)

Digital Instruments, Inc. (Santa Barbara, CA)

Dow Corning Asia (Yamakita, Japan)

DuPont Corporation (Wilmington, DE)

Los Alamos National Laboratory, NM

Lucent Corporation (Bell Laboratories; Murray Hill, NJ)

Oak Ridge National Laboratory, TN

Weizmann Institute, Tel Aviv, Israel

University of Bristol, UK

University of Frankfurt, Germany

University of Tokyo, Japan

Tokyo University of Fisheries, Japan

Melbourne University, Australia

**References:**


Publications:


