BE.342/442 Tuesday, October 20, 2005
Topic: Biomineralization and Sea Creatures

Seashells

Hands-on demo: observation of seashells.
Consider the many biological process evident from an abalone shell. The outside is covered with barnacles: next time, we'll look at the chemistry of how barnacles bind. The composite of protein and calcite of the shell is 3,000 times more fracture resistant than a single crystal of the pure mineral. Why is this material so much tougher and more resistant to fracture?

Seashells are formed in a process called biomineralization. First, a sea creature builds a protein scaffolds: just like an architect, it must build a scaffold before the building. In a complex and dynamic process, the scaffold attracts inorganic ions, often layer by layer, organizing them into nanoclusters. The final material is often highly organized, with great strength, flexibility, and multifunctionality. We materials scientists are far from achieving what nature has done effortlessly and efficiently! A new journal on "biomimetics" exists to address this question.

Understanding the details of the formation process is tremendously important for the construction of nanoscale devices. Instead of attracting calcite and silica ions, we might attract conducting or semiconducting ions to build multifunctional nanodevices!

Studies of seashell formation were pioneered by Daniel E. Morse (Letter to Nature: "Molecular mechanistic origin of the toughness of natural adhesive, fibres, and composites.") and is studied here at MIT by Prof. Angela Belcher. Morse studied fracture and crack propagation in 5% protein/ 95% calcium carbonate matrices.

In a shell (nacre), the protein matrix is composed of Lustrin A, a protein that folds quite differently in the absence of calcium carbonate.

Composition of Lustrin A		
Amino acid	# residues	Mol. %
Ser	234	16.39
Pro	198	13.87
Gly	191	13.38
Cys	131	9.17
Val	89	6.23
Ala	85	5.95
Thr	76	5.32
Arg	70	4.90
Leu	63	4.41
Gln	56	3.92
Asp	55	3.85
Lys	32	2.24
Glu	30	2.10

Ile	29	2.03
Tyr	28	1.96
Phe	24	1.68
Asn	18	1.26
Trp	12	0.84
His	5	0.35
Met	2	0.14
Total	1428	

Looking at this table, or directly at the DNA sequence, what conclusions could we draw about the properties of this protein?

The sequence is suggestive of the protein's organization: the "GS" domain of the protein is rich in glysine and serine, which make it highly flexible, highly soluble, and rich in serine, able to form intermolecular bonds to other GS units. The high cysteine content of the overall protein allows it to form covalent disultide bonds between molecules, which allows the protein to cross-link and act like a glue that remains stable over a wide range of temperatures. Serine and threonine can also become phosphorylated, giving them two negative charges, which increases their ability to attract positively-charged ions.

Growth of pearls

Letter to *Nature* by Monika Fritz, Angela M. Belcher, et al. "Flat pearls from biofabrication of organized composites of inorganic substrates."
By understanding how proteins can nucleate the growth of the pearl material, scientists were able to grow a "flat pearl" deposited on a glass cover slip, and could follow the growth of the pearl by observing the development of the middle pearl-inner layer, the epithelial cells at the mantle edge, and the pigmented protein matrices. SEM images of these synthetically grown materials reveal stacks of coin-like plates separated by organic sheets.

Letter to *Nature* by A. M. Belcher, X. H. Wu, et al. "Control of crystal phase switching and orientation by soluble mollusc-shell proteins."
Comparing the morphology and diffraction patterns from crystals grown with out without proteins, we see that proteins change the crystalline nature of the crystal. During growth of pure calcite, the grain spirals out in straight, well-defined steps. Upon addition of calcite proteins, the steps become highlighted and rounded. Upon addition of aragonite proteins, the steps become highly convoluted in comparison with the sharply defined step edges seen with calcite crystals.

Brittlestars

Letter to *Nature* by Joanna Aizenberg, et al. "Calcitic microlenses as part of the photoreceptor system in brittlestars."

Proteins direct the formation of dome-shaped calcite precipitates that collect light despite the opacity of the calcite material. These lenses collectively allow the animal to sense light. This discovery offers a new approach to nanoscale optical devices.

Sponges

The diversity of structures seen is sponges is due to the regulatory behavior of proteins. Sponges have performed biomineralization processes over 650 million years ago! The Dutch portion of Indonesia is located in an ocean ecosystem rich in diverse, colorful sponges, and has become a key location for research about sponges.

Source: Aizenberg, *et. al.,* PNAS, March 2004.
A sponge is composed of spicules, approximately 1 cm long, which can collect light and light up at one end. On the ~20 nm scale, we can see the long, thin spicule contains large barbs at the lighted end, which shrink to make the spicule smooth at the other end. The backbone of the spicule is composed of a central cylinder supported by organic filaments, surrounded by coaxial "striated shells." On the single-micron scale, we can see that the more is composted of nanoparticles: it's a composite crystal with a variety of structures to be observed on different length scales, from the scale of mineral nanocrystals to the struts that weave together a supporting mesh for the sponge. Aizenberg, et al. coupled light to a free-fractured spicule. Bell labs is currently investigating possible telecommunications applications of this material.

Source: Daniel E. Morse. "Silicon biotechnology: harnessing biological silica production to construct new materials." *Trends in Biotechnology*: 17 (6) 1999.
Researchers looked at the sequence alignment of silicatein and the protease Cathepsin. A mechanism for the catalysis of peptide bond cleavage (and sometimes formation) by silicatein was proposed based on the protease crystal structure and analysis of the catalytic activity of the protein with specific point mutation. (Chemical mechanism shown in *Biochemistry*: Cha *et al*, pg. 364.) A new mechanism was proposed based on observation of the fractal nature of silicatein. Based on the hydrophobic and hydrophilic regions in the final structure, a mechanism for catalysis was proposed.

Diatoms

Diatoms have many regular, repeating structures. Some diatoms have the same geometric patterns as some architectural works! Many diatoms appear regular, but have some random variation in structure (e.g., 5-ringed vs. 6-ringed skeletal cage structure).

The cage structures are amazingly intricate and sophisticated, mediated by the protein scaffold coded in the DNA of the diatom. E.g., silaffin can induce silica to form uniform nanoparticles at a neutral pH (whereas no nanoparticles would form in the absence of silaffin). Silaffin has shown promise in the field of holographic printing!

Look up diatoms in Google Image Search and admire the regularity of their nanoscale features.