Understanding Biosilicification: Diatoms as Bioarchitects

SAND2007-5845P

LDRD
LABORATORY DIRECTED RESEARCH & DEVELOPMENT

Sandia National Laboratories
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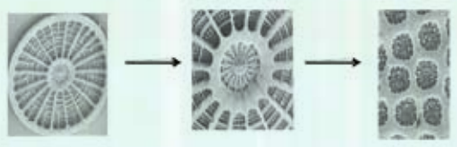
Problem: Understanding Diatom Silica Biomineralization

- Biomineralization occurs during reproductive cell cycle/frustule division
- Believed to occur along the silica deposition vesicle
- Robust, fault-tolerant process driven by genomics and proteomics in the organism
- Silica transport proteins identified by Hildebrand et al.
- Sumper et al. identifed frustule associated protein, silaffin, from one species (C. fusiformis) shown to polymerize silica in vitro
- Wright et al. have shown that primary amine containing dendrimers also spontaneously polymerize silica
- No demonstration of multi-scale phenomenon and/or control as of yet

Diatoms

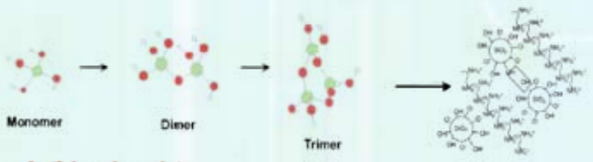
Known proteins: silaffins



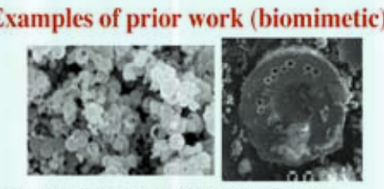
Range from nm to 100 µm

Chemistry

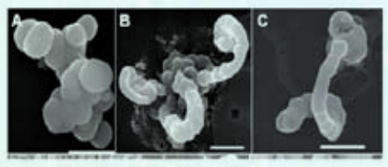
Polyamines and proteins coordinate in biogenesis



Examples of prior work (biomimetic)



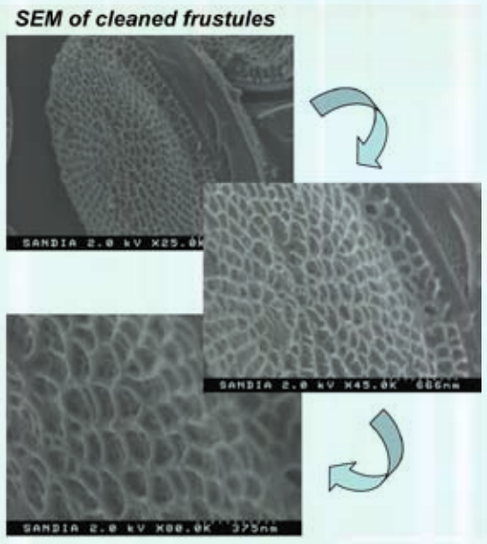
http://www.chem.tue.nl/smo/MBE/biomineralisation.htm



Naik et al, 2002, *Chem Comm*

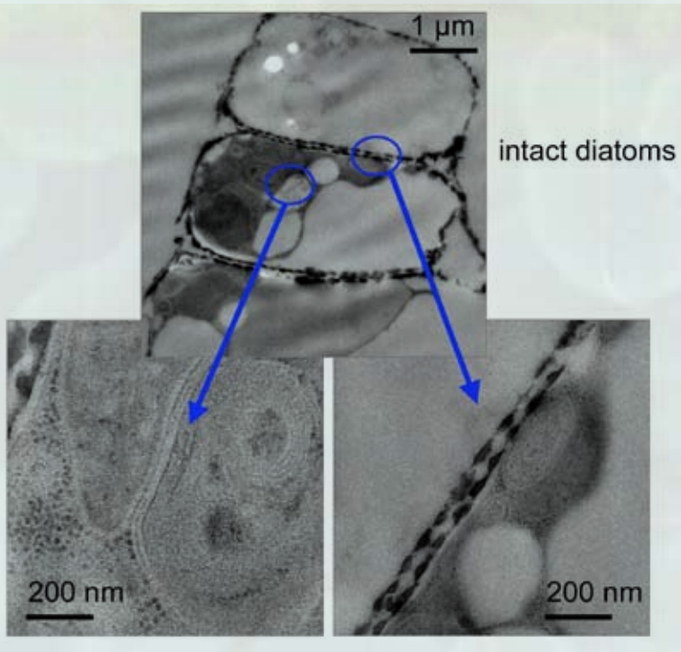
Research Approach

- Use an integrated approach to discovery that combines materials science, biochemistry, computational modeling, proteomics, as well as molecular and cellular biology
- Develop a basic understanding of the role of amination of peptides and polyamines in silica biocatalysis
- Investigate the role of confinement in the templating of silica hierarchical structures in order to mimic the silica deposition vesicle
- Use synthetic and native proteins to explore the parameter space of



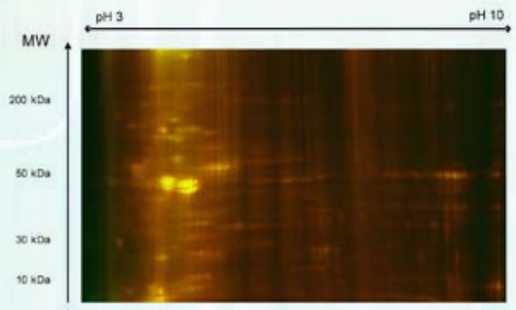
Structural Biology of a Diatom

- Images taken of T. pseudonana by collaborator Manfred Auer (LBNL) using a hi-pressure cryo-TEM technique
- Offered new insight into the interaction between the silica deposition vesicle and the silica frustule
- Work continues to investigate dynamics of frustule division and interactions with the silica deposition vesicle



Proteomic Analysis

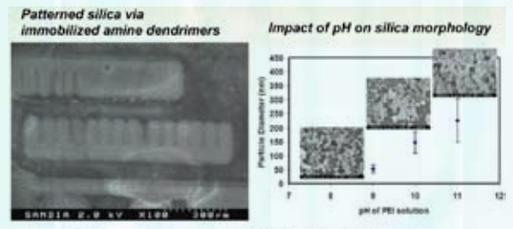
- Use 2-dimensional protein analysis (differential in-gel electrophoresis) to investigate protein expression
- Analyzed different stages of cell growth/ frustule division
- Different colors present in bands indicate different expression levels as a function of time
- Used to isolate and identify proteins that are expressed during frustule formation
- Form the basis of "knock-out" mutations by blocking protein expression

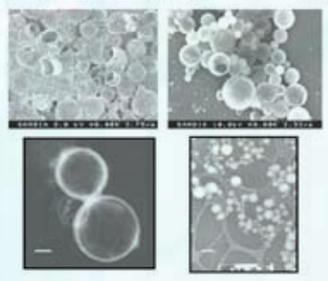


Comparison of the marine diatom Thalassiosira pseudonana (Tp) membrane proteins grown in the absebce of silica (0 hr sample; Cy3 label- green) and 6 hours after induction with Silica (6 hr sample; Cy5 label- red). The proteome was prepared for labeling by acetone precipitation of the extract followed by resuspension in a denaturing buffer containing 7M urea, 2M thiourea, 4% (w/v) CHAPS detergent, 0.5% (v/v) Triton X-100 and ampholytes in the pH 3-10 range. The IEF was performed on IPGPhor (Amersham Biosciences) followed by denaturing polyacrylamide gel electrophoresis on a 10% acrylamide/bisacrylamide gel. The gel was scanned using variable mode scanner imager Typhoon 9400 (Amersham Biosciences). The green protein spots shows the Tp proteome which is expressed in the absence of silica where as the red spots indicate the fraction of the proteome which is expressed only after induction with silica. Yellow spots are the proteins that are present in both conditions- as is the case here for majority of the proteins.

Confined Amine Silicification

- Investigated the structure and composition of different synthetic amines as they impact observed silica structures
- Two different modes of confinement
  Surface immobilization of PAMAM dendrimers
  Microemulsions (reverse micelles, water-in-isooctane, dioctyl sulfosuccinate)
- Planar structures with nanoscale topography observed on patterned surfaces
- Spherical structures with porous structures produced by water-in-oil microemulsions (SANS and QELS used to characterize reverse micelle deformation)
- Hollow silica structures observed as a function of water content

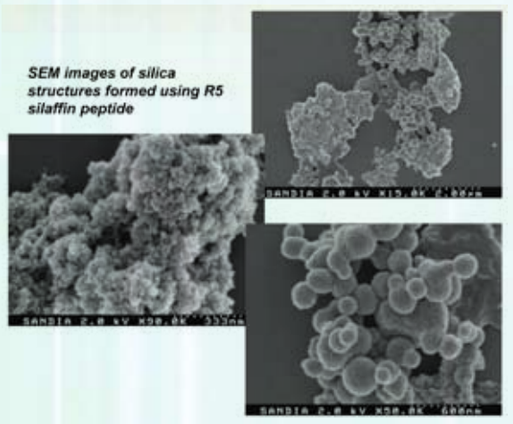




Impact of reverse micellar water content on silica morphology; formation of hollow spheres

Confined Peptide Silicification

- Determined the impact of different amine moieties and molecular weight on silica morphology
- Baseline: silaffins isolated from diatoms
- Used water-in-oil microemulsions
- Observed direct dependence on pH
- Relative hydrophobicity of the protein impacted silica morphology, believed to be caused by self-aggregation during nucleation and growth
- Phosphate counterion dependence verified
- Results obtained are very similar with synthetic catalyst analogues
- Suggest amine placement and density are key to catalysis; structure and porosity determined by environment



Significance

- Fundamental investigations yielded new insight into the role of amination on silicification
- Determined that confinement alters the templated silica structures produced (layers ⟶ spheres ⟶ hollow spheres) over multiple length scales
- Observed differential protein expression in diatoms during cell division
- RNA inhibition studies underway to down-regulate silica transporters
- SANS, QELS and reflectivity studies indicate there is a strong interaction between peptides and phospholipid bilayers (first time this has been reported)
- Four summer interns worked on project over three-year period (MIT, UC-Davis, Univ. of Colorado, Georgia Tech)
- Publications
  - Two peer-reviewed publications
  - One manuscript under review
  - Three manuscripts in preparation
- Presentations
  - Five conference presentations (MRS, Particles, ACS)
  - PI co-chairing peptide-based materials session (NN) at Fall 2007 MRS National Meeting
- Patents
  - One patent application
- Commercial Impact
  - Communications underway with a potential CRADA partner

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