Spatial Proteomics in Diatoms: Towards a Systems View of Biosilicification and Beyond

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Abstract

Diatoms are **unicellular photosynthetic algae** with intricately ornamented **species-specific silica cell walls**. To elucidate unknown, but speculated, proteins required for **diatom cell wall biosynthesis**, we aim to apply proximity-based proteomic mapping to the model diatom species ***Thalassiosira pseudonana*** using the engineered ascorbate peroxidase APEX2. Advancing our understanding of how naturally occurring silica-based materials such as diatom cell walls are constructed could inform future **biotechnological and biomimetic strategies to produce self-assembled nanomaterials**.

Diatoms

Importance

- Biomonitoring tool for aquatic environments.
- Source of ice nuclei in the atmosphere.
- Produce about **20% of the oxygen we breathe**.
- Crucial for C, N and **Si** biogeochemical cycles.

Model organisms

- Evolution (secondary endosymbiosis).
- Photosynthesis (pyrenoid biology).
- Diatom-bacteria interactions and signaling.
- Extracellular matrix (underwater adhesives).
- **Silica (SiO_2) biomineralization.**

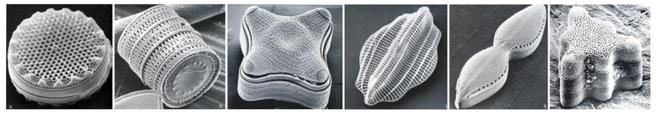

Figure 1 | Morphological diversity of diatoms. It is estimated that there are approximately **100,000 extant diatom species** each encasing itself in a unique glassy shell (Kröger, 2007).

Biosilicification in Diatoms

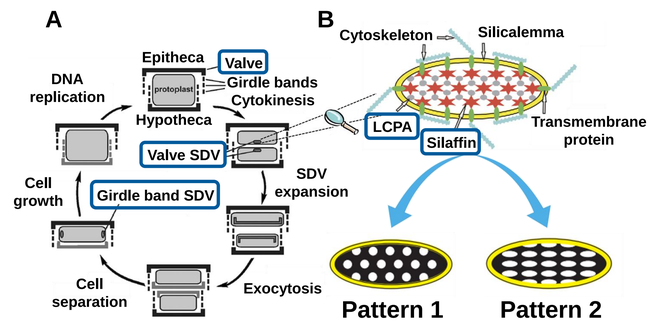

Figure 2 | Biosilicification in diatoms. (A) Diatom cell wall is synthesized inside an acidic compartment called the **silica deposition vesicle** (SDV). (B) It is hypothesized that the **interaction of silaffins with long-chain polyamines (LCPA)** inside the SDV leads to the formation of an organic matrix, the structure of which is controlled by the silaffins (Kröger, 2007; Kröger & Poulsen, 2008).

Spatial Proteomics in *Thalassiosira pseudonana*

Thalassiosira pseudonana

- Marine centric diatom.
- **1st sequenced diatom species** (~11,800 genes).

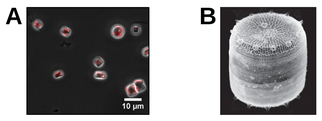

Figure 3 | *Thalassiosira pseudonana.* (A) Fluorescence microscopy image of *T. pseudonana* (red, plastids). (B) SEM image of *T. pseudonana* (courtesy: Nils Kröger).

Overview of the Approach

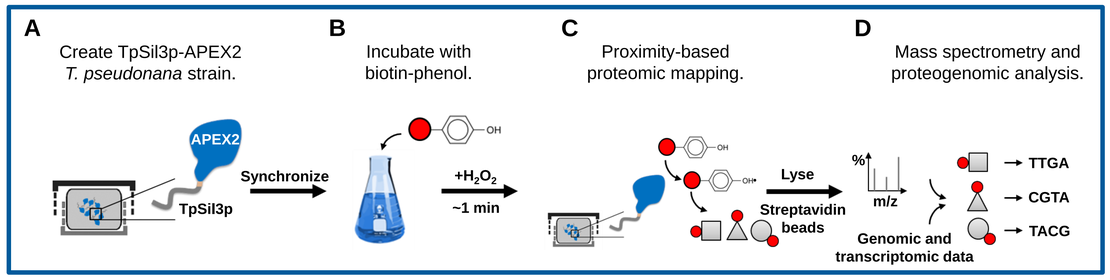

Figure 6 | Spatial proteomics in *Thalassiosira pseudonana*. (A) *T. pseudonana* strain expressing a TpSil3p-APEX2 protein. (B) Synchronized *T. pseudonana* strain is supplemented with **biotin-phenol** and (C) **hydrogen peroxide** to initiate **biotin labeling** (red, biotin). (D) Cell lysate is enriched for biotinylated proteins followed by their identification with **mass spectrometry**. Some expected hits: silaffin-modifying enzymes, SDV-associated receptors and proteins involved in vesicle transport and interactions with cytoskeleton, $Si(OH)_4$-interacting proteins, and lysyl oxidases.

Silaffin TpSil3

- **Silica precipitating, cell wall associated protein.**

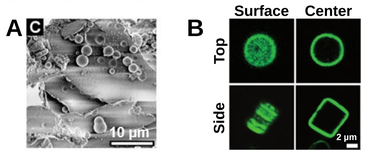

Figure 4 | Silaffin TpSil3. (A) TpSil3, the mature form of the precursor protein TpSil3p, precipitates silica *in vitro* (Poulsen & Kröger, 2004) and (B) its fusion with GFP localizes to the silica cell wall (Poulsen et al., 2007).

APEX2

- 2nd generation **engineered peroxidase** for spatial proteomics.

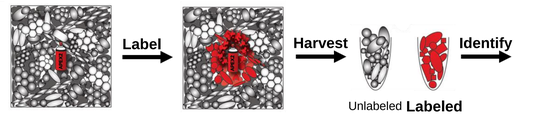

Figure 5 | "Molecular painting" with APEX2. APEX2 can be genetically targeted to a cellular compartment where it catalyzes attachment of biotin to proximal endogenous proteins. Labelled proteins can be harvested and identified with mass spectrometry (adapted from Marx, 2015).

Results

Conjugation of *T. pseudonana*

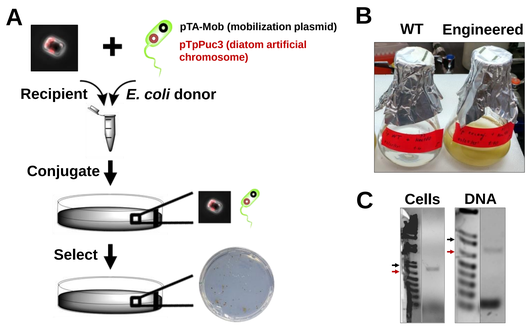

Figure 7 | Conjugation of *T. pseudonana*. (A) Engineered *T. pseudonana* cells appear on agar plates supplemented with 100 ng/µL nourseothricin after ~10 days. (B) Transgenic cells (right) growing in the presence of antibiotic. Wild type control (left). (C) Transgene-specific PCR run on engineered *T. pseudonana* cells (left) and on DNA purified from them (right) yields the expected 0.8 kb product. Black and red arrows: ladder bands at 0.85 and 0.65 kb, respectively.

TpSil3-APEX2 Donor Strain

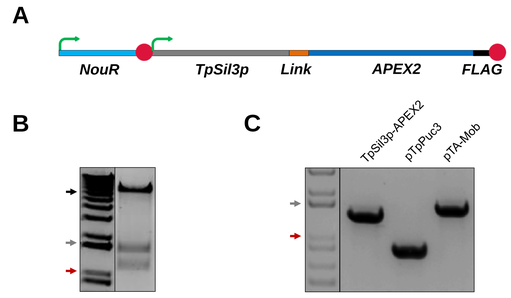

Figure 8 | *E. coli* donor for spatial proteomics. (A) Diatom artificial chromosome expressing TpSil3p-APEX2. Green arrows and red circles: constitutive promoters and associated terminators, respectively. (B) Digestion of the constructed chromosome yields the expected 7.78, 1.72 and 1.26 kb fragments. (C) Diagnostic PCR on a doubly transformed *E. coli* strain confirms the presence of the mobilization plasmid and the TpSil3p-APEX2 encoding diatom artificial chromosome. Arrows: 6 kb (black), 1.65 kb (grey), 1 kb (red).

Endogenous Protein Biotinylation in *T. pseudonana*

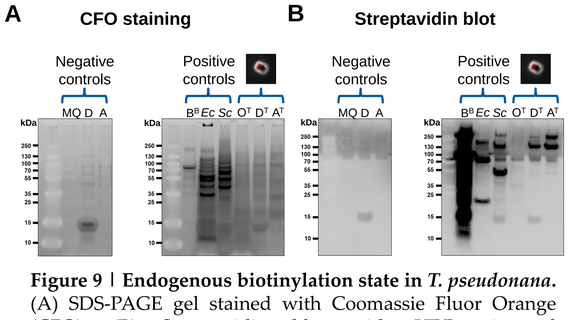

Figure 9 | Endogenous biotinylation state in *T. pseudonana*. (A) SDS-PAGE gel stained with Coomassie Fluor Orange (CFO). (B) Streptavidin blot with HRP-conjugated streptavidin. Details: MQ: MQ water; D, A: lysis buffers; O^T, D^T, A^T: *T. pseudonana* lysates using three different lysis protocols; B^B: 0.4 µg biotinylated BSA (~66.5 kDa); *Ec*: *E. coli* (NEB5α) lysate; *S. cerevisiae* (BY4743) lysate. *E. coli* contains 1 trimeric biotinylated protein complex, *S. cerevisiae* 5 biotinylated proteins whereas at least 4 proteins in *T. pseudonana* that are predicted to contain biotin are yet to be characterized.

Ongoing Work

- **Optimizing conjugation conditions** using TpSil3p-APEX2 *E. coli* donor strain.
- Designing and building **alternative diatom artificial chromosomes for spatial proteomics**.
  - BirA- and HRP- tagged TpSil3p
- **Streptavidin pull-down** and identification of endogenous biotinylated proteins in *T. pseudonana.*

Funding

This project is funded by the Gordon and Betty Moore Foundation through Grant GBMF4958 to Julie Theriot (Stanford University), Andrew Fire (Stanford University) and Pamela Silver (Harvard University).

Contact