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**. The complete understanding of the molecular mechanism guiding the **morphogenesis** of this hybrid biomaterial remains elusive. 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**. In addition to advancing our understanding of the long-standing biological problem of diatom cell wall biogenesis, these efforts will provide a platform to start **cataloging proteomes of other partially understood biological processes in diatoms**.

Diatoms

Importance

- **Biomonitoring tool** for aquatic environments.
- Produce about **20% of the Earth's oxygen**.
- Crucial for **C, N and Si biogeochemical cycles**.

Model organisms

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

Figure 1 | Morphological diversity of diatoms. (A) It is estimated there are **100,000 diatom species** each encasing itself in a unique glassy shell (Kröger, 2007). (B) Diatom star made by Klaus Kemp, a specialist in diatom microslides and diatom arrangements.

Biosilicification in Diatoms

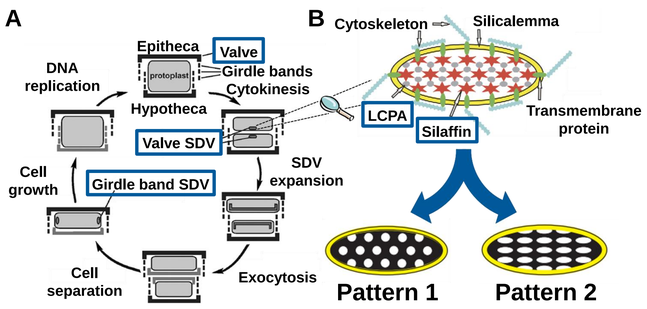


Figure 2 | Biosilicification in diatoms. (A) Diatom cell wall is synthesized inside an acidic compartment called the **silica deposition vesicle** (SDV). (B) It is hypothesized that the **interaction of silaffins with long-chain polyamines (LCPA)** inside the SDV leads to the formation of an organic 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).

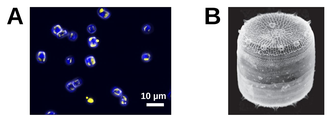


Figure 3 | *Thalassiosira pseudonana.* (A) Fluorescence microscopy image of *T. pseudonana* (yellow, plastids; blue, PDMPO-stained silica). (B) SEM image of *T. pseudonana* (courtesy: Nils Kröger).

Silaffin TpSil3

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

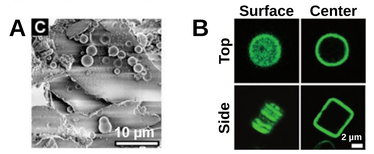


Figure 4 | Silaffin TpSil3. (A) TpSil3, the mature form of the precursor protein TpSil3p, precipitates silica *in vitro* (Poulsen & Kröger, 2004) and (B) its fusion with GFP localizes to the silica cell wall (Poulsen et al., 2007).

APEX2

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

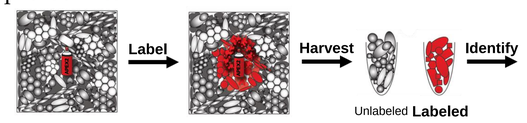
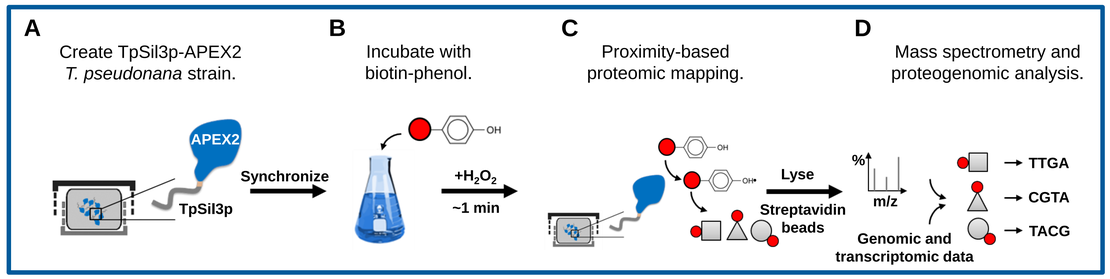


Figure 5 | "Molecular painting" with APEX2. APEX2 can be genetically targeted to a cellular compartment where it catalyzes attachment of biotin to proximal endogenous proteins. Labelled proteins can be harvested and identified with mass spectrometry (adapted from Marx, 2015).

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.



Results

Conjugation of *T. pseudonana*

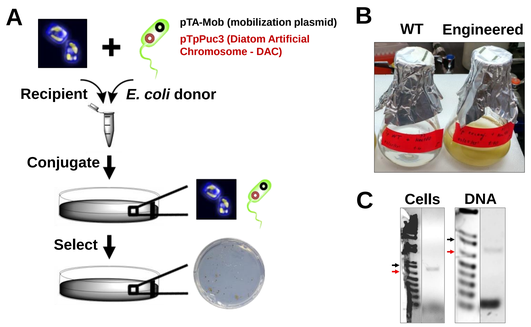


Figure 7 | Conjugation of *T. pseudonana*. (A) Genetically modified *T. pseudonana* cells appear on agar plates supplemented with 50 or 100 ng/µL nourseothricin after ~10 days. (B) Transgenic cells (right) growing in the presence of antibiotic. Wild type control (left). (C) pTpPuc3-specific PCR run on conjugated *T. pseudonana* cells (left) and on DNA purified from them (right) yields the expected 0.8 kb product. Side arrows: 0.85 kb (black), 0.65 kb (red).

TpSil3p-APEX2 *T. pseudonana* Strain

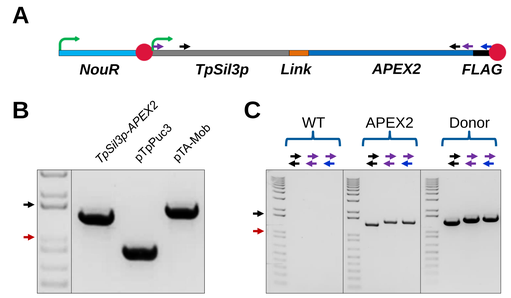


Figure 8 | TpSil3p-APEX2 *T. pseudonana* strain. (A) Diatom artificial chromosome (DAC) expressing TpSil3p-APEX2. Green arrows and red circles: constitutive promoters and associated terminators, respectively. (B) Diagnostic PCR on a doubly transformed *E. coli* donor strain confirms the presence of the mobilization plasmid and the TpSil3p-APEX2 encoding DAC. (C) Diagnostic PCRs confirm the presence of intact TpSil3p-APEX2-FLAG gene in *T. pseudonana* exconjugant. Side arrows: 1.65 kb (black), 1 kb (red).

Endogenous Protein Biotinylation in *T. pseudonana*

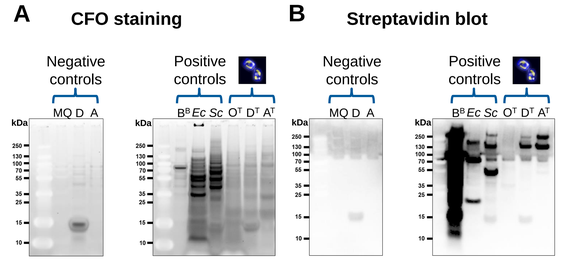


Figure 9 | Endogenous biotinylation state in *T. pseudonana*. (A) SDS-PAGE gel stained with Coomassie Fluor Orange (CFO). (B) Streptavidin blot with HRP-conjugated streptavidin. Details: MQ: MQ water; D, A: lysis buffers; O^T, D^T, A^T: *T. pseudonana* lysates using three different lysis protocols; B^B: 0.4 µg biotinylated BSA (~66.5 kDa); *Ec*: *E. coli* (NEB5α) lysate; *Sc*: *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

- **Western blot** and **Amplex UltraRed assay** to confirm TpSil3p-APEX2 expression and activity.
- Designing and building **alternative diatom artificial chromosomes for spatial proteomics**.
  - BirA-, HRP- and 3xFLAG-tagged TpSil3p
- **Streptavidin pull-down** and identification of endogenous biotinylated proteins in *T. pseudonana*.

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Contact