

Illuminating *Phaeodactylum tricornutum* Cell Biology with a Genetically Encodable Tag for Electron Microscopy and Subcellular Proteomics

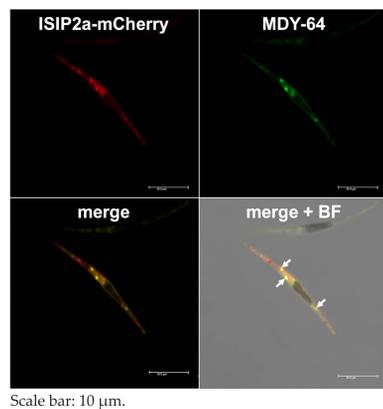


1. Background

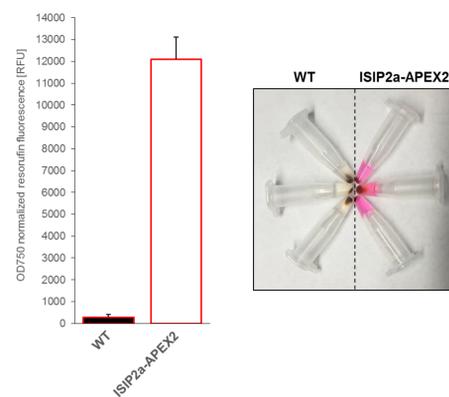
Iron plays a crucial role in many key enzymes linked to photosynthesis, respiration and nitrogen fixation. **Iron availability limits primary productivity in ~30% of the modern oceans.** Our laboratory has identified **phytotransferrins** as a new group of high affinity ferric iron-binding proteins widespread among marine microeukaryotes. **Phydotransferrin ISIP2a** from a model diatom *Phaeodactylum tricornutum* **internalizes ferric iron via endocytosis**, but the molecular details behind ion liberation, chemical speciation and intracellular allocation remain elusive.

3. Results

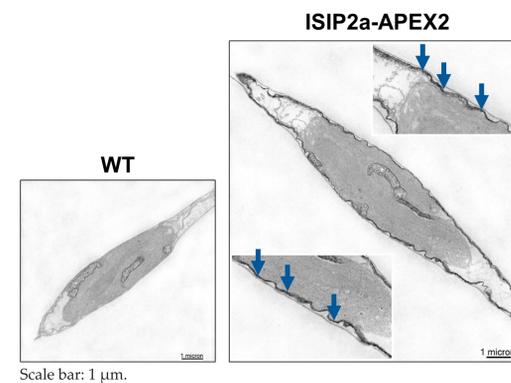
3.1 ISIP2a-mCherry colocalization with MDY-64 (membrane stain) further supports ISIP2a endocytosis model.



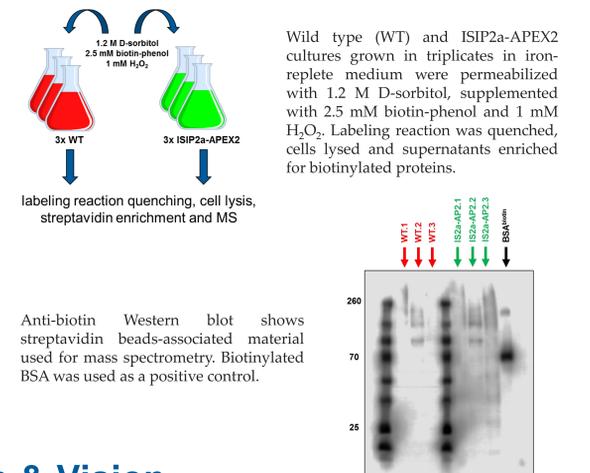
3.2 Confirming *in vivo* peroxidase activity of APEX2 with Amplex UltraRed assay.



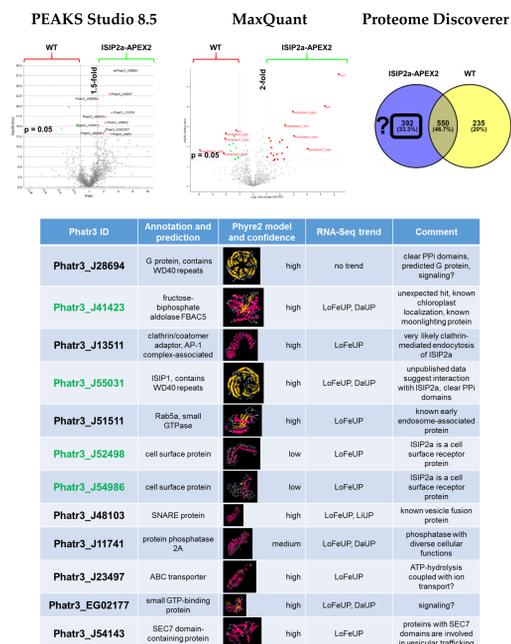
3.3 Visualizing ISIP2a-APEX2 using transmission electron microscopy (TEM).



3.4 Subcellular proteomic experiment summary.

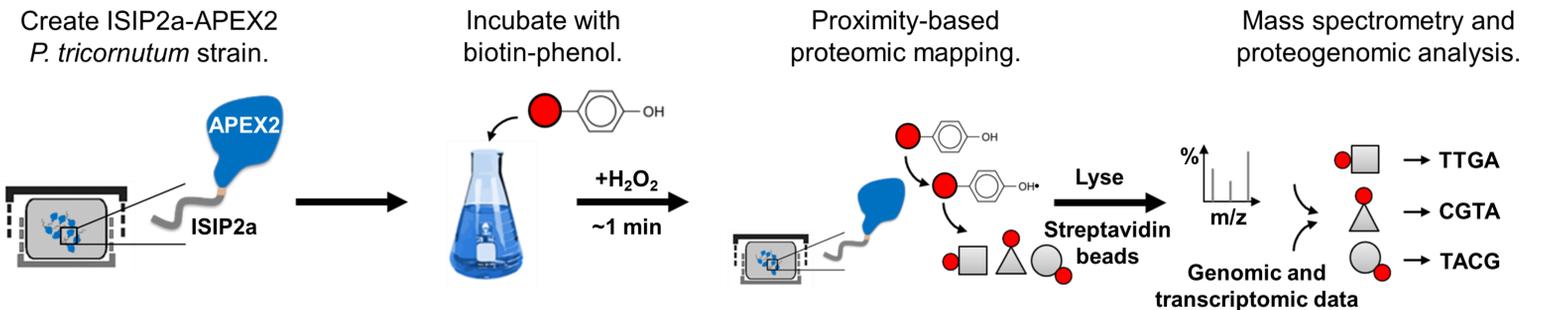


3.5 Label-free quantification, putative ISIP2a interactors and vicinal proteins.

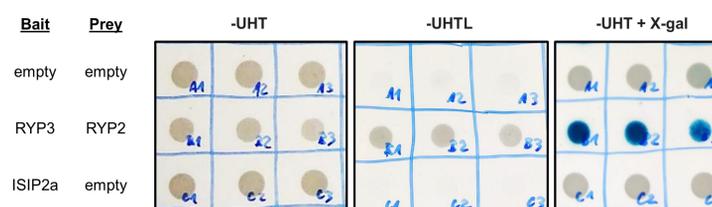


Highlighted in green are proteins that were highly enriched (at least 2-fold above WT) in ISIP2a-APEX2 strains in an independent Tandem Mass Tag proteomic experiment performed in quintuplicates. LiUP: upregulated in light, DaUP: upregulated in dark, LoFeUP: upregulated at low Fe (20 or 40 pM Fe').

2. Approach



3.6 Successful implementation of a yeast two-hybrid (Y2H) screen to study protein interactions with ISIP2a.



In our Y2H screen, a protein-protein interaction leads to restored ability of yeast cells to grow in the absence of leucine as well as their ability to break down X-gal. Cells were spotted on each of the following three plates: -UHT (all strains will grow), -UHTL (only strains where protein-protein interaction occurs will grow), -UHT + X-gal (all strains will grow; strains where protein-protein interaction occurs will turn blue). Plates were incubated for 2 days at 30 °C and imaged. U: uracil, H: histidine, T: tryptophan, L: leucine. RYP3 and RYP2 are known interacting proteins from a fungal pathogen. ISIP2a alone doesn't activate reporter genes.

4. Next steps

- Y2H screen using a prey library containing proteins identified with MS is in progress.
- Co-localization studies in *Phaeodactylum tricornutum*.
- His tag/Strep-tag II pull-downs for additional cross-validation.

Funding

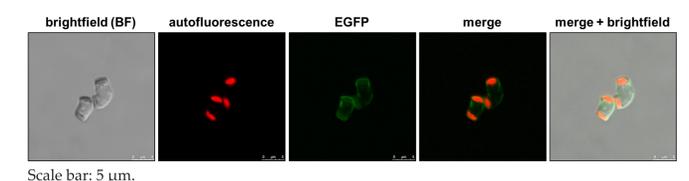
This research is supported by the Department of Energy, Office of Biological and Environmental Research (BER), grant DE-SC0018344 (AEA), and by the Gordon and Betty Moore Foundation (GBMF), grant GBMF4958 (JT).

5. Conclusions & Vision

We have implemented APEX2, a genetically encodable ascorbate peroxidase, in *Phaeodactylum tricornutum*, to promote high resolution protein imaging and cataloging of biological pathway-specific proteomes. This work represents the first application of APEX2 technology in a photosynthetic host. We envision it will allow us to dissect a range of outstanding cell biology questions in *Phaeodactylum tricornutum* and other diatoms as they relate to their prominent role in global biogeochemical cycles, unique evolutionary history, and biotechnological potential.

6. Future applications of APEX2 in diatoms

Proteomic characterization of a silica deposition vesicle (SDV) in *Thalassiosira pseudonana* (Tp) – a biomineralization model system.



Ornamented silicified cell walls are the hallmark of diatom biology. Biosilica morphogenesis proceeds inside a silica deposition vesicle (SDV). A revisited *Thalassiosira pseudonana* conjugation protocol was used to localize a known SDV-associated protein. We are currently preparing strains expressing this protein fused to APEX2.

Acknowledgements

We thank Pardis Gholami for help with diatom culture work; Tom Deerinck, Mason Mackey, Andrea Thor, Daniela Boassa and Mark Ellisman for help with TEM; Majid Ghassemian, Yanbao Yu, Marian Kalocsay and Steven Gygi for help with mass spectrometry and proteomic data analysis; Sinem Beyhan for help with Y2H work.

Affiliations

¹Harvard Medical School, Department of Systems Biology, Boston, MA 02115
²Microbial and Environmental Genomics, J. Craig Venter Institute, La Jolla, CA 92037
³Integrative Oceanography Division, Scripps Institution of Oceanography, UC San Diego, La Jolla, CA 92037

Contact

