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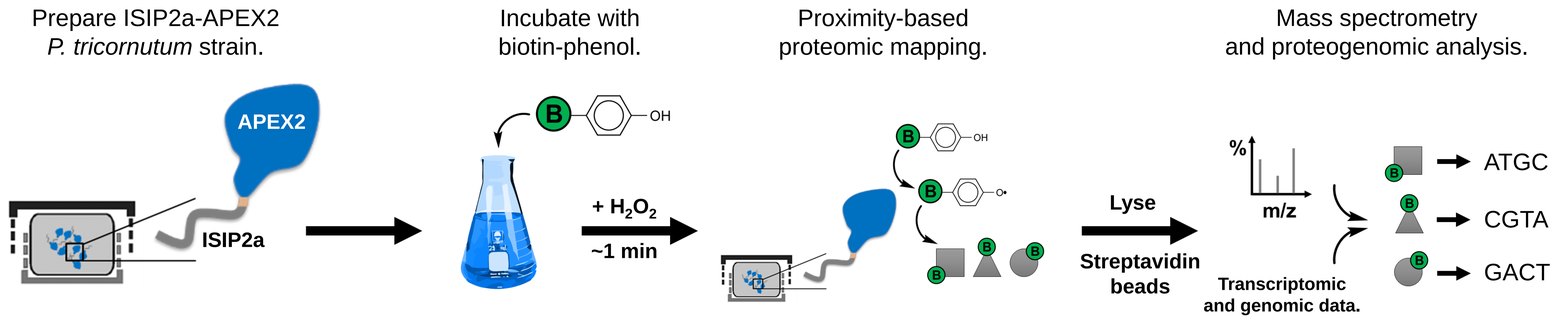
Illuminating *Phaeodactylum tricornutum* Cell Biology with a Genetically Encodable Tag for Electron Microscopy and Subcellular Proteomics

1. Background

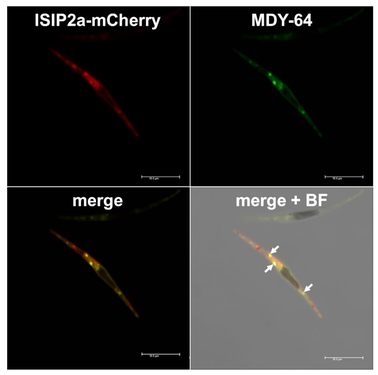
Iron plays a crucial role in many key enzymes linked to photosynthesis, respiration and nitrogen fixation. **Iron availability limits primary productivity in ~30% of the modern oceans**. Our laboratory has identified **phytotransferrins as a new group of high affinity ferric iron-binding proteins** widespread among marine microeukaryotes. **Phytotransferrin 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.

2. Approach



3. Results

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



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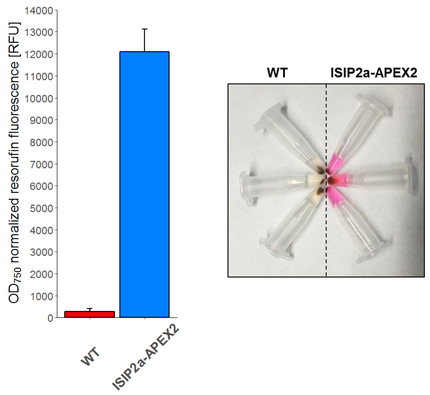
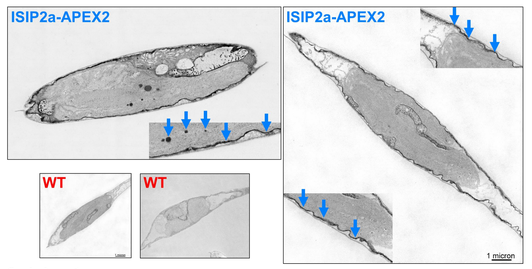
3.2 Confirming *in vivo* peroxidase activity of APEX2 with Amplex UltraRed assay.



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



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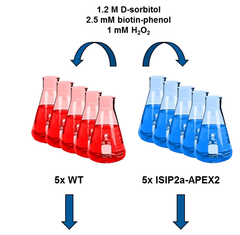
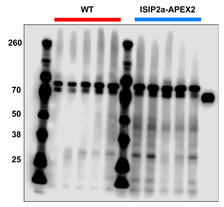
3.4 Subcellular proteomic experiment summary.

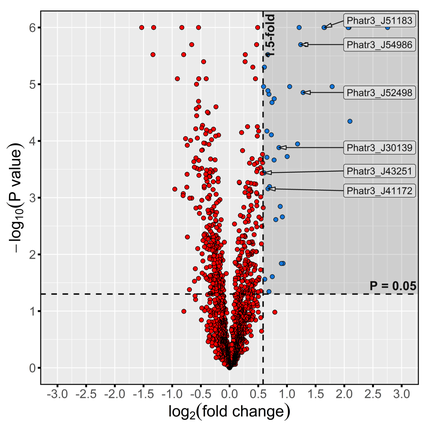


Wild type (WT) and ISIP2a-APEX2 cultures grown in quintuplicates in iron-replete medium were permeabilized with 1.2 M D-sorbitol, supplemented with 2.5 mM biotin-phenol and 1 mM H_2O_2. Labeling reaction was quenched, cells lysed and supernatants enriched for biotinylated proteins.

Anti-biotin Western blot after the labeling reaction. Biotinylated BSA was used as a positive control (rightmost lane).



3.5 Identification of putative ISIP2a interactors and vicinal proteins.



Gene ID	Annotation and prediction	RNA-Seq trend	Comment
Phatr3_J54986	cell surface protein	LoFeUP	ISIP2a is a cell surface receptor protein
Phatr3_J52498	cell surface protein	LoFeUP	ISIP2a is a cell surface receptor protein
Phatr3_J51183	CREG1	LoFeUP	known excreted protein
Phatr3_J30139	Sec4	LoFeUP	secretory Rab GTPase
Phatr3_J41172	calreticulin	/	multifunctional Ca^{2+} storage protein
Phatr3_J43251	Arf1 GTPase	LiUP	phospholipase D activator

Tandem Mass Tag (TMT) proteomic data revealed **38 candidate proteins interacting with or vicinal to ISIP2a**. 6 of them will be co-localized with ISIP2a and used in co-immunoprecipitation experiments. LiUP: upregulated in light, LoFeUP: upregulated at low Fe (20 or 40 pM Fe').

4. Next steps

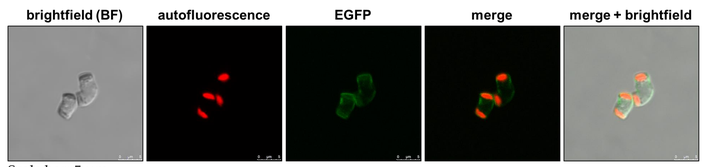
- **His tag pull-downs** and mass spectrometry for additional cross-validation and identification of tightly interacting proteins.
- **Co-localization** and **co-immunoprecipitation** studies.

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 silica deposition vesicles.



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Silicified cell walls with nanosized features are a hallmark of diatom biology. **Biosilica morphogenesis** proceeds inside a **silica deposition vesicle (SDV)**. A revisited conjugation protocol was used to localize a known SDV-associated protein in ***Thalassiosira pseudonana***—biomineralization model diatom species. Proximity proteomic labeling from such proteins will allow us to further characterize **this elusive eukaryotic compartment**.

7. References

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