



Exploring Metabolite Production in *Pseudo-nitzschia* Diatoms

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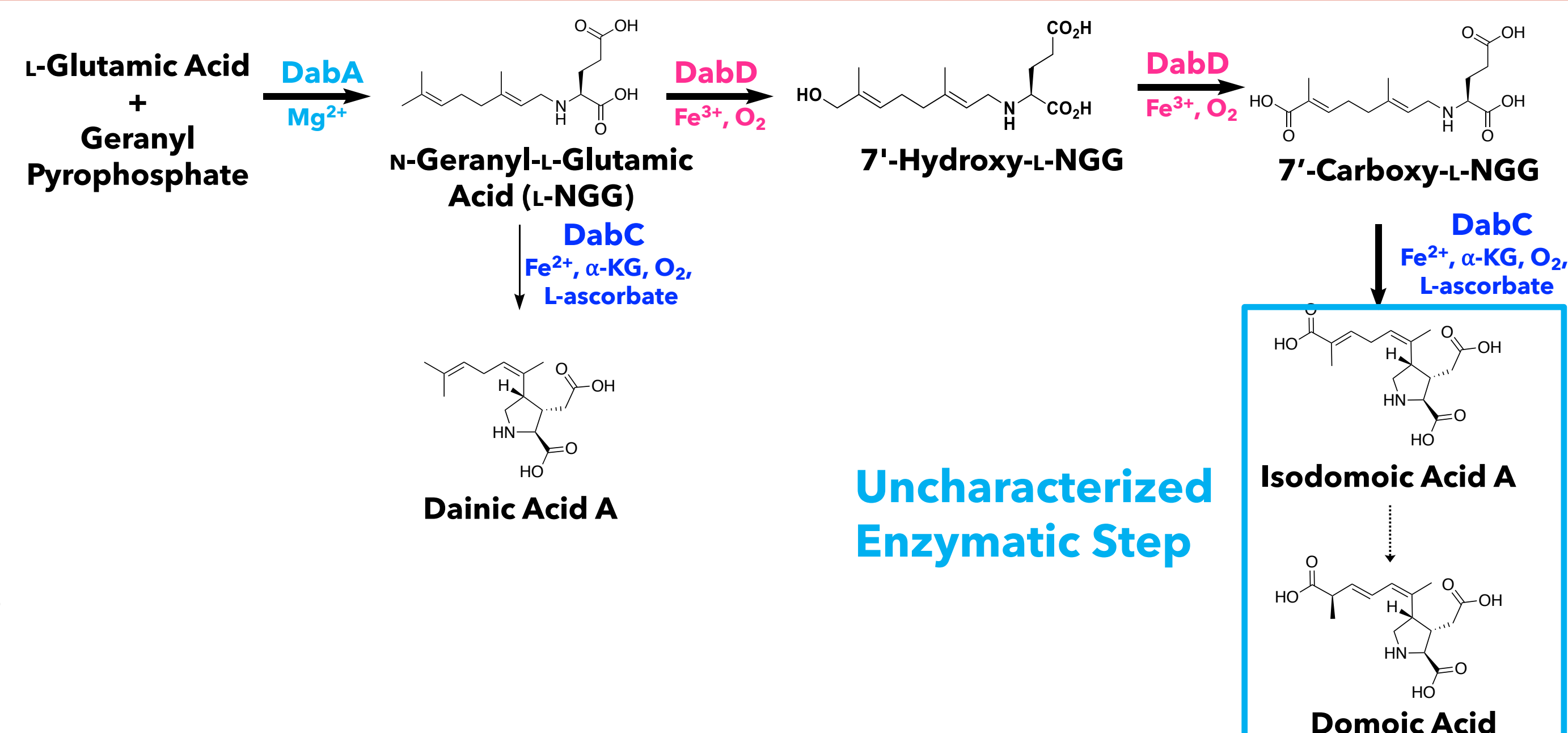
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Pseudo-nitzschia Diatoms Biosynthesize Domoic Acid

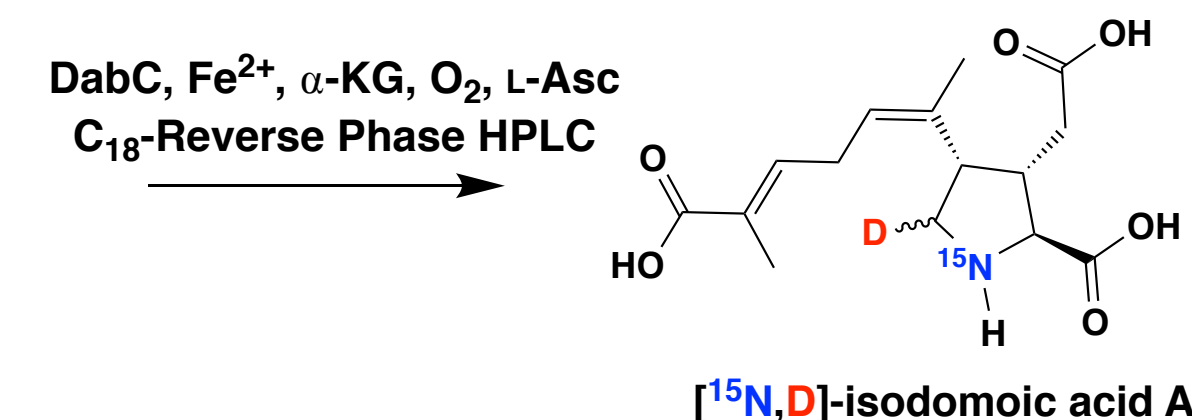
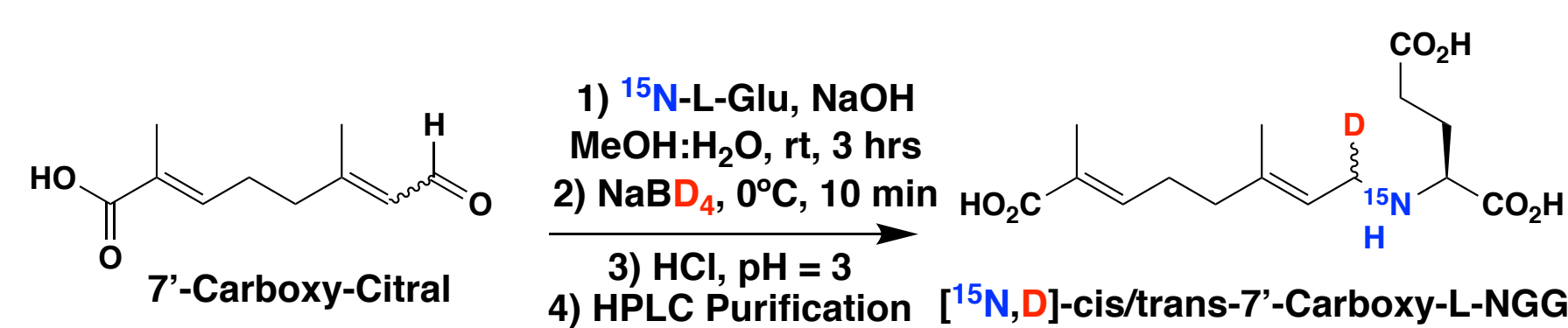
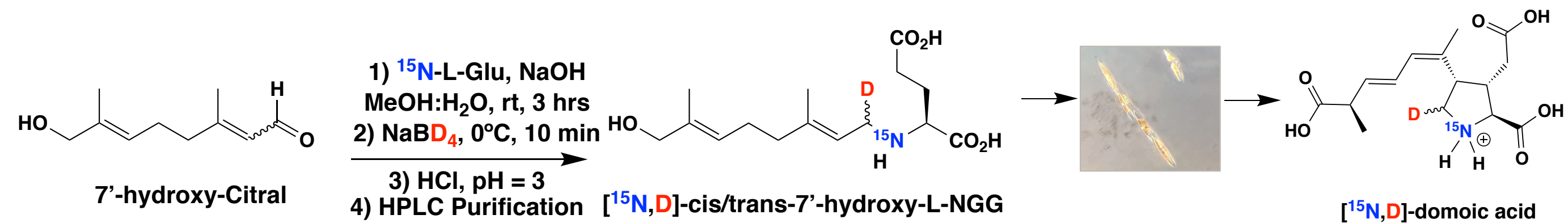
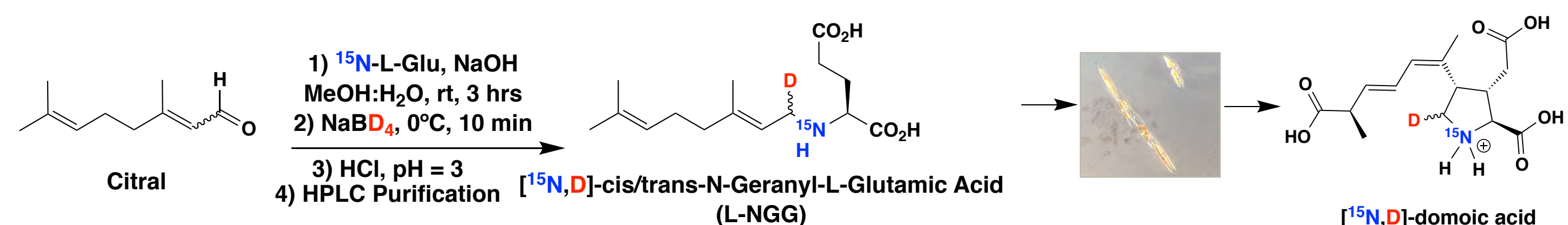
Pseudo-nitzschia is a diatom genus in coastal oceans worldwide. Blooms of *Pseudo-nitzschia* often produce domoic acid (DA), a potent neurotoxin that accumulates in shellfish and can cause serious illnesses in humans and marine mammals.

DA biosynthesis was recently decoded by our group, establishing a cluster of transcriptionally co-regulated genes (*dabA*, *dabB*, *dabC*, *dabD*) that encode enzymes that perform all but one step of the biosynthesis (Brunson *et al.*, *Science* 2018).



Confirming Biosynthetic Intermediates *in vivo*

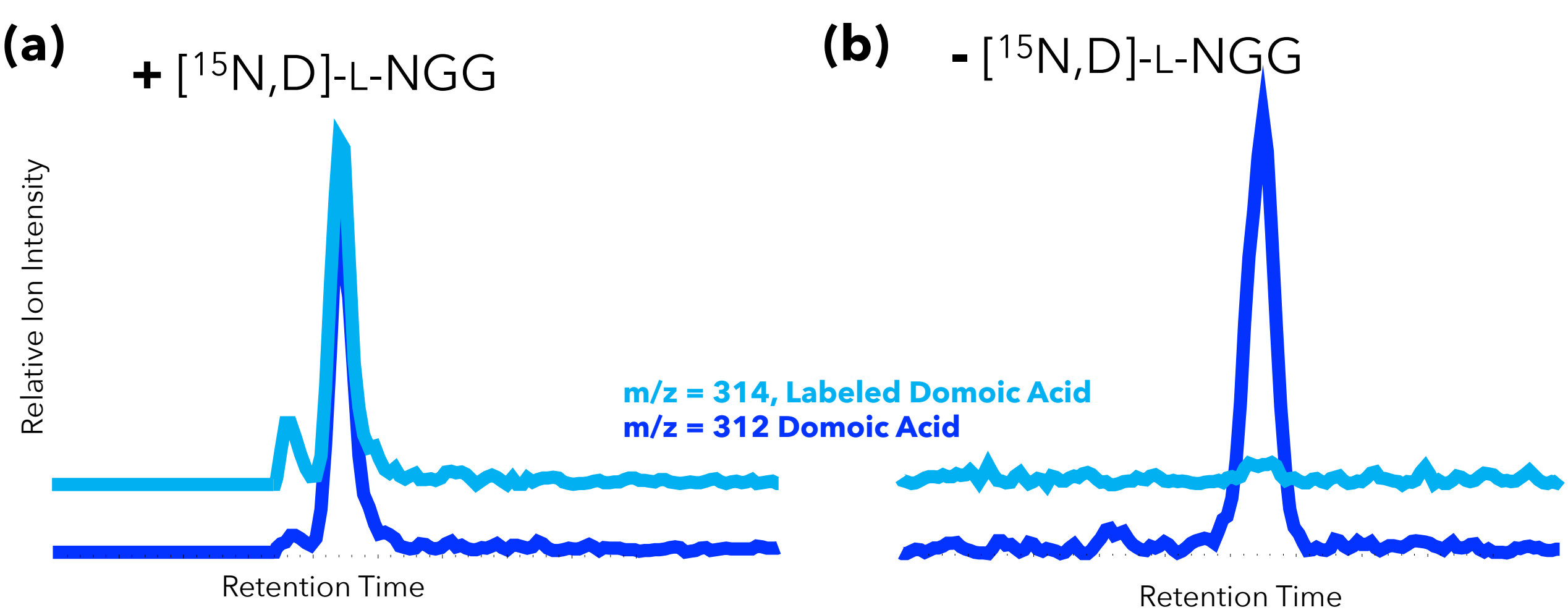
Synthesis of Labeled Intermediates & Feeding Experiments



Experiments in progress.

Labeled intermediates were incubated with a live culture of *P. australis* under silicate starvation. Silicate stress is used to induce DA production. L-NGG and 7'-hydroxy-L-NGG have been confirmed as biosynthetic intermediates.

Feeding Experiments yield [¹⁵N,²D]-Domoic Acid



An example of raw data. *P. australis* culture fed with **(a)** [¹⁵N,²D]-L-NGG and **(b)** solvent control.

Objectives

To confirm each proposed step of DA biosynthesis *in vivo* through stable isotope labeled feeding experiments.

To explore DA intermediate and analog metabolites *in vivo* using molecular networking.

Future Work

Feeding experiments with modified 7'-carboxy-L-NGG, dainic acid A, and isodomoic acid A.

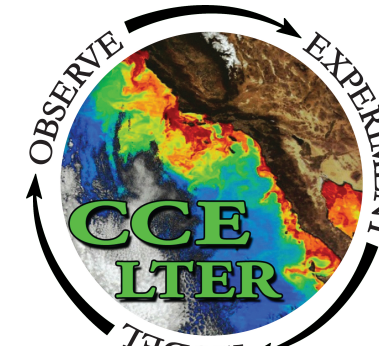
Species comparison through feeding experiments in a non-DA producer *Pseudo-nitzschia delicatissima*.

References & Support

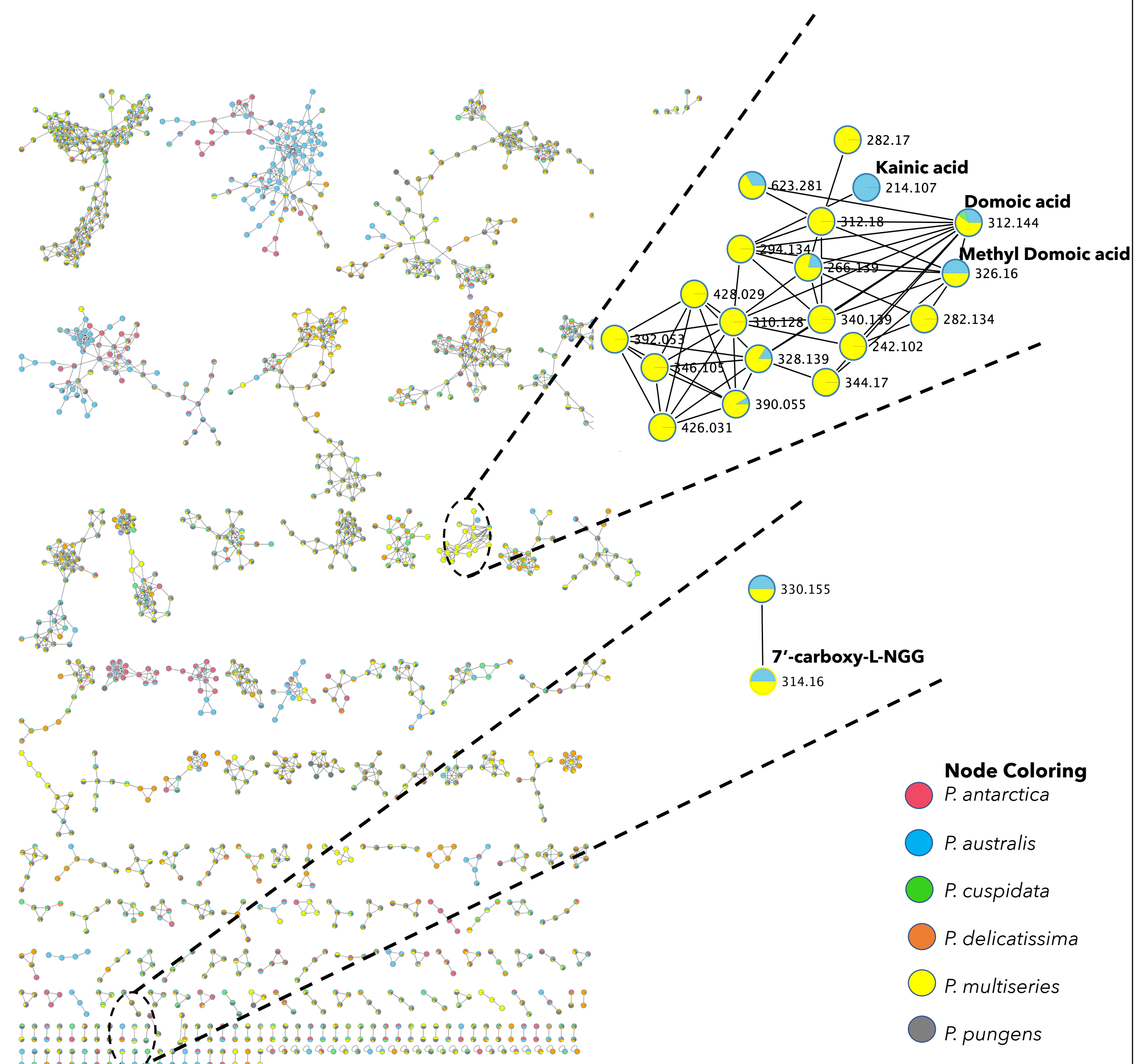
We thank Dr. Jason Smith (Moss Landing Marine Laboratories) for contributing the *P. australis* strain and our SIO colleague Dr. Lihini Aluwihare for helpful discussions and data sharing.

Brunson, J. K. & S. M. K. McKinnie, *et al.*, (2018), Biosynthesis of the neurotoxin domoic acid in a bloom-forming diatom. *Science*.

Maeno, Y., *et al.*, (2018). Six domoic acid related compounds from the red alga, *Chondria armata*, and domoic acid biosynthesis by the diatom, *Pseudo-nitzschia multiseries*. *Scientific Reports*.



Metabolites in Cultures



Metabolites in cultures of six species of *Pseudo-nitzschia*, three of which produce DA. Network is generated using Global Natural Products Social Molecular Networking (GNPS), where each node represents one molecule and clustered nodes form networks of similar molecules.