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Supporting information for article:

Structural and biochemical characterization of novel carbonic anhydrases from *Phaeodactylum tricornutum*

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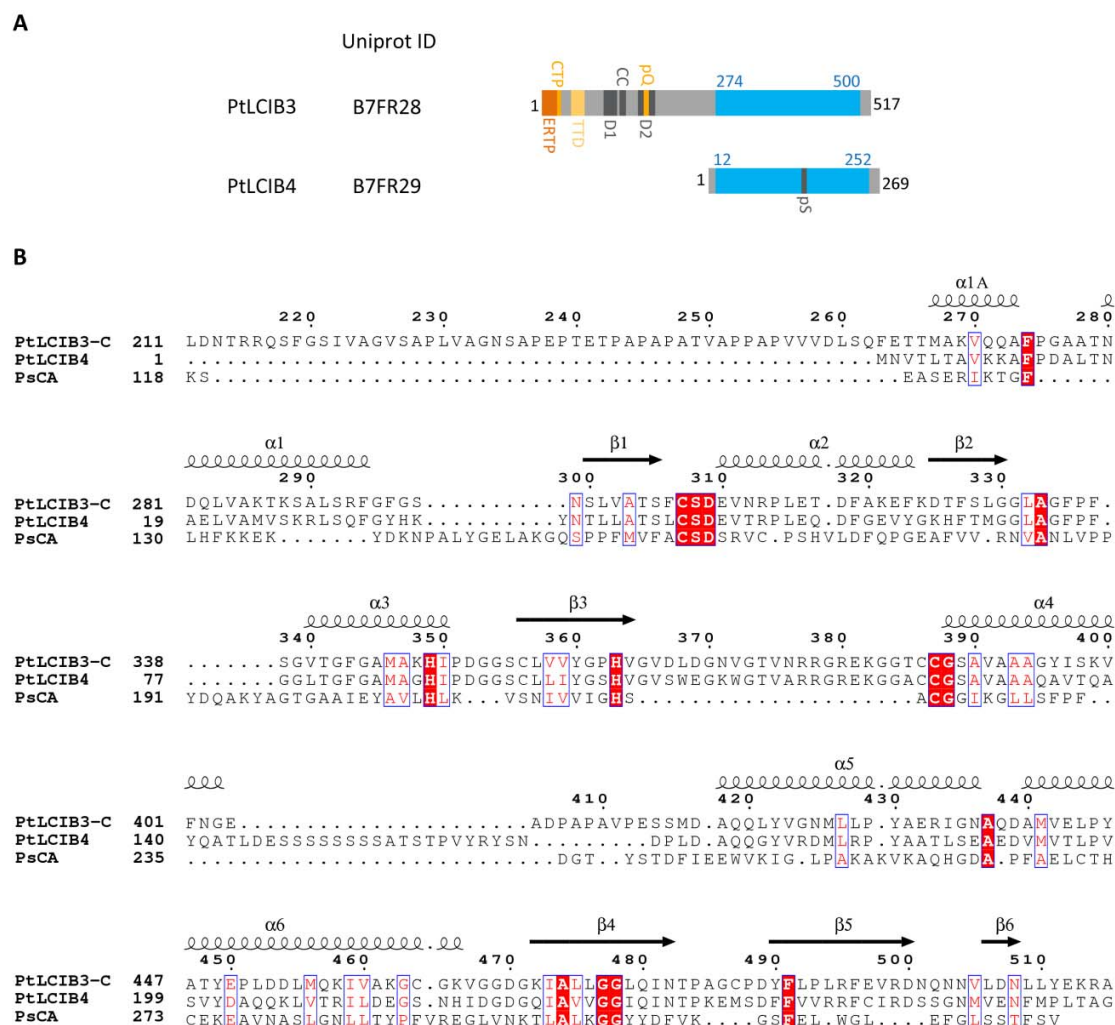


Figure S1 Construct of PtLCIB3 and PtLCIB4 proteins. **(A)** Schematic drawing of the PtLCIB3 and PtLCIB4 genes. On PtLCIB3, the predicted ER transit peptide (ERTP), chloroplast transit peptide (CTP), thylakoid-targeting domain (TTD), disordered regions (D1 and D2), coiled coil region (CC), and poly-Q region (pQ) are labelled. On PtLCIB4, the poly-S region (pS) is labelled. LCIB-homologous domains in both proteins are represented by blue bars. **(B)** Amino acid sequences of PtLCIB3, PtLCIB4, and PsCA are aligned based on their structure alignment using PROMALS3D (Pei *et al.*, 2008) and drawn using ESPrpt 3.0. Sequence on top of the alignment and secondary structure elements are labeled with respect to PtLCIB3.

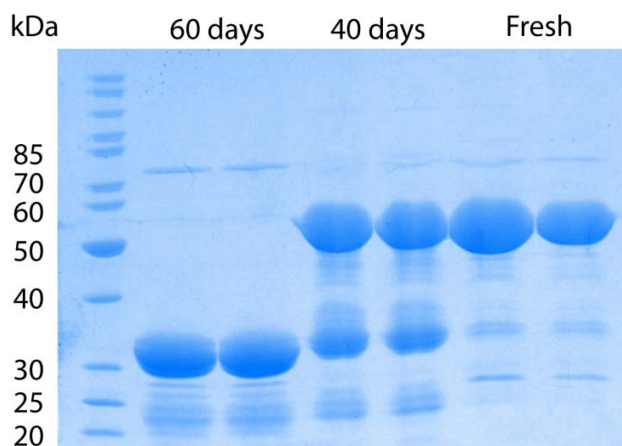


Figure S2 Degradation of the PtLCIB3 protein. Purified PtLCIB3 protein are stored at 4 °C for various periods of time and checked on SDS-PAGE. Duplicate of each sample is present on the gel.

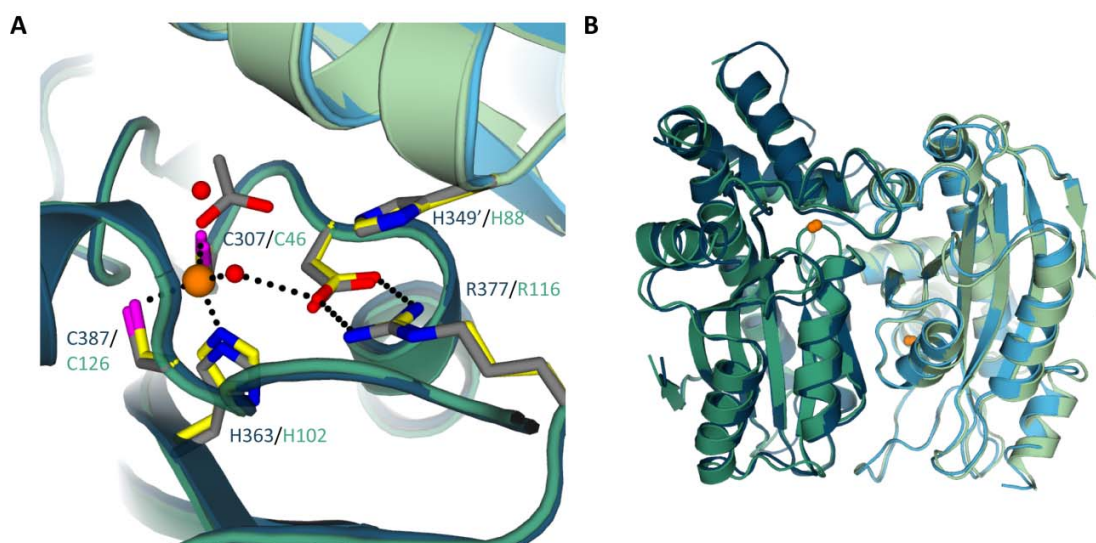


Figure S3 Comparison of PtLCIB3-C and PtLCIB4 structure. **(A)** PtLCIB3-C is shown in dark and pale blue cartoon. The superimposed PtLCIB4 structure is shown in dark and pale green. Active site zinc and waters are from PtLCIB3-C, whereas the acetate is from PtLCIB4. Selected PtLCIB3/4 active site residues are shown in yellow/grey sticks, respectively. Polar interactions are indicated by black dashes. **(B)** Superposition of PtLCIB3-C (dark and pale blues) and PtLCIB4 (dark and pale greens) dimers shows close structural homology between the two proteins.

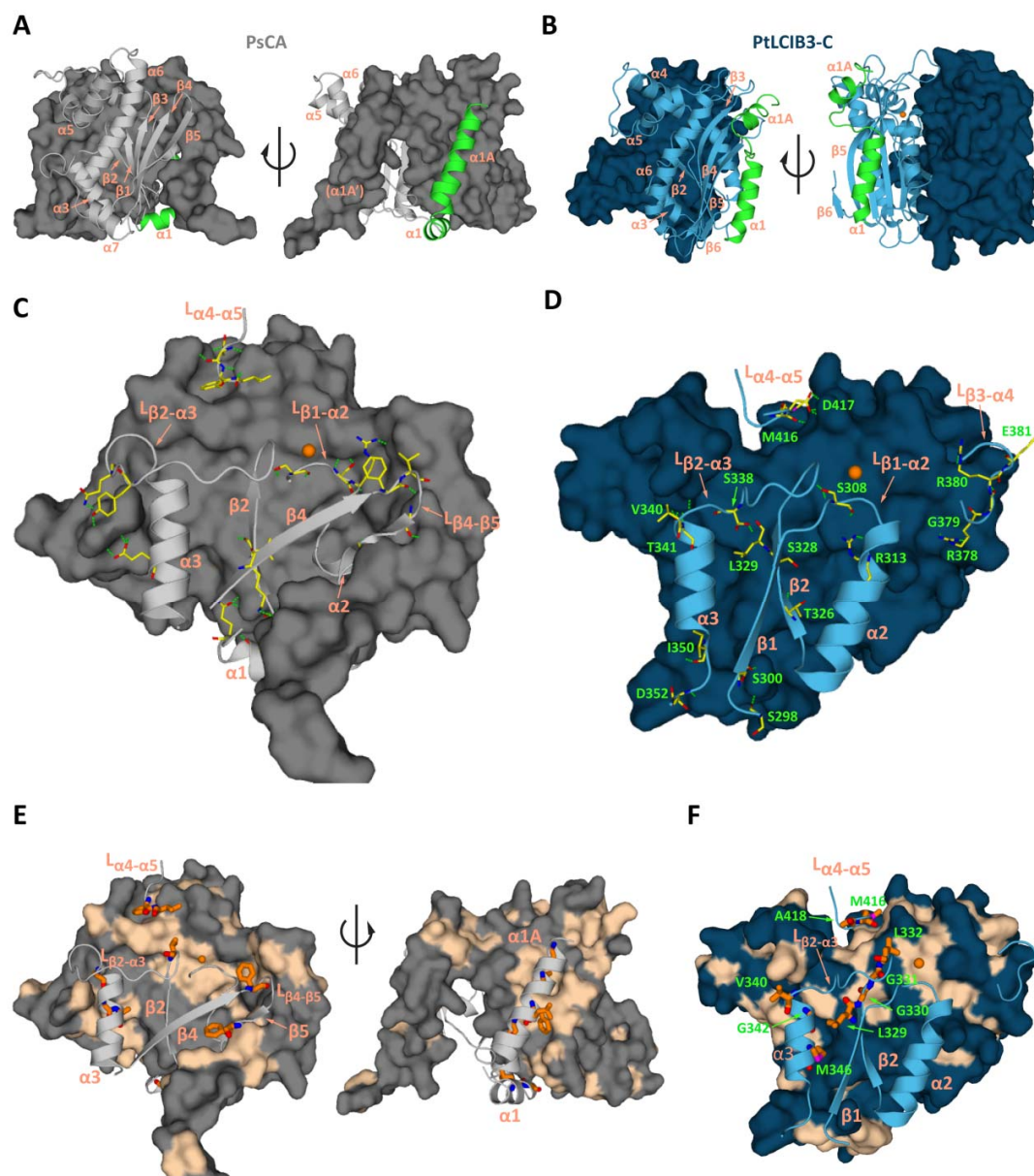
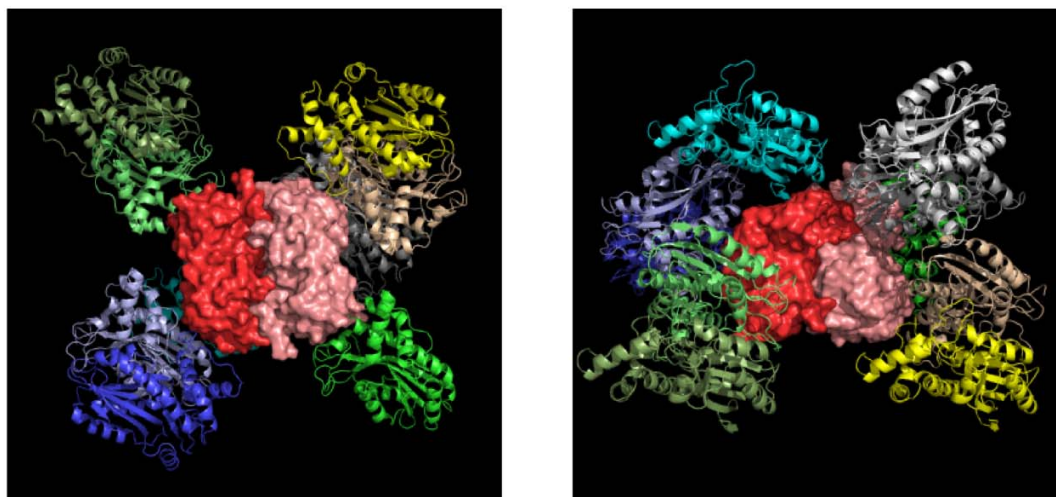


Figure S4 Comparison between PtLCIB3-C and PsCA (PDB 1EKJ). In all figures, Zn²⁺ ions are shown as orange spheres and rotations indicated by the arrows are all 90°. Wheat label: secondary structure elements; Green label: residues. In figure (C-F), only residues and secondary structure elements at the dimer interface are shown for clarity. Stereo views of the PsCA (A) and PtLCIB3-C (B) dimer are shown with one protomer in surface rendering. The two N-terminal helices of the two proteins are colored green. The polar interactions between the dimers are shown in (C) for PsCA and in (D) for PtLCIB3-C. Hydrophobic interactions between the dimers are shown in (E) for PsCA and in (F) for PtLCIB3-C. The interface hydrophobic residues in one

protomer are shown in wheat surface color and the ones on the other protomer are shown as orange sticks.

A



B

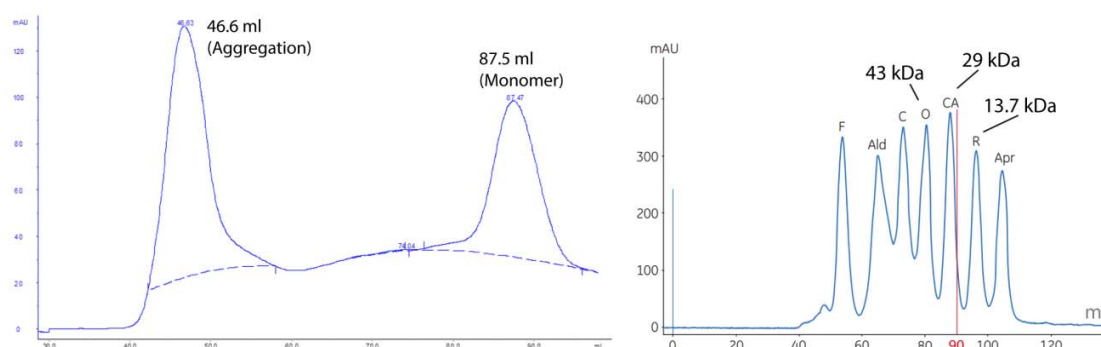
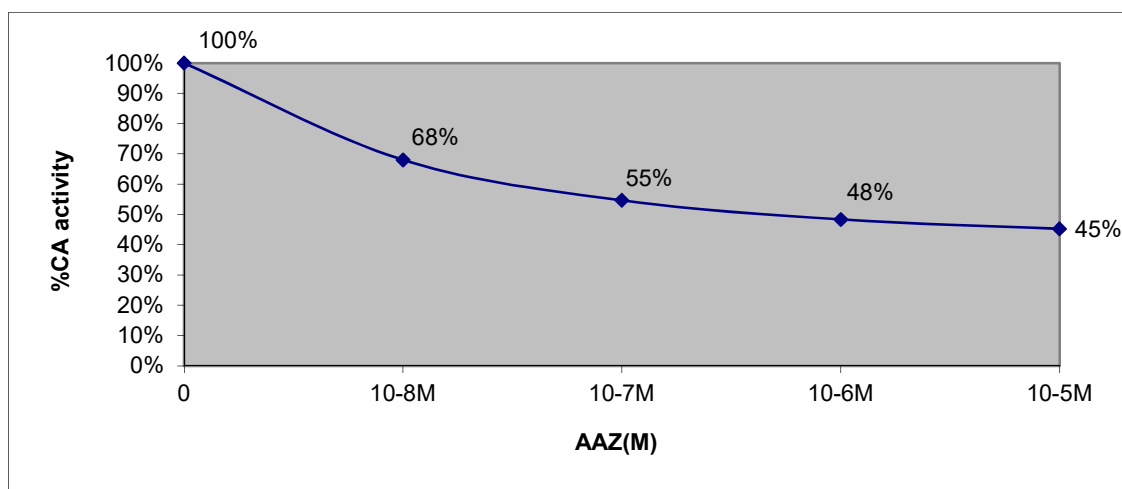


Figure S5 (A) Two stereo views of the symmetry related molecules in the PtLCIB3-C crystal structure. The proposed dimer in our analysis is shown as red and pink surfaces. (B) Gel filtration profile of PtLCIB3-C S308R. The gel filtration column used was HiLoad Superdex 200 16/60. Elution volumes of standard proteins of the gel filtration calibration kit (GE Healthcare, Data file 28-4073-84 AA) are shown on the right.



Compound	Inhibitor		%Free Enzyme	%Inhibition
	concentration	Slope		
PtLCIB3-WT 10^{-7} M	0	0.6782	100%	0%
AAZ	10^{-8} M	0.4613	68%	32%
AAZ	10^{-7} M	0.3708	55%	45%
AAZ	10^{-6} M	0.3279	48%	52%
AAZ	10^{-5} M	0.307	45%	55%

Figure S6 Acetazolamide (AAZ) inhibition of the PtLCIB3 wild type protein.