

## **SUPPORTING INFORMATION**

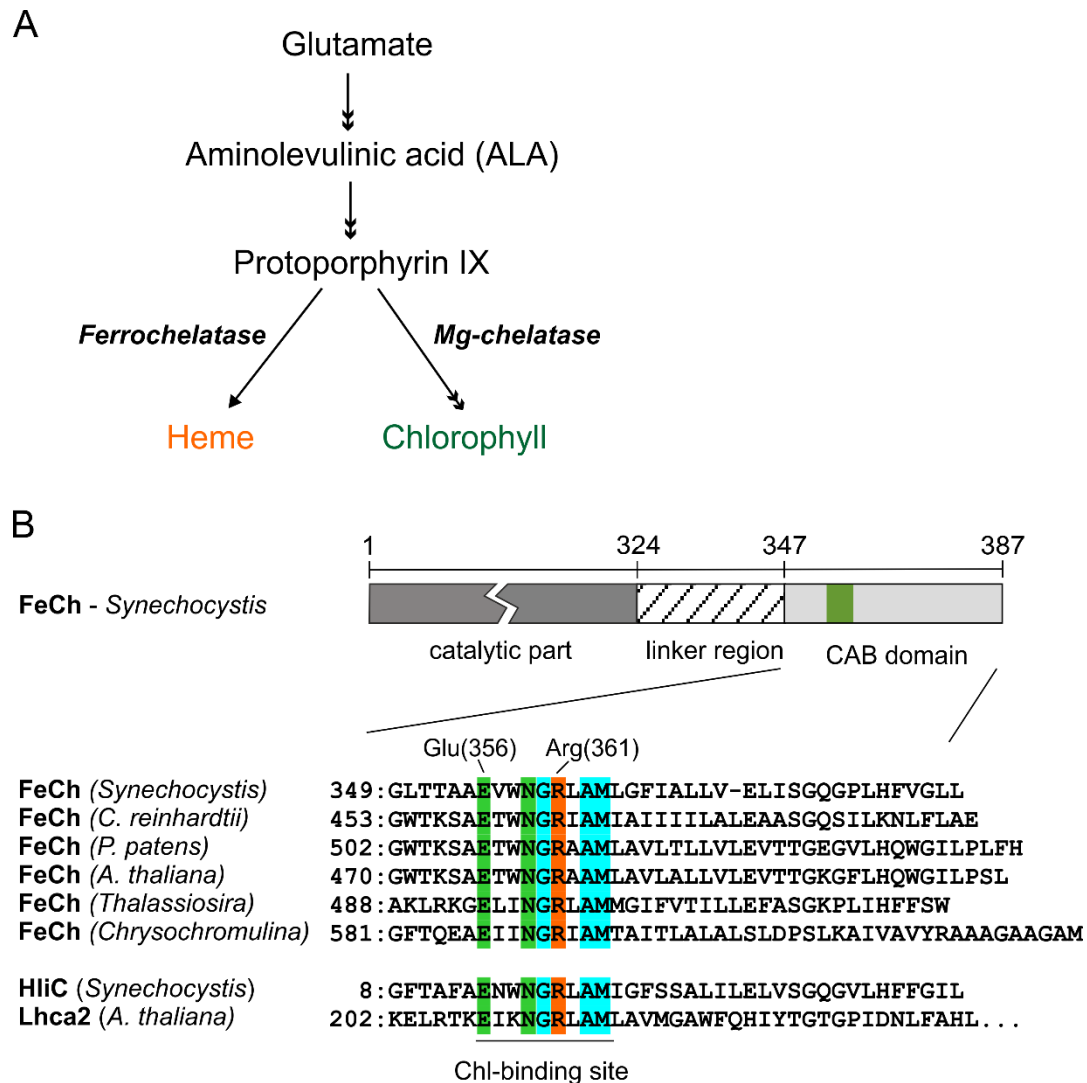
### **Antenna-like domain of the cyanobacterial ferroxidase can bind chlorophyll and carotenoids in an energy-dissipative configuration**

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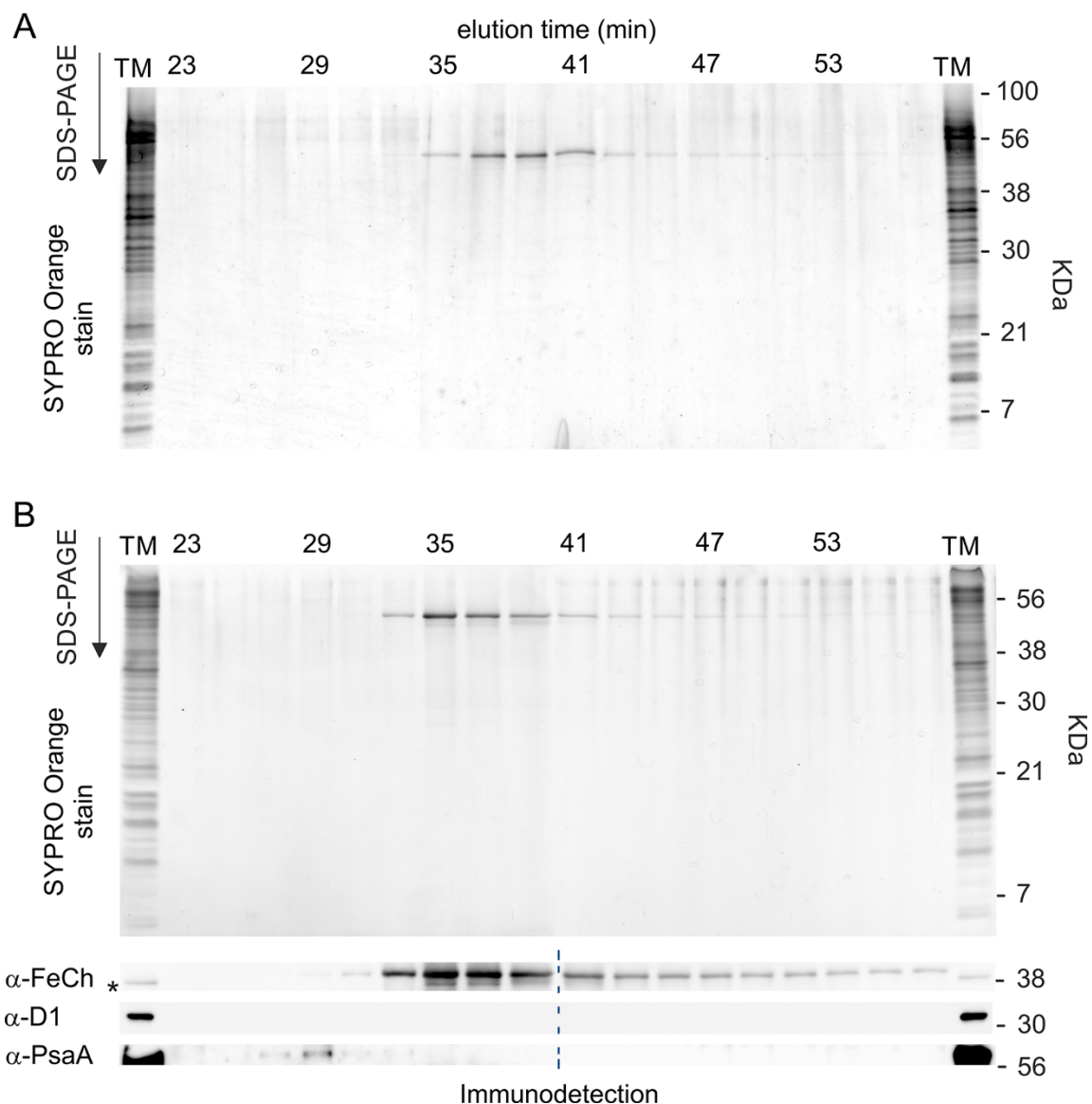
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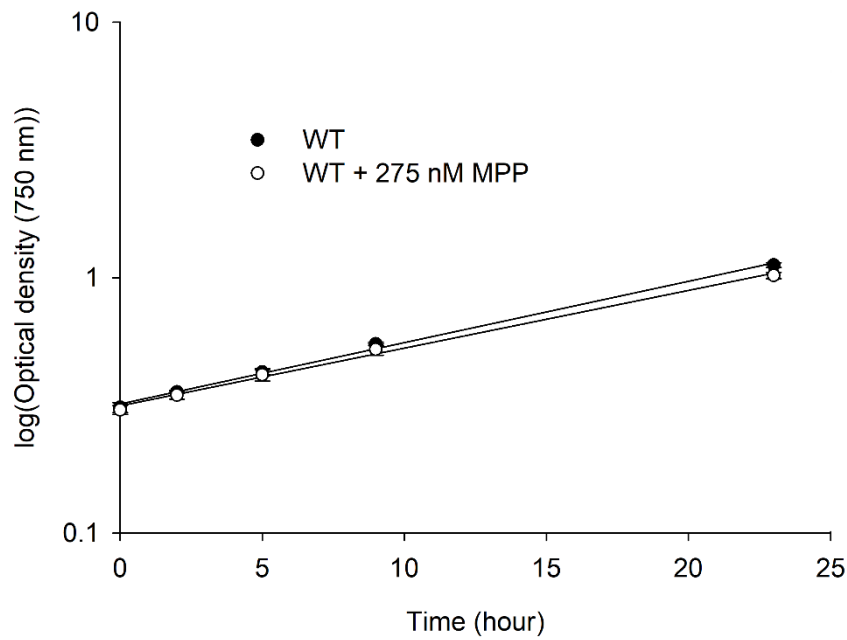
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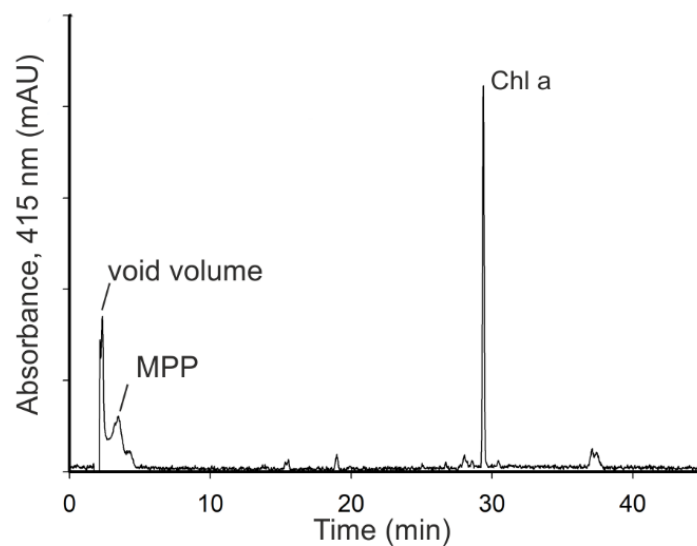
**Figure S1. The heme-producing FeCh enzyme contains a conserved LHC motif.** A) A simplified scheme of the tetrapyrrole biosynthetic pathway highlighting the positions of FeCh and magnesium chelatase at the branch-point between heme and Chl biosynthesis. Protoporphyrin IX, the substrate of both chelatases, is produced in several steps from aminolevulinic acid (ALA). B) Sequence alignment of the FeCh C-terminal CAB domain from *Synechocystis* sp. PCC 6803 (BAA10523.1); *Chlamydomonas reinhardtii* (AAK16728.1); *Physcomitrella patens* (XP\_024401127.1); *Arabidopsis thaliana* (CAA73614.1); *Thalassiosira pseudonana* CCMP1335 (EED96635.1); *Chrysochromulina* sp. CCMP291 (KOO22639.1); the HliC protein from *Synechocystis* (BAA17603.1) and the LHca2 protein from *Arabidopsis thaliana* (OAP01790.1). Amino-acid residues coordinating Chl in LHC proteins are in green, a charged arginine residue is in red. Blue denotes residues with non-polar side-chains.



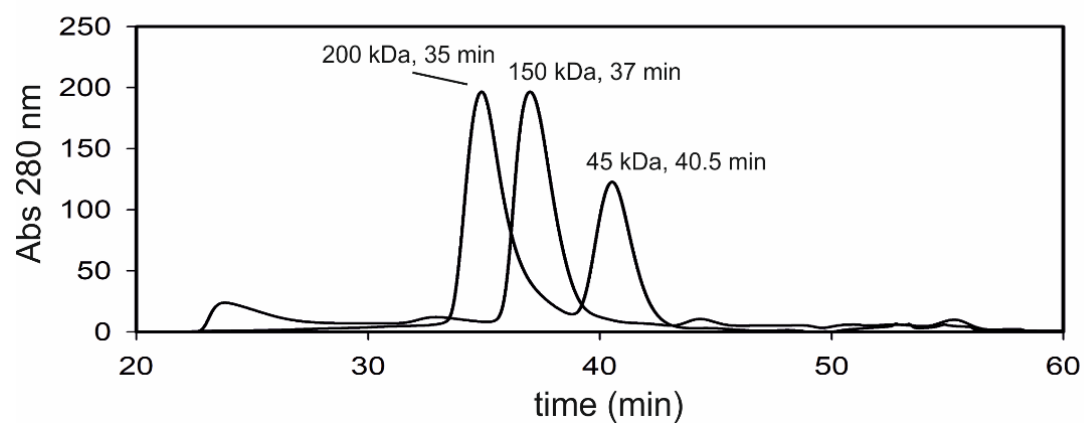
**Figure S2. SDS-PAGE and immunoblot analysis of the f.FeCh eluate separated by SEC.** A) Minute fractions collected during the SEC separation of f.FeCh eluate (see Figure 2A in the main text) were further separated by SDS-electrophoresis and stained by SYPRO Orange. Values on the top of the gel indicate the elution time from the column. B) The identical analysis described in (A) but for the f.FeCh eluate pulled down from cells treated with MPP (Figure 2B). In this case, the stained gel was blotted and the f.FeCh protein immunodetected. To exclude possible contamination of the pigmented f.FeCh complex by Chl-rich photosystem complexes the blot was also re-probed for the presence of PsaA and D1 core subunits. TM denotes solubilized membrane fraction loaded as a positive control. Asterisk denotes the native FeCh present in wild-type membranes migrating slightly faster than the f.FeCh; dashed line indicates blot splicing.



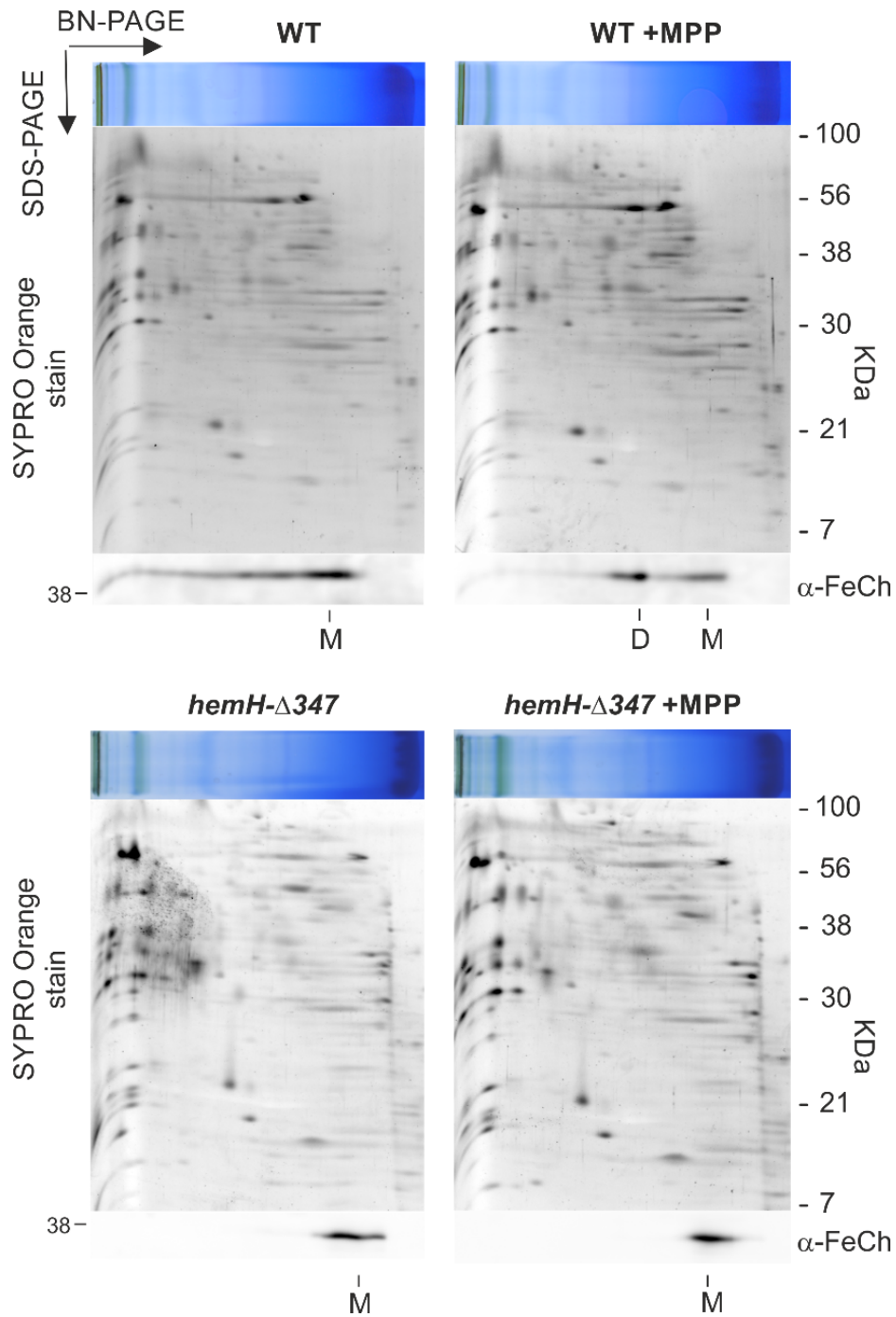
**Figure S3. Effect of 275 nM FeCh inhibitor (MPP) on the growth rate of *Synechocystis* wild-type (WT) cell culture.** Liquid cultures were grown under standard conditions (29 °C, 40  $\mu\text{mol photons s}^{-1} \text{m}^{-2}$ ) and the optical density of cells was measured at indicated time periods. MPP was added to the cultures at time zero. Error bars give s.d., n = 3 biological replicates.



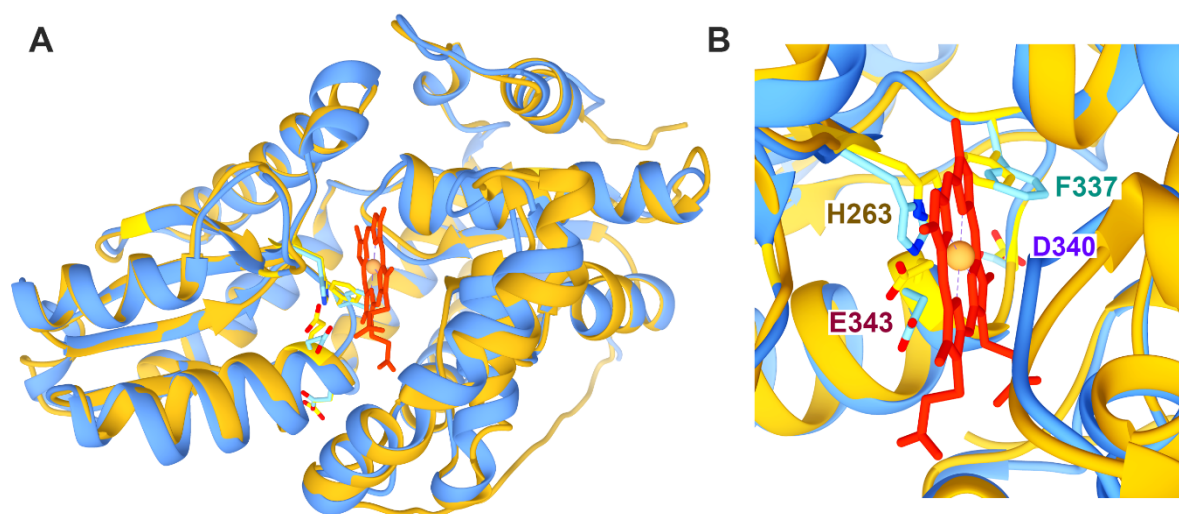
**Figure S4. The FeCh inhibitor (MPP) is co-purified with the dimeric FeCh-pigment complex.** Pigments from the isolated f.FeCh complex (Figure 2B) were extracted by methanol and separated on a C18 column. MPP is quite a hydrophilic compound and it is eluted near the void volume of the C18 column.



**Figure S5. Calibration of the SEC column used in this study; 200 kDa  $\beta$ -amylase, 150 kDa alcohol dehydrogenase, 45 kDa albumin.**



**Figure S6. Immunodetection of the FeCh monomeric and dimeric forms on the 2D blue-native/SDS-gel.** Membrane fractions were isolated from wild-type (WT), *hemH*- $\Delta$ 347 and from the same strains treated for 4 hours with MPP. Membrane complexes were solubilized, separated by 2D blue-native/SDS-electrophoresis, blotted and the FeCh immunodetected. The position of monomeric and dimeric FeCh is indicated.



**Figure S7. Structure alignment of human FeCh and predicted structure of the FeCh-like protein present in many cyanobacteria.** A) Structure of the FeCh-like protein from *Cyanothece* sp. PCC 8801 (ACK65781.1) was predicted using I-TASSER (1; the score obtained = 0.54) and aligned with the crystal structure of human FeCh (PDB code 3HCP; 2) using Chimera software (3). FeCh-like structure is depicted in gold, human FeCh in blue, deuteroporphyrin IX in red. B) Residues, essential for the catalytic activity of human FeCh, are conserved in cyanobacterial FeCh-like protein (see main text for details) Conformations of protein side chains were manually adjusted using a rotamer library (4).

## REFERENCES

1. Yang, J. Y., Yan, R. X., Roy, A., Xu, D., Poisson, J., and Zhang, Y. (2015) The I-TASSER Suite: protein structure and function prediction. *Nat Meth* **12**, 7-8
2. Medlock, A. E., Carter, M., Dailey, T. A., Dailey, H. A., and Lanzilotta, W. N. (2009) Product release rather than chelation determines metal specificity for ferrochelatase. *J Mol Biol* **393**, 308-319
3. Pettersen, E. F., Goddard, T. D., Huang, C. C., Couch, G. S., Greenblatt, D. M., Meng, E. C., and Ferrin, T. E. (2004) UCSF Chimera--a visualization system for exploratory research and analysis. *J Comput Chem* **25**, 1605-1612
4. Dunbrack, R. L., Jr. (2002) Rotamer libraries in the 21st century. *Curr Opin Struct Biol* **12**, 431-440