

Exploring the structural diversity and evolution of the D1 subunit of photosystem II using AlphaFold and Foldtree

Tom Dongmin Kim^{1,2,*}, Daniella Pretorius², James W. Murray², and Tanai Cardona^{1,2,*}

¹School of Biological and Behavioural Sciences, Queen Mary University of London, United Kingdom

²Department of Life Sciences, Imperial College London, United Kingdom

*Corresponding authors

Supplementary material

Table S1. Full list of 24 representative structures

Group	Species origin
G0	<i>Gloeobacter morelensis</i>
G0	<i>Gloeobacter kilaueensis</i>
G1	<i>Chroococcidiopsis</i> sp. CCALA 051
G1	<i>Synechococcus</i> sp. PCC 7335
G1	<i>Fischerella thermalis</i>
G2	<i>Anabaena</i> sp. PCC 7108
G2	<i>Synechococcus</i> sp. PCC 7336
G2	<i>Nostoc</i> sp. ATCC 53789
G3	<i>Synechococcus</i> sp. PCC 7310
G3	<i>Pseudanabaena</i> sp. PCC 6802
G3	<i>Leptolyngbya</i> sp. PCC 7375
D1 ^{FRL}	<i>Chroococcidiopsis</i> sp. CCALA 051
D1 ^{FRL}	<i>Chlorogloeopsis fritschii</i>
D1 ^{FRL}	<i>Synechococcus</i> sp. PCC 7335
G4Glo	<i>Gloeobacter morelensis</i>
G4Glo	<i>Gloeobacter kilaueensis</i>
G4Glo	<i>Candidatus Cyanoaurora vandensis</i>
G4Bac	<i>Fischerella</i> sp. PCC 9605
G4Bac	<i>Calothrix</i> sp. 336/3
G4Bac	<i>Synechococcus</i> sp. PCC 7002
G4Euk	<i>Flintiella sanguinaria</i>
G4euk	<i>Pharus lappulaceus</i> YP 008
G4euk	<i>Cannabis sativa</i>
Reference	<i>Synechocystis</i> sp. PCC 6803 (G4)

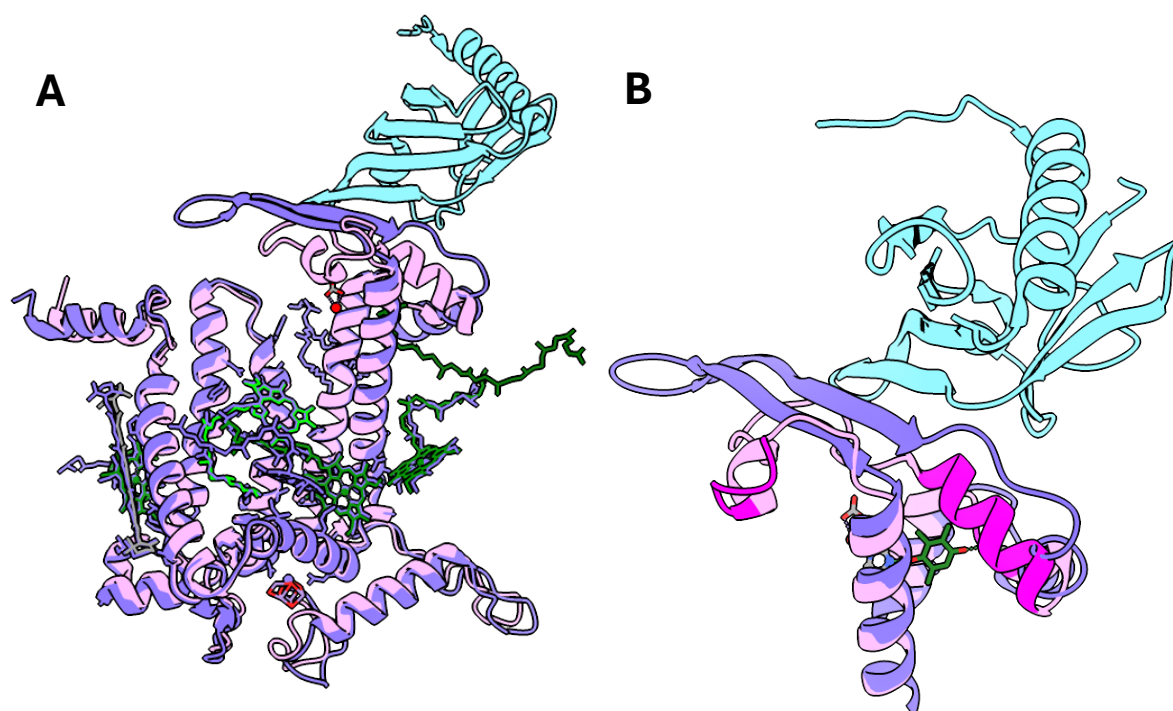


Figure S1. Experimentally determined structure of Psb28 bound D1 protein in an intermediate Photosystem II and the DE loop region in detail. The structure was determined by cryo-EM using *Thermosynechococcus vestitus* BP-1, PDB ID: 7NHP (Zabret et al., 2021). D1 is shown in purple and Psb28 in cyan. The structure is aligned to a reference structure: the D1 (PsbA2) subunit of *Synechocystis* sp. PCC 6803, PDB ID: 7N8O (Gisriel et al., 2022) in pink.

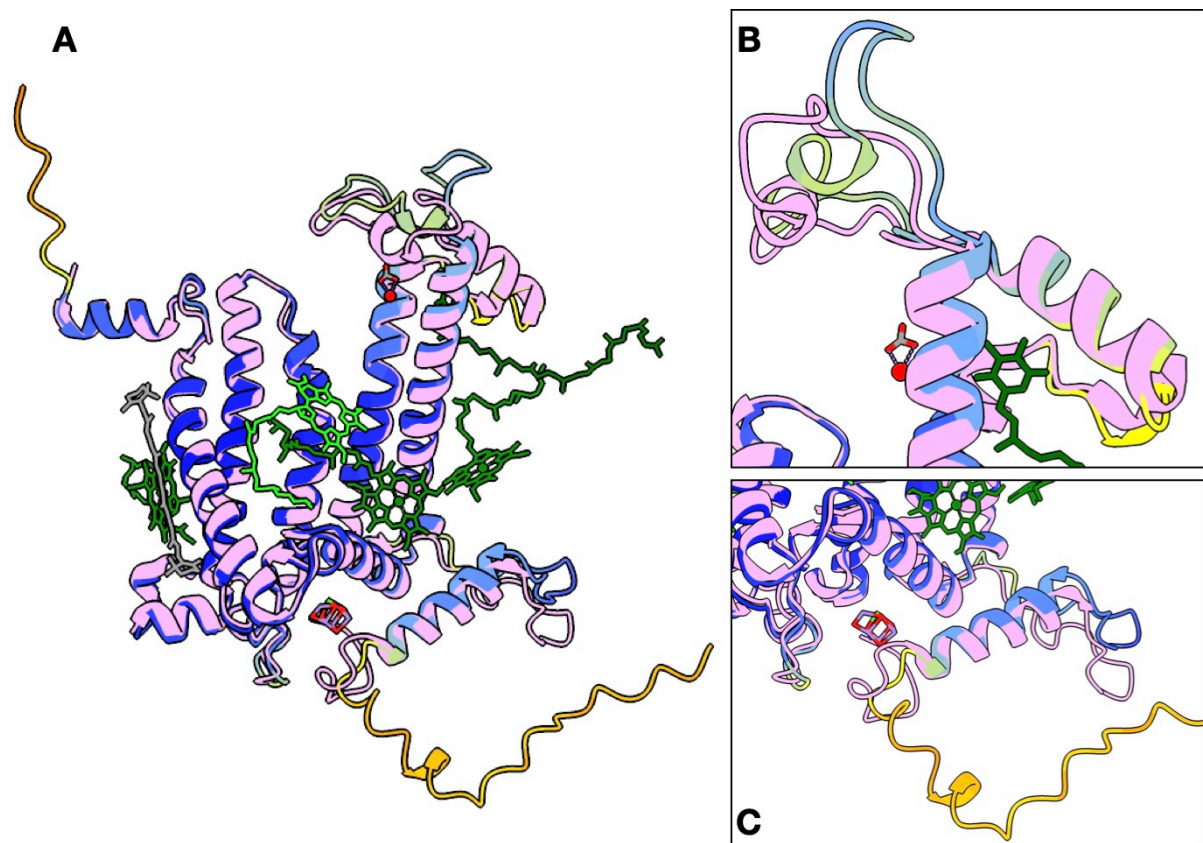


Figure S2. Comparison between experimentally determined and AlphaFold-predicted D1 protein. The experimentally determined structure (cryo-EM) of D1 in *Synechocystis* sp. PCC 6803 is shown in pink, and the AlphaFold prediction of the protein is depicted in pLDDT colouring. **A** shows the global alignment of the two structures; **B** highlights the DE loop region in detail and the luminal region and C-terminus are shown in panel **C**.

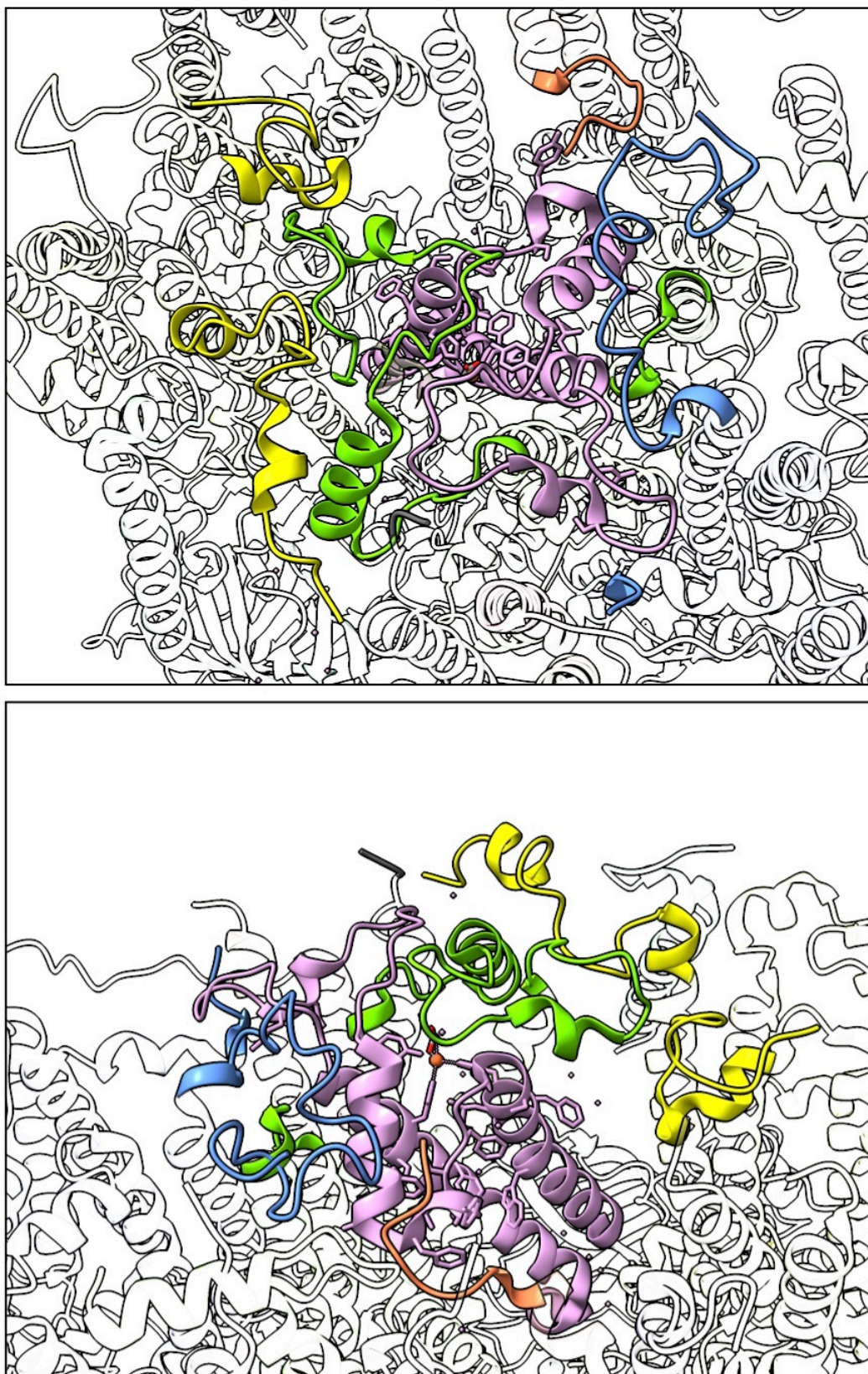


Figure S3. Surroundings of the DE loop in detail (PDB 7N8O). D1 is depicted in pink, CP43 in yellow, D2 in green and CP47 in blue. PsbE is depicted in orange. Top panel shows a view from the top of the enzyme (stromal side), while the bottom side has been rotated to show a different perspective on the site.

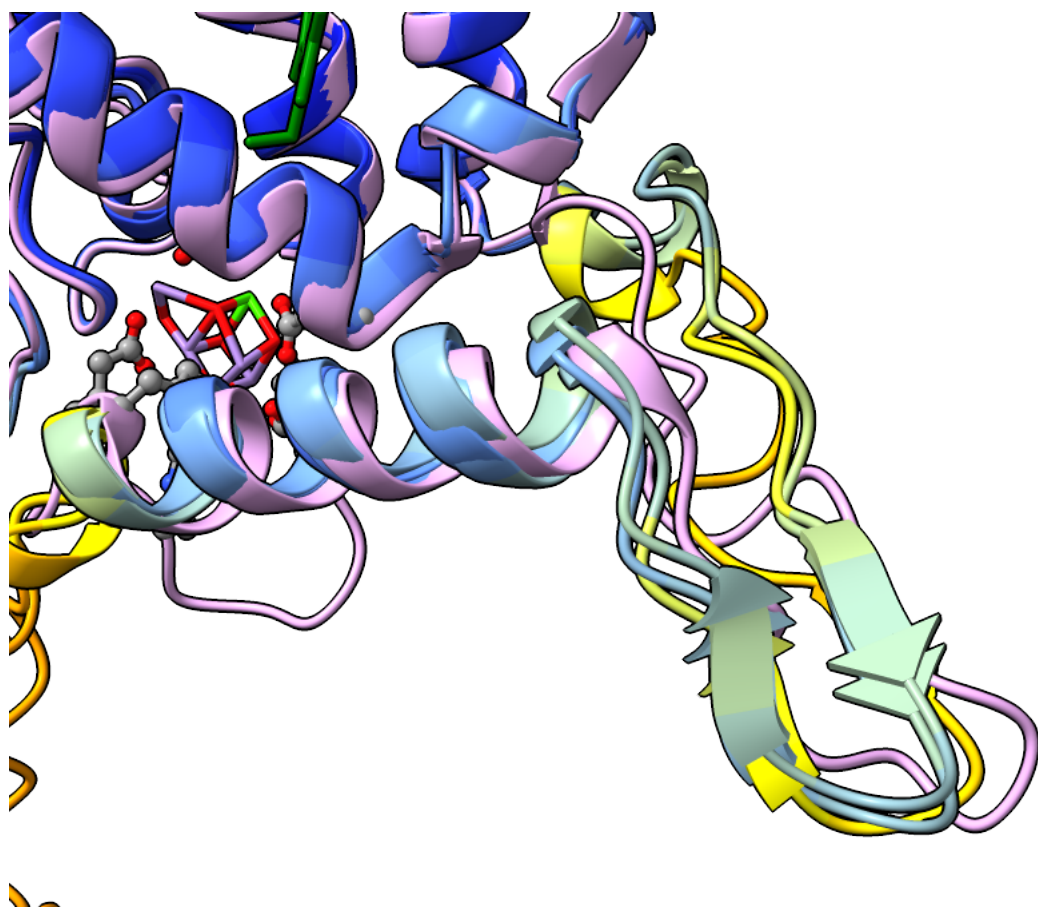


Figure S4. Close up of the beta-hairpin found in the C-terminal region of the G1 D1 structures. The reference structure is depicted in pink, and the other three G1 AF structures are in pLDDT colouring.

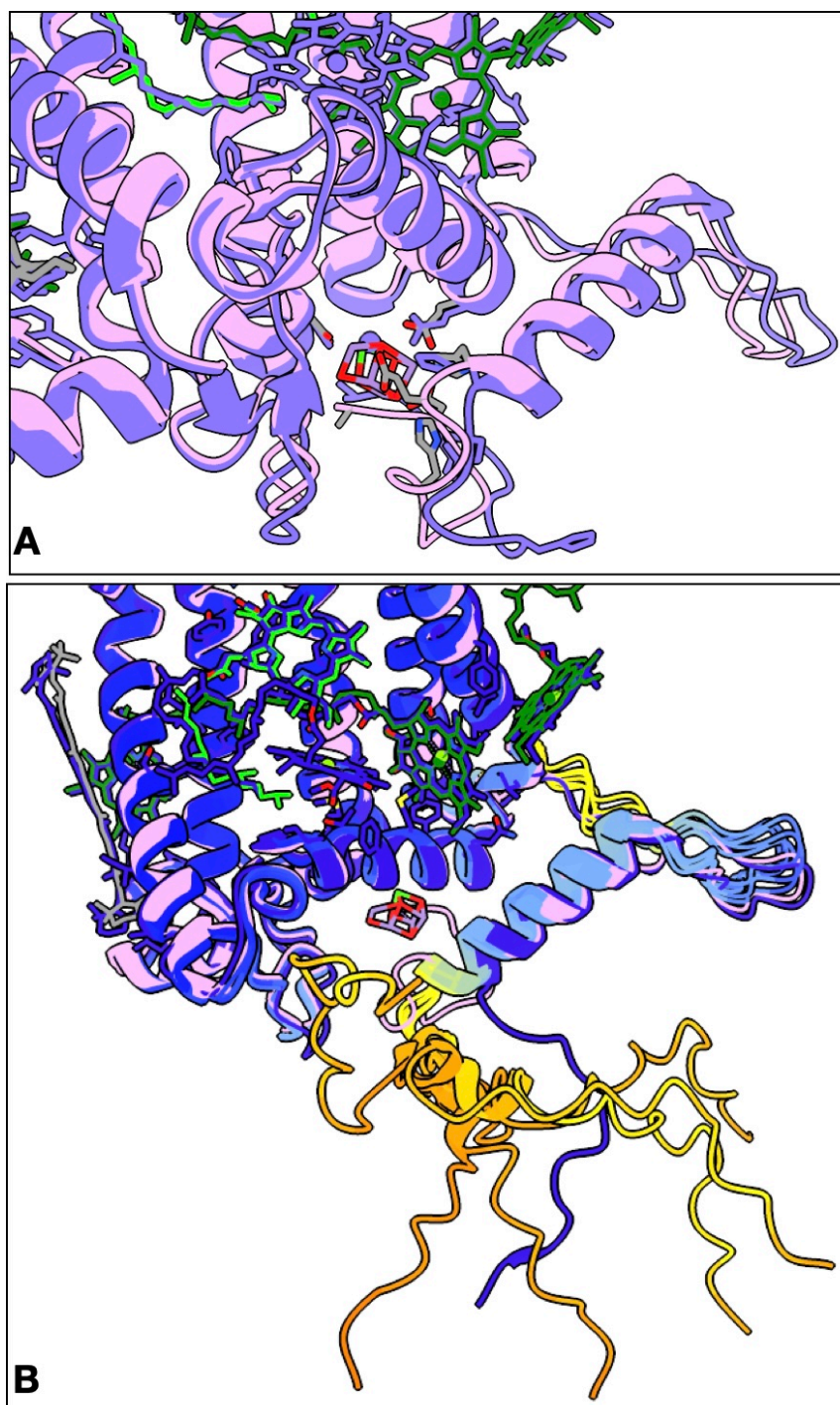


Figure S5. Comparison of the C-terminus of D1 from different PSII complexes during intermediate steps of assembly. **A** shows the C-terminus of D1 from an intermediate PSII with bound assembly factors Psb27, Psb28 and Psb34 reported by Zabret et al. (2021), PDB ID: 7NHP. The assembly factors are not shown for clarity. The reference structure is coloured pink. **B** shows the C-terminus from a PSII complex with bound Ycf48, instead of the extrinsic polypeptides PsbO, V, and U as reported by Zhao et al. (2023), PDB ID: 8AM5. This is shown in deep blue and the reference structure is in pink. G4Euk and G4Bac structures are shown in pLDDT colouring for comparison.

Reference

- Gisriel, C. J., Wang, J., Liu, J., Flesher, D. A., Reiss, K. M., Huang, H. L., Yang, K. R., Armstrong, W. H., Gunner, M. R., Batista, V. S., Debus, R. J. & Brudvig, G. W. 2022. High-resolution cryo-electron microscopy structure of photosystem II from the mesophilic cyanobacterium, *Synechocystis* sp. PCC 6803. *Proc Natl Acad Sci U S A*, 119, e2116765118.
- Zabret, J., Bohn, S., Schuller, S. K., Arnolds, O., Moller, M., Meier-Credo, J., Liauw, P., Chan, A., Tajkhorshid, E., Langer, J. D., Stoll, R., Krieger-Liszkay, A., Engel, B. D., Rudack, T., Schuller, J. M. & Nowaczyk, M. M. 2021. Structural insights into photosystem II assembly. *Nat Plants*.
- Zhao, Z., Vercellino, I., Knoppova, J., Sobotka, R., Murray, J. W., Nixon, P. J., Sazanov, L. A. & Komenda, J. 2023. The Ycf48 accessory factor occupies the site of the oxygen-evolving manganese cluster during photosystem II biogenesis. *Nat Commun*, 14, 4681.