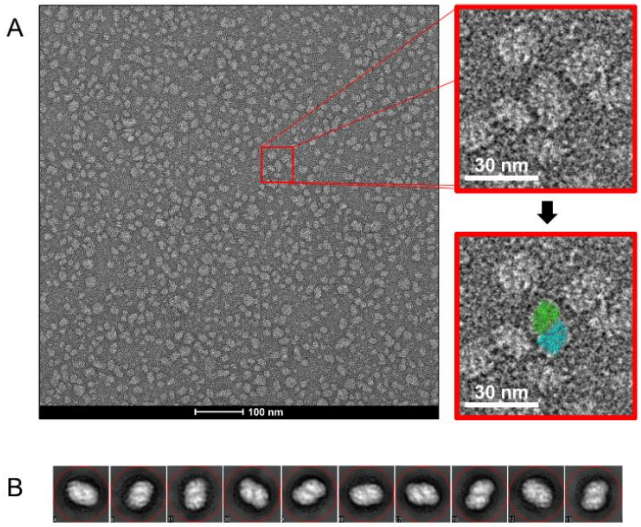

Fig. 1. Negative stain TEM (Thermofisher Talos L120C, 120 kV, CCD camera) of FRL-PSII. **A** shows a typical image of FRL-PSII particles at ~10 µM concentration. A section is enlarged where one of the dimers can be observed. Below this, a FRL-PSII homology model is inserted to scale in a probable orientation. **B** shows various 2D classes from the cisTEM software suite.

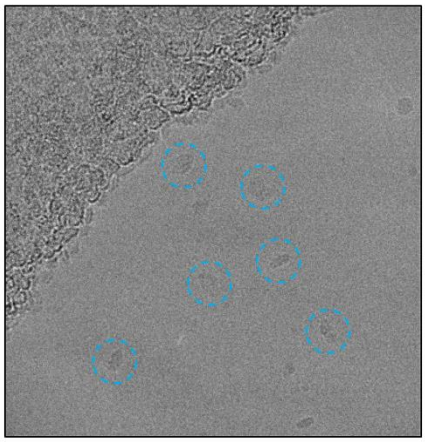

Fig. 2. Cryo-EM example micrograph (Thermofisher Glacios, 200 kV, K2 detector). Most protein is aggregated on the carbon grid rather than in the hole. The few FRL-PSII particle that are identified in the hole are circled in dashed blue lines.

Table 1. Parameters varied so far in cryo-EM plunge conditions for FRL-PSII.

Blot time (s)	4, 3, 2
Blot force	0, -4
Buffer	100 mM tricine pH=8.0, 10 mM HEPES pH=7.5, 10 mM HEPES pH=7.0, 50 mM MES pH=6.5
Protein concentration	1 µM, 10 µM
Salt*	100 mM NaCl, 5 mM $CaCl_2$, 15 mM $MgCl_2$
Grid type^	Au C-flat, Cu C-flat, Cu Quantifoil
Grid treatment	Glow discharge for 10 sec at 25 mA, glow discharge for 60 sec at 25 mA, no glow discharge but dope with detergent-containing buffer (0.03% β-DDM) for 1 minute

* Salts have been tried individually and in combinations.
^ Grids have so far always been 300 mesh and 2/2.