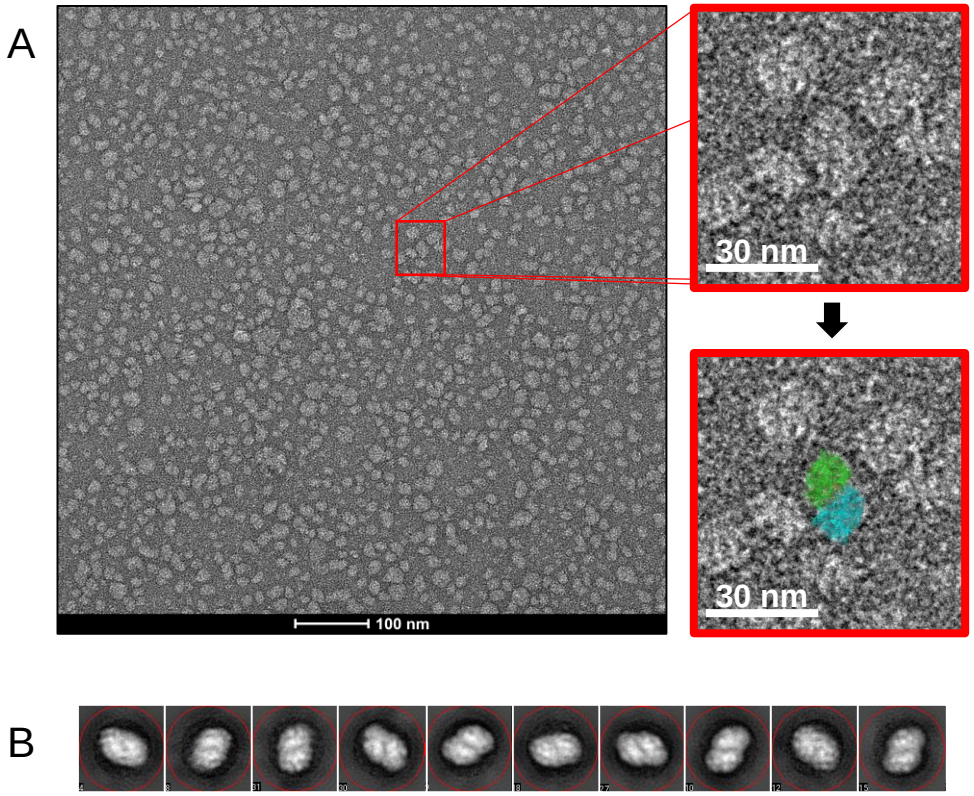

Fig. 1. Negative stain TEM (Thermofisher Talos L120C, 120 kV, CCD camera) of FRL-PSII. **A** shows a typical image of FRL-PSII particles at ~10 µM concentration. A section is enlarged where one of the dimers can be observed. Below this, a FRL-PSII homology model is inserted to scale in a probable orientation.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

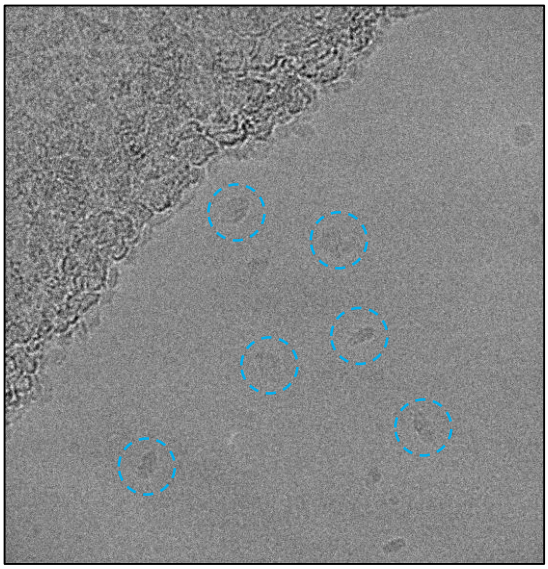

Fig. 2. CryoEM example micrograph (Thermofisher Glacios, 200 kV, K2 detector). Most protein is aggregated on the carbon grid rather than in the hole. The few FRL-PSII particle that are identified in the hole are circled in dashed blue lines.

Table 1. Parameters varied so far in cryoEM 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.