Activity Report CM01 mx2340 – 12 to 14 May 2021

We aim at solving the structure of temporal supercomplexes formed around Photosystem I (PSI) during steady state and under stress conditions by the cryo EM technique. So far our visits yielded two high-resolution structures of two distinct forms of Photosystem I (PSI) from the halotolerant green algae *Dunaliella salina*. We solved the crystal structure of this supercomplex at 3.2 A resolution (Perez-Boerema A, Klaiman D, Caspy I, Netzer-El S, Amunts A, Nelson N., 2020. Structure of a minimal photosystem I from a green alga. Nature Plants 6:321-327 PDB 6RHZ). The second visit yielded structure at 2.84 A resolution of a much larger form of PSI containing 8 additional subunits and about 40 additional prosthetic groups (Caspy I, Malavath T, Klaiman D, Fadeeva M, Shkolnisky Y, Nelson N. (2020) Structure and Energy Transfer Pathways of the *Dunaliella Salina* Photosystem I Supercomplex. BBA - Bioenergetics, in press PDB 6SL5). In the next experiment we solved the structure of a larger PSI complex isolated from TSP4 temperature sensitive mutant of *Chlamydomonas reinhardtii*. PSI was isolated from cells grown at 37^{O}C where PSII is absent (Caspy et al., Dimeric and high-resolution structures of *Chlamydomonas* Photosystem I from a temperature-sensitive Photosystem II mutant (Communications Biology, under review). Using a high-resolution (2.54 A PDB 7BGI) cryogenic electron microscopy (cryo-EM) PSI structure a conserved network of water molecules - dating back to cyanobacteria - was uncovered, mainly in the vicinity of the electron transport chain (ETC). The high-resolution structure illustrated that the water molecules served as a ligand in every chlorophyll that was missing a fifth magnesium coordination in the PSI core and in the light-harvesting complexes (LHC). The asymmetric distribution of the water molecules near the ETC branches modulated their electrostatic landscape, distinctly in the space between the quinones and FX. The data also disclosed the first observation of eukaryotic PSI oligomerization through a low-resolution PSI dimer that was comprised of PSI-10LHC and PSI-8LHC (7BGI; 7BLX). In our last visit we attempted to solve the structure of plant PSI in association with additional complexes. 3D classification of the particles clearly revealed the presence of such assemblies (see figure 1) and we now attempt to improve the resolution and solve the structure of at least one of them.

In the future experiment we would like to solve the structure of *Chlamydomonas* PSI from a temperature-sensitive mutant that gown at 37^{O}C where cytochrome b6f complex is totally absent. Under this condition PSI operates without active linear or cyclic electron transport and is likely to be modified accordingly. The purification procedure worked very well and the grids are ready for data collection.

The 3 days experiment exceeded my expectations.

Figure 1. Initial structure of a large plant PSI and built-in model of PSI with 3 LHCII attached.

Figure legend: Left – low resolution 3D reconstruction of Photosystem I in complex with an unidentified protein. Map is colored in grey. Right – mesh display of the cryo-EM map and a placed model of a PSI-LHCI-LHCII complex in cyan, showing the unidentified density can accommodate several LHCII trimers.