

# Structural characterization of protein supercomplexes for a bioinspired electrochemical cell

Maksym Golub<sup>1</sup>, Adrian Kölsch<sup>2</sup>, Artem Feoktystov<sup>3</sup>, Athina Zouni<sup>2</sup>, and Jörg Pieper<sup>1,\*</sup>

<sup>1</sup>*Institute of Physics, University of Tartu, Wilhelm Ostwald str. 1, 50411, Tartu, Estonia*

<sup>2</sup>*Department of Biology, Humboldt–Universität zu Berlin, Philippstrasse 13, 10115, Berlin, Germany*

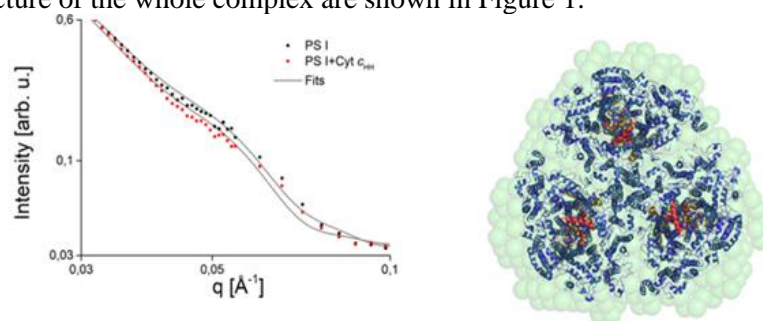
<sup>3</sup>*Jülich Centre for Neutron Science (JCNS), Lichtenbergstr. 1, 85748, Garching, Germany*

\**pieper@ut.ee*

Photosynthesis is a fundamental natural process capable of converting solar radiation into storable energy-rich carbohydrates. Therefore, it is highly attractive to use this process as an inspiration for the development of photoelectrochemical cells exploiting sustainable energy sources.<sup>1</sup> The primary processes of oxygenic photosynthesis involve the cooperation of several protein-cofactor complexes including photosystems I (PSI) and II (PSII), which can perform the light-induced charge separation. The trimeric PSI from the cyanobacterium *Thermosynechococcus elongatus* (TePSI) is a remarkably stable intrinsic membrane protein, which converts solar energy into electrical energy with high quantum yield by oxidizing the soluble redox mediator cytochrome *c6* (Cyt *c6*) at the luminal side. PSI can be integrated into an artificial biohybrid electrode.<sup>1</sup> To design electrode surfaces that efficiently interact with PSI, structural characterization of the protein complexes formed by Cyt *c6* with PSI is needed. Different approaches of protein supercomplex formation of PSI with Cyt *c6* can be effectively verified directly in detergent solution using small-angle neutron scattering (SANS) before high-resolution structures become available.

Recently, we analyzed the interaction of PSI with cyt *c6* and the non-native mitochondrial cytochrome from horse heart (Cyt *cHH*).<sup>2</sup> We found that Cyt *cHH* is strongly bound to the luminal side of PSI. In contrast, Cyt *c6* is also bound, but the binding affinity is weaker than for Cyt *cHH*. A co-crystal structure of PSI with Cyt *c6* or Cyt *cHH* could not be determined.

In the absence of high-resolution structures, SANS is the technique of choice to investigate the binding of Cyt *c6* and Cyt *cHH* to TePSI in solution – i.e. under the same conditions required for biotechnological applications. We have used SANS at KWS-1 at MLZ before to show that PSI in buffer solution exhibits a structure close to the trimeric crystal structure.<sup>2,3</sup> In the same experiment, we successfully proved a complex formation of TePSI-Cyt *cHH* consistent with the expected structure.<sup>2</sup> The latter SANS data, the data fit, and the simulated structure of the whole complex are shown in Figure 1.



**Figure 1:** Left: SANS data of TePSI (black dots) and of TePSI-Cyt *cHH* complexes (red dots) obtained at a contrast of 5% D<sub>2</sub>O matching the hydrophobic tails of the detergent. The full lines correspond to simulations based on the TePSI crystal structure and a model for the TePSI-Cyt *cHH* complex. Right: Comparison of the structure of the TePSI-Cyt *cHH* complex reconstructed from the SANS data (light blue spheres) with a crystal structure of the PSI-cytochrome complex.

## Acknowledgements

We gratefully acknowledge financial support by the Estonian Research Council (Grant PRG 539). We also thank JCNS and MLZ Garching (Germany) for the generous allocation of SANS beamtime at KWS-1.

## References

1. Ciornii et. al. 2017, J. Am. Chem. Soc. 2017, 139, 16478-16481
2. Kölsch et. al. 2020, Curr.Res.Struct.Biol.2: 171.
3. Golub et al. J.Phys.Chem.B 2022,126, 2824.