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POSTER ABSTRACT PRESENTATIONS

SESSION TITLE: STRUCTURE OF MEMBRANE PROTEINS

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Abstract P-11: Conformational Study of an Archaeal Photoreceptor/Transducer Complex from *Natronomonas pharaonis* Assembled in Styrene-Maleic Acid Lipid Particles Using Electron Paramagnetic Resonance Spectroscopy

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Background: The membrane-embedded 2:2 sensory rhodopsin II/transducer complex, NpSRII/NpHtrII, plays a key role in the photophobic response of the halophilic archaeon *Natronomonas pharaonis*. Photon absorption induces transient structural changes in the transmembrane part of NpSRII, which are conducted to the transducer NpHtrII. The subsequent signal propagates along the cytoplasmic part of NpHtrII to the intracellular signaling pathway, which modulates the rotation of the flagellum. Here, we studied the conformation and dynamics of the NpSRII/NpHtrII complex after reconstitution into cell membrane-mimicking nanoparticles, namely, styrene maleic acid lipoprotein particles (SMALPs) (Fig. 1), and in comparison with those of NpSRII/NpHtrII reconstituted in liposomes and nanodiscs.

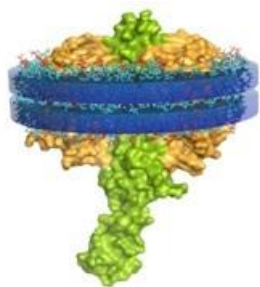


Fig. 1. Schematic SRII/HtrII complex in SMALPs.

Methods: We utilized the SMA copolymer with a 3:1 styrene-to-maleic acid molar ratio (3:1 SMA) to isolate, in a detergent-free manner, the NpSRII/NpHtrII complex from preformed proteoliposomes. We

characterized the resulting NpSR_{II}/NpHtr_{II}-SMALPs using a set of optical and electron paramagnetic resonance (EPR, DEER) spectroscopy methods as well as dynamic light scattering (DLS) and transmission electron microscopy (TEM).

Results: The cw-EPR data show unaffected spin-label dynamics of the L159R1 side chain in the interior of NpSR_{II} reconstituted into SMALPs. The DEER data indicate that NpSR_{II}/NpHtr_{II} incorporates into SMALPs as a native-like 2:2 dimer with the distances between L159R1 sites corresponding to the “V”-shaped conformation. Time-resolved optical and EPR spectroscopy data reveal light-induced spectral and conformational changes for NpSR_{II}/NpHtr_{II} reconstituted in SMALPs. The DLS data indicate the monodispersity of SMALP preparations. TEM images reveal well-defined individual particles of 12–14 nm in diameter.

Conclusion: We showed that the NpSR_{II}/NpHtr_{II} complex retains its integrity and functionality upon reconstitution into SMALPs although with restricted dynamics. We conclude that the SMALP approach could be suitable for preparation of stable membrane protein samples for further structural and functional studies.

Key Words: photoreceptor/transducer complex • detergent-free reconstitution • nanolipoprotein particles

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