A088-07-250823

SAXS determination of the conformational information of VPPase embedded in a phospholipid nanodisc environment

Orion Shih[1], Yi-Qi Yeh[1], Kuei-Fen Liao[1], Chun-Jen Su[1], Kun-Mou Li[2], Chieh-Chin Li[3], Yuh-Ju Sun[2], Yun-Wei Chiang[3], and U-Ser Jeng[1,4,*]

[1]National Synchrotron Radiation Research Center, Hsinchu 30076, Taiwan
[2]Department of Life Science and Institute of Bioinformatics and Structural Biology, College of Life Science, National Tsing Hua University, Hsinchu 30013, Taiwan
[3]Department of Chemistry, National Tsing Hua University, Hsinchu 30013, Taiwan
[4]Department of Chemical Engineering, National Tsing Hua University, Hsinchu 30013, Taiwan
E-mail: shih.orion@nsrrc.org.tw

Keywords: SAXS, nanodisc, membrane protein

Vigna radiata H+-PPase (VPPase) is a proton pump that hydrolyzes pyrophosphate (PPi) to drive proton transportation across cellular membranes against the electrochemical gradient [1]. These enzymes are found in plants and various unicellular organisms and are essential for survival under different stress conditions [2]. However, the detailed mechanisms underlying the translocation reactions and structural changes between other conformational states of VPPase (ligand-free or PPi-binding) are still unclear. In this report, high-performance-liquid-chromatography, small-angle X-ray scattering (SAXS), UV−Vis absorption, differential refractive index (RI) detections, and modified core-shell bicelle model fitting are integrated to probe the structural information of VPPase-incorporated POPC nanodiscs (Fig. 1). The results indicate that VPPase is stable in the POPC nanodiscs, and the length of VPPase slightly thickens when changing from resting state (R-state, ligand-free) to initiated state (I-state, PPi-binding). This integrated analysis scheme can be applied to other membrane protein/detergents/lipids complexes and provides a new approach to membrane protein studies.

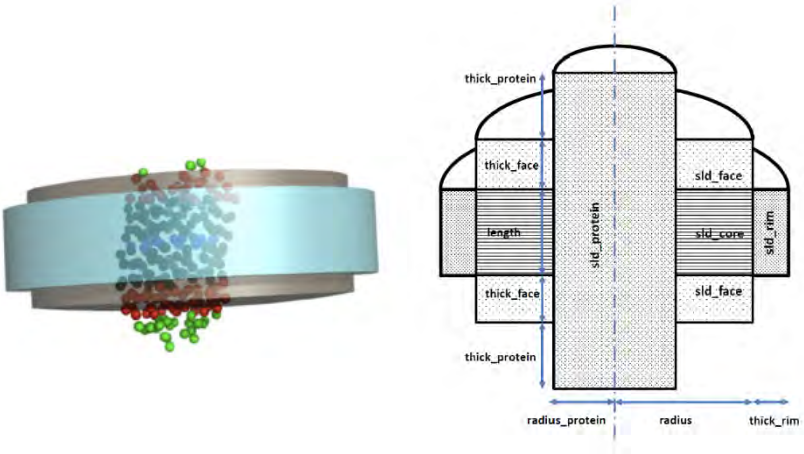

Figure 1. (left) Schematic diagram showing VPPase loaded in a nanodisc, and (right) the simulation model for this complex.

[1] Luoto, H. H., Baykov, A. A., Lahti, R. & Malinen, A. M. (2013) *Proc. Natl Acad. Sci. USA* **110**, 1255–1260.
[2] Maeshima, M. (2000) *Biochim. Biophys. Acta* **1465**, 37–51.