Supplementary Material (ESI) for Soft Matter

Electronic **Supplemental Information for: Evidence that Membrane Curvature Distorts the Tertiary Structure of Bacteriorhodopsin**

Chandrashekhar V. Kulkarni[a], Annela M. Seddon[b] , Oscar Ces [a], and Richard H. Templer*[a]
[a]Department of Chemistry, Imperial College London, Exhibition Road, South Kensington, London, SW7 2AZ, United Kingdom. [b]H.H.Wills Physics Laboratory, Tyndall Avenue, University of Bristol, Bristol BS8 1TL, UK

A. Materials

Bacteriorhodopsin (bR) from *Halobacterium salinarum* in the form of purple membrane (PM) was obtained from Munich Innovative Biomaterials GmbH, Germany and ACTILOR GmbH, Germany or grown in house according to the protocol of Oesterhelt *et al.*[1] Lyophillsed protein was kept at -20°C in the dark and stored at 4°C in aqueous solution. Monoolein (MO) was obtained from Sigma-Aldrich (U.K.), stored at -20°C and used without further purification. It was lyophilized under vacuum for ~48 hours prior to use and weighed as a function of time to ensure that any traces of water were removed. Samples were prepared with HPLC-grade water (Sigma-Aldrich UK).

B. Experimental Methods

All experiments were carried out at 40:60 (wt/wt) of protein solution/MO. According to the phase diagram for MO in water [2-6], MO is in the D phase in equilibrium with excess water at this hydration, ensuring that the lattice parameters remain constant with any subsequent further increase in hydration. Any additional swelling of the cubic phase can thus be attributed to protein incorporation.

1. UV-spectroscopy:

UV-Vis measurements were carried out on a Lambda-25 UV-Vis spectrophotometer (Perkin-Elmer Life and Analytical Sciences Inc. MA, USA) in a quartz Ultra-Micro cell cuvette (CXA-145-410S,

Fisher Scientific). The conformational state of bR in OG can be characterized by UV-Vis as described in Allen *et al* [7] and a standard spectrum is given in figure S1. bR concentration was determined spectroscopically [8] or using a standard protein concentration determination method based on the bicinchoninic acid (BCA) assay [9]. Protein solution of known concentration was added to the pre-weighed dry MO in the ratio given above. Homogenization was then achieved via immediate centrifugation of the cuvette (2000rpm, 5 minutes) at 20±0.5°C, in the dark. The data was corrected for scattering due to the presence of MO by shifting the subsequent curves obtained during the measurements to a control measurement of lipid alone using the absorbance values at 750nm.

2. Small angle x-ray scattering (SAXS):

Kinetic studies of protein insertion were performed on an in-house SAXS beamline with X-rays generated by a FR591 rotating anode generator (Enraf-Nonius, Netherlands) running at 40kV and 30mA and focused using Franks Optics. Diffraction patterns were detected by an image intensified Charge Coupled device (CCD) camera (Photonic Science Ltd, UK). The capillaries were held in a custom built brass sample holder. Exposure time for a single image was typically 150 seconds. Six images were collected per data point and summed prior to image analysis. Diffraction patterns were analyzed using an in-house developed software package, AXcess, using IDL language (ITT Visual Information Solutions, USA) [10]. Lattice parameter values (in Å) were calibrated with silver behenate (d_{001} = 58.38 Å).

Aqueous solutions of PM were vortexed prior to addition to dry MO at 40:60 wt/wt directly into the x-ray capillaries (1.5 mm diameter; Gulmay Medical Ltd. Berkshire, UK). Homogenization was achieved by centrifuging the mixture for 2 minutes at 2000 rpm, followed by mechanical mixing with a needle spatula and a further centrifugation step. The capillaries were flame sealed then additionally sealed

with a heat shrink material (RS Components, UK) to avoid dehydration and were weighed to confirm no losses of water between the sample preparation and subsequent SAXS measurements.

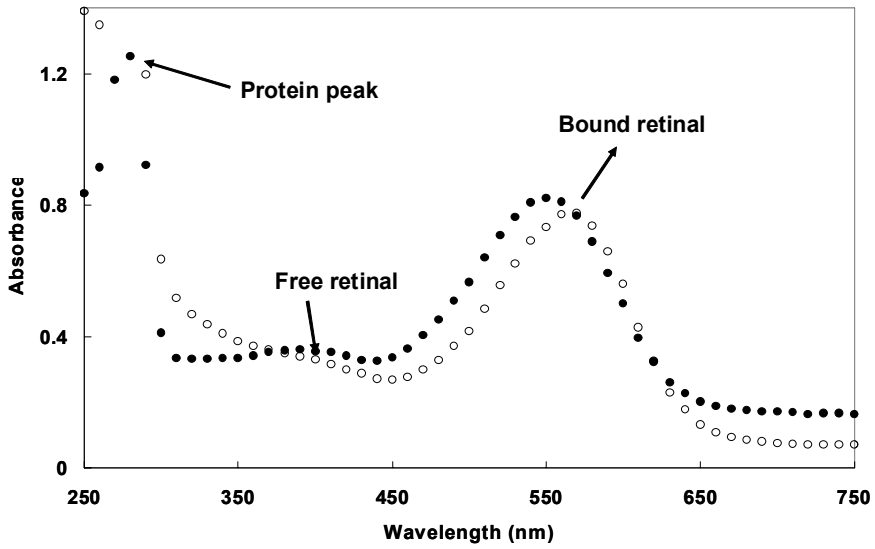

Figure S1: The UV-Vis spectra of purple membrane (hollow circles) and bacteriorhodopsin (dissolved in OG, solid circles). The peaks in the range of 250-280nm, 380-420nm and 540-570nm correspond to the presence of total protein (with a contribution from retinal), unbound i.e. free retinal and retinal bound to protein (folded bacteriorhodopsin chromophore), respectively. [7]

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