Supplemental Information for

Uniformly Aligned Full-length Membrane Proteins in Lipid Crystalline Bilayers for Structural Characterization

Conggang Li,[†] Philip Gao,[‡] Huajun Qin[†], Rose Chase[†], Timothy A. Cross*[,†,‡, §]

Department of Chemistry and Biochemistry, Florida State University, Tallahassee, Florida, 32306. Institute of Molecular Biophysics, Florida State University, Tallahassee, Florida, 32306, and National High Magnetic Field Laboratory, Tallahassee, Florida 32310.

Sample preparation for Solid-state NMR

Preparation of lipid stocks. 1,2-Dimyristol-sn-Glyero-3-Phosphocholine (DMPC) and 1,2-Dimyristoyl-*sn*-Glycero-3-[Phospho-*rac*-(1-glycerol)] (Sodium Salt) (DMPG) were dissolved in chloroform at a molar ratio of 4:1 in a round-bottom flask and the solvent evaporated in a rotary evaporator followed by vacuum drying overnight. The DMPC/DMPG film was then dissolved in deionized water at a final concentration 20mg/ml, bath sonicated followed by three freeze (liquid-nitrogen)-thaw (37°C) cycle for 3 times.

Reconstitution. The preformed liposomes were added to the purified protein solution in DPC to a final ratio of 10:1 (w:w – lipid to protein dry weight ratio), 20% octyl-glucoside (OG) is then added to the protein liposomes mixtures until the solution clarifies, usually the final OG concentration is about 50 to 100mM. After overnight incubation at 37°C, OG was removed by dialysis.

Aligned sample preparation. Proteoliposomes were pelleted by ultracentrifugation from the dialyzed solution (196000*g*, 90min) in a 70 Ti rotor. The 50 mg pellet was suspended in 1 mL of HPLC-grade water. Aliquots were spread on 33 glass slides (12×5.7×0.07 mm) dehydrated in a 78% relative humidity chamber at room temperature for a day. The slides were stacked into a sample cell followed by incubation at 97% relative humidity in a small chamber for one week at 37 °C to achieve a hydration level of approximately 50%. The sample cell was sealed with wax for solid-state NMR experiments.

Oligomeric state of Rv1861

28 mg of Rv1861 per L of minimal media culture are typically achieved in the form of inclusion bodies. Purification via a reconstitution protocol[1] has resulted in high purity samples on SDS PAGE gels. On PFO gels a large oligomeric structure is implicated, potentially an octamer (Figure S1).

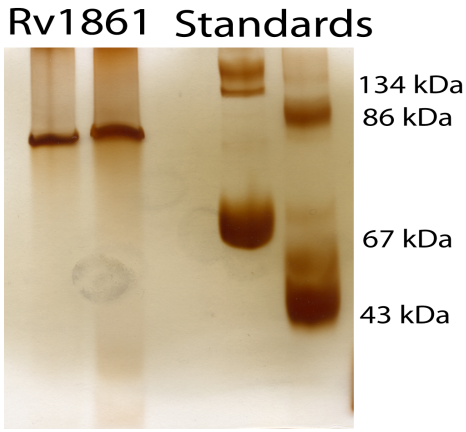

Figure S1: PFO- gel of Rv1861 showing that the molecular weight is approximately 80 kDa suggesting an octamer.

PISA wheel as a function of CSA:

Chemical shift tensor anisotropy (CSA) variation will blurr the resonance pattern in the PISEMA spectrum that forms the PISA wheel. Figure S2 shows the PISA wheel at 2 tilt angles with a distribution of 20 ppm for each of the tensor elements. When the tilt angle is small, σ_{11} and σ_{22} have little effect on the shape of the wheel while σ_{33} shifts the position of the wheel. However, when the tilt angle is large, σ_{11} and σ_{22} have a significant influence on the shape of the wheel, while σ_{33} has little effect.

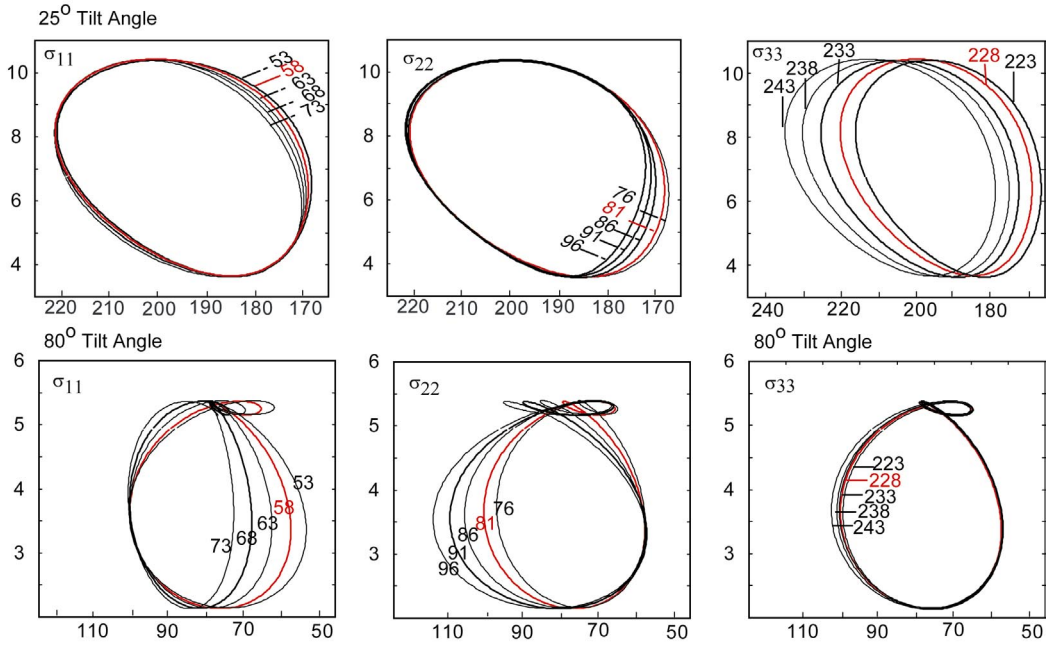

Figure S2: PISA wheel as a function of CSA tensor variation.

Reference:

(1) Page, R. C.; Moore, J. D.; Nguyen, H. B.; Sharma, M.; Chase, R.; Gao, F. P.; Mobley, C. K.; Sanders, C. R.; Ma, L.; Sonnichsen, F. D.; Lee, S.; Howell, S. C.; Opella, S. J.; Cross, T. A. *J Struct Funct Genomics* **2006**, *7*, 51-64.