Supporting Information for

Nanodisc *vs.* Macrodisc for NMR of Membrane Proteins

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Sample preparation

Preparation of micelle, isotropic bicelle, and magnetically alignable bicelle samples of Pf1 [1], p7 [2], and CXCR1 [3-5] were described previously.

The 14-residue peptide (>98% purity, Ac-DYLKAFYDKLKEAF-NH_2) was purchased from NEO Peptide (www.NEO-Peptide.com). DMPC was purchased from Anatrace (www.affymetrix.com), DHPC from Avanti Polar Lipids (www.avantilipids.com), and Triton X-100 from Sigma-Aldrich (www.sigmaaldrich.com). The procedure for preparation of nanodisc and macrodisc samples is similar to that of bicelle samples. Lyophilized powder of the 14-residue peptide was dissolved in an aqueous buffer at a concentration of 80mg/ml. Nanodisc and macrodisc samples were prepared by adding appropriate amounts of an aqueous solution of the 14-residue peptide to DMPC liposomes (for empty bilayer discs) or DMPC proteoliposomes (for protein-containing bilayer discs) to a final molar ratio (DMPC/peptide) of 1.67 and 13.3, respectively. Final lipid contents in the nanodiscs and macrodiscs were 5% (w/v) and 10% (w/v), respectively.

Pf1, p7, and MerE proteoliposomes was prepared by mixing 2-3 mg protein solubilized in 500 μl trifluoroethanol with 20 mg DMPC solubilized in 500 μl chloroform at a ratio of 1:1 (v/v). The organic solvent was then evaporated under a stream of nitrogen gas to obtain a thin, transparent protein-lipid film, which was placed under high vacuum overnight. An aqueous solution of the 14-residue peptide was added to the dry film and vortexed and cool-heated (ice/40°C) several times and then allowed to equilibrate to room temperature. Preparation of CXCR1 proteoliposomes was described previously [6]. Upon macrodisc formation, the previously white dispersion of proteoliposomes becomes a clear and non-viscous solution between 0°C and 20°C.

Weakly aligned interleukin-8 (IL-8) sample was prepared by mixing IL-8 with macrodiscs in 20 mM HEPES, pH 5.5 at a final concentration of 0.5 mM IL-8 and 10% DMPC (w/v) macrodiscs.

NMR Spectroscopy

The solution NMR experiments were performed at 50°C on a Bruker DRX 600 MHz spectrometer equipped with 5 mm triple-resonance cold probe and z-axis gradient. ^{15}N edited ^{1}H NMR experiments were performed on uniformly ^{15}N labeled samples incorporated in 100 mM DHPC micelles, isotropic DMPC:DHPC bicelles, and nanodics with a protein concentration of 50-100 μM. IPAP-HSQC spectra [7] obtained on isotropic and weakly aligned IL-8 were used to measure the $^{1}H–^{15}N$ residual dipolar couplings.

The ^{15}N and ^{31}P solid-state NMR spectra were obtained on a Bruker Avance spectrometer with a ^{1}H resonance frequency of 700 MHz. The homebuilt $^{1}H/^{15}N$ double-resonance probe had 5 mm inner diameter solenoid coil tuned to the ^{15}N frequency, and an outer MAGC coil tuned to the ^{1}H frequency [8]. The ^{31}P solid-state NMR spectra were obtained using a homebuilt $^{1}H/^{31}P$ double-resonance probe equipped with double-tuned scroll coil [9]. The one-dimensional ^{31}P NMR spectra were obtained by direct excitation with a single pulse using a 6 s recycle delay. 64 scans were signal averaged with a 10 ms acquisition time for each spectrum. Continuous wave ^{1}H decoupling utilized a B_1 radio frequency field strength of 42 kHz during the acquisition period. The one-dimensional ^{15}N solid-state NMR spectra were obtained by spin-lock cross-polarization with a contact time of 1 ms, a recycle delay of 6 s, and an acquisition time of 10 ms. 2048 transients were co-added, and an exponential function corresponding to 50 Hz of line broadening was applied prior to Fourier transformation.

Electron Microscopy

A 2.5 ml solution of the sample was absorbed to a carbon-coated copper grid, washed with deionized water and stained with 0.75% uranyl acetate. Micrographs were taken in a 120 kV CM120 electron transmission electron microscope at a magnification of 50,000X and a defocus value of 1.5 mm. Images were digitized with a Nikon SuperCool 9000 scanner using a step size of 6.25 mm. All digitized micrographs were initially binned over 3 x 3 pixels yielding a pixel size on the specimen level of 3.81 Å. Particles were manually selected using the display program WEB and the SPIDER program was used for the subsequent image-processing steps [10]. A total of 3064 nanodisk particles

were selected and windowed into 80 x 80 pixels and 2754 150 x 150 pixels macrodisks particles. Images were normalized and eight cycles of multi-reference alignment, multivariant statistical analysis and classification into 50 class averages were performed.

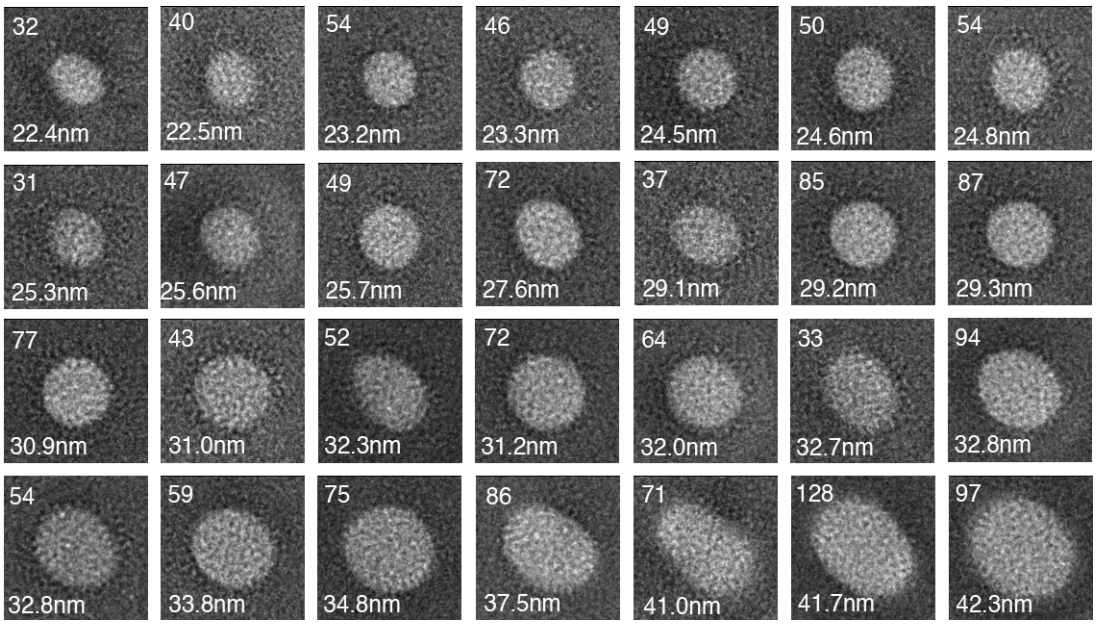

Figure S1. Single particle electron microscopy of macrodiscs. The length of the panels is 57.2 nm per side. The molar ratio of DMPC to 14-residue peptide was 13.3. The number of particles (top) and average size (bottom) of macrodiscs are shown in each panel.

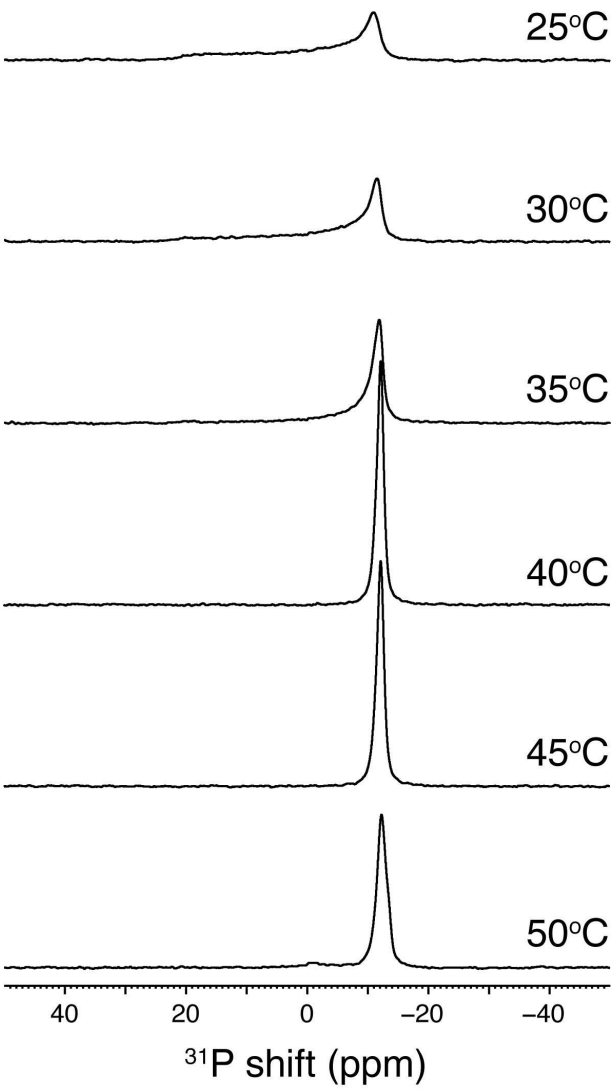

Figure S2. One-dimensional solid-state ^{31}P NMR spectra of the membrane protein-containing macrodisc at various temperatures recorded at 284 MHz. 64 acquisitions were collected for each spectrum.

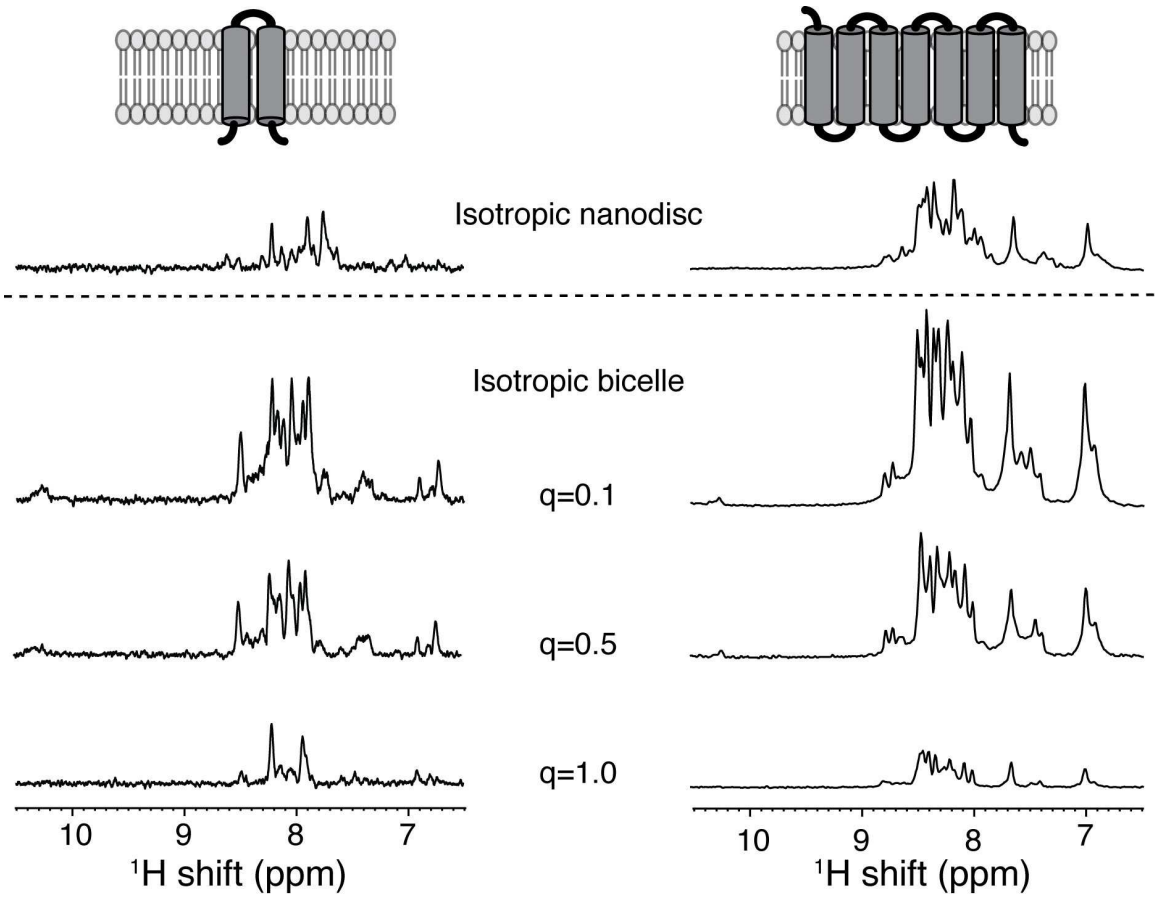

Figure S3. Comparison of ^{15}N edited ^{1}H solution NMR spectra of two transmembrane protein MerE (left) and seven transmembrane protein CXCR1 (right) reconstituted into isotropic nanodiscs and isotropic bicelles.

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