Supporting Information

In Situ Structural Characterization of a Recombinant Protein in Native *Escherichia coli* Membranes with Solid-State MAS NMR

Riqiang Fu[†], Xingsheng Wang[‡], Conggang Li[§], Adriana N. Santiago-Miranda[⊥], Gary J. Pielak[Δ], and Fang Tian[‡]

[†]National High Magnetic Field Laboratory, Tallahassee, FL
[‡]Department of Biochemistry and Molecular Biology, The Pennsylvania State University, Hershey, PA
[§]State Key Laboratory of Magnetic Resonance and Atomic and Molecular Physics, Wuhan Institute of Physics and Mathematics, Chinese Academy of Sciences, Wuhan, P. R. China
[⊥]Department of Chemical Engineering, University of Puerto Rico, Mayaguez, Puerto Rico
[Δ]Department of Chemistry, The University of North Carolina, Chapel Hill, NC

Methods

Sample preparation. Expression and isolation of the fully ^{13}C, ^{15}N enriched LR11 TM in native *E. coli* membranes using a new MBP expression vector has been described (1). For the preparation of $^{13}C_{\alpha,\beta}$-Ala labeled LR11 TM, M9 medium was mixed with NH_4Cl (1 g/L), D-glucose (4 g/L), $MgSO_4$ (0.2 mM), $(NH_4)_2Fe(SO_4)_2.6H_2O$ (7 mg/L), thiamine hydrochloride (0.01 mg/L), Tyr (50 mg/L), Phe (50 mg/L), Val (50 mg/L), Leu (50 mg/L), Ile (50 mg/L) and Met (125 mg/L) and 8X amino acid mixture (62.4 ml/L) consisting of Glu (3.2 g/L), Gln (3.2 g/L), Arg (3.2 g/L), Asp (1 g/L), Asn (0.8 g/L), Gly (0.8 g/L), His (0.8 g/L), Lys (0.8 g/L), Pro (0.8 g/L), Ser (12.8 g/L), Thr (0.8 g/L), Trp (0.4 g/L), and Cys (0.4 g/L). Cells were grown in 1 to 2 mL LB medium overnight and then inoculated in 250 mL of the above medium mixed with ^{13}C-$Ala_{\alpha,\beta}$ (0.2 g/L, ISOTEC). Cells were induced at A_{600nm} 0.6 to 0.9 with 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) at 16°C for ~24 h after adding 0.8 g/L $^{13}C_{\alpha,\beta}$-Ala.

E. coli membrane pellets were prepared as described in our previous publication [1]. Briefly, the cell lysate was centrifuged at ~30,000g for 30 min first to remove insolubles (cell debris, inclusion bodies, etc.) The low speed supernatant fraction, which included the soluble fraction and membrane fraction, was further centrifuged at ~160,000g for 1 h to obtain the membrane pellet. An example of the SDS-PAGE result is shown in Figure S1. To this membrane fraction, 20 mL PBS buffer (140 mM NaCl, 10 mM phosphate, pH 7.3) was added, and the sample was incubated at 4°C for overnight. The membrane fraction was then resuspended and mixed with ~500 U thrombin (GE Healthcare, 27-0846-01). The solution was slowly rotated at room temperature for 1 to 2 days to cleave the MBP fusion protein. The membrane fraction was isolated with ultracentrifugation at ~160,000g for 1 h. The membrane pellet was washed several times with PBS to further remove the cleaved MBP for solid-state MAS NMR experiments.

MBP-LR11TM pull down experiment. ~5 μg MBP and MBP-LR11TM were mixed with ~5 μg LR11TM in the column buffer (337 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, 1 mM EDTA, 2 mM β-mercaptoethanol, pH 7.4, 1% Triton X-100), respectively. These two mixtures (total 200 μl each) were incubated at room temperature for ~30 min. 200 μl amylose resin (New England BioLabs) were added to each mixture, and the solutions were incubated at room temperature for 5 min. The samples were centrifuged at ~16,000 g for 2 min.

The supernatants (flow-through) were saved for the SDS-PAGE. The amylose resin was washed twice with 1 ml column buffer, then incubated with 0.5 ml column buffer with 20 mM maltose. The resin was centrifuged and the supernatant was concentrated about 10 times. 10 μl of the samples (elutions) were loaded on the SDS-PAGE (Figure S2).

NMR spectroscopy and data analysis. All NMR measurements were carried out on a Bruker Avance 600MHz NMR spectrometer using a home-made low-E 3.2 mm triple-resonance MAS probe [2]. About 18 mg of the sample was packed into a 3.2 mm MAS rotor. The sample spinning rate was 10 kHz ±3 Hz controlled by a Bruker pneumatic MAS unit. During measurement the sample temperature was set to 305 K. The ramped cross polarization (CP) was used to enhance the ^{13}C magnetization in which the ^{1}H spinlock field of 50 kHz was used while the ^{13}C rf amplitude was ramped from 38 to 56 kHz with a CP contact time of 1 ms. The ^{13}C 90° pulse length was 4.6 μs. A SPINAL64 decoupling sequence [3] was used for ^{1}H decoupling during the t_1 and t_2 dimensions with a ^{1}H rf amplitude of 78 kHz. The quadrature detection in the t_1 dimension was achieved by TPPI. During the ^{13}C-^{13}C mixing time, a PARIS irradiation [4] was used with a ^{1}H rf amplitude of 10 kHz. The acquisition times for the indirect and direct dimensions were 4.8 and 10.3 ms, respectively. The FIDs were apodized with a Gaussian window function (LB=-50 Hz and GB=0.15) in both dimensions and zero-filled into a 2048 x 2048 matrix before Fourier transform using Bruker Topspin software. The ^{13}C chemical shifts were referenced to the carbonyl carbon of glycine at 176.4 ppm.

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Table S1: Chemical shifts of LR11 TM in native *E. coli* membranes from solid-state MAS NMR and in DPC micelles from solution NMR.

Residue number	C^{α} (ppm)	C^{β} (ppm)	$\Delta\delta^{E.coli}$ (ppm)	$\Delta\delta^{dpc}$ (ppm)
V25	-	-	-	1.5
A26	53.1	16.5	3.1	3.8
A27	53.1	16.5	3.1	2.5
V28	-	-	-	-0.7
V29	-	-	-	2.7
V30	-	-	-	9.6
P31	64.4	29.7	3.5	4.5
I32	63.9	35.8	3.0	3.5
L33	-	-	-	3.1
F34	60.7	36.8	5.3	4.4
L35	-	-	-	2.7
I36	-	-	-	3.3
L37	-	-	-	3.3
L38	-	-	-	2.6
S39	56.5	62.3	-1.4	-
L40	56.2	40.7	1.7	2.3
G41	46.1	-	1.1	2.7
V42	64.6	29.6	3.7	4.3
G43	46.1	-	1.1	2.7
F44	60.4	37.1	3.7	4.5
A45	48.4	21.9	-7.0	3.9
I46	-	-	-	-
L47	-	-	-	1.6

Note: The secondary shift, $\Delta\delta$, is defined as:

$$\Delta\delta = \{\delta_{C_\alpha}(observed) - \delta_{C_\alpha}(random\_coil)\} - \{\delta_{C_\beta}(observed) - \delta_{C_\beta}(random\_coil)\}$$

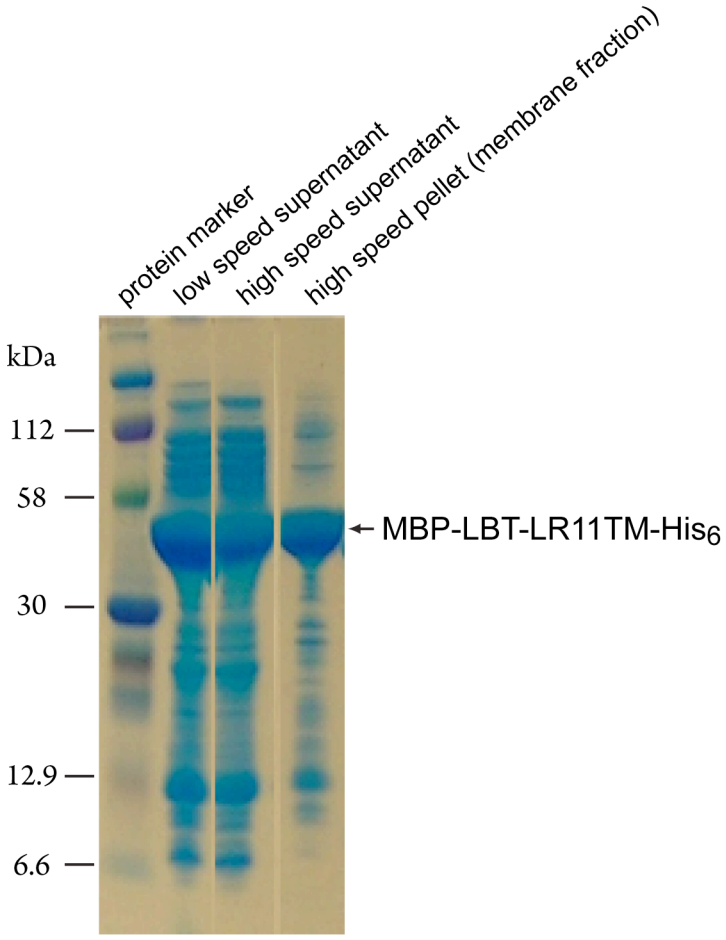

Figure S1: Preparation of *E. coli* membrane fraction.

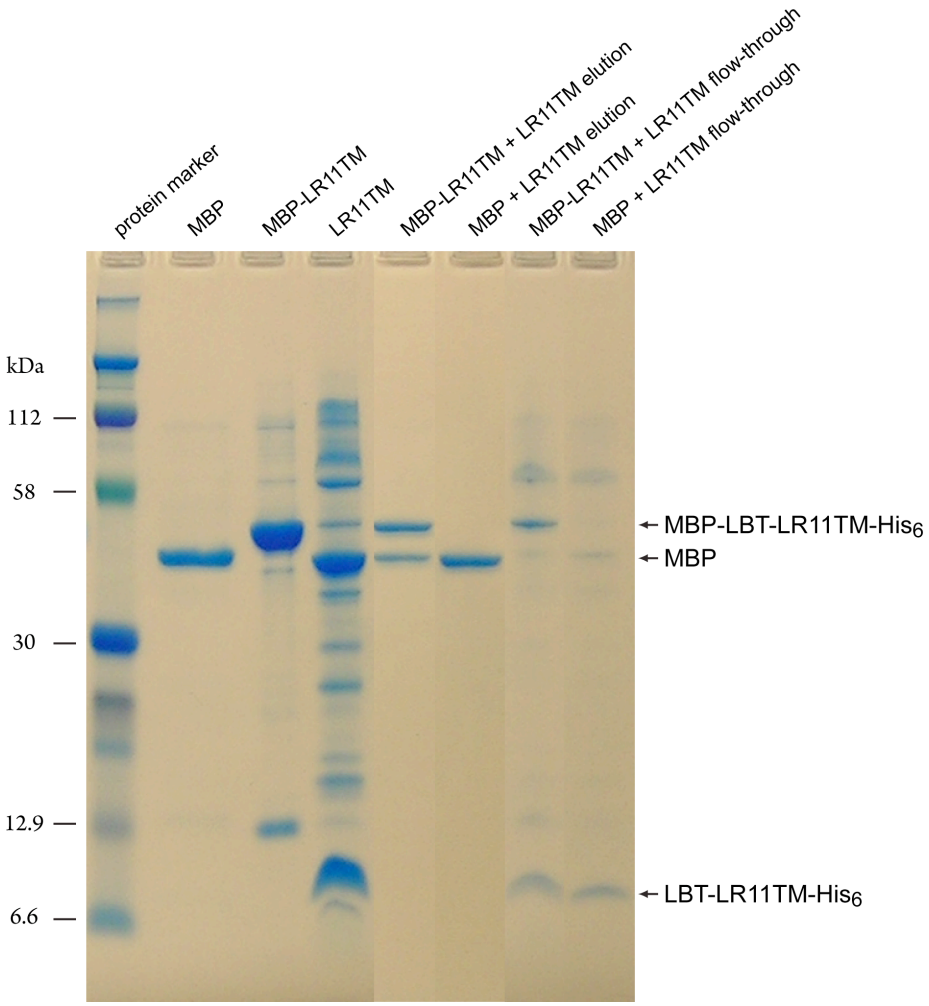

Figure S2: LR11 TM does not form exchangable oligomers. Lanes: 1, protein marker; 2: purified MBP; 3, isolated *E. coli* membrane fraction with MBP-LR11TM; 4, thrombin cleavage of the sample in lane 3; 5, elution of MBP-LR11TM pull down of LR11 TM; 6, elution of MBP pull down of LR11 TM; 7, flow-through of MBP-LR11TM pull down of LR11 TM; 8, flow-through of MBP pull down of LR11 TM.

Note: For pull down experiment LR11 TM in *E. coli* membrane fraction (lane 4) was washed only once, thus some cleaved MBP remain.