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Supporting Information

Micelles, Bicelles, and Nanodiscs: Comparing the Impact of Membrane Mimetics on Membrane Protein Backbone Dynamics

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Expression of OmpX in M9 Medium and formation of DPC micelles

E. coli Outer membrane protein X (OmpX) was expressed using a pET-28b, which was generously provided by the Hiller group (Biozentrum Basel, Switzerland). OmpX was expressed in *E. coli* strain BL21(DE3) Star (Invitrogen, Carlsbad) using M9 minimal medium containing 72% D_2O and ^{15}NH4Cl. The culture was grown at 37°C until OD_{600}= 0.7 Overexpression of OmpX was induced by adding 1mM isopropyl β-D-1-thiogalactopyranoside (IPTG; Invitrogen, Carlsbad). After 15h of overexpression, the cells were harvested by centrifuging at 5000 g, 4 °C for 10 min and were resuspended in 100 mL lysis buffer (20mM Tris, 5mM EDTA, pH 8). After gently stirring for 30 min at 4 °C, the cells were lysed by passing twice the Microfluidizer at a pressure of 42 bar. OmpX was purified from inclusion bodies as reported previously.[1]

OmpX was refolded in a dropwise manner by addition of 5mL, 500uM OmpX solution using a peristaltic pump (0.3 mL) to 50mL of refolding buffer (50 mM Tris, 100mM NaCl, 0.5% dodecylphosphocholine (FC-12 (Avanti Polar lipids), 500mM L-arginine, pH 8.5). After gently stirring for 2h at room temperature OmpX was dialyzed three times against 4L buffer (20 mM Tris, 100 mM NaCl, pH 7.4) using a 6-8 kDa membrane. OmpX was concentrated using an Amicon Ultra-4 10.000 Da molecular weight cut-off (MWCO) concentrator (Millipore) to a concentration of ~600uM and stored after freezing at -80°C.

Expression of MSPΔH5

The plasmid (pET-28a) with the coding sequence of truncated apoA-I missing residues 1- 54 and 121-142 (MSPΔH5), with an N-terminal TEV (Tobacco Etch Virus) protease cleavable histidine tag, was a generous gift of the Zerbe lab (University of Zurich) given with permission from Gerhard Wagner (Harvard University). MSPΔH5 (pET-28a) was expressed in *E. coli* strain BL21(DE3) Star (Invitrogen, Carlsbad) using Terrific Broth medium. The culture was grown at 37 °C, until an OD_{600}= 0.8 was reached. Overexpression was induced by addition of 0.5mM IPTG (Invitrogen, Carlsbad). After 30min, the temperature was lowered to 28°C for 4h. The cells were harvested by centrifuging at 5000g, 4°C, 10min and were resuspended in 100mL Lysis Buffer (20 mM Tris, pH 8, 5mg Protein Inhibitor Tablets). After gently stirring at 4°C for 30min, the cells were lysed by passing twice the Microfluidizer at a pressure of 4 bar. Purification of MSPΔH5 followed previous protocols.[2] Finally, MSPΔH5 was concentrated using an Amicon Ultra-4 10.000 Da molecular weight cut-off (MWCO) concentrator (Millipore) to a concentration of 500uM and was stored at -80°C.

Reconstitution of OmpX Nanodiscs

For the reconstitution of OmpX in Nanodiscs we followed established protocols.[3] In brief a molar ratio of 1: 2 : 80 : 160 of OmpX : MSPdH5 : DMPC : sodium cholate provided the best incorporation results. Either deuterated d_{54}-DMPC (1,2-ditetradecanoyl-sn-glycero-3-phosphocholine, FB reagents) or protonated DMPC (Avanti polar lipids) was used. For the reconstitution OmpX, MSPΔH5 and lipids were mixed in a glass vial and were shaken for 12h at 27°C, 220 rpm. Afterwards 1g Biobeads SM-2 (Biorad) per ml of assembly mixture was added shaken for 5h at 27°C, 220 rpm to remove any detergents. The supernatant was separated from Biobeads through low speed centrifugation. The supernatant was further purified with pre equilibrated (20 mM Tris, 100mM NaCl, pH 7.4) size exclusion column (Superdex 200 GL 10/30). The collected fractions were concentrated using an Amicon Ultra-4 10.000 Da molecular weight cut-off (MWCO) concentrator (Millipore) to a final concentration of ~ 650uM.

Reconstitution of OmpX in DHPC/DMPC bicelles

OmpX was refolded in a dropwise manner by addition of 1mL, 500uM OmpX solution using a peristaltic pump (0.3 mL) to 10mL of refolding buffer (50mM Tris, 100mM NaCl, 5% 1,2-dihexanoyl-*sn*-glycero-3-phosphocholine (DHPC-6, Avanti Polar Lipids), 500mM L-arginine, pH 7.4). After gently stirring for 2h at room temperature OmpX was dialyzed against 4L buffer (20 mM Tris, 100 mM NaCl, pH 7.4) for 20min using a 6-8 kDa membrane (Spectrum Laboratories, Inc). The OmpX solution was concentrated to 2mL using an Amicon Ultra-4 10.000 Da molecular weight cut-off (MWCO) concentrator (Millipore). Subsequently, the solution was diluted to 10 mL with 20mM Tris, 100mM NaCl, 3% DHPC-6, pH 7.4 and concentrated again to 2mL using an Amicon Ultra-4 10.000 Da molecular weight cut-off (MWCO) concentrator (Millipore). The OmpX solution was diluted to 20mL using a 20mM Tris, 100mM NaCl, pH 7.4 and then concentrated to an OD_{280}=3. The detergent concentration was estimated with ^{1}H- NMR of OmpX solution. The lipid bilayer was formed by addition of DMPC (Avanti Polar Lipids) in a q ratio of 0.5 and going through the transition temperature by cooling (4°C) and warming cycles (36°C).[4] The OmpX solution was concentrated to ~ 300uM using Amicon Ultra-4 10.000 Da molecular weight cut-off (MWCO) concentrator (Millipore).

NMR Spectroscopy

All experiments were recorded at 316K on a BRUKER 600 MHz Avance III HD spectrometer with a cryogenic probehead. The number of scans was adjusted for each sample individually to achieve a satisfactory signal-to-noise ratio.

All relaxation experiments were recorded in an interleaved manner using different relaxation delays.[5,6] For the R1, R2α, R2β and $R2_{R1\rho}$ experiments, the relaxation rates were determined by sampling for 4 different delay durations (see table S1), which were pseudo-randomized with a recovery delay of 7s for R1 and 3s for R2α, R2β and $R2_{R1\rho}$. The strength of the spin-lock field during the R1ρ measurement was 2000 Hz. R2 rates from the R1ρ measurement were calculated by correcting the off-resonance tilted field using the relation $R2 = R1\rho/\sin^2\theta - R_1/\tan^2\theta$, with $\theta = \tan^{-}$

[1](ω/Ω), where ω is the spin-lock field strength and Ω the offset from the ^{15}N carrier frequency. The residue-specific tumbling time (rotational correlation time) τ_c was calculated according to Lee et al.[7], η_{xy} was obtained from the difference between $R2_\alpha$ and $R2_\beta$.[5,6] The [^1H]^{15}N-hetNOE experiment was recorded with a 7s ^1H saturation time for the NOE experiment and the equivalent recovery time for the reference experiment, each preceded by an additional 1s recovery time. The error for hetNOE values was determined using Gaussian error propagation. Errors for R1, R2α, R2β and $R2_{R1\rho}$ are one standard deviation from a single exponential decay fit.

Experiment	OmpX Micelle [ms]	OmpX in MSPΔH5 [ms]	OmpX in Bicelle [ms]
R1	0, 1400, 800, 400	0, 2000, 1000, 400	0, 400, 1040, 1600
R1ρ	1, 50, 35, 20	1, 25, 15, 8	1, 20, 10, 5
$R2_\beta$	0, 50, 25, 14	0, 25, 14, 3.5	0, 45, 30, 15
$R2_\alpha$	0, 20, 10 ,5	0, 10, 6, 3	0, 15, 10, 5

Table S1: Delay times for Relaxation Measurements

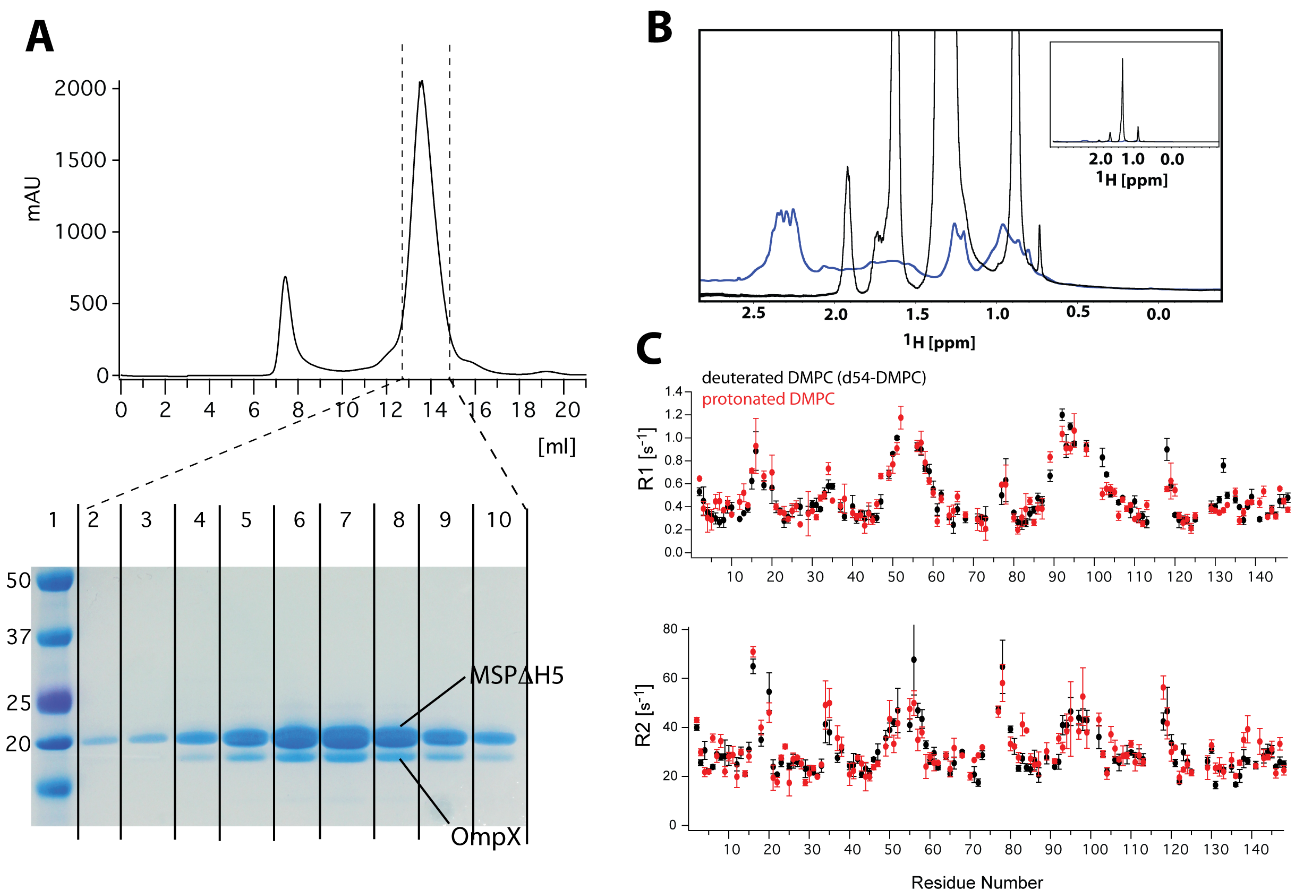

Figure S1. Reconstitution of OmpX in MSPΔH5 nanodiscs. (A) Size Exclusion Chromatography (Superdex 200 GL 10/30) profile of OmpX reconstituted in MSPΔH5 using DMPC lipids after the removal of cholate and FC12. A molar ratio of 1: 2 of OmpX:MSPΔH5 resulted in optimal reconstitution (lower panel), whereby the lipid concentration did not exceed 16 mM. (B) 1D proton spectra of FC12 (black spectrum) and OmpX reconstituted in MSPΔH5 (blue spectrum). No residual FC12 could be detected after 5h of BioBeads treatment. Spectra were recorded on a Bruker 600 MHz spectrometer. (C) Longitudinal (R_1) and transverse ($R_{2\beta}$) relaxation rates in deuterated d54-DMPC and protonated DMPC showed no differences.

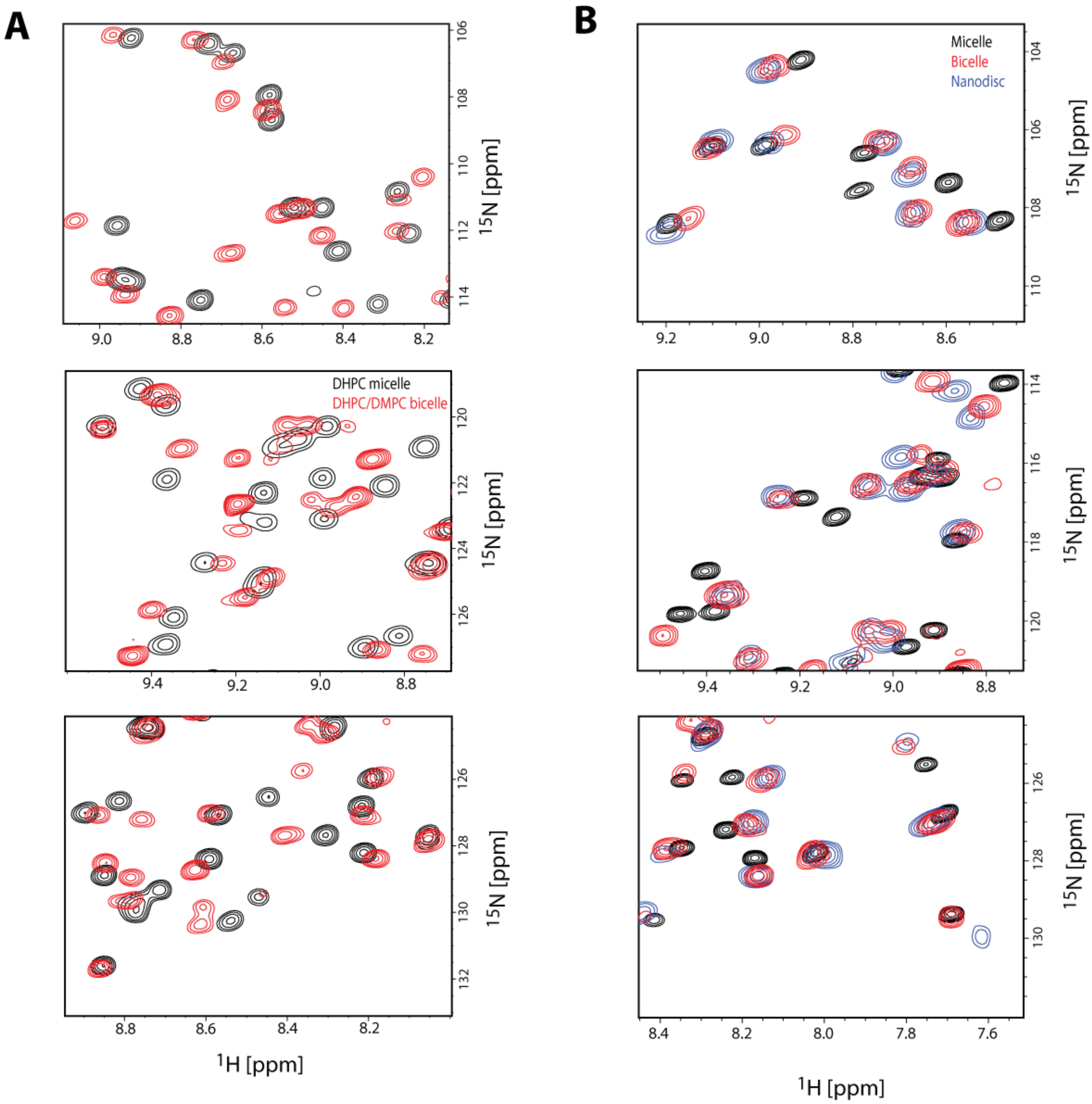

Figure S2. Chemical shift changes of OmpX in different membrane mimetics. (A) Overlay of 2D ^{1}H-^{15}N TROSY-HSQC experiments reveal chemical shift changes for OmpX after the formation of DHPC/DMPC bicelles (red spectrum) from DHPC micelles (black spectrum). (B) Overlay of 2D ^{1}H-^{15}N TROSY-HSQC experiments of OmpX in DPC micelles (black spectrum), DHPC/DMPC bicelles (red spectrum) and DMPC nanodiscs. Chemical shifts of OmpX in DHPC/DMPC bicelles and DMPC nanodiscs overlap, indicating that DMPC molecules in both membrane mimetics are in direct contact with OmpX. All spectra were recorded on a Bruker 600 MHz spectrometer.

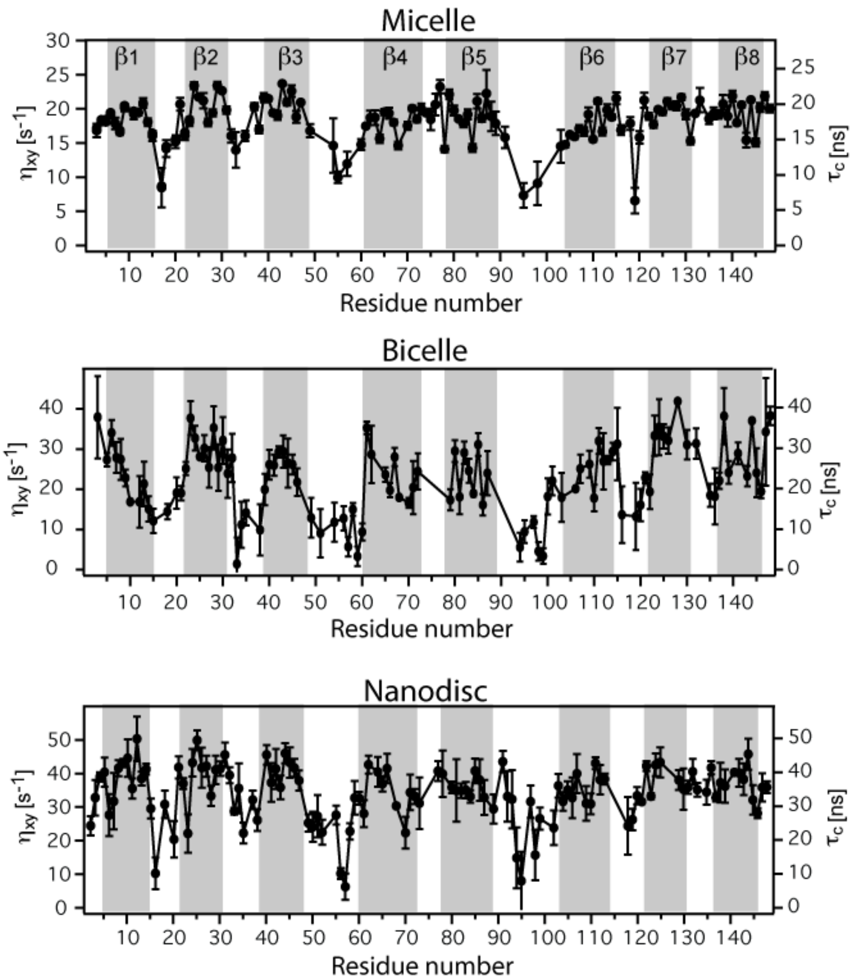

Figure S3. Transverse cross-correlated relaxation rates (η_{xy}) were determined from measurements of the fast and slowly relaxing [^{1}H]-^{15}N doublet component.[5-7] Calculation of the residue-specific rotational correlation time (τ_c) was according to Lee et al.[7] For clarity, the left and the right axis were adjusted so that η_{xy} data points overlap with their corresponding τ_c data points.

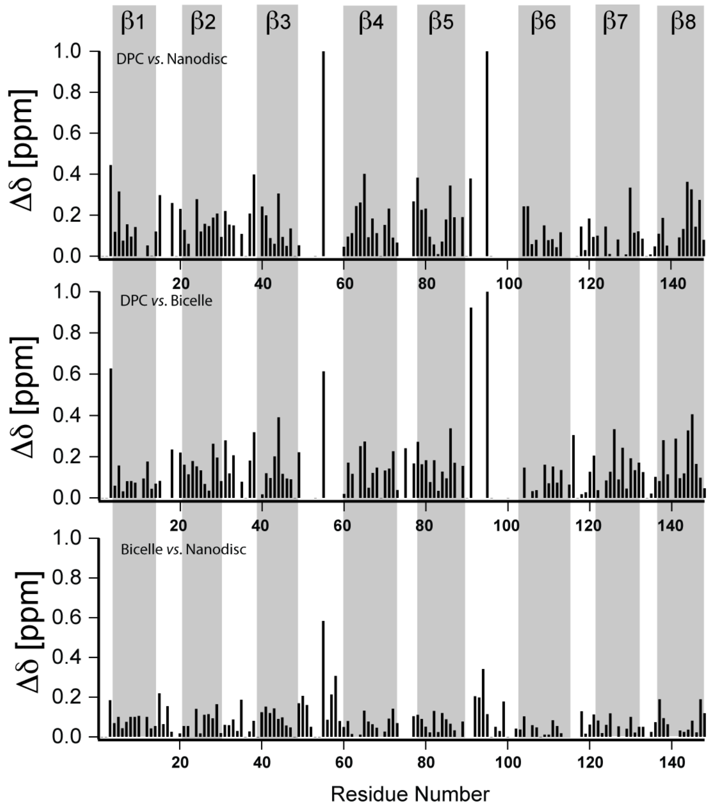

Figure S4. Chemical shift differences of OmpX in DPC micelles, DHPC/DMPC bicelles and DMPC nanodiscs were calculated using the formula: $\Delta\delta = \sqrt{\left(\frac{\Delta\,^{15}N}{5}\right)^2 + \left(\Delta\,^{1}H\right)^2}$

Table S2.

Relaxation Rates of OmpX in Bicelles

Residue	R1 [s^{-1}]	$R2_{(R1\rho)}$ [s^{-1}]	$R_{2\beta}$ [s^{-1}]	hetNOE	η_{xy}
2					
3	0.69		31.5	0.57	37.9
4	0.7		22.8	0.51	
5	0.27	49.7	15.8	0.87	27.4
6	0.26	61.1	15.2	0.73	34.1
7	0.24	39.6	20.2	0.81	27.9
8	0.41	45.8	17.6	0.61	27.5
9	0.4	44.1	15.9	0.65	22.8
10	0.26	44.4	17.9	0.67	16.9
11	0.2		23.8	0.55	
12	0.24		32.9	0.64	16.9
13	0.31	44.2	23.3	0.52	21.4
14	0.34	43.3	32.3	0.61	15.1
15	0.62	19.8	34.5	0.25	12.2
16	0.67	31.6	52.6	0.3	
17	0.6	26.1	46.3	0.53	
18	0.6	27.5	37	0.51	14.5
19	0.77	20.9		0.36	
20	0.6	74.7	35.7	0.45	19.1
21	0.64	37.1	26.6	0.64	19.1
22	0.38	68.6	14.8	0.52	25.2
23	0.44	70.2	15.2	0.9	37.7
24	0.37	62.5	18.6	0.77	32.8
25	0.47	60	16.5	0.71	28.2
26	0.46	55.3	14.2	0.8	30.2
27			17.2	0.84	25.4
28	0.4	51	20	0.7	35.3
29	0.37	63.4	17.5	0.74	25.4
30	0.3	56.9	15.7	0.81	32.2
31	0.56	74.4	18.8	0.83	23.9
32	0.59	35.9		0.56	27.8
33	0.57	22.2	53.1	0.19	1.5
34	0.58	29	45	0.18	11.2
35	0.33	36	31.3	0.45	14.2
36					
37	0.37	43.3	44.1	0.44	
38	0.4	38.5	57.1	0.69	9.9
39	0.41	49.2	19.6	0.68	20.0
40	0.37	47	17.9	0.58	26.1
41	0.41	51.8	21	0.69	26.0
42	0.48	45	20.1	0.86	29.3
43	0.43	44.9	21.2	0.9	29.4
44	0.52	46.3	19.3	0.81	26.5
45	0.52	52	14.2	0.94	26.2
46	0.61	37.4	28.6	0.53	21.8
47					
48				0.36	
49	0.67	19.4	30.3	0.51	12.9
50	0.76	18.9	50	0.48	
51	0.94	24.4	46.8	0.33	9.1
52					
53					
54	1	15.9	40	0.22	11.8
55	1.04	17.3	36.7	0.19	
56	0.94	23.1	24.4	0.28	12.8
57	0.97	13.1	28.6	0.25	5.7
58	0.82	9.5	35.7	0.35	15.0
59	0.95	20	33.3	0.26	3.3
60	0.6	24.2	30.3	0.41	9.4
61	0.68	49.4	24.3	0.47	35.3
62	0.63	39.8	31.8	0.86	28.7
63					
64		40.6		0.74	
65	0.53	39.4	20	0.91	23.6
66	0.36	48	20.8	0.9	19.8
67	0.42	42.6	17.8	0.85	28.0
68	0.34	53.5	25.7	0.9	18.0

69					
70					16.6
71	0.42	72.3	17.6	0.9	20.4
72	0.43	91.5	17.7	0.9	24.5
73					
74					
75	0.5			0.81	
76					
77	0.53	80.8	21.1	0.86	
78					
79	0.53	41	15.8	0.6	17.4
80	0.66	41	17.1	0.51	29.5
81	0.33	43.3	22.1	0.9	18.1
82	0.34	45.5	18.6	0.77	29.1
83	0.3	67.5	18.5	0.66	24.6
84	0.36	58.3	19.2	0.75	18.9
85	0.57	58.1	15.4	0.73	31.1
86	0.55	50.8	28.6	0.71	16.1
87	0.6	26	33.3	0.49	24.0
88					
89	0.88	10	31.3	0.45	
90					
91	0.77	16.1	37.9	0.29	
92	0.89	27		0.29	
93	0.87	15.5		0.31	
94	0.83	20.1	44.4	0.4	5.6
95	0.95		37.5	0.3	9.4
96					
97	0.93	20	23.5	0.29	11.9
98	0.99	7.3	24.2	0.37	4.6
99	0.83	15	28.6	0.44	3.4
100	1.03	10	26.1	0.46	18.2
101	0.8	21	22.5	0.57	22.1
102	0.99			0.52	
103	0.8	22.5	24.6	0.59	18.0
104	0.54	25.8	23.4	0.45	
105		55.2		0.72	
106	0.65	58.4	20.2	0.7	20.2
107	0.43	40	18.6	0.61	25.1
108					
109	0.41	45	19.9	0.65	26.3
110	0.45	50.5	24.3	0.9	17.8
111					32.0
112	0.4	46.7	19.8	0.83	27.0
113	0.35	54.4	17.7	0.65	27.3
114	0.28	70	18.1	0.65	29.4
115	0.38	70.2	26.5	0.8	31.3
116	0.61	29	50	0.68	13.7
117					
118	0.8	32.4		0.44	
119	0.49	40	45.7	0.64	13.3
120	0.54	41.3	34.5	0.7	16.1
121	0.26	64.1	16.7	0.71	22.9
122	0.35	70	20	0.8	19.4
123	0.27	60	22.7	0.84	33.3
124	0.4	62.5	20	0.8	35.5
125	0.46	67.4	19	0.92	33.2
126	0.48	60	20.6	0.77	32.2
127		47		0.88	
128	0.41	42		0.64	41.9
129		41	21.1	0.67	
130	0.39	40.8	25.4	0.58	31.1
131	0.36	44.4	19.9	0.43	
132	0.5	15.7	17.7	0.63	31.4
133	0.41	32	20	0.76	
134					
135	0.46	38	18.5	0.73	18.5
136	0.45	36.6	22.3	0.66	18.3
137	0.6	33.4	17.9	0.5	22.2
138	0.51	48.5	18.2	0.81	38.2
139	0.41	41.5	18.3	0.8	24.2
140					
141	0.32	35	19.2	0.81	28.9

142	0.35	40	23.4	0.8	
143	0.31	42.8	19.2	0.8	23.3
144	0.38	20		0.56	37.1
145	0.37	71	17.3	0.59	24.1
146					19.5
147	0.42	38.9	31.3	0.9	34.3
148	0.56	63.9	30.3	0.89	38.3

Relaxation Rates of OmpX in Nanodisc

Residue	R1 [s^{-1}]	$R2_{(R1\rho)}$ [s^{-1}]	$R_{2\beta}$ [s^{-1}]	hetNOE	η_{xy}
2	0.65	57.8	43.0	0.71	24.5
3	0.38	48.3	29.9	0.70	32.9
4	0.30	49.8	21.8	0.75	39.1
5	0.29	55.3	22.0	0.90	40.6
6	0.45	52.8	35.7	0.83	27.6
7	0.45	52.1	28.4	0.66	31.8
8	0.37	55.1	34.5	0.72	41.8
9	0.43	50.9	22.1	0.71	43.3
10	0.33	61.3	28.1	0.75	44.8
11			28.8		35.6
12	0.44	64.6	19.9	0.69	50.6
13	0.52	50.4	30.0	0.84	38.6
14	0.42	59.3	21.2	0.75	41.4
15	0.71	32.0	28.2		29.6
16	0.93	41.0	70.9	0.47	10.1
17					
18	0.67	41.9	40.0		30.8
19					
20	0.70	29.0	46.1	0.42	20.4
21	0.40	57.7	19.5		42.0
22	0.35	47.6	17.7	0.64	37.1
23	0.34	58.0	27.8	0.84	22.1
24	0.29	55.8	32.3		43.4
25	0.37	54.9	17.4	0.73	50.1
26	0.41	49.7	27.1	0.79	41.9
27	0.25	51.6	27.8	0.93	42.3
28		53.5	25.3		33.4
29	0.34	57.9	17.5	0.76	41.2
30	0.36	53.8	21.2	0.54	41.6
31	0.31	54.1	23.4	0.50	45.8
32	0.48	46.9	19.9	0.31	39.6
33	0.65	39.4	24.9	0.16	28.8
34	0.73	34.7	49.2	0.41	35.6
35	0.70	45.2	50.0	0.33	22.2
36					
37	0.50	51.9	35.7	0.47	32.2
38	0.47	57.4	32.1	0.52	26.2
39					
40	0.35	54.5	20.8	0.72	45.8
41	0.31	56.2	22.3	0.66	37.4
42	0.33	53.4	27.8	0.83	41.5
43	0.24	60.8	25.3	0.81	36.0
44	0.34	50.5	20.0	0.80	46.2
45	0.28	50.8	19.9	0.74	43.4
46	0.42	56.5	23.8	0.60	41.9
47	0.67	41.6	25.2	0.55	38.0
48					
49	0.69	45.8	32.3	0.50	25.3
50	0.77	32.1	43.5	0.60	24.1
51	0.91	31.7	33.3	0.40	27.3
52	1.18		41.7		22.5
53					
54					
55		27.1	47.6		27.7
56	0.93	19.7	50.0	0.27	10.1

57	0.96	24.1	35.0		6.1
58	0.78	30.2	38.0	0.35	22.8
59	0.63	32.6	24.1		32.8
60	0.52	51.1	28.0		32.7
61	0.27	45.7	25.6	0.88	28.1
62	0.47	52.0	25.6	0.84	42.8
63				0.83	
64	0.32	54.0	28.6	0.79	40.7
65	0.37	57.7	23.1	0.77	37.0
66	0.49	50.1	31.6		41.7
67		42.7		0.82	
68	0.33	58.9	28.6	0.80	30.4
69					
70			20.2		22.3
71	0.29	66.4	26.7	0.75	34.5
72	0.26	62.8	28.7	0.93	33.3
73	0.21	53.8	31.9	0.94	31.2
74					
75					
76					
77	0.59	63.0	46.5	0.54	40.9
78	0.59	52.6	58.2	0.57	40.0
79					
80	0.30	55.1	33.3	0.77	36.0
81	0.20	63.4	32.3	0.83	35.0
82	0.31	52.6	27.8	0.87	34.9
83	0.38	48.9	41.3	0.94	35.2
84	0.45	47.3	38.8	0.82	33.2
85	0.27	54.3	27.3	0.86	40.9
86	0.39	45.0	24.8	0.92	38.2
87	0.38	29.4	25.2	0.92	32.9
88					
89	0.83	24.5	30.3	0.73	29.5
90					
91			26.3	0.49	43.7
92	1.03	28.6	35.6		33.3
93	0.91		31.8	0.17	32.4
94	0.91	29.5	38.5	0.36	14.8
95	1.06		43.5	0.40	7.9
96					
97		25.7			31.7
98	0.90	31.0	52.6	0.35	15.7
99			38.0		26.6
100					
101					
102	0.51	38.0	43.3	0.26	23.8
103	0.56	43.5	29.1	0.48	36.5
104	0.56	38.4	21.3	0.53	31.8
105	0.53		37.2		35.1
106	0.32	52.3	31.4	0.67	32.9
107	0.46	59.8	26.9	0.70	40.1
108	0.38	45.8	28.5		
109	0.42	61.8	28.6	0.79	31.2
110	0.29	59.2	33.3	0.80	31.0
111	0.32	46.7	25.8	0.81	43.3
112	0.25	64.0	28.7	0.95	38.3
113	0.41	49.3	25.8	0.81	38.5
114		33.7			
115					
116					
117					
118	0.56	33.7	56.4	0.50	24.4
119	0.63	37.7	41.7	0.69	26.3
120	0.55	56.6	30.0	0.78	33.4
121	0.33	49.7	31.8	0.76	31.7
122	0.26	56.7	19.6	0.76	42.4
123	0.29	47.4	22.8	0.83	33.3
124	0.21	53.2	25.0	0.76	42.7
125	0.32	56.3	21.5	0.95	43.5
126					
127					

128					
129	0.38	62.4	23.6	0.78	38.2
130	0.37	53.6	32.8	0.76	35.4
131	0.35	45.5	23.5	0.70	36.0
132	0.37	36.2	23.9	0.77	40.7
133	0.42	36.3	22.0	0.49	35.3
134					
135	0.53	51.4	26.8	0.52	34.5
136	0.28	54.4	20.3	0.63	41.9
137	0.35	53.6	25.6	0.80	32.9
138	0.44	44.4	35.0	0.70	37.4
139	0.44	46.7	39.3	0.80	36.3
140					
141	0.44	54.9	24.3	0.83	40.5
142	0.53	55.2	34.3		40.3
143	0.31	45.3	27.5	0.78	38.4
144	0.37	50.5	27.9	0.87	46.0
145	0.32	60.8	31.3	0.70	32.2
146	0.56	44.2	21.3	0.71	28.2
147	0.45	56.6	33.3	0.72	36.0
148	0.37	54.4	22.6	0.60	36.0

Relaxation Rates of OmpX in DPC Micelles

Residue	R1 [s^{-1}]	$R2_{(R1\rho)}$ [s^{-1}]	$R_{2\beta}$ [s^{-1}]	hetNOE	η_{xy}
2	0.63	11.1	40.0	0.56	
3	0.57	22.3	17.5	0.69	16.8
4	0.59	23.7	15.4	0.77	18.1
5	0.58	25.6	16.7	0.83	18.4
6	0.54	23.4	15.7	0.83	19.1
7	0.54	26.0	13.7	0.82	17.9
8	0.57	24.9	14.7	0.86	16.6
9	0.61	25.6	13.2	0.80	20.2
10					
11	0.60	24.3	13.5	0.83	19.3
12	0.62	22.5	11.7	0.87	19.4
13	0.55	25.5	12.5	0.90	20.9
14	0.65	23.5	10.6	0.76	18.1
15	0.75	18.9	15.9	0.64	16.1
16					
17	1.11	17.2	25.0		8.5
18	0.81	21.4	22.2	0.63	14.2
19					
20	0.76	20.9	16.6	0.57	15.2
21	0.67	23.6	12.9	0.74	20.7
22	0.59	23.3	13.1	0.64	16.1
23	0.58	24.0	15.4	0.76	18.1
24	0.65	25.0	13.7	0.86	23.4
25	0.56	25.6	14.3	0.83	21.7
26	0.55	24.8	12.4	0.85	21.2
27	0.61	23.5	15.7	0.81	18.0
28	0.61	24.4	13.6	0.86	19.4
29	0.59	25.9	12.0	0.90	23.4
30	0.60	25.4	11.4	0.78	22.7
31	0.61	22.9	10.8	0.83	19.8
32	0.64	21.9	11.1	0.65	16.1
33	0.84	17.9	30.0	0.43	14.0
34					
35	0.73	17.3	19.6	0.49	16.1
36					
37	0.66	25.9	16.4	0.65	20.4
38	0.66	22.0	14.1	0.75	17.0
39	0.63	25.6	12.9	0.80	21.6
40	0.65	24.9	11.7	0.75	21.5
41	0.58	26.0	14.1	0.78	19.4
42	0.58	22.8	15.0	0.84	19.0

43	0.56	26.2	11.8	0.84	23.7
44	0.59	23.6	14.1	0.91	21.0
45	0.56	26.9	12.4	0.89	22.6
46	0.60	25.3	16.7	0.87	18.8
47	0.55	27.2	14.1	0.87	21.0
48					
49	0.67	22.3	18.9	0.73	16.8
50					
51					
52					
53					
54	0.81	18.5	36.5		14.6
55	0.90	18.3		0.60	10.0
56					
57	1.10	18.0	28.8	0.29	12.0
58					
59					
60	0.75	19.6	17.0	0.71	14.8
61	0.65	23.5	13.2	0.72	17.5
62	0.59	22.7	14.4	0.81	18.7
63	0.59	23.8	15.0	0.94	18.7
64	0.65	24.4	14.9	0.83	15.6
65	0.60	24.5	14.7	0.84	19.5
66	0.60	25.3	15.7	0.78	19.4
67	0.62	23.3	14.5	0.77	18.0
68	0.61	26.0	16.6	0.90	14.6
69					
70	0.61	21.7	14.7	0.78	17.5
71	0.61	27.2	15.9	0.77	20.1
72	0.57	24.8	15.4	0.81	18.5
73	0.60	25.9	14.7	0.71	20.0
74	0.52	24.2	16.0	0.76	19.4
75	0.51	24.3	23.9	0.84	18.5
76	0.70	23.7	15.6	0.81	20.6
77	0.53	28.2	15.7	0.88	23.3
78	0.51	23.9	18.5	0.79	14.1
79	0.61	25.4	13.5	0.84	22.1
80	0.63	25.9	14.0	0.80	19.8
81	0.59	24.5	14.1	0.81	18.5
82	0.60	23.1	14.2	0.81	17.9
83	0.60	23.7	14.8	0.84	19.2
84	0.63	24.1	16.0	0.76	14.3
85	0.62	25.5	11.7	0.79	21.1
86	0.60	23.3	13.5	0.74	18.6
87	0.60	24.7	12.6	0.89	22.2
88	0.60	25.2	12.7	0.86	18.9
89	0.72	21.4	14.9	0.70	17.9
90					
91	0.80	19.6	15.8	0.64	15.8
92					
93					
94					
95	1.36	12.4	21.7	0.40	7.4
96					
97					
98	1.03	13.0	33.6	0.40	9.1
99					
100					
101					
102					
	0.93	17.7	32.8	0.63	14.5
104	0.87	19.1	16.4	0.71	14.8
105	0.60	20.7	14.3	0.84	16.2
106	0.66	23.9	16.2	0.81	15.9
107	0.64	21.0	14.1	0.55	17.1
108	0.64	22.1	14.6	0.82	16.7
109	0.55	25.8	15.0	0.83	19.2
110	0.60	25.5	17.9	0.84	15.6
111	0.60	25.1	12.5	0.79	21.1
112	0.50	26.1	15.2	0.71	16.7

113	0.59	24.1	12.9	0.80	19.9
114	0.51	23.3	14.7	0.85	18.8
115	0.63	26.1	13.3	0.77	21.6
116	0.63	22.9	13.8	0.80	16.9
117					
118	0.67	23.2	14.9	0.80	17.9
119	0.77	10.2	24.1	0.65	6.6
120	0.73	22.0	19.8	0.66	15.8
121	0.63	24.4	13.7	0.79	21.3
122	0.63	24.8	12.8	0.90	18.9
123	0.57	22.7	16.0	0.82	17.7
124	0.59	24.6	13.7	0.86	19.9
125	0.60	24.1	14.2	0.89	19.6
126	0.60	23.8	14.4	0.85	21.1
127	0.62	24.7	15.2	0.86	20.4
128	0.61	25.1	13.9	0.79	20.4
129	0.60	25.4	13.9	0.79	21.7
130	0.59	24.6	14.3	0.84	19.2
131	0.60	22.0	15.4	0.75	15.3
132	0.60	24.1	13.9	0.71	19.3
133	0.65	25.7	12.4	0.72	21.3
134					
135	0.64	22.5	12.5	0.75	18.6
136	0.56	22.3	10.2	0.73	19.4
137	0.63	23.3	11.7	0.84	19.2
138	0.57	24.9	12.4	0.85	20.7
139	0.55	24.3	13.7	0.84	18.9
140	0.57	25.4	14.2	0.85	21.9
141	0.66	23.0	15.6	0.81	18.0
142	0.60	26.7	12.8	0.83	20.6
143	0.62	25.4	14.2	0.87	15.4
144	0.62	24.8	12.6	0.82	21.3
145	0.61	23.5	16.6	0.83	15.1
146	0.62	24.4	12.3	0.89	20.2
147	0.63	25.7	11.2	0.73	21.9
148	0.76	25.0	12.0	0.76	20.1

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