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Supporting Information

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Interactions with hydrophobic clusters in the urea-unfolded membrane protein OmpX

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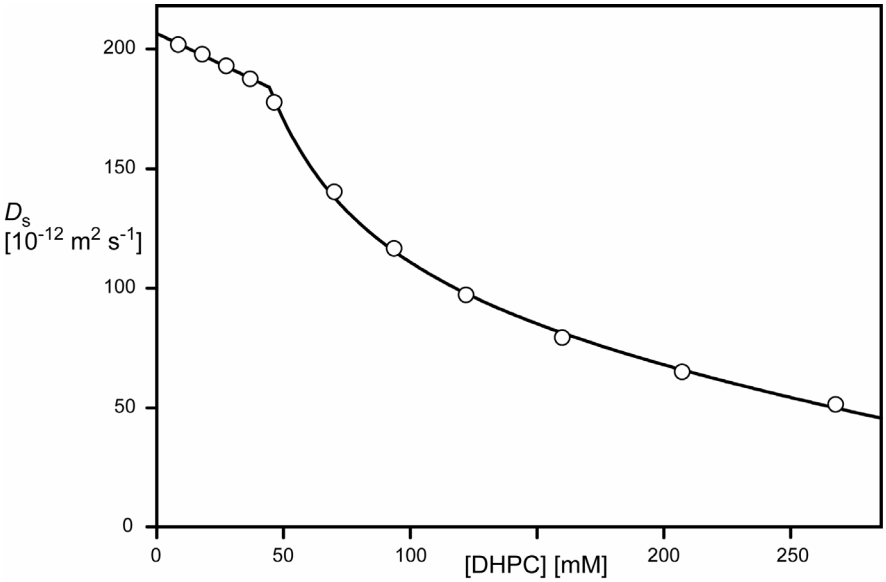

Figure S1. Diffusion constant, D_S, of DHPC in 8M aqueous urea solution measured with BPP-LED NMR diffusion experiments [1]. The circles are experimental data points, the line is the result of a least-squares fit of the data. The transition at 45(±3) mM is indicative of micelle formation. The diffusion constant of DHPC micelles in 8 M urea at infinite dilution was determined to be $D_0 = 62 \cdot 10^{-12}$ m^2 s^{-1} in this experiment.

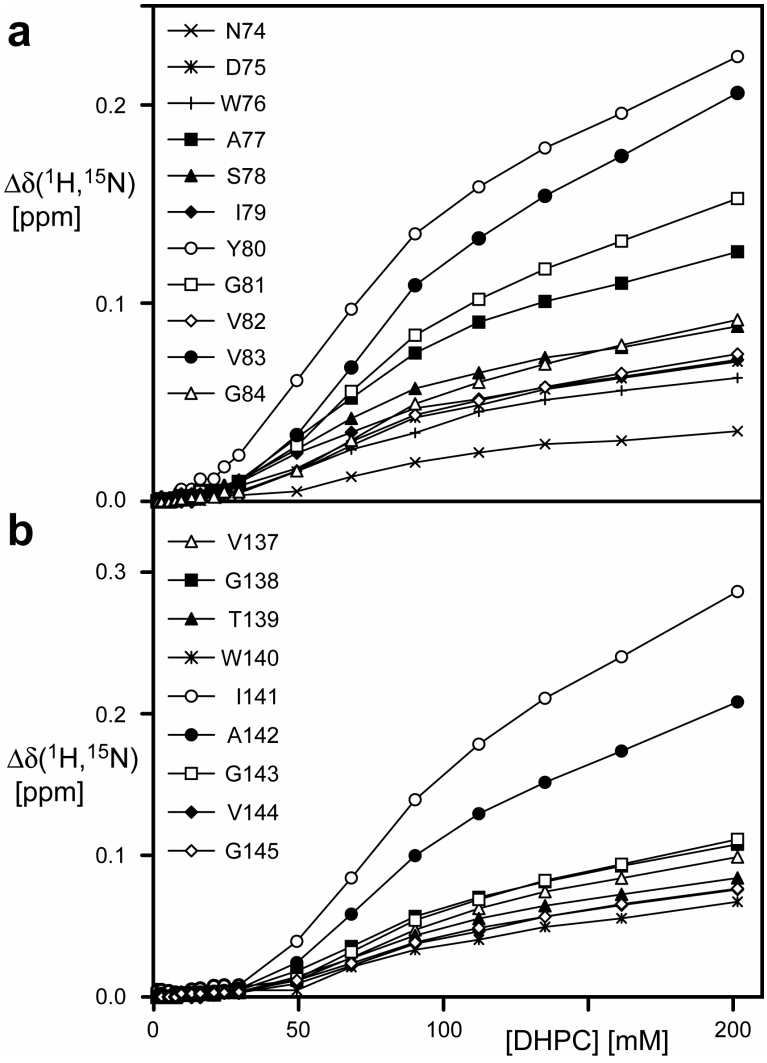

Figure S2. Chemical shift differences, $\Delta\delta(^{1}H,^{15}N)$, of all the amide moieties in the two clusters of urea-unfolded OmpX at variable DHPC concentrations. (a) Cluster I. (b) Cluster II.

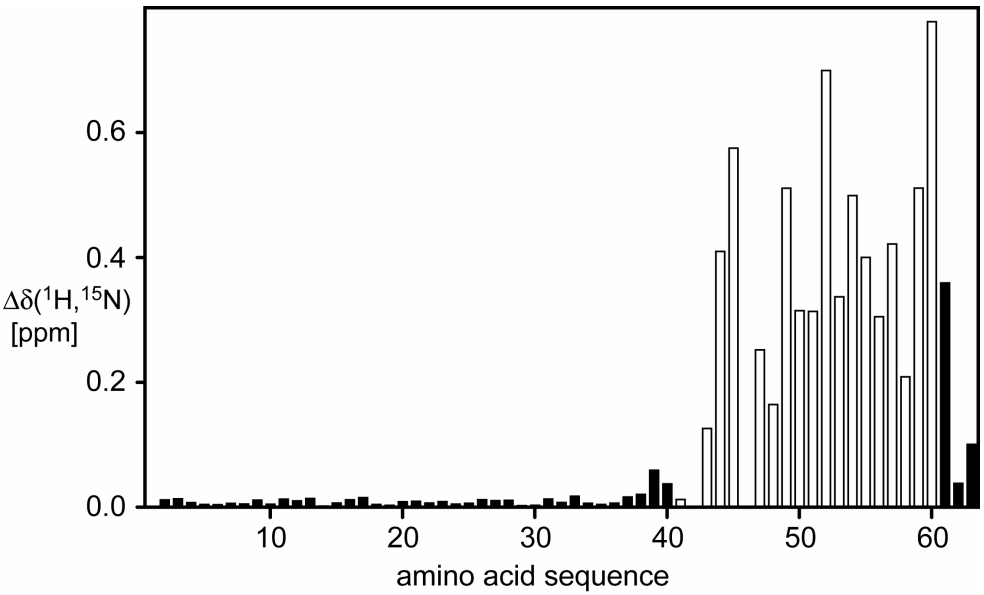

Figure S3. Chemical shift differences $\Delta\delta(^{1}H,^{15}N)$, of the amide moieties in urea-denatured 434-repressor(1–63) with and without addition of 200 mM DHPC. The open bars represent data for the residues which were previously shown to be involved in hydrophobic cluster formation [2, 3].

Methods

NMR spectroscopy. All NMR experiments required for the sequence-specific backbone and side chain resonance assignments and the structure determination of denatured wild-type OmpX in complex with DHPC micelles were measured at 15°C on a Bruker DRX 750 spectrometer. The following experiments were recorded: 2D [^{15}N,^{1}H]-HSQC [4], 2D *ct*-[^{13}C,^{1}H]-HSQC [5], 3D *ct*-HNCA [6], 3D CBCA(CO)NH [7], 3D H(CCO)NH-TOCSY [8], 3D (H)C(CO)NH-TOCSY [8], 3D ^{15}N-resolved-[^{1}H,^{1}H]-NOESY [9], two 3D ^{13}C-resolved-[^{1}H,^{1}H]-NOESYs for aliphatic and aromatic protons, respectively [10].

2D [^{15}N,^{1}H]-HSQC spectra [4] with a WATERGATE sequence for water suppression [11, 12] were recorded with an interscan delay of 1s. 2k complex points were recorded with an acquisition time of 194 ms, and prior to Fourier transformation the FID was multiplied with a 75°-shifted sine bell and zero-filled to 4k complex points. In the ^{15}N-dimension, 256 complex points were measured, with a maximal evolution time of 112 ms, and the data was multiplied with a 75°-shifted sine bell and zero-filled to 512 complex points before Fourier transformation. The baseline was corrected using the IFLAT method [13] in the ω_2(^{1}H)-dimension, and polynomials of 5th order in the ω_1(^{15}N)-dimension.

With the peptide OmpX(70–87), a 2D [^{1}H,^{1}H]-TOCSY experiment [14] and a 2D [^{1}H,^{1}H]-NOESY experiment [15] with a mixing time of τ_m = 120 ms were recorded. For both experiments, the following parameters were used: The interscan delay was 1s; 4k complex points were recorded with an acquisition time of 388 ms, and prior to Fourier transformation the FID was multiplied with a 75°-shifted sine bell and zero-filled to 8k complex points. In the ω_1(^{1}H)-dimension, 512 complex points were measured, with a maximal evolution time of 195 ms, and the data was multiplied with a 75°-shifted sine bell and zero-filled to 1024 complex points before Fourier transformation. The baseline was corrected using the IFLAT method [13] in the ω_2(^{1}H)-dimension, and polynomials of 5th order in the ω_1(^{1}H)-dimension.

Data analysis. The proton chemical shifts were referenced to internal DSS [16], and those for nitrogen-15 and carbon-13 were indirectly referenced [12, 17]. All spectra were processed with the software PROSA [18] and analyzed using the softwares XEASY [19] and CARA [20]. The sequence-specific resonance assignments for wild-type OmpX obtained with standard triple resonance experiments [21] were the starting platform for the present spectral analyses. Sequence-specific resonance assignments for the amide moieties of OmpX[W76A] and OmpX[W140A] were derived from these reference assignments with 3D ^{15}N-resolved [^{1}H,^{1}H]-NOESY experiments [9] recorded with τ_m = 120 ms. Unambiguous sequence-specific resonance assignments could thus be obtained, since identical chemical shifts to those of wild-type OmpX were found for the majority of the residues. The titrations from 0 mM to 200 mM DHPC were done in steps of 20 or 40 mM (Figs. S2 and 3A), and the sequence-specific resonance assignment of the amide moieties was carried over during the stepwise titration from one spectrum to the next one. At the DHPC concentration of 200 mM, the assignment was independently verified, using a 3D *ct*-HNCA spectrum [6] for 434-repressor(1–63), and a 3D ^{15}N-resolved [^{1}H,^{1}H]-NOESY spectrum [9] for OmpX.

The overall chemical shift difference of the amide moiety, $\Delta\delta(^{1}H,^{15}N)$, was defined as $\Delta\delta\left(^{1}\mathrm{H},^{15}\mathrm{N}\right)=\sqrt{\left(\Delta\delta\left(^{1}\mathrm{H}^{\mathrm{N}}\right)\right)^{2}+\left(0.2\cdot\Delta\delta\left(^{15}\mathrm{N}\right)\right)^{2}}$, where $\Delta\delta(^{1}H^{N})$ and $\Delta\delta(^{15}N)$ are the chemical shift differences for $^{1}H^{N}$ and ^{15}N, respectively, between the urea-unfolded protein at 0 mM DHPC and at 200 mM DHPC: $\Delta\delta(^{1}H^{N}) = \delta(^{1}H^{N})$ [200 mM DHPC] - $\delta(^{1}H^{N})$ [0 mM DHPC]. $\Delta\delta(^{15}N) = \delta(^{15}N)$ [200 mM DHPC] - $\delta(^{15}N)$ [0 mM DHPC]. The weighting factor of 0.2 reflects the difference in chemical shift dispersion of $^{1}H^{N}$ and ^{15}N in denatured proteins [22, 23].

Diffusion measurements. Measurements of the diffusion constants of DHPC in 8M urea were done using 1D BPP-LED experiments with varying field gradient strength [1]. The data was fitted to the equation $\ln [I(f) / I(f_0)] = -(\gamma \cdot \delta \cdot G_{max})^2 (f^2 - f_0^2) \cdot (\Delta + 2/3 \cdot \delta + 3/2 \cdot \tau) \cdot D_s$,

where I is the integrated intensity of the NMR signal, f is the fractional gradient strength, f_0 is the fractional gradient strength of the reference spectrum (0.02), γ is the gyromagnetic ratio of ^{1}H, G_{max} is the maximal gradient strength at f = 1.0, Δ is the diffusion time, δ is the gradient length, τ is gradient recovery delay, and D_s is the diffusion constant. The gradient strengths were calibrated on the residual ^{1}H signal in a 99.8% D_2O sample, using the published value of $1.902 \cdot 10^{-9}$ m^2 s^{-1} for the self-diffusion coefficient of HDO at 25°C [1, 24].

Titrations with detergents. For the DHPC titrations of OmpX and its variants, the samples contained 0.5 mM protein. For 434-repressor(1–63), a 1 mM sample was used. For both proteins, 50 mM DHPC stock solutions in the corresponding NMR buffers were used for titrations up to a final concentration of 30 mM DHPC; for further steps, solid DHPC was added to the sample. The detergent was purchased from Avanti Polar Lipids (Alabaster, AL) and used without further purification. Detergent concentrations were monitored with 1D ^{1}H-NMR experiments.

Population analysis of ensembles of conformers. In the limiting situation of rapid interconversion of conformers i on the chemical shift time scale, the chemical shift δ for a given nucleus in an ensemble of species is given by Equation (1), where the "unstructured" random coil is considered to be a single state representing all random conformations of the polypeptide chain [25-27]. Non-random structures, such as hydrophobic clusters, are treated as a second state [25]. With these assumptions, the chemical shift, δ, of a nucleus in a hydrophobic cluster with population q is given by

$$\delta = \delta_{rc} \cdot (1-q) + \delta_{hc} \cdot q, \qquad \text{(S1)}$$

where δ_{rc} and δ_{hc} are the chemical shifts in the random coil state and in the hydrophobic cluster, respectively. The chemical shift difference between the wild type protein with cluster population q and a variant protein with cluster population q', $\Delta\delta$, then is

$$\Delta\delta = \Delta\delta_{hc} \cdot (q' - q), \qquad \text{(S2)}$$

with $\Delta\delta_{hc} = \delta_{hc} - \delta_{rc}$. Hence,

$$q' = q + \Delta\delta / \Delta\delta_{hc}. \qquad \text{(S3)}$$

In the two OmpX variants studied in this paper, significant chemical shift changes are observed exclusively for residues in the modified clusters. For the calculation of upper limits of the population differences between wild type OmpX and the variant proteins we derived the values $\Delta\delta_{hc} \cdot q > 0.1$ ppm and q = 25% from the experimental data (see main text), resulting in $\Delta\delta_{hc} \geq 0.4$ ppm. With the experimentally determined standard deviation of 0.004 ppm for the measurement of $\Delta\delta$, Equation (S3) yields $q' = q \pm 0.01$ for both variant proteins studied.

References of Supporting Information

[1] J. J. Chou, J. L. Baber, A. Bax, *J. Biomol. NMR* **2004**, *29*, 299–308.

[2] D. Neri, M. Billeter, G. Wider, K. Wüthrich, *Science* **1992**, *257*, 1559–1563.

[3] D. Neri, G. Wider, K. Wüthrich, *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 4397–4401.

[4] G. Bodenhausen, D. J. Ruben, *Chem. Phys. Lett.* **1980**, *69*, 185–189.

[5] G. W. Vuister, A. Bax, *J. Magn. Reson.* **1992**, *98*, 428–435.

[6] T. Yamazaki, W. Lee, M. Revington, D. L. Mattiello, F. W. Dahlquist, C. H. Arrowsmith, L. E. Kay, *J. Am. Chem. Soc.* **1994**, *116*, 6464–6465.

[7] S. Grzesiek, A. Bax, *J. Am. Chem. Soc.* **1992**, *114*, 6291–6293.

[8] S. Grzesiek, J. Anglister, A. Bax, *J. Magn. Reson. B* **1993**, *101*, 114–119.

[9] S. Talluri, G. Wagner, *J. Magn. Reson. B* **1996**, *112*, 200–205.

[10] D. R. Muhandiram, N. A. Farrow, G. Y. Xu, S. H. Smallcombe, L. E. Kay, *J. Magn. Reson. B* **1993**, *102*, 317–321.

[11] V. Sklenar, M. Piotto, R. Leppik, V. Saudek, *J. Magn. Reson. A* **1993**, *102*, 241–245.

[12] G. Wider, *Prog. Nucl. Magn. Reson. Spectrosc.* **1998**, *32*, 193–275.

[13] C. Bartels, P. Güntert, K. Wüthrich, *J. Magn. Reson. A* **1995**, *117*, 330–333.

[14] L. Braunschweiler, R. R. Ernst, *J. Magn. Reson.* **1983**, *53*, 521–528.

[15] A. Kumar, R. R. Ernst, K. Wüthrich, *Biochem. Biophys. Res. Commun.* **1980**, *95*, 1–6.

[16] J. L. Markley, A. Bax, Y. Arata, C. W. Hilbers, R. Kaptein, B. D. Sykes, P. E. Wright, K. Wüthrich, *J. Biomol. NMR* **1998**, *12*, 1–23.

[17] D. S. Wishart, C. G. Bigam, J. Yao, F. Abildgaard, H. J. Dyson, E. Oldfield, J. L. Markley, B. D. Sykes, *J. Biomol. NMR* **1995**, *6*, 135–140.

[18] P. Güntert, V. Dötsch, G. Wider, K. Wüthrich, *J. Biomol. NMR* **1992**, *2*, 619–629.

[19] C. Bartels, T. Xia, M. Billeter, P. Güntert, K. Wüthrich, *J. Biomol. NMR* **1995**, *6*, 1–10.

[20] R. Keller, *The Computer Aided Resonance Assignment Tutorial*, Cantina, Goldau, **2004**.

[21] H. Tafer, S. Hiller, C. Hilty, C. Fernández, K. Wüthrich, *Biochemistry* **2004**, *43*, 860–869.

[22] D. Braun, G. Wider, K. Wüthrich, *J. Am. Chem. Soc.* **1994**, *116*, 8466–8469.

[23] K. Wüthrich, *NMR of Proteins and Nucleic Acids*, Wiley, New York, **1986**.

[24] R. Mills, *J. Phys. Chem.* **1973**, *77*, 685–688.

[25] K. Wüthrich, *Curr. Opin. Struct. Biol.* **1994**, *4*, 93–99.

[26] L. J. Smith, K. M. Fiebig, H. Schwalbe, C. M. Dobson, *Fold. Des.* **1996**, *1*, R95–R106.

[27] R. Mohana-Borges, N. K. Goto, G. J. A. Kroon, H. J. Dyson, P. E. Wright, *J. Mol. Biol.* **2004**, *340*, 1131–1142.