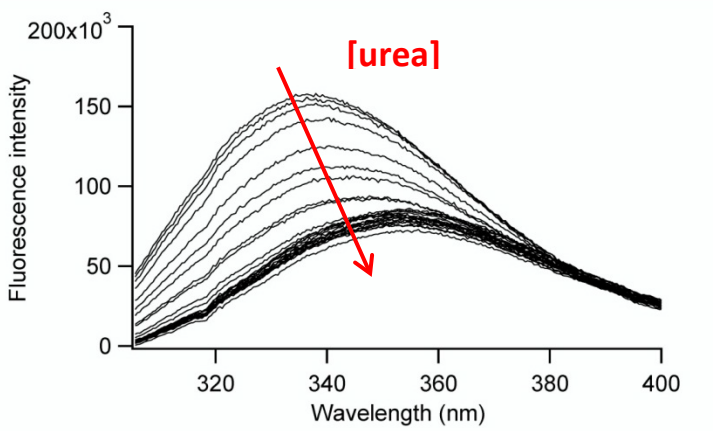
Supporting Information

Fitting procedures to obtain free energies of unfolding from equilibrium unfolding curves monitored by weighted average wavelength (<λ>)

Here we describe the procedure to obtain free energies of unfolding of membrane proteins by nonlinear least-square fitting of denaturant-induced equilibrium unfolding curves to the two-state model (see Eq. (S2) below). The unfolding of membrane proteins with buried Trps often results in large red-shifts and a reduction of the emission intensity of Trp fluorescence spectra. More precise fit values can be obtained when unfolding is monitored by the change of the intensity weighted average emission wavelength (<λ>) instead of fluorescence intensity changes at a single wavelength.

Step 1: Collect the Trp fluorescence spectra at various denaturant concentrations.



Step 2: Construct the unfolding curve by plotting the total fluorescence intensity versus denaturant concentration. The total fluorescence intensity of each spectrum is obtained by summing the fluorescence intensity at each measuring step (4 nm interval) over a 300 nm~400 nm wavelength range according to

$$F = \sum_i F_i \qquad \text{Eq. (S1)}$$

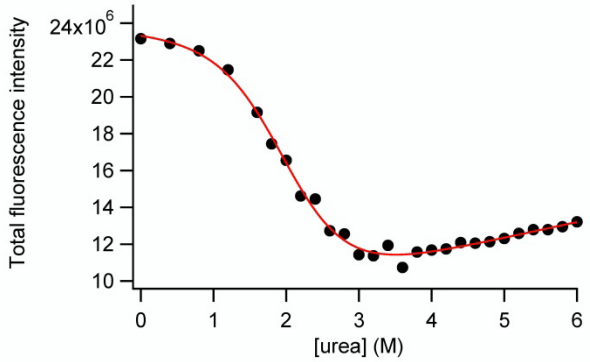


Table 1

Parameter	Fitted value
C_m (M)	1.9 ± 0.1
m (cal/mol M^{-1})	1300 ± 100
m_{folded} (M^{-1})	6.4 ± 9.6×10^5
FL_{folded}	2.3± 0.03×10^7
$m_{unfolded}$ (M^{-1})	9.2 ±1.4×10^5
$FL_{unfolded}$	7.7 ± 0.7×10^6

The unfolding curve is fitted to Eq. (S2) to obtain the urea-dependent total fluorescence intensity changes of the folded (F_{folded}) and unfolded ($F_{unfolded}$) states, respectively. F_{folded} and $F_{unfolded}$ are assumed to be linearly dependent upon denaturant concentration as defined in Eq. (S3).

$$F = \frac{F_{folded} + F_{unfolded} \exp[\frac{m([urea] - C_m)}{RT}]}{1 + \exp[\frac{m([urea] - C_m)}{RT}]} \qquad \text{Eq. (S2)}$$

m: slope of the free energy of unfolding on denaturant concentration
C_m: midpoint of unfolding transition
R: gas constant (1.987 cal/mol)
T: absolute temperature (310 K)

$$F_{folded} = m_{folded}[urea] + F_{0M,\,folded} \qquad \text{Eq. (S3)}$$
$$F_{unfolded} = m_{unfolded}[urea] + F_{0M,\,unfolded}$$

m_{folded}: slope of the fluorescence intensity change of the folded state as a function of denaturant
$F_{0M,folded}$: total fluorescence intensity of the folded state extrapolated to 0 M denaturant
$m_{unfolded}$: slope of the fluorescence intensity change of the unfolded state as a function of denaturant
$F_{0M,unfolded}$: total fluorescence intensity of the unfolded state extrapolated to 0 M denaturant

The ratio (Q_R) of the total fluorescence intensity of the folded state to that of the unfolded state is obtained at each denaturant concentration by

$$Q_R = \frac{F_{folded}}{F_{unfolded}} = \frac{\left(6.4\times10^5[urea] + 2.3\times10^7\right)}{\left(9.2\times10^5[urea] + 7.7\times10^6\right)} \qquad \text{Eq. (S4)}$$

Step 3: Construct the unfolding curve by plotting the intensity weighted average emission wavelength ($<\lambda>$) versus denaturant concentration. $<\lambda>$ is defined as the sum of the product of the wavelength (λ_i) and the corresponding intensity (F_i) at each measuring step divided by the total fluorescence intensity from step 1.

$$\langle\lambda\rangle = \frac{\sum_i (F_i\lambda_i)}{\sum_i F_i} \qquad \text{Eq. (S5)}$$

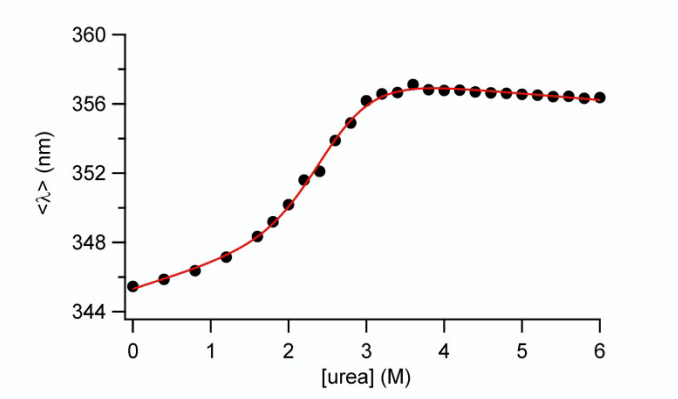


Table 2

Parameter	Fitted value
C_m (M)	2.2 ± 0.1
m (cal/mol M^{-1})	1700 ± 100
m_f (M^{-1})	1.4± 0.2
$<\lambda>_F$	345.3± 0.2
m_u (M^{-1})	–0.4±0.1
$<\lambda>_U$	358.6± 0.4

The unfolding curve is fitted to Eq. (S6) to obtain the midpoint of unfolding (C_m) and the m-value.

$$\langle\lambda\rangle = \frac{\langle\lambda\rangle_F + \langle\lambda\rangle_U \frac{1}{Q_R}\exp[\frac{m([urea]-C_m)}{RT}]}{1+\frac{1}{Q_R}\exp[\frac{m([urea]-C_m)}{RT}]} \qquad \text{Eq. (S6)}$$

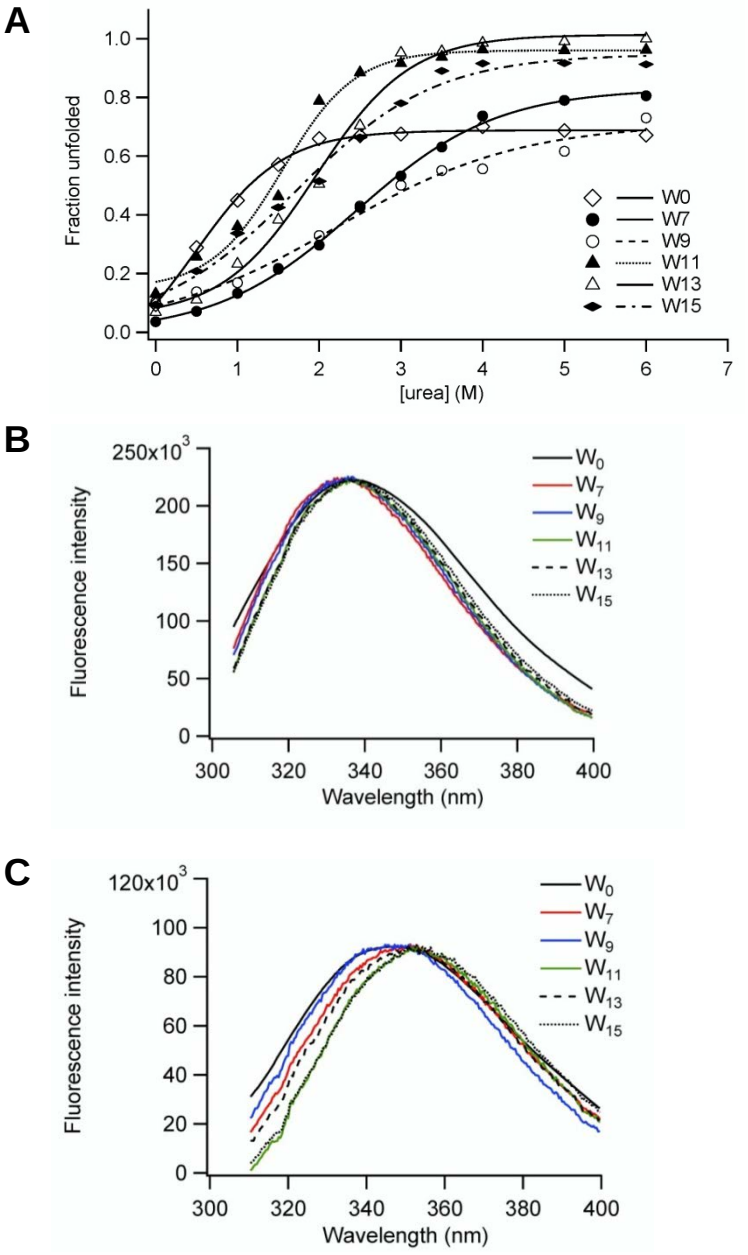
$$\langle\lambda\rangle_F = m_F[urea]+\langle\lambda\rangle_{0M,F}$$
$$\langle\lambda\rangle_F = m_U[urea]+\langle\lambda\rangle_{0M,U} \qquad \text{Eq. (S7)}$$

$<\lambda>_F$: $<\lambda>$ of the folded state as a function of denaturant
$<\lambda>_U$: $<\lambda>$ of the unfolded state as a function of denaturant
m_F: slope of $<\lambda>$ of the folded state as a function of denaturant
$<\lambda>_{0M,\,F}$: $<\lambda>$ of the folded state extrapolated to 0 M denaturant
m_U: slope of $<\lambda>$ of the unfolded state as a function of denaturant
$<\lambda>_{0M,\,U}$: $<\lambda>$ of the unfolded state extrapolated to 0 M denaturant

The free energy of unfolding at 0 M denaturant ($\Delta G_{Unfolding,\,H2O}$) is calculated by the product of the fitted C_m and m-value.

$$\Delta G^o_{Unfolding,H_2O} = mC_m \qquad \text{Eq. (S8)}$$

Supplementary Figures



Supplementary Figure S1. (A) Urea-induced unfolding of OmpA mutants in DPoPC/POPG (9:1) lipid bilayers monitored by SDS-PAGE without sample-heating. The fitted lines are just for guidance. (B) Trp-fluorescence spectra of OmpA mutants refolded in the lipid bilayers. The maximum emission wavelengths of mutants were 333.6 nm for W_7, 334.4 nm for W_9, 335.6 nm for W_{11}, 335.6 nm for W_{13} and 336.0 nm for W_{15}, respectively. (C) Trp-fluorescence spectra of OmpA mutants unfolded in 6 M urea. The maximum emission wavelengths of mutants were 350.8 nm for W_7, 346.4 nm for W_9, 352.8 nm for W_{11}, 350.8 nm for W_{13} and 353.2 nm for W_{15}, respectively.