Supplementary information

SI Figure 1

Equilibrium unfolding curves for His-tagged PagP (HT PagP, circles) and untagged PagP (PagP, diamonds). The dark grey dashed lines represent the global fit of the HT PagP curves to a two-state model (described below). The fit parameters obtained ($\Delta G^\circ_{\text{UN}} = -60.26 \pm 6.24 \text{ kJ mol}^{-1}, M_{\text{UN}} = 6.92 \pm 0.74 \text{ kJ mol}^{-1} \text{ M}^{-1}$) are in agreement with those previously measured for HT PagP. The untagged construct did not fold reversibly under these conditions and the data therefore are not fitted. The black symbols indicate the $<\lambda_{320-370}\text{nm}>$ for each PagP construct denatured in 10 M urea in the absence of liposomes. All experiments were carried out at a final protein concentration of 0.4 $\mu$M, in $\text{dilC}_{12}:\text{PC}$ liposomes, LPR 3200:1, 25 ºC, pH 8.0.

The average wavelength, $<\lambda>$, was calculated according to the following equation:

$$<\lambda> = \frac{\sum \lambda_i I_i}{\sum I_i}$$

in which $<\lambda>$ is the average wavelength, $\lambda_i$ the wavelength and $I_i$ the fluorescence intensity at $\lambda_i$. The $<\lambda>$ is calculated over the range $i = 320-370$ nm.

Since $<\lambda>$ has been shown to vary non-linearly with protein population, the fitting equation used must take into account the quantum yield ratio of the folded and unfolded states of PagP ($Q_R$)\(^2;3\). $Q_R$ was calculated according to the following equation:

$$Q_R = \frac{\Sigma I_i (F)}{\Sigma I_i (U)}$$

where $\Sigma I_i (F)$ is the sum of the intensities of the folded state spectrum over the range of wavelengths used to calculate $<\lambda>$, $\Sigma I_i (U)$ is the sum of the intensities of the unfolded state spectrum over the range of wavelengths used to calculate $<\lambda>$. This term is then incorporated into the equation for a two-state folding model as previously described\(^2;3\), and the data fitted using this equation:
where $S_{obs}$ is the observed signal, $a$ and $c$ are the signals of the native and denatured states, respectively, in the absence of denaturant, $b$ and $d$ are the denaturant dependence of the signal of the native and denatured states, respectively, $[D]$ is the denaturant concentration, $\Delta G_{\text{UN}}^{0}$ is the free energy of unfolding in the absence of denaturant, $M_{\text{UN}}$ is the m-value (which reflects the denaturant dependence of $\Delta G_{\text{UN}}^{0}$) and $Q_{R}$ is the quantum yield ratio as previously defined.

**SI Table 1**

| Lipid         | [Urea] (M) | Rate Constant (×10^{-3} s^{-1}) | Error in Global Fit (×10^{-5} s^{-1}) |
|---------------|------------|---------------------------------|-------------------------------------|
| $diC_{12:0}$PC | 2.0        | 1.37                            | 0.48                                |
| $diC_{12:0}$PC | 3.0        | 2.90                            | 1.41                                |
| $diC_{12:0}$PC | 4.0        | 7.02                            | 6.22                                |
| $diC_{12:0}$PC: $diC_{12:0}$PG | 2.0 | 0.32 | 0.24 |
| $diC_{12:0}$PC: $diC_{12:0}$PG | 3.0 | 0.92 | 0.48 |
| $diC_{12:0}$PC: $diC_{12:0}$PG | 4.0 | 1.20 | 0.49 |

Urea dependence of PagP folding into $diC_{12:0}$PC and 80:20 $diC_{12:0}$PC:$diC_{12:0}$PG liposomes. Rate constants were obtained by measuring Trp fluorescence emission at 335 nm over time, and fitting the observed transients to a single exponential function (see Methods). Four replicate samples were globally fitted to a single exponential function to obtain the reported rate constants.

**SI Figure 2**

Urea-dependence of PagP folding monitored using cold SDS-PAGE. All samples contained 4 µM PagP ($diC_{12:0}$PC liposomes, LPR 3200:1) in 10 mM glycine buffer, pH 9.5, 2 mM EDTA and were refolded at 37 ºC for 15 hours prior to 1:1 dilution with 2 × SDS-PAGE loading buffer. Lanes on the
SDS-PAGE gel are numbered to indicate the final concentration of urea (M) in each sample. The unfolded and folded forms of PagP are denoted by U and F, respectively.

**SI Table 2**

| PagP Concentration (µM) | Average Rate Constant (×10^{-3} s^{-1}) | Standard Deviation (×10^{-4} s^{-1}) | Standard Error of Mean (×10^{-4} s^{-1}) |
|-------------------------|-----------------------------------------|--------------------------------------|------------------------------------------|
| 0.4                     | 1.61                                    | 7.43                                 | 3.71                                     |
| 0.1                     | 2.35                                    | 7.98                                 | 3.99                                     |
| 0.04                    | 1.56                                    | 12.1                                 | 6.03                                     |

Concentration dependence of PagP folding into diC_{12:0}PC liposomes. Rate constants were obtained by measuring Trp fluorescence emission at 335 nm over time, and fitting the observed transients to a single exponential function (see Methods). The mean of four transients was calculated, and the standard deviation and standard error (n = 4) were calculated.

**SI Figure 3**

Concentration dependence of PagP folding into diC_{12:0}PC liposomes. Kinetic traces are normalised to the final fluorescence signal. Samples contained 0.4 µM PagP (red lines), 0.1 µM (blue lines) or 0.04 µM PagP (green lines). All samples contained 50 mM glycine, pH 9.5, 2 M urea and were measured at 37 °C at an LPR of 3200:1. Note that an additional slow phase visible for 0.04 µM PagP was not observed reproducibly in replicate experiments and was thus not considered further.
SI Figure 4

PagP populates a single conformation under the conditions used for chaperone binding. 10 µM PagP in 50 mM glycine, pH 9.5 containing 0.24 M urea was incubated for 5 min at room temperature before injection on to a Superdex 75 10/300 GL column (see Methods).

SI Figure 5

Equilibrium unfolding of SurA monitored using tryptophan fluorescence emission. SurA dissolved in 50 mM glycine buffer, pH 9.5 was diluted into separate aliquots of 50 mM glycine buffer containing different concentrations of urea (0–8 M in 0.2 M increments). The final concentration of protein was 2.5 µM. The aliquots were equilibrated overnight (16 h) at 37 °C before measurement. The fluorescence emission intensity was measured at 335 nm (after excitation at 280 nm) due to the large
intensity difference between the folded and unfolded states at this wavelength, for 60 s and the average signal calculated.

**SI Figure 6**

PagP and SurA do not interact under the conditions used in the kinetic folding assays. (a) 10 µM PagP was added to 60 µM SurA in 50 mM glycine, pH 9.5 containing 0.24 M urea and incubated for 5 min at room temperature before injection on to a Superdex 75 10/300 GL column (see Methods). Samples containing either 10 µM PagP or 60 µM SurA in 50 mM glycine, pH 9.5 containing 0.24 M urea were also analysed. (b) Fractions were collected and analysed by SDS-PAGE.
Equilibrium unfolding of Skp monitored using far-UV CD. Skp dissolved in 50 mM glycine buffer, pH 9.5 was diluted into separate aliquots of 50 mM glycine buffer containing different concentrations of urea (0–8 M in 0.2 M increments). The final concentration of protein was 15 µM Skp. The aliquots were equilibrated overnight (16 h) at 37 °C before measurement by far-UV CD spectroscopy. The CD signal of each sample was measured at 222 nm due to the difference in intensity between the folded and unfolded states at this wavelength, for 60 s and the average signal calculated.

Skp retards the folding of PagP into diC_{12:0}PC liposomes over a 2 h time course. (a) PagP folding into diC_{12:0}PC liposomes in the absence of Skp. (b) PagP folding into diC_{12:0}PC liposomes in the presence of Skp. 12 µM PagP was incubated (with a 2-fold excess of Skp trimers, if appropriate) for 5 min at room temperature in 50 mM glycine, pH 9.5 containing 0.24 M urea before a 3-fold dilution into diC_{12:0}PC liposomes (LPR 3200:1) in 50 mM glycine, pH 9.5 containing 2 M urea. Samples were removed at 0, 2 and 16 hours and analysed by cold SDS-PAGE immediately.
Lysozyme interacts with PagP under the conditions used in the kinetic folding assays as shown by (a) analytical gel filtration and (b) nickel affinity chromatography. For analytical gel filtration, 2 µM PagP was added to 6 µM lysozyme in 50 mM glycine, pH 9.5 containing 0.24 M urea and incubated for 5 min at room temperature before injection on to a Superdex 75 10/300 GL column (see Methods). Samples containing either 2 µM PagP or 6 µM lysozyme in 50 mM glycine, pH 9.5 containing 0.24 M urea were also analysed. For the nickel affinity assay, 10 µM HT PagP was immobilised on nickel Sepharose resin before incubation with 10 µM lysozyme in 50 mM glycine buffer, pH 9.5. Control experiments containing HT PagP or lysozyme only were conducted under identical conditions for comparison. Lysozyme alone does not bind to the resin and is present in the unbound (U) fraction, while in the presence of HT PagP, lysozyme co-elutes in the bound (B) fraction.
SI Table 3

| Lipid | Experiment | Ave Rate Constant $(×10^{-3} \text{ s}^{-1})$ | Std Dev $(×10^{-4} \text{ s}^{-1})$ | Std Error of Mean $(×10^{-4} \text{ s}^{-1})$ |
|-------|------------|---------------------------------------------|---------------------------------|---------------------------------|
| $\text{diC}_{12:0}\text{PC}$ | PagP alone (2M urea) | 1.34 | 0.77 | 0.45 |
| | PagP & SurA (2M urea) | 1.41 | 2.26 | 1.31 |
| | PagP & Skp (2M urea) | 0 | N/A | N/A |
| | PagP & Lysozyme (2M urea) | 0 | N/A | N/A |
| | PagP & NaCl (2M urea) | 1.20 | 2.49 | 1.44 |
| | PagP, NaCl & Skp (2M urea) | 0.81 | 1.35 | 0.96 |
| | PagP & LPS (2M urea) | 0.89 | 1.17 | 0.67 |
| | PagP, LPS & Skp (2M urea) | 1.89 | 5.75 | 3.32 |
| $\text{80:20 diC}_{12:0}\text{PC: diC}_{12:0}\text{PG}$ | PagP alone (3M urea) | 0.79 | 2.00 | 1.41 |
| | PagP alone (2M urea) | 0.28 | 0.32 | 0.18 |
| | PagP & SurA (3M urea) | 0.63 | 2.46 | 1.74 |
| | PagP & Skp (2M urea) | 2.41 | 6.05 | 4.28 |
| | PagP & Lysozyme (3M urea) | 1.52 | 2.60 | 1.84 |
| | PagP & NaCl (2M urea) | 0.56 | 1.07 | 0.62 |
| | PagP, NaCl & Skp (2M urea) | 1.53 | 1.62 | 0.94 |
| | PagP & LPS (2M urea) | 0.68 | 0.12 | 0.07 |
| | PagP, LPS & Skp (2M urea) | 1.00 | 2.62 | 1.51 |

Measured rates of PagP folding into liposomes in vitro. Rate constants were obtained by measuring Trp fluorescence emission at 335 nm over time, and fitting the observed transients to a single exponential function (see Methods). Global fits were obtained over four replicates from a single batch of liposomes, and the average of the global fits from folding reactions into three batches of liposomes calculated. The standard error of the mean was calculated by taking the number of liposome replicates to be 3.

References

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