Supplementary Information

Dodine as a Protein Denaturant: The Best of Two Worlds?

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CMC measurements Critical micelle concentration (CMC) was measured by monitoring spectral changes of pyrene (Sigma-Aldrich, St. Louis, MO) fluorescence due to solubilization by detergent micelles [ref. 26 in the main text] (Figure S1A). Pyrene was titrated with dodine and the intensity ratio between the 1st and 3d peaks was used as indicative of micelle formation (Fig.1B). Pyrene emission spectra were measured using a PC1 Spectrofluorimeter (ISS, Champaign, Il). The temperature of the sample chamber was controlled by a water bath circulator RTE-111 (Neslab, Thermo Scientific, Waltman, MA). The pyrene concentration was 4 μM, and pyrene was excited at 334 nm. Spectra were recorded from 350 to 500 nm with 1 nm step size. The emission slit was set at 2 nm, the excitation slit at 16 nm. To obtain CMC intensity ratio of peaks 3 and 1 as a function of dodine concentration was fitted using a 2-state thermodynamic fit (see Methods section of the main text).

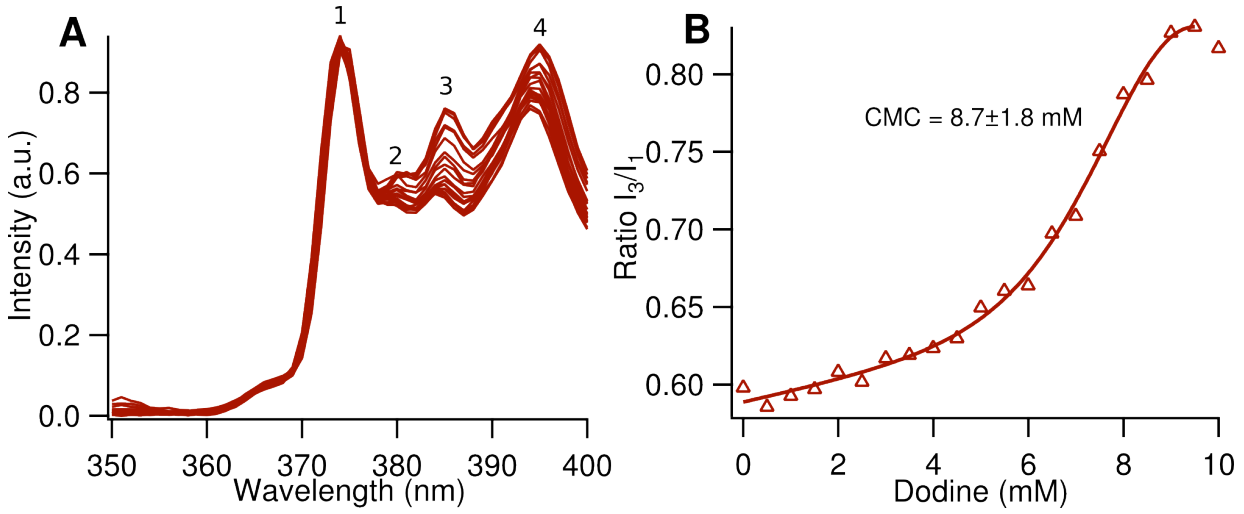

Figure S1. Pyrene titration by dodine monitored by fluorescence spectroscopy at 45 °C, near the midpoint of the lowest thermal denaturation transitions. (A) Fluorescence emission spectra of pyrene undergoing titration with dodine. Each spectrum is normalized by the intensity of the 1st peak. As the concentration of dodine increases, the relative intensity of the 3rd peak goes up. (B) Variation of the intensity ratio of peaks 3 and 1 as a function of dodine concentration. Triangles represent experimental points. Solid line is a two-state thermodynamic fit to the data. CMC value resulting from the fit is shown.

Reversibility measurements To check reversibility of the thermal unfolding of $\lambda_{6\text{-}85}$ and WW-domain we performed protein melts in water, 2-2.5 M GuHCl and 1 mM dodine and monitored unfolding using CD spectroscopy. Temperature was only increased up to unfolding baseline of the protein in the particular chemical environment to avoid aggregation. WW-domain melts were started at 30 °C to avoid dodine solubility problems due to the presence of salts in the protein solution, $\lambda_{6\text{-}85}$ melts were started at 20 °C. Then temperature was decreased in 5°C steps, with a 5-7 min waiting time between measurements. Reversibility percentage was calculated as percentage of MRE restored after return to initial temperature at a characteristic wavelength (222 nm for $\lambda_{6\text{-}85}$ and 227 nm for WW-domain). In case of $\lambda_{6\text{-}85}$ in 2.5M GuHCl MRE at 222 nm upon return to 20 °C was lower than the initial one, which resulted in higher than 100% reversibility percentage. Both proteins were 70-100% reversible in all of the three solutions (Table S1).

To measure isothermal reversibility of GuHCl and dodine denaturation, we monitored titrations of $\lambda_{6\text{-}85}$ by fluorescence (Figure S2). As in the case of thermal reversibility measurements, denaturant concentration was only increased up to unfolding baseline. The resulting solution was then diluted 3 times consecutively by 2x to reach the folding baseline. $\lambda_{6\text{-}85}$ isothermal reversibility is close to 100% in both denaturants. Only the isothermal reversibility of $\lambda_{6\text{-}85}$ was tested because WW-domain unfolding was monitored by measuring peak intensity rather than peak wavelength shift.

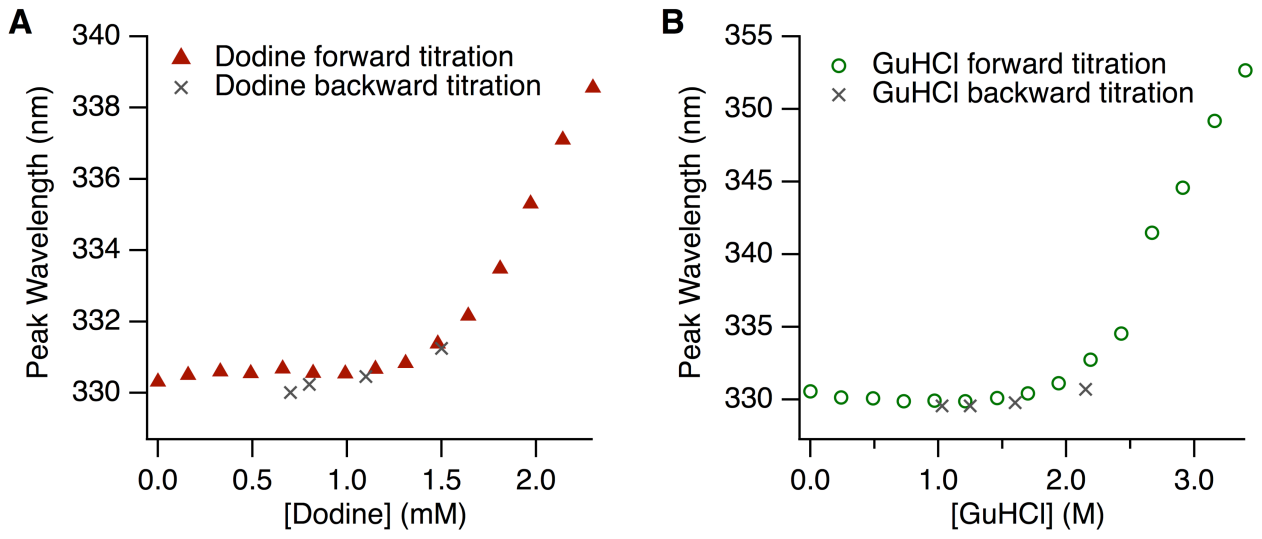

Figure S2. Isothermal denaturation and refolding of $\lambda_{6\text{-}85}$ in (A) dodine and (B) guanidinium solution. Full native fluorescence is recovered within experimental uncertainty.

Table S1: Thermal reversibility percentage of $\lambda_{6\text{-}85}$ and WW-domain.

	$\lambda_{6\text{-}85}$, % reversible	WW-domain, % reversible
Water	93	73
Guanidinium	105 (2.5M)	98 (2M)
1 mM dodine	99	93

The melting temperature of $\lambda_{6\text{-}85}$ is sensitive to small variations in protein and dodine concentration

The thermal unfolding of $\lambda_{6\text{-}85}$ in the presence of dodine is sensitive to the concentration of both protein and denaturant in the 'cooperative' region of concentrations. Even a small variation in concentration can cause noticeable changes in the melting temperature of the protein (Figure S3). As can be seen in Figure S3, T_m needs to be verified for a given protein solution to avoid shifts due to protein and dodine concentration.

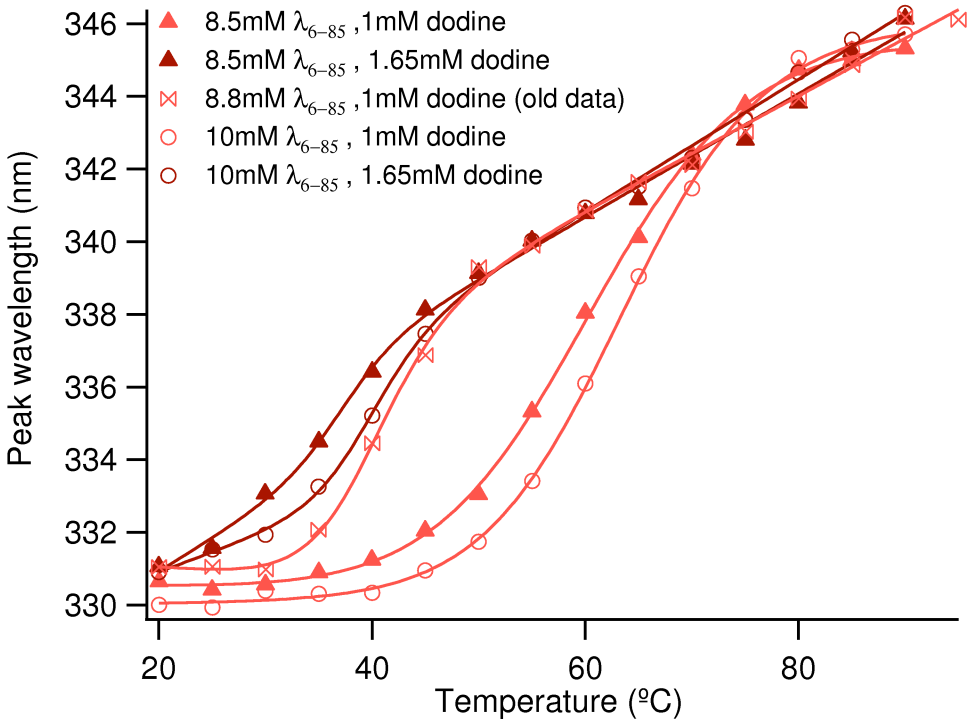

Figure S3. Thermal denaturation of $\lambda_{6\text{-}85}$ in the presence of different concentrations of dodine and protein monitored by fluorescence. Circles, squares and triangles represent experimental points; solid lines are thermodynamic fits to the data. 10-20% variations of protein and dodine concentration result in different melting temperatures.