Electronic Supplementary Information for

A dry molten globule-like intermediate during the base-induced unfolding of a multidomain protein

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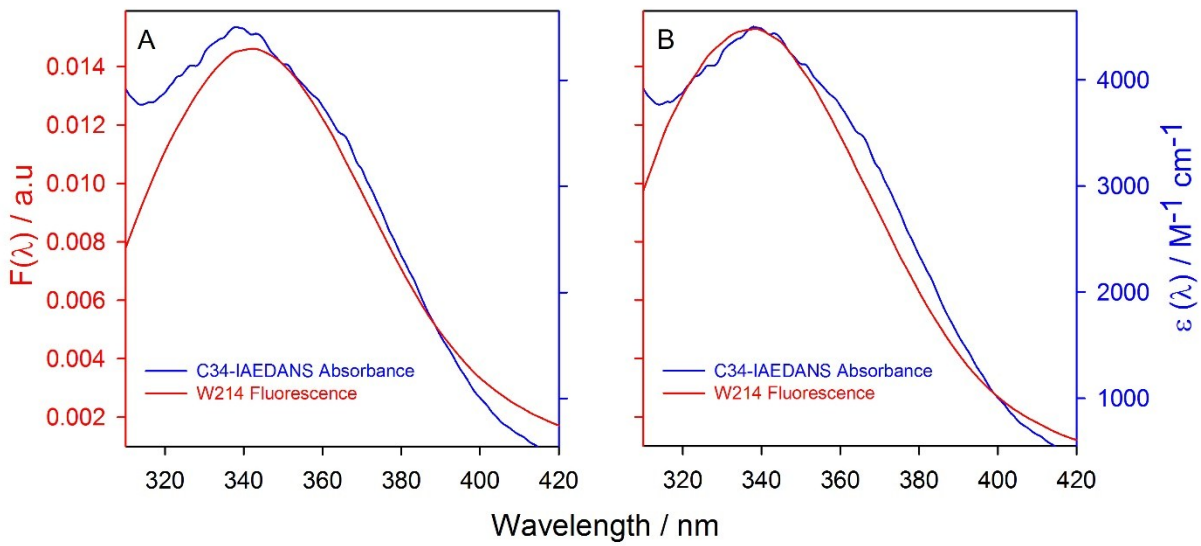

Figure S1. Determination of the overlap integral, *J*, in the N form (A) and in the B form (B). In both the panels, the left y-axis represents the fluorescence emission spectra (F(λ)) of W214 and the right y-axis represents absorbance spectra ($\varepsilon(\lambda)$) of C34-IAEDANS, respectively. The total area under each fluorescence emission spectrum has been normalized to unity. Each absorbance spectrum has been divided by the respective molar protein concentration to obtain $\varepsilon(\lambda)$.

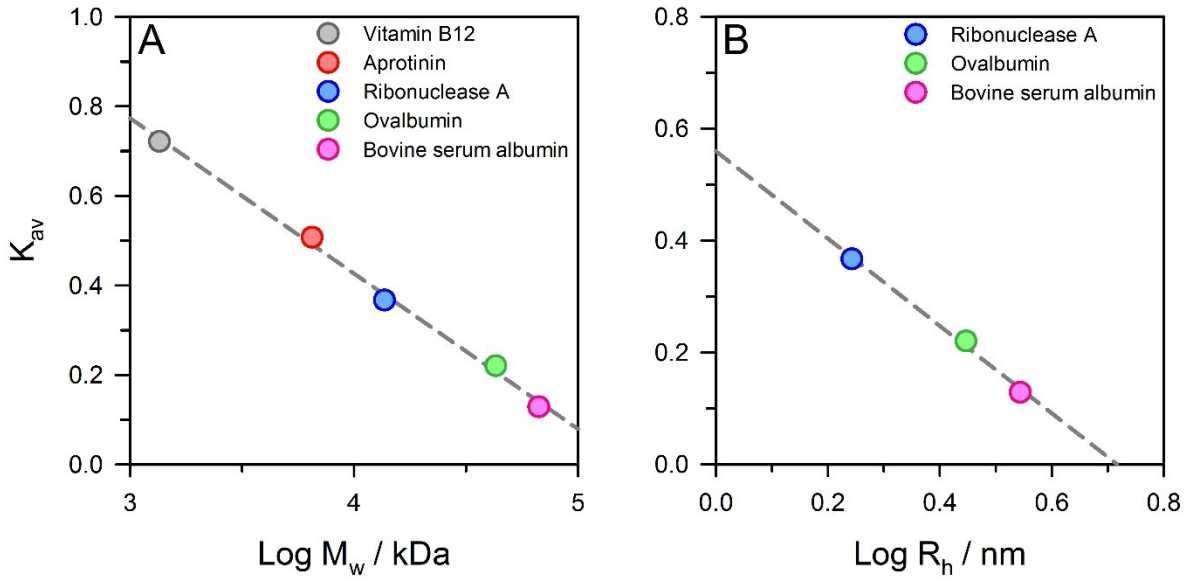

Figure S2. Calibration curves for the determination of the molecular weight (M_w) and the hydrodynamic radius (R_h) of the N form and the B form from size exclusion chromatography. Panel A shows the partition coefficient (K_{av}) plotted against log M_w of five different standard biomolecules. Panel B shows the K_{av} plotted against log R_h of three different standard proteins. The K_{av} values for all the standards were calculated from the respective elution volumes, given in the manufacturer provided manual for GE Superdex 75 10/300 GL high performance gel filtration column, using equation 4 (main text). The R_h values in panel B for ribonuclease A, ovalbumin and bovine serum albumin were taken from a previous study.[1] The dashed lines in panel A and B are linear fits to the data and used to calculate the M_w and R_h values of the N form and the B form of HSA (Figure 2C (top and bottom inset), main text).

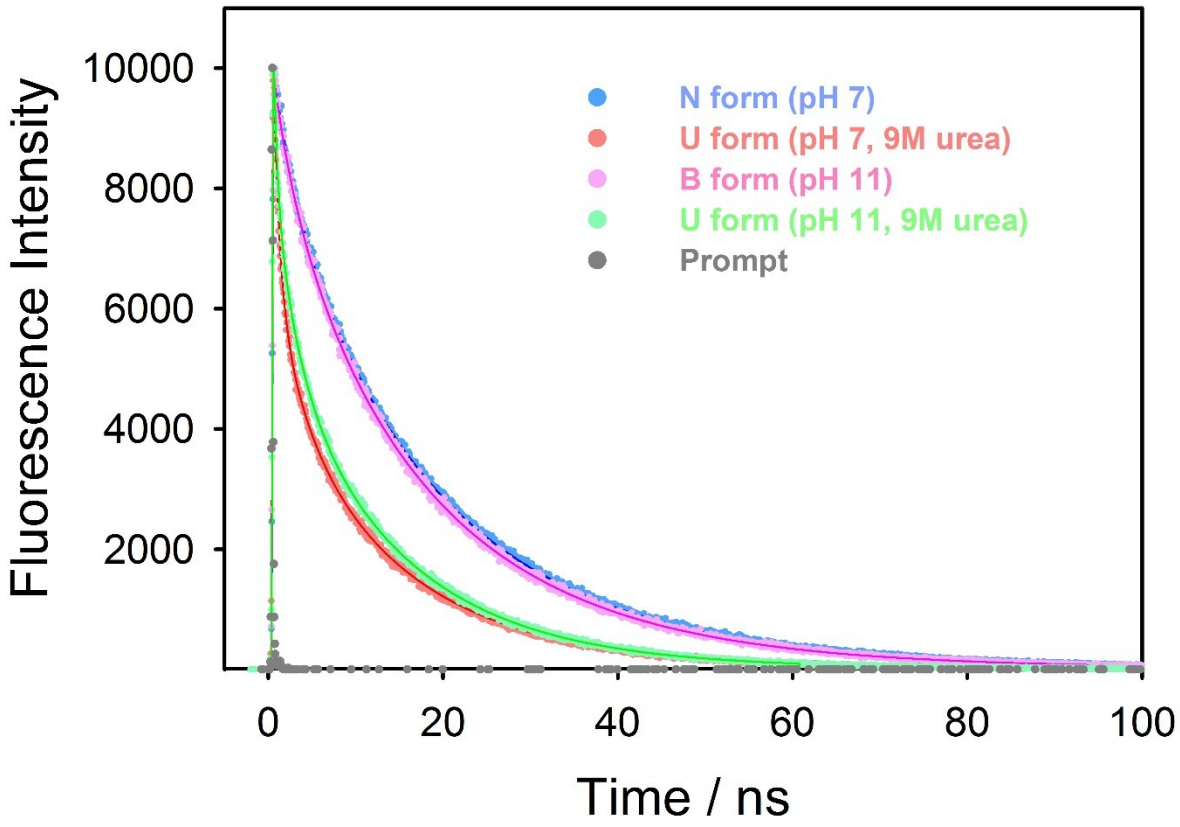

Figure S3. Fluorescence intensity decay kinetics of C34-IAEDANS.

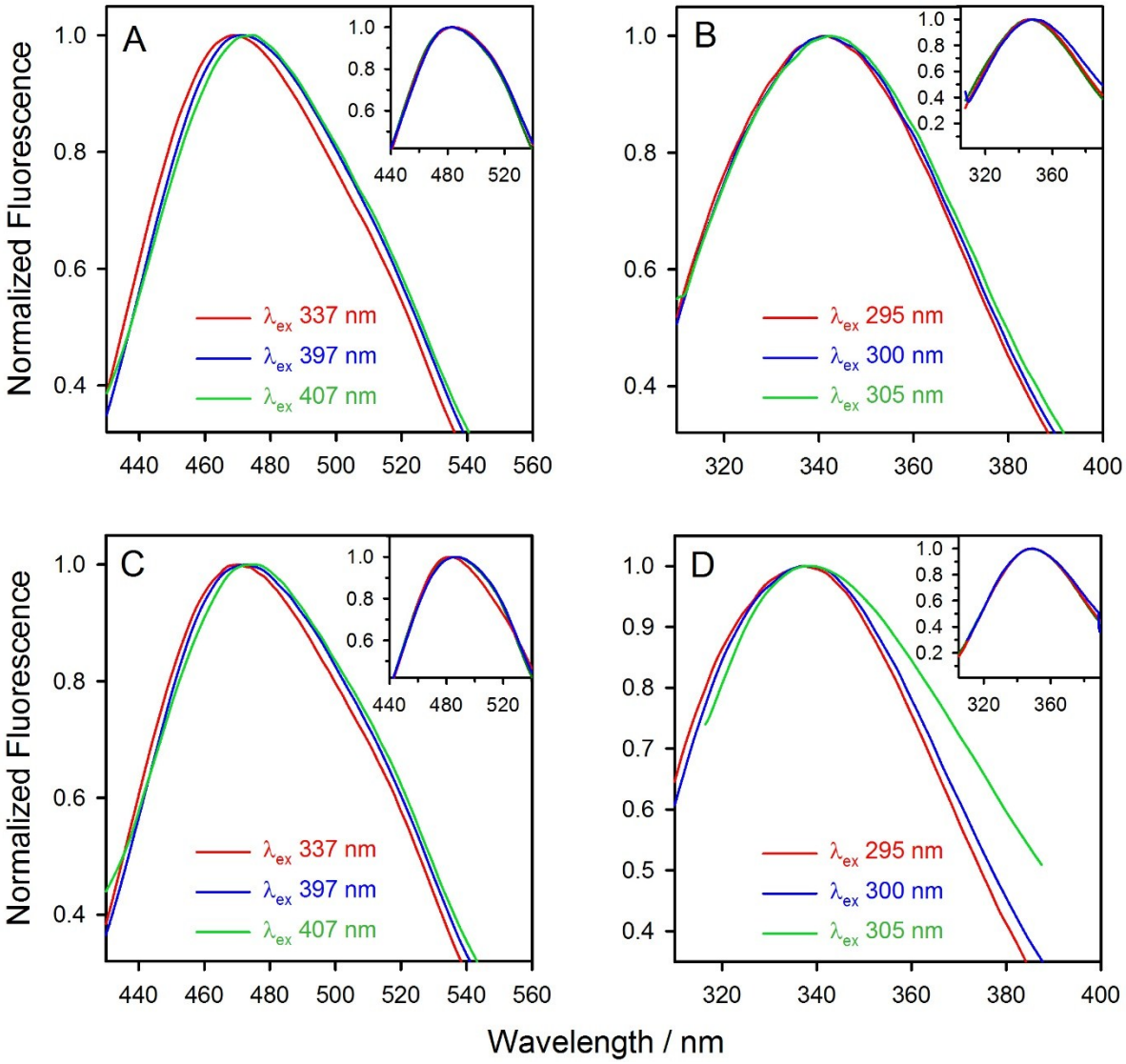

Figure S4. Dependence of λ_{max}^{em} of C34-IAEDANS and W214 on λ_{ex}. Panel A and B respectively show the representative fluorescence scan of C34-IAEDANS and W214 in the N form, when excited at different λ_{ex}. Panel C and D respectively show the representative fluorescence scan of C34-IAEDANS and W214 in the B form, when excited at different λ_{ex}. In each panel, the inset represents the fluorescence scans of the respective U forms when excited at different λ_{ex}. (N form: HSA at pH 7; B form: HSA at pH 11; U form: HSA in 9M urea accordingly at pH 7 or pH 11)

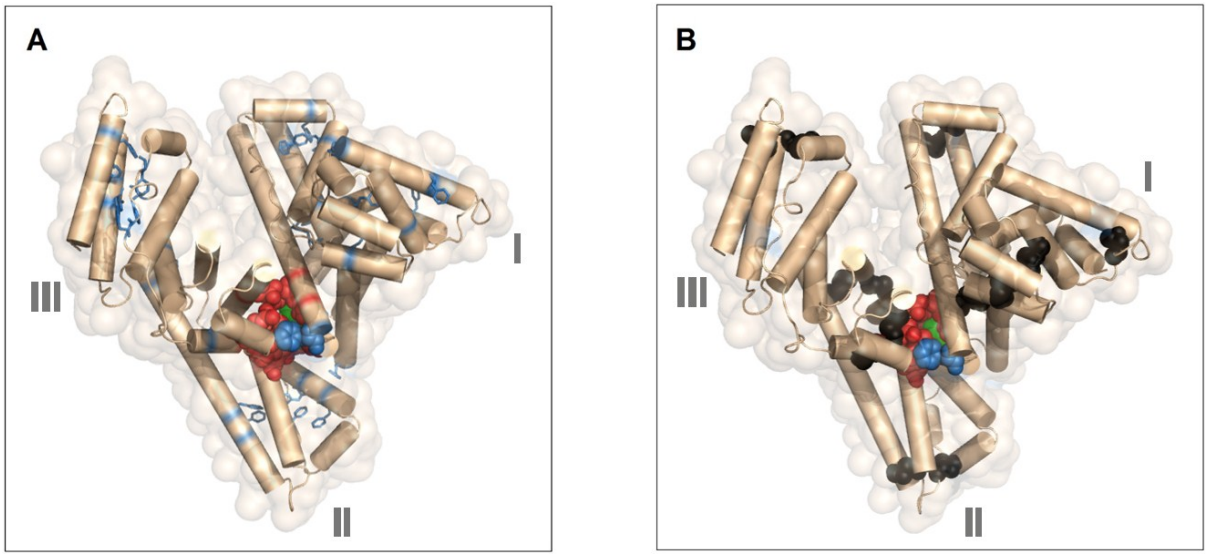

Figure S5. Structure of HSA. Panel (A) shows all the Phe residues (blue), the inter-domain region (red) between domain I-II and domain II-III along with the sole tryptophan residue (green). It is important to note that although there are 31 Phe residues distributed throughout the protein, there is only single Phe residue, F206 (shown as blue sphere), in the inter-domain region. Panel (B) shows 17 disulfide bonds (black spheres) distributed in all the 3 intra-domain regions.

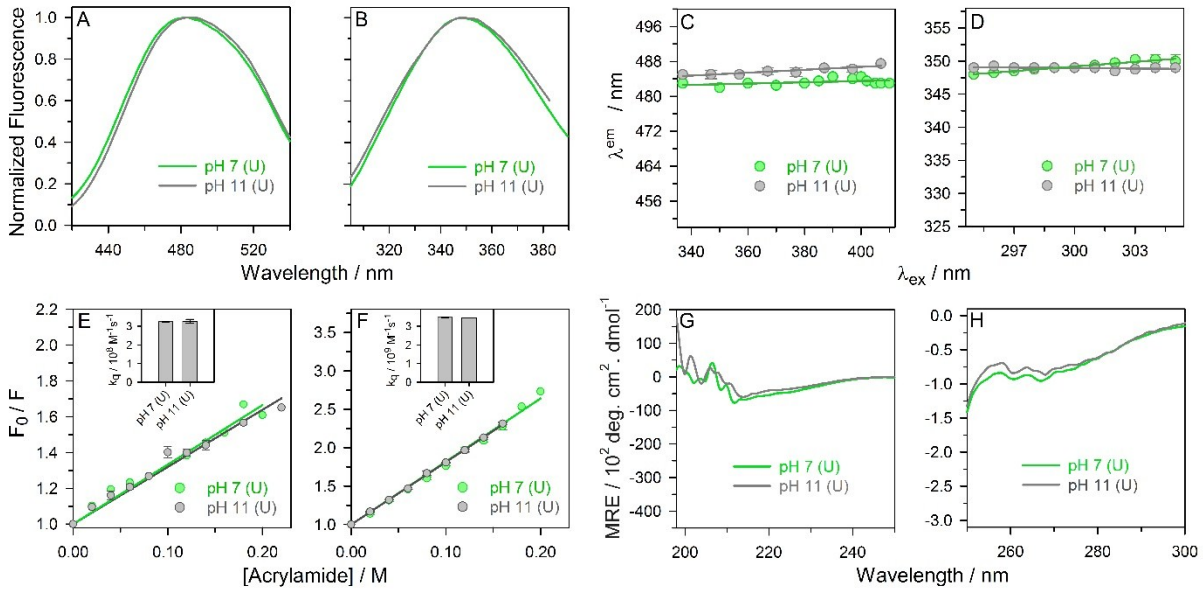

Figure S6. Comparison of the spectroscopic properties of the U form (unfolded in 9M urea) at pH 7 and pH 11. Panel (A) and (B) shows the fluorescence spectra of C34-IAEDANS and W214, respectively. The wavelength of maximum emission (λ_{max}^{em}) of each spectrum was normalized to 1 for comparison. Panel (C) and (D) show the effect of the change in excitation wavelength (λ_{ex}) on the wavelength of maximum emission (λ_{max}^{em}). The λ_{max}^{em} of (C) C34-IAEDANS and (D) W214 is plotted as a function of respective λ_{ex}, where the solid lines are drawn to guide the eyes. Panel (E) and (F) show the Stern-Volmer plots for dynamic quenching of C34-IAEDANS and W214 fluorescence, respectively. The solid lines through the data are the least-square fits to equation 10 (main text). The values of bimolecular quenching constant, k_q, are compared in the insets of panel (E) and (F). The errors in the values of k_q are less than 5%. Panel (G) and (H) show the global secondary structure and the tertiary structure, respectively.

Table S1. Determination of FRET parameters and D-A distances. Values of FRET efficiency (E), quantum yield (Q_D), overlap integral (J), Forster's distance (R_0), and D-A distance (R) for FRET between W214 and C34-IAEDANS pair.

Conditions	$^{*}E$	$^{\S}Q_D$	$^{\dagger}J$/ 10^{13}M^{-1}cm^{-1} nm^4	$^{\ddagger}R_0$ / Å	$^{\ddagger}R$ / Å
N form	0.31 ± 0.01	0.31	5.1	25.8	29.6 ± 0.1
B form	0.20 ± 0.01	0.23	6.1	24.5	31.0 ± 0.4

$^{*}E$ was determined using the fluorescence spectra shown in Figure 2A and 2B as described in the main text using equation 8 (main text). The standard error was estimated from three separate measurements.

$^{\S}Q_D$ was calculated as described previously.[2]

$^{\dagger}J$ was calculated using equation 3 (main text).

‡ R_0 was calculated using equation 2 (main text); with the following values: $\kappa^2 = 2/3$; $n = 1.332$.

‡ R was calculated using equation 9 (main text). The standard error was estimated from three separate measurements.

Table S2. Parameters for dynamic fluorescence quenching experiments. Values of Stern-Volmer constants (K_{sv}), intensity averaged fluorescence lifetimes (τ_0) and bimolecular quenching rate constants (k_q) for C34-IAEDANS and W214.

	C34-IAEDANS			W214		
Conditions	K_{sv} /M^{-1}	τ_0 /ns	$k_q/10^8$ $M^{-1}s^{-1}$	K_{sv} /M^{-1}	$^*\tau_0$ /ns	k_q /10^9 $M^{-1}s^{-1}$
N form	1.6	19.3	0.8	6.1	7.1	0.9
B form	1.6	18.9	0.8	6.5	6.3	1.0
U form	3.3	14.2	3.2[§]	8.2	3.3	3.4[§]

[*] These values are taken from previous studies.[3, 4]

[§] These values are corrected for the effects of viscosity of 9 M urea, as described in the 'Experimental' section of the main text.

Note: Errors in the values of different parameters are less than 5%.

References

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2. N. Acharya, P. Mishra and S. K. Jha, *J. Phys. Chem. Lett.*, 2016, **7**, 173-179.
3. M. Amiri, K. Jankeje and J. R. Albani, *J. fluoresc.*, 2010, **20**, 651-656.
4. M. Amiri, K. Jankeje and J. R. Albani, *J. Pharm. Biomed. Anal.*, 2010, **51**, 1097-1102.