Supplementary Information

The effect of Ficoll 70 on thermally-induced and chemically-induced conformational transitions of an RTX protein is quantitatively accounted for by a unified excluded volume model

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1. Relationship between relative abundance of conformational states and an experimentally measured quantity

2. Table 1 – Best-fit parameter values obtained via modeling data plotted in Text Figure 1 using unconstrained and constrained models described in Text Section III.

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1. Relationship between relative abundance of conformational states and an experimentally measured quantity

In the context of a two-state model, the generic relationship between the fraction of state 1 and the value of an experimentally measured quantity termed signal *S* is given by

$$S(x) = f_1(x)S_1(x) + \left[1 - f_1(x)\right]S_2(x) \qquad \text{[S1]}$$

Here *x* denotes the value of the independent variable that is varied in a controlled fashion during the experiment, and $S_i(x)$ denotes the possible dependence of the signal of pure state *i* upon the value of *x*, which may or may not exist depending upon the particular measurement. In the Ca titration experiments analyzed in Section II, *x* represents the thermodynamic activity of Ca, and *S* the ratio of intrinsic tryptophan fluorescence at 360/320 nm (FIR) relative to that in the absence of Ca, on an arbitrary scale of 0 to 100. No dependence of *S* upon Ca activity is evident in the data plotted in Figure 3E[1], either below or above the binding transition. In the thermal stability experiments analyzed in Section III, *x* represents temperature and *S* either FIR or mean residual ellipticity (MRE) at 201 nm. Inspection of the data plotted in Figures S4 and S5 of SP indicate that the pure states at temperatures below and above the unfolding transition do exhibit temperature dependent responses, which appear to be linear in *x*. Thus, for a two-state transition we accordingly write

$$\begin{aligned} S_1 &= s_{11}(x_{ref}) + s_{12}\left(x - x_{ref}\right) \\ S_2 &= s_{21}(x_{ref}) + s_{22}\left(x - x_{ref}\right) \end{aligned} \qquad \text{[S2]}$$

where x_{ref} denotes a fixed value of *x* that is selected for convenience in modeling. ‡

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‡ Since equations [S2] are empirical, it is possible to expand S_i to quadratic terms in *x* (see SI reference [2]), but we have found in practice that such expansion does not improve the quality of model fitting while adding two extra undetermined parameters.

2. Table 1 – Best-fit parameter values obtained via modeling data plotted in text Figure 2 using Independent L and Constrained L models described in Text Section III.

Parameter name	**Independent L model**	**Constrained L model**
n fixed equal to 9		
log K_A (μM^{-1})	-3.4	-3.1
log z	1.04	0.85
ln L (Ficoll 0)	-9.9	-10.15
ln L (Ficoll 0.1)	-7.4	NA
ln L (Ficoll 0.2)	-6.5	NA
ln L (Ficoll 0.3)	-4.5	NA
ln L (Ficoll 0.4)	-3.3	NA
α	NA	15.4 (-2, + 4) †
x_{off} fixed equal to 1.0		
s_{11} (Ficoll 0)	3.2	-1.1
s_{11} (Ficoll 0.1)	7.6	13.0
s_{11} (Ficoll 0.2)	8.4	5.9
s_{11} (Ficoll 0.3)	11.3	15.0
s_{11} (Ficoll 0.4)	1.9	4.2
# of variable parameters	12	9
Best fit SSR	535	1210

† Indicated uncertainties correspond to one standard error of estimate, calculated via parameter scanning[3].

3. Table 2 – Best-fit parameter values obtained via modeling data plotted in text Figures 4A and 4B with models described in Text Section IV.

Parameter name	**5 mM Ca (H → H*)**	**No Ca (A → A*)**
ΔH_{Tm} (cal/mol)	69,300	28,600
ΔC_p (cal/mol-deg)	1313	334
T_m (°C)	72.1	34.1
α (cm^3/g Ficoll)	-10.9 (-4, +3) †	-6.15 (-2, +1.5) †
x_{off}	20°C (fixed)	0°C (fixed)
s_{11}	1.13	2.06
s_{12}	-0.00476	0.00869
s_{21}	-0.465	3.76
s_{22}	0.00515	-0.00119
# of variable parameters	8	8
Best fit SSR	0.053	1210

Bibliographic references

1 A. C. Sotomayor-Perez, O. Subrini, A. Hessel, D. Ladant and A. Chenal, *J Am Chem Soc*, 2013, **135**, 11929–34.

2 Yadav, S. and Ahmad, F., *Anal. Biochem.*, 2000, **283**, 207–213.

3 Saroff, H. A., *Anal. Biochem.*, 1989, **176**, 161–169.