

Supporting Information

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SI Text

Preparation of TNB-Labeled Proteins. The TNB-labeled proteins were obtained by incubating the guanidinium chloride (GdmCl)-unfolded protein with a 100-fold molar excess of 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) in 6 M GdmCl at pH 8.5. After completion of the reaction, the labeled protein was separated from free dye and GdmCl by the use of a PD-10 column (GE Health Care Life Sciences). All of the proteins were found by mass spectrometry to be >98% labeled, with an expected 197 Da increase in the mass because of the addition of the TNB moiety. Protein concentrations were determined by measurement of the absorbance at 280 nm, using an extinction coefficient of 40,500 M⁻¹·cm⁻¹ for the unlabeled proteins. Because the TNB group contributes to the absorbance measured at 280 nm, a correction for its contribution was done for the labeled proteins, as described earlier (1).

The Donors and Acceptors Appear to Rotate Freely Faster than the Fluorescence Decay Rate of Donor. The value of R_0 , the Forster's distance, in Eq. 1 can be determined by using the equation:

$$R_0 = 0.211 [Q_D J \kappa^2 n^{-4}]^{\frac{1}{6}} \quad [S1]$$

In Eq. S1, Q_D is the quantum yield of donor fluorescence, J is the overlap integral, κ^2 is the orientation factor, and n is refractive index of the medium (2). Most of these parameters except the

orientation factor can be determined experimentally and have been evaluated for Trp-TNB FRET pair in previous studies (1, 3). The Forster's distance, R_0 , of the Trp-TNB FRET pair has been shown to lie between 22 and 23 Å in [GdmCl] ranging between 0 and 6 M for several different proteins, assuming a value of 2/3 for κ^2 .

The value of κ^2 depends on the relative orientation of the transition dipoles of the donor and the acceptor. At present, there is no direct way to measure κ^2 in nonrigid experimental setup. If the donor and the acceptor are oriented randomly with respect to each other, the value of κ^2 is 2/3 (2). This condition is typically met when at least one of the two chromophores is rotating freely at rates faster than the fluorescence decay.

In the case of RNase H, all of the tryptophan donors and the residues S36 and L136 to which acceptor TNB moiety is attached are on the surface of the protein. Side chains of the surface residues of proteins rotate between different rotamers on the timescale of tens of picoseconds (4). This timescale is faster than the time constant of fluorescence decay of tryptophans (~4–5 ns). During unfolding, the segmental flexibility of dry molten globule is likely to make the averaging of the orientations of donor and acceptors even faster. Hence, using a value of two-thirds for κ^2 appears reasonable. This is more justified in view of the fact that tryptophan has multiple absorption transition dipoles, and therefore mixed polarizations (5).

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2. Lakowicz JR (2006) *Energy transfer. Principles of Fluorescence Spectroscopy* (Springer, New York), 3rd Ed, pp 443–472.
3. Sridevi K, Udgaonkar JB (2003) Surface expansion is independent of and occurs faster than core solvation during the unfolding of barstar. *Biochemistry* 42(6):1551–1563.

4. Mccammon JA, Lee CY, Northrup SH (1983) Side-Chain Rotational Isomerization in Proteins - a Mechanism Involving Gating and Transient Packing Defects. *J Am Chem Soc* 105:2232–2237.
5. Haas E, Katchalski-Katzir E, Steinberg IZ (1978) Effect of the orientation of donor and acceptor on the probability of energy transfer involving electronic transitions of mixed polarization. *Biochemistry* 17(23):5064–5070.

