

**Table 2.** FRET peak widths for unfolded proteins

GdmCl conc., M	Prot. L,* $\langle E_m \rangle$	Prot. L,* $\langle E \rangle$	Prot. L, <sup>†</sup> $\sigma_m$	Prot. L, <sup>‡</sup> $\sigma_{sn}$	CspTm,* $\langle E_m \rangle$	CspTm,* $\langle E \rangle$	CspTm, <sup>†</sup> $\sigma_m$	CspTm, <sup>‡</sup> $\sigma_{s.n.}$
2	--	--	--	--	0.56	0.51	0.136	0.083
2.5	0.55	0.50	0.135	0.082	0.52	0.47	0.116	0.084
3	0.53	0.48	0.120	0.082	0.49	0.44	0.120	0.084
4	0.47	0.42	0.131	0.082	0.47	0.42	0.120	0.084
6	0.42	0.36	0.125	0.080	0.42	0.36	0.109	0.081
7.2	0.39	0.33	0.121	0.079	0.39	0.33	0.106	0.080

\*Errors for mean FRET efficiencies are  $\pm 0.01$ .

<sup>†</sup>Errors for FRET peak widths are  $\pm 0.006$ .

<sup>‡</sup>The width due to shot noise is  $\sigma_{sn} = \sqrt{\langle E_m \rangle (1 - \langle E_m \rangle) \langle N^{-1} \rangle}$ , where  $\langle N^{-1} \rangle$  is the mean of the reciprocal number of the donor and acceptor photons. The mean is calculated using the bursts that contribute to the FRET efficiency peak of the unfolded protein. For a photon trajectory divided by equally spaced time bins, this result follows from equation 4.29 of Gopich and Szabo (1). It can be readily extended to the FRET efficiency determined from bursts with variable duration.

1. Gopich I, Szabo A (2005) *J Chem Phys* 122:014707.