Supplementary Information

Derivation of the 'same molecule' probability

The two bursts separated by time t can originate form the same molecule or from different molecules. Therefore, the joint probability $p \equiv p(\{b_1,t\},\{b_2,t+\tau\})$ can be expressed as

$$p = p_{i=j} + p_{i\neq j}$$

where we have introduced the notations for the joint probability to have two bursts at *t* and *t*+τ that originate form the same, $p_{i=j}$, or from different, $p_{i\neq j}$, molecules.

The probability to have two bursts from the same molecule is the fraction of the bursts that are from the same molecule:

$$p_{same} = \frac{p_{i=j}}{p} = 1 - \frac{p_{i\neq j}}{p}$$

When the bursts originate from different molecules, they are independent, therefore

$$p_{i\neq j} = p(\{b_1,t\})\,p(\{b_2,t+\tau\})$$

Using this in the above equation and using the definition of $g(\tau)$, Eq. 1, we get Eq. 2.

The proof is fully analogous for the more general case that the two bursts b_1 and b_2 belong to two subsets B_1 and B_2. We have then $p_{same}(\tau,B_1,B_2) = 1 - 1/g_{B_1B_2}(\tau)$.

Alternative procedure for obtaining $p_{same}(\tau)$

The example of Csp*Tm* in 1.1 M GdmCl (Fig. 9) shows that in the absence of interconversion between subpopulations on the recurrence time scale, the change of the subpopulations in a kinetic recurrence analysis can be described exclusively by the appearance of non-recurring molecules (Fig. 9d). Due to the irreversibility of photobleaching, the subpopulation of molecules without an active acceptor does not interconvert to other subpopulations (see main text). The only effect that decreases the fraction of bursts from molecules with an inactive acceptor[1] (AI) over time ($p_{AI}(\tau)$) is the appearance of non-recurring molecules. In analogy to equation 4 we can write

$$p_{AI}(\tau) = p_{same}(\tau)\,p_{AI}^{i=j} + \left[1 - p_{same}(\tau)\right] p_{AI}^{i\neq j}, \qquad \text{[s1]}$$

where the fraction of bursts from acceptor-inactive molecules from the initial transfer efficiency range ($p_{AI}(0)$) and from the complete histogram ($p_{AI}(\infty)$) can be extracted from the complete histogram (see main text). Note that the fraction of bursts with an inactive

[1] Note that in contrast to the fraction of unfolded bursts, we define the fraction of bursts from molecules with an inactive acceptor as the area of the peak representing these bursts divided by *all* bursts in the histogram.

acceptor from recurring molecules is independent of the recurrence time. Rearranging equation s1 yields

$$p_{same}(\tau) = \frac{p_{AI}(\tau) - p_{AI}(\infty)}{p_{AI}(0) - p_{AI}(\infty)}, \qquad [s2]$$

We can thus calculate p_{same} in a second way independent of burst time correlation analysis. As shown in Fig. 9d, both methods agree well. We expect a small error for the second procedure because the fractions of acceptor-inactive molecules extracted from the complete histograms are apparently too high, as they also contain bursts from molecules that bleach during detection. This explains why the same molecule probabilities obtained with equation s4 are slightly lower than the values obtained from burst correlation analysis.

References

1. M. M. Santoro and D. W. Bolen, *Biochemistry*, 1988, **27**, 8063-8068.
2. D. Nettels, S. Müller-Späth, F. Küster, H. Hofmann, D. Haenni, S. Rüegger, L. Reymond, A. Hoffmann, J. Kubelka, B. Heinz, K. Gast, R. B. Best and B. Schuler, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 20740–20745.
3. I. V. Gopich and A. Szabo, *J. Phys. Chem. B*, 2009, **113**, 10965-10973.

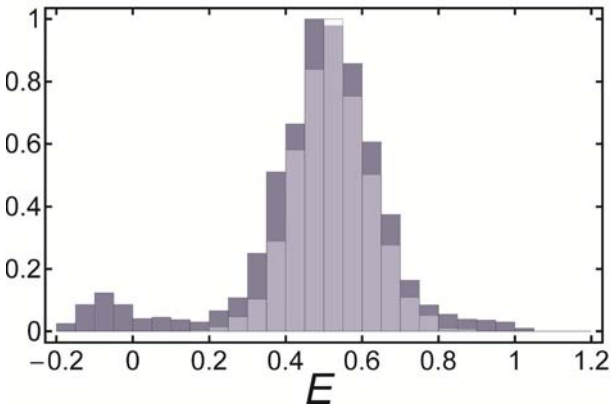
Supplementary Table

	Csp*Tm*	**Polyproline**	**Csp*Tm***	**R15**	**BdpA**
buffer	1.1 M GdmCl, 50 mM NaP, pH 7	3.5 M GdmCl, 50 mM NaP, pH 7	3.5 M GdmCl, 50% EG, 50 mM NaP, pH 7	1.1 M GdmCl, 50 mM NaP, pH 7	2.5 M GdmCl, 20 mM NaAc, 100 mM NaCl, pH 5, 37°C[2]
Laser power (μW)	100	100	100	100	300
1st threshold	25	50	60	25	80
2nd threshold	25	20	20	20	15
Bin size (ms)	1	1	0.5	1	0.05
Measurement time (h)	20	15	8	14	14
Relative burst detection efficiency ε	0.11	--	--	0.43	0.23

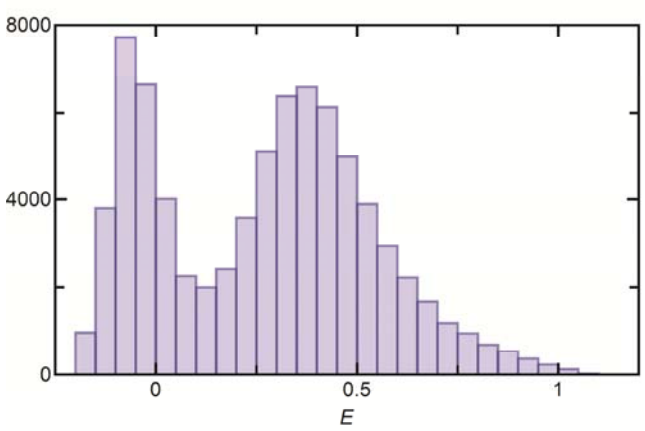
Supplementary Table 1: Experimental conditions and analysis parameters. '1st threshold' refers to the first burst identification step, '2nd threshold' and the bin size to the second step (see "Experimental" for details on burst identification). All experiments were performed at 22° C if not noted otherwise.

[2] Temperature was adjusted and calibrated as described previously [2].

Supplementary Figures



Suppl. Figure 1: Comparison of the measured transfer efficiency peak (Csp in 1.1 M GdmCl) of the unfolded state (dark blue) with the expected shot noise only broadened peak (light blue). The measured histogram is the recurrence histogram of Fig.4a with the initial transfer efficiency range ΔE_1=(0.4,0.45). For calculating the purely shot noise-broadened peak, the measured bursts were 'recolored' assuming a fixed transfer efficiency of 0.52 according to the procedure described by Gopich and Szabo[3]. The advantage of this method is that the original burst size distribution is used. The value of 0.52 was determined using a maximum likelihood method also described in Ref. [3]. For comparison, the peak heights are normalized to one.



Suppl. Figure 2: Transfer efficiency histogram of Csp in 3.5 M GdmCl and 50% ethylene glycol. The unusually broad and asymmetric unfolded state peak suggests the existence of unresolved subpopulations. The recurrence transfer efficiency contour plots in Fig. 8 of the main text reveal more details.