Supporting Material

Single molecule study of the intrinsically disordered FG-repeat Nucleoporin 153

Sigrid Milles, Edward A Lemke

EMBL, Structural and Computational Biology Unit, Meyerhofstrasse 1, 69117 Heidelberg, Germany

Table S1: Nup153FG labeling mutants. Donor positions describe the point mutations to the unnatural AA AcF that were used to link D. Acceptor positions describe the point mutations to Cys that were used to link A in order to probe the various segments. AA positions refer to AA position within Nup153FG. Our Nup153FG is similar to the FG domain introduced by Lim et al (AA residues 875-1475 of the full length human Nup153 protein (1)).

Segment	Donor position (AcF)	Acceptor position (Cys)	$R_E/R_{E,RC}$ PBS	$R_E/R_{E,RC}$ 4M GdmCl
α	Ser-61	Ser-9	0.74	0.87
β	Ser-64	Ser-116	0.82	1.03
γ	Ser-168	Ser-116	0.94	1.28
δ	Ser-220	Ser-168	0.68	0.87
ε	Ala-438	Gly-480	0.73	0.93
ζ	Ala-475	Ser-517	0.82	0.98

All FRET efficiencies (E_{FRET}) that are measured with a protein behaving like a random-coil underlie fast fluctuations and can therefore be described according to

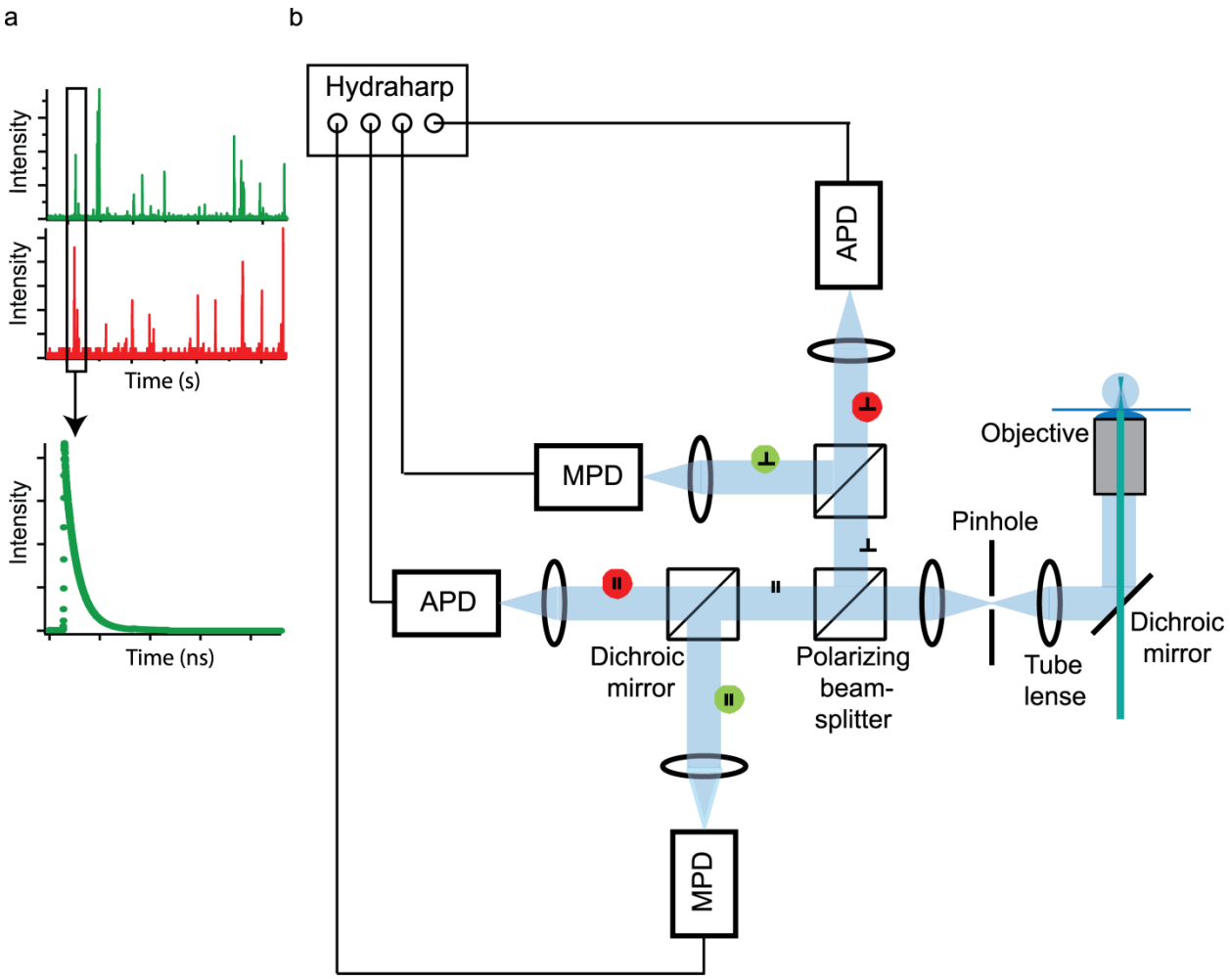
$$\langle E \rangle \approx E_{FRET} = \int_0^\infty E(r)P(r)dr,$$

with E(r) as given in the main text in equation (6) and the radial probability distribution of a Gaussian chain (see (2-5) for this and other possible convolution operations).

$$P(r) = 4\pi r^2 \left(\frac{3}{2\pi\langle r^2\rangle}\right)^{3/2} \exp\left(-\frac{3r^2}{2\langle r^2\rangle}\right).$$

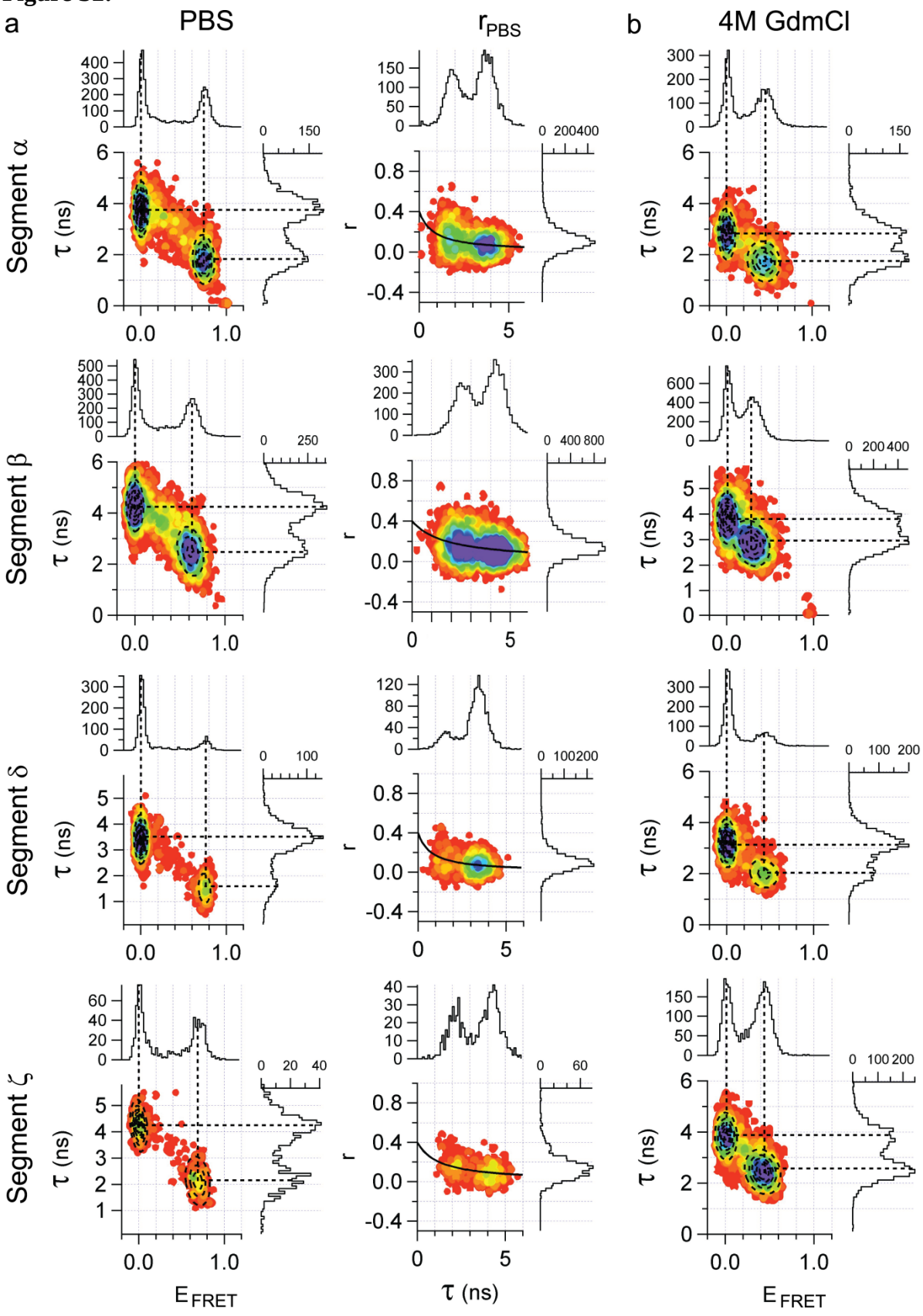
$R_E = \sqrt{\langle r^2 \rangle}$ values satisfying this equation were determined numerically using IgorPro (Wavemetrics, Lake Oswego, OR).

Figure S1:



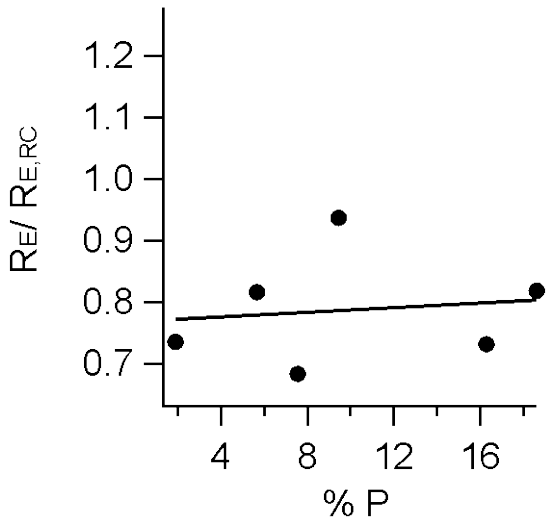
Single-molecule fluorescence setup. (a) Typical time trace of a single-molecule measurement in the D (green) and A (red) channel. The lower panel shows a typical fluorescence lifetime distribution. (b) Emission and detection pathway. Laser light is indicated in turquoise, emitting light in light blue. Light is split into parallel (II) and perpendicular (⊥) light, then into green and red light and detected by either APDs or MPDs.

Figure S2:


a
PBS
r PBS
b
4M GdmCl
Segment α
Segment β
Segment δ
Segment ζ
τ (ns)
r
E FRET
τ (ns)
E FRET

Single molecule MFD analysis of Nup153FG segments α, β, δ (FxFG) and ζ (PxFG). (a) The left columns show burst integrated fluorescence lifetime (BIFL) analysis under native conditions (PBS). The two dimensional plots are color coded for frequency of occurrence, while the top and right histograms are maximum projections of the data along the vertical (lifetime, τ) axis and along the horizontal (E_{FRET}) axis. Dashed circles outline the result from 2D Gaussian fits of both the 0-peak and the FRET-peak population. Dashed lines relate the centers of the fits to their position in the one-dimensional representation of E_{FRET} and τ data. The relationship between donor fluorescence anisotropy (r) and the corresponding τ is shown in the right columns for each studied segment. The black line shows the expected trend according to the Perrin equation. (b) BIFL histograms analogous to (a) under unfolding conditions (4 M GdmCl).

Figure S3:



Dependence of $R_E/R_{E,RC}$ on proline content (%).

Supporting References:

1. Lim, R. Y., N. P. Huang, J. Koser, J. Deng, K. H. Lau, K. Schwarz-Herion, B. Fahrenkrog, and U. Aebi. 2006. Flexible phenylalanine-glycine nucleoporins as entropic barriers to nucleocytoplasmic transport. Proc Natl Acad Sci U S A 103:9512-9517.

2. Merchant, K. A., R. B. Best, J. M. Louis, I. V. Gopich, and W. A. Eaton. 2007. Characterizing the unfolded states of proteins using single-molecule FRET spectroscopy and molecular simulations. Proc Natl Acad Sci U S A 104:1528-1533.

3. Hoffmann, A., A. Kane, D. Nettels, D. E. Hertzog, P. Baumgartel, J. Lengefeld, G. Reichardt, D. A. Horsley, R. Seckler, O. Bakajin, and B. Schuler. 2007. Mapping protein collapse with single-molecule fluorescence and kinetic synchrotron radiation circular dichroism spectroscopy. Proc Natl Acad Sci U S A 104:105-110.

4. Gopich, I. V. and A. Szabo. 2003. Single-macromolecule fluorescence resonance energy transfer and free-energy profiles. Journal of Physical Chemistry B 107:5058-5063.

5. Laurence, T. A., X. Kong, M. Jager, and S. Weiss. 2005. Probing structural heterogeneities and fluctuations of nucleic acids and denatured proteins. Proc Natl Acad Sci U S A 102:17348-17353.