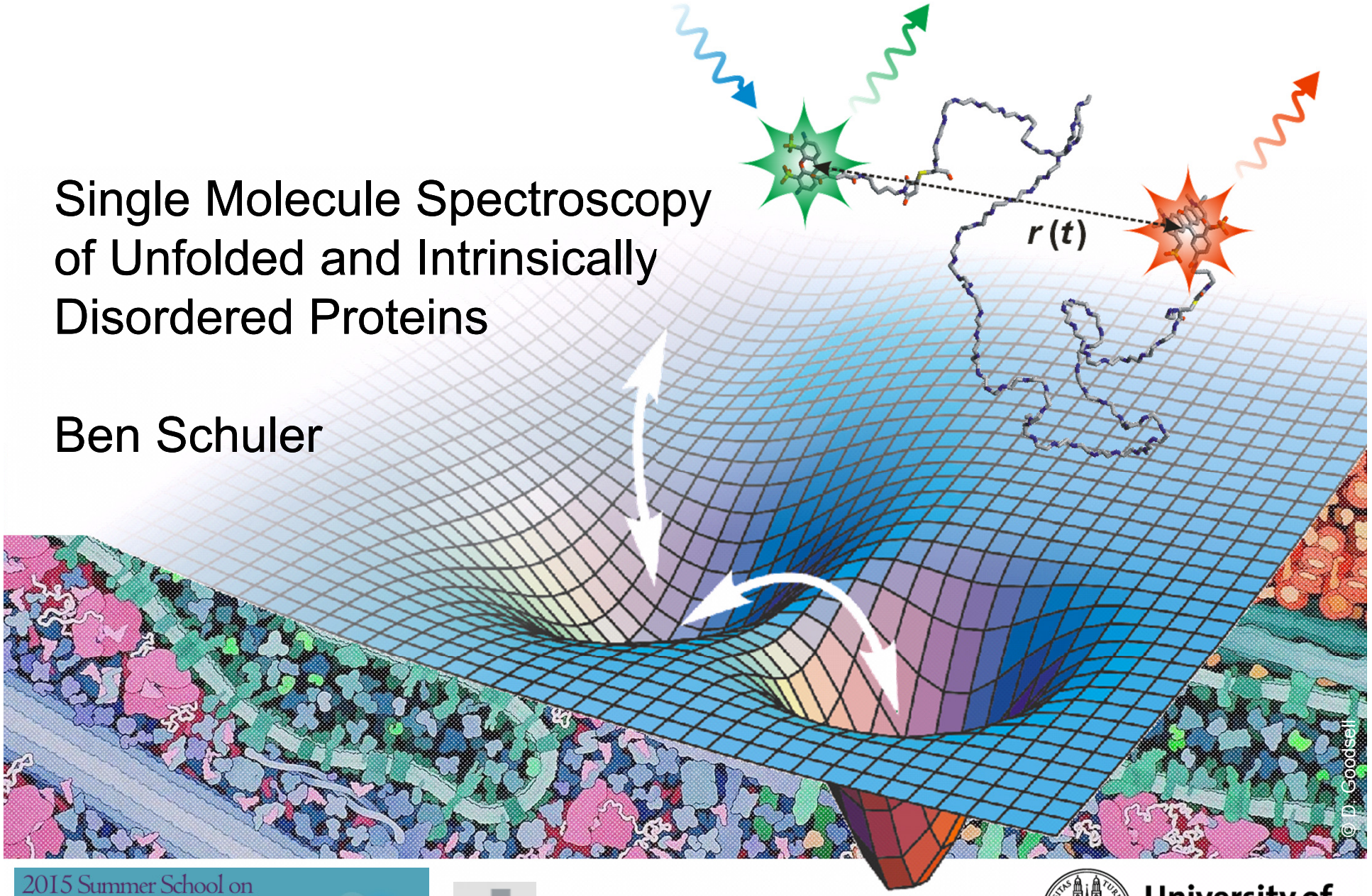
Single Molecule Spectroscopy of Unfolded and Intrinsically Disordered Proteins

Ben Schuler



2015 Summer School on Polymers in Biology

KIAS

University of Zurich[UZH]

Outline

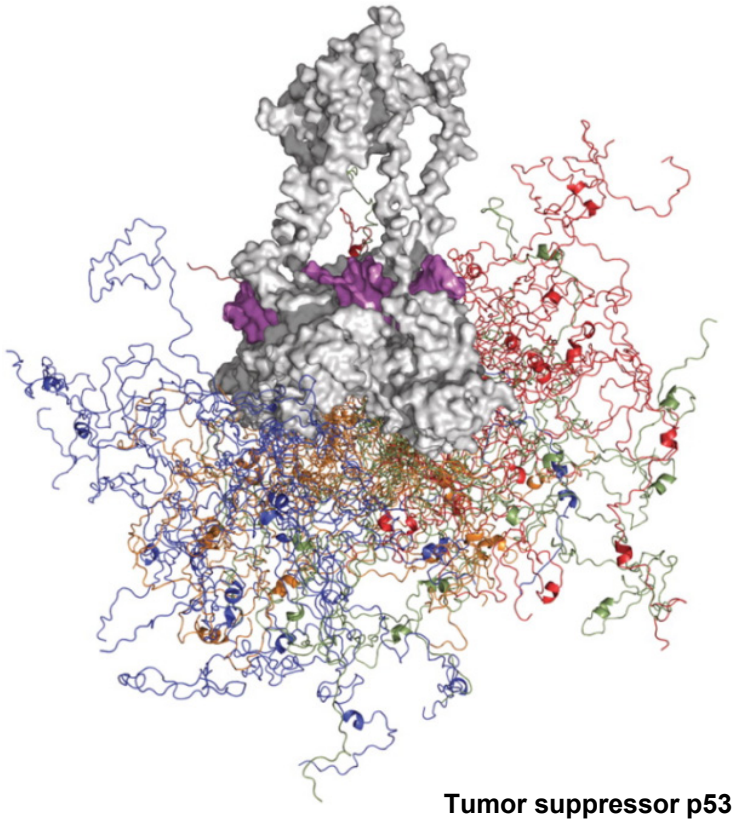
- Unfolded and intrinsically disordered proteins
- Single Molecule Fluorescence Spectroscopy and FRET
- Distances and distance distributions from single-molecule FRET
- Fluorescence Correlation Spectroscopy (FCS)
- Unfolded state dynamics from FCS

Unfolded and intrinsically disordered proteins

Protein Folding

Voet & Voet, Biochemistry

Intrinsically disordered proteins (IDPs)



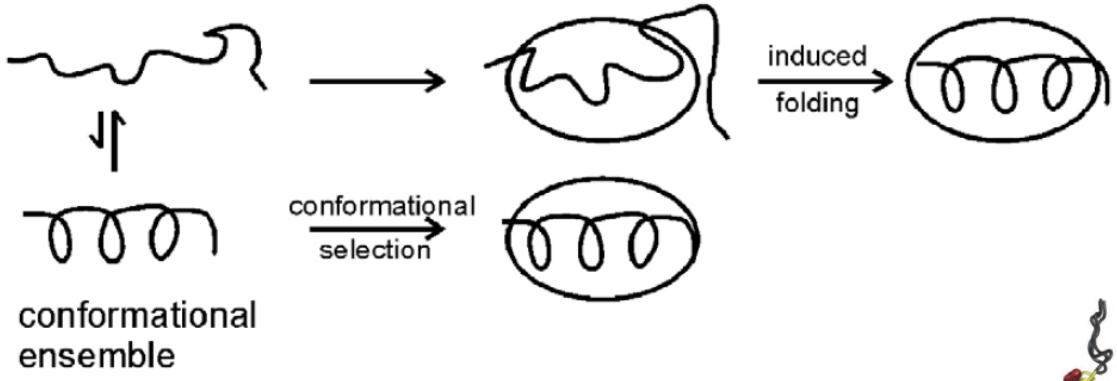
Tumor suppressor p53

Wells M *et al. PNAS* 2008;105:5762-5767

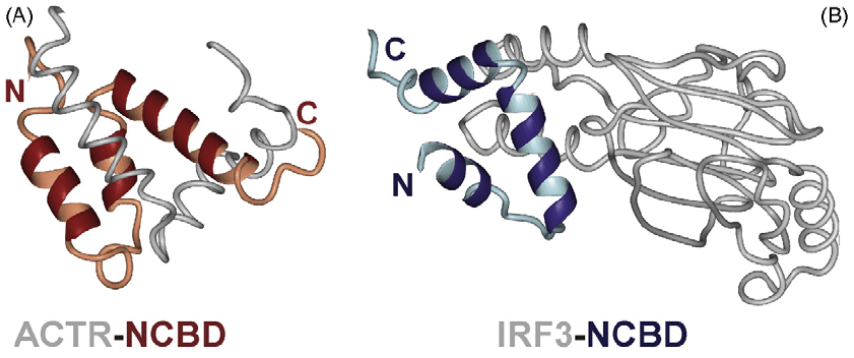
→ ~30% of the human proteome are estimated to be IDPs

Intrinsically disordered proteins

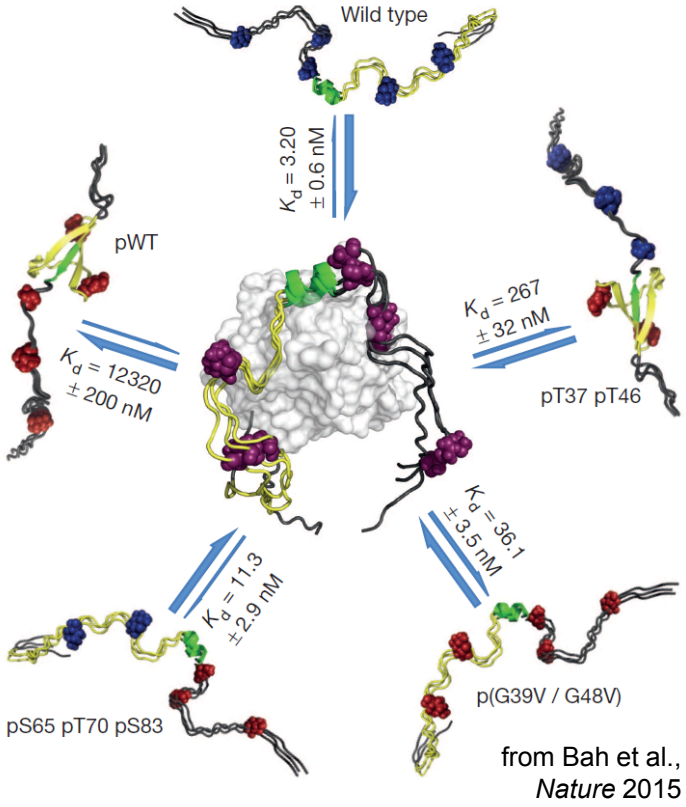
Coupled folding & binding



Structural plasticity/promiscuity



from Wright & Dyson, *Curr Opin Struct Biol* 2009

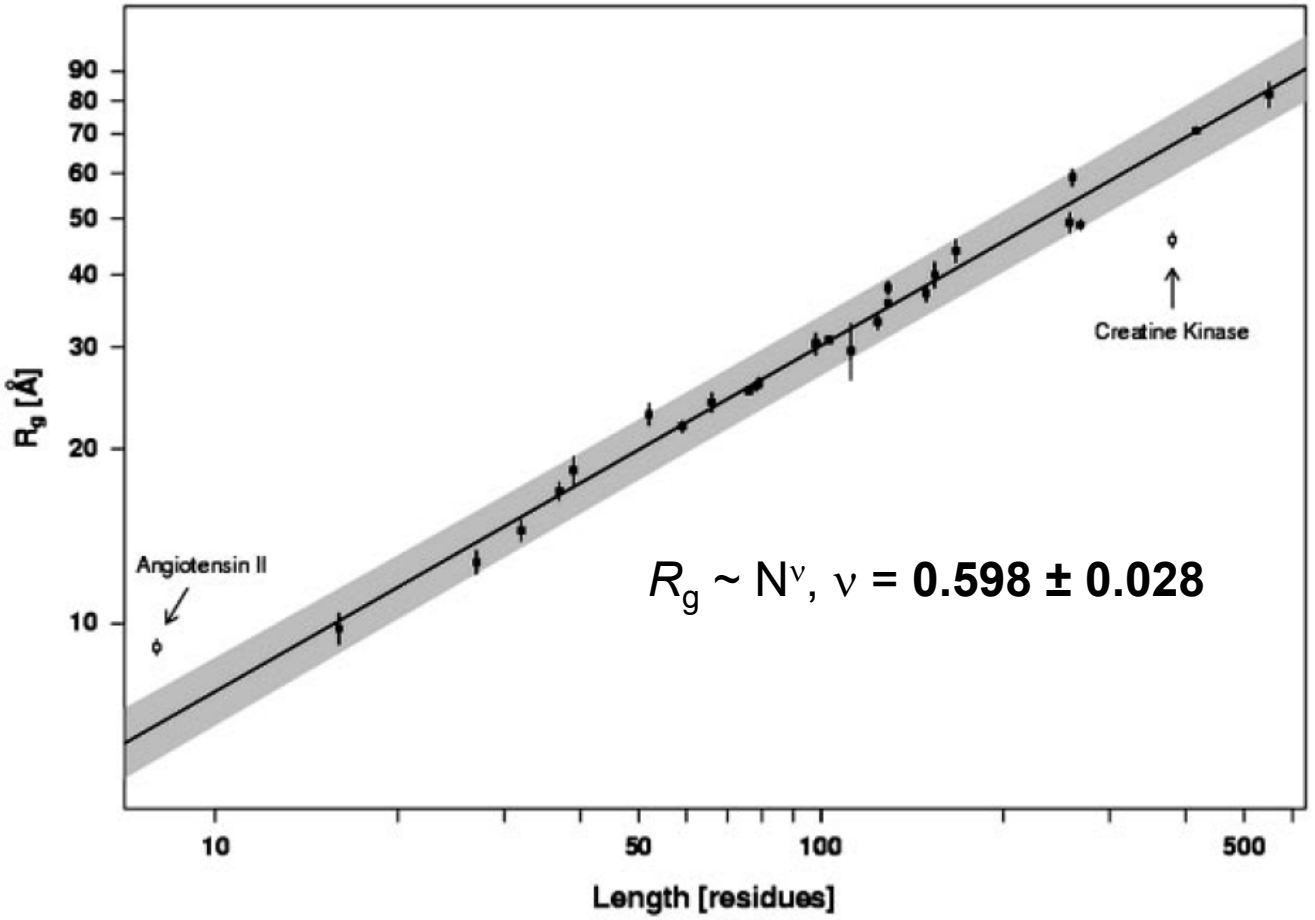


from Bah et al., *Nature* 2015

'Fuzzy' complexes,
regulation by chemical modifications

Polymeric properties of unfolded proteins

By and large only accessible in high concentrations of denaturant by ensemble methods



Kohn *et al.* PNAS 2004

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Fluorescence: a reminder

Timescales:

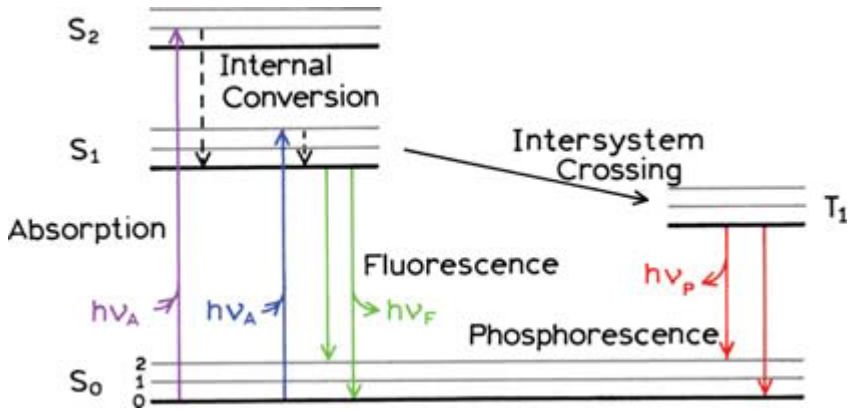
Excitation/Absorption
~10^{-15} s

Internal Conversion (IC)
~10^{-14} s to 10^{-11} s

Fluorescence
~10^{-9} s to 10^{-7} s

Phosphorescence
~10^{-3} s to 10^{2} s

Jablonski Diagram



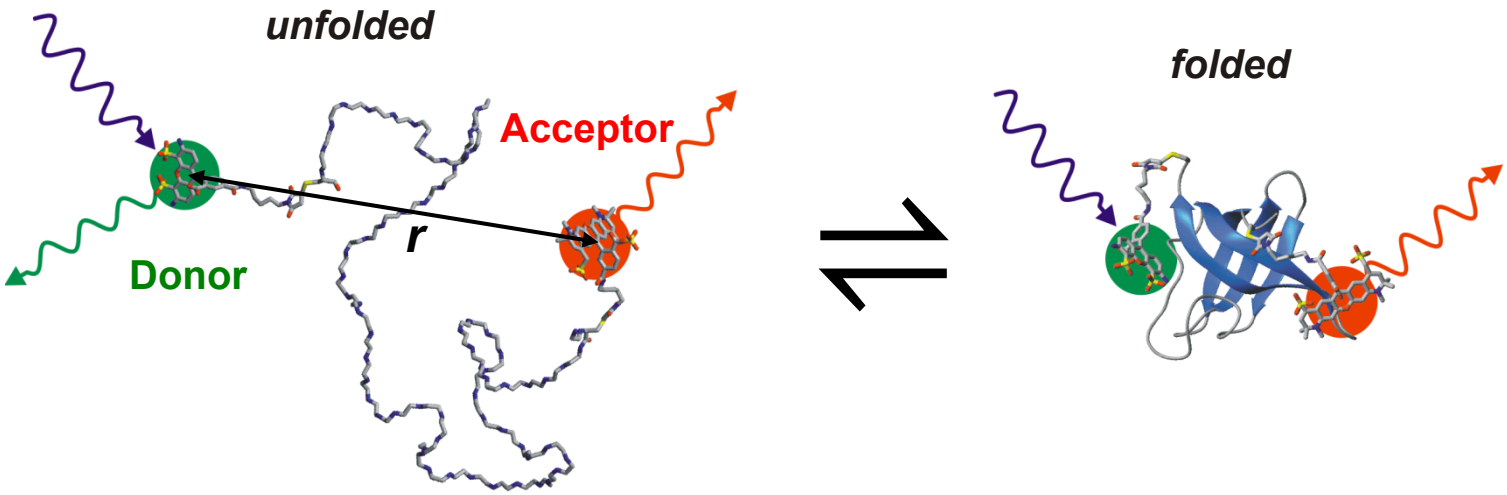
Fluorescence quantum yields depend on relative rates of radiative and nonradiative processes

$$\Phi = \frac{k_{rad}}{k_{rad} + \sum k_{non-rad}}$$

Fluorescence lifetimes depend on the rates of all decay processes

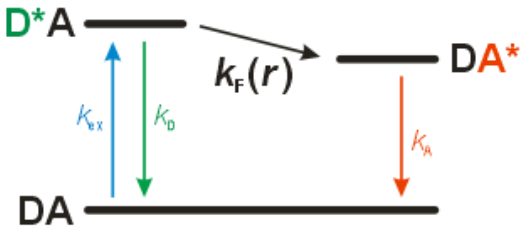
$$\tau = \frac{1}{k_{rad} + \sum k_{non-rad}}$$

Förster Resonance Energy Transfer (FRET)



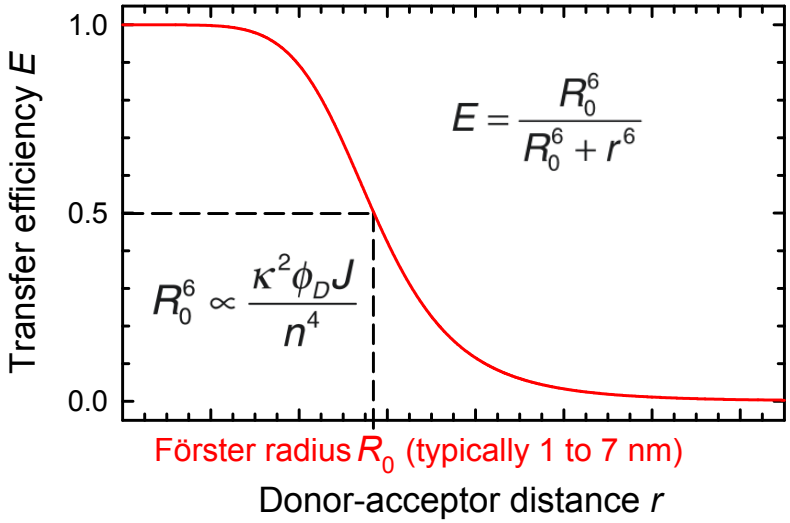
$$k_F = k_D \left(\frac{R_0}{r}\right)^6$$

$$= \frac{1}{\tau_D}\left(\frac{R_0}{r}\right)^6$$

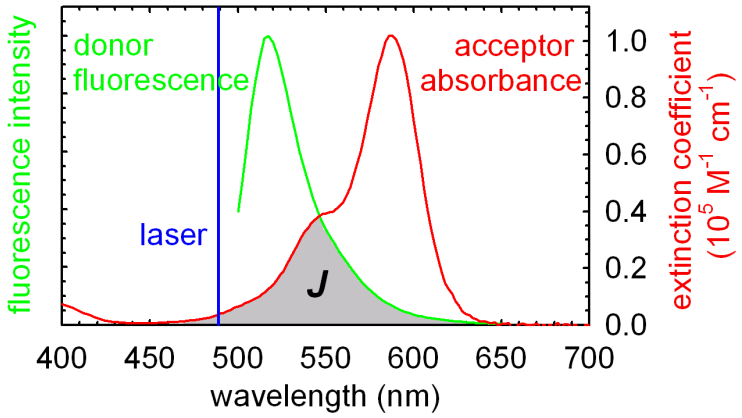


$$E = \frac{k_F}{k_F + k_D}$$

$$= \frac{n_A}{n_A + n_D} = 1 - \frac{\tau_{DA}}{\tau_D}$$



Förster Resonance Energy Transfer (FRET)



THEODOR FÖRSTER

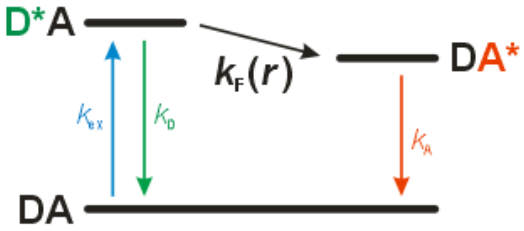
15. 5. 1910 – 20. 5. 1974

$$J = \int_0^\infty f_D(\lambda)\varepsilon(\lambda)\lambda^4 d\lambda$$

$$\kappa^2 = \left(\cos\theta_T - 3\cos\theta_D\cos\theta_A\right)^2$$

= 2/3 for rapid orientational averaging

$$k_T = \frac{1}{\tau_D}\left(\frac{R_0}{r}\right)^6$$



$$E = \frac{k_F}{k_F + k_D}$$

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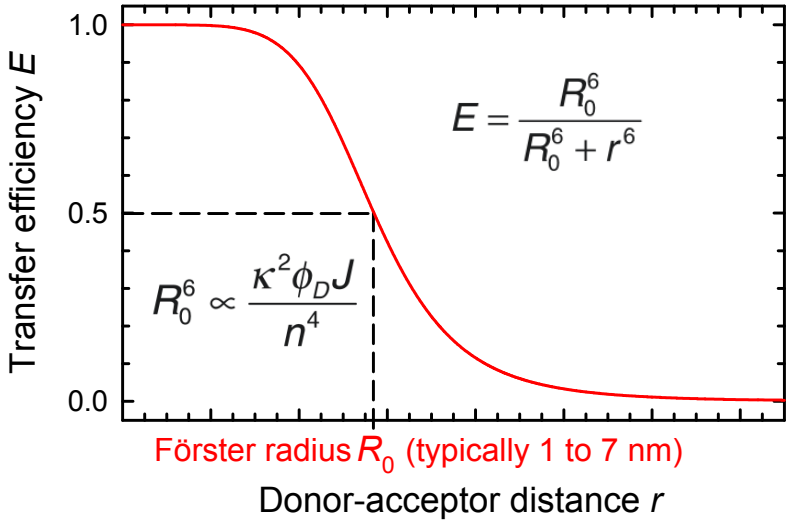
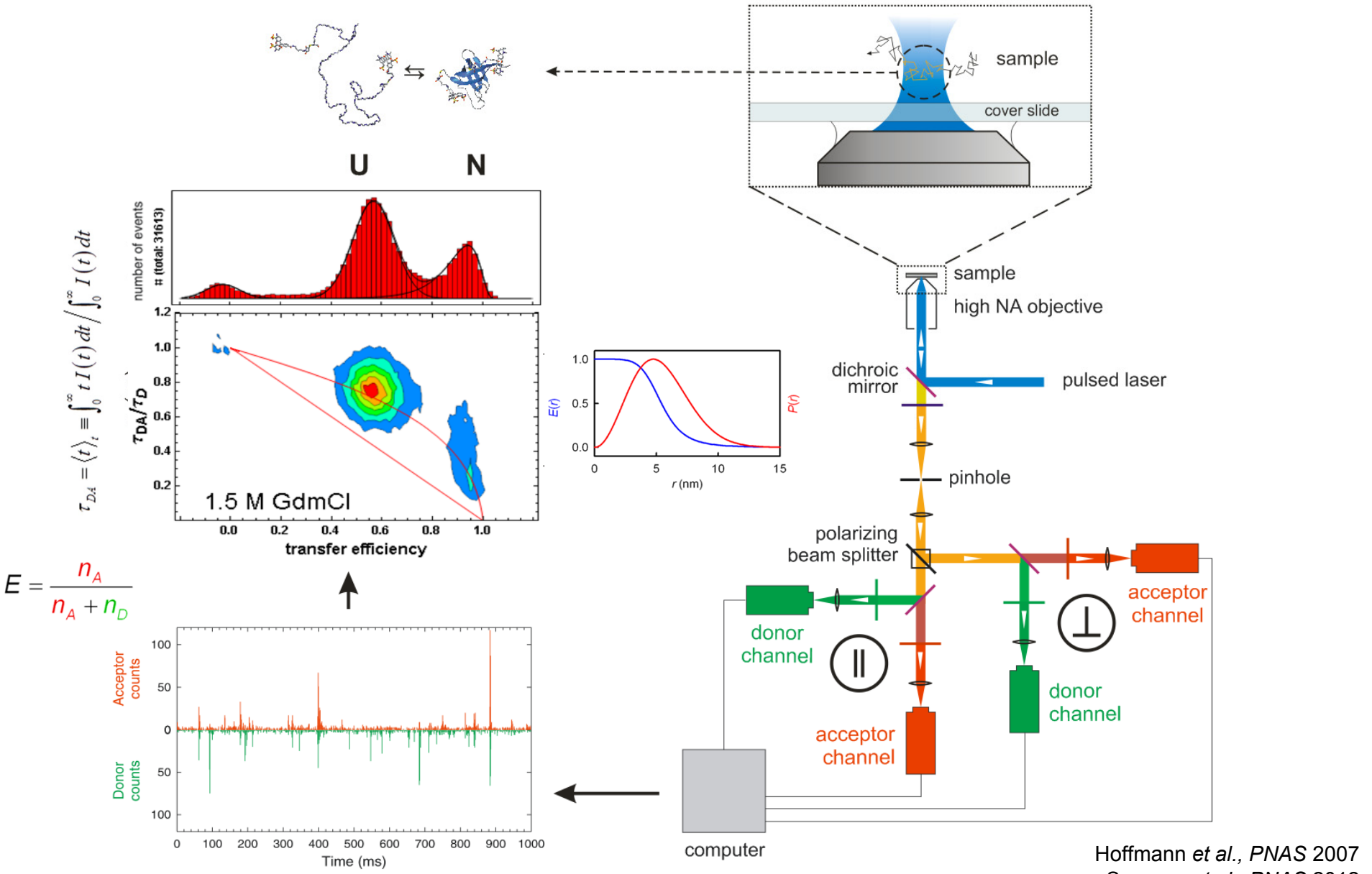
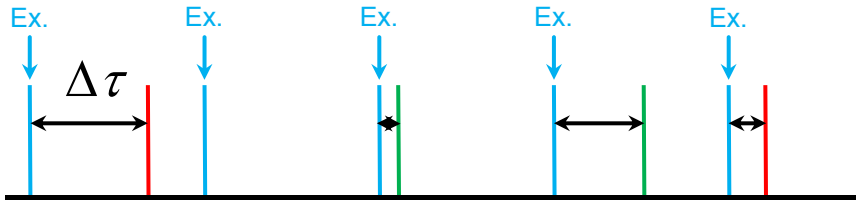


Confocal single molecule fluorescence spectroscopy

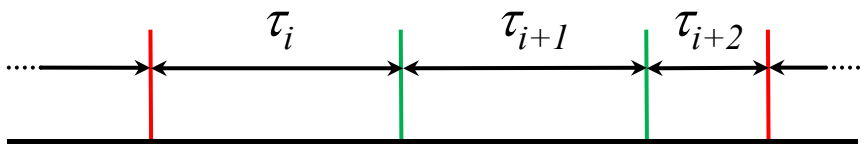


Hoffmann *et al.*, *PNAS* 2007
Soranno *et al.*, *PNAS* 2012

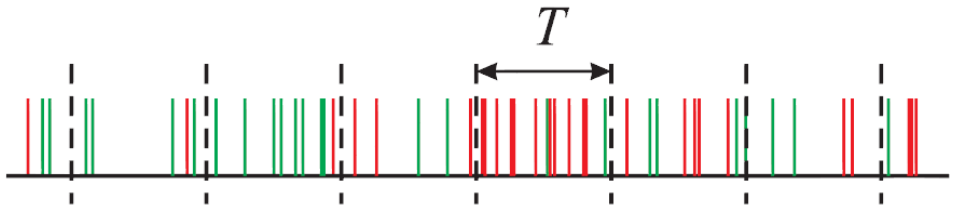
Basics of photon statistics



Fluorescence lifetimes $\Delta\tau$ (~1 ns)
from time-correlated
single-photon counting (TCSPC)



Single photon counting
Interphoton times τ_i (~1..10 μs)



Photon binning time T (~1 ms)
Count rate n (~100 ms^{-1})

Simplest case:
single fixed distance or all dynamics faster than mean interphoton time (~10 μs)

⇒ Photons uncorrelated, i.e. Poissonian photon statistics (good approximation):

$$p(N) = (nT)^N \frac{e^{-nT}}{N!}, \quad \text{with} \quad nT = \langle N \rangle \quad \text{mean number of photons per bin}$$

Exponential interphoton time distribution $p(\tau) = n e^{-n\tau} \quad \Rightarrow \quad \langle \tau \rangle = n^{-1}$

Photon shot noise in single molecule FRET

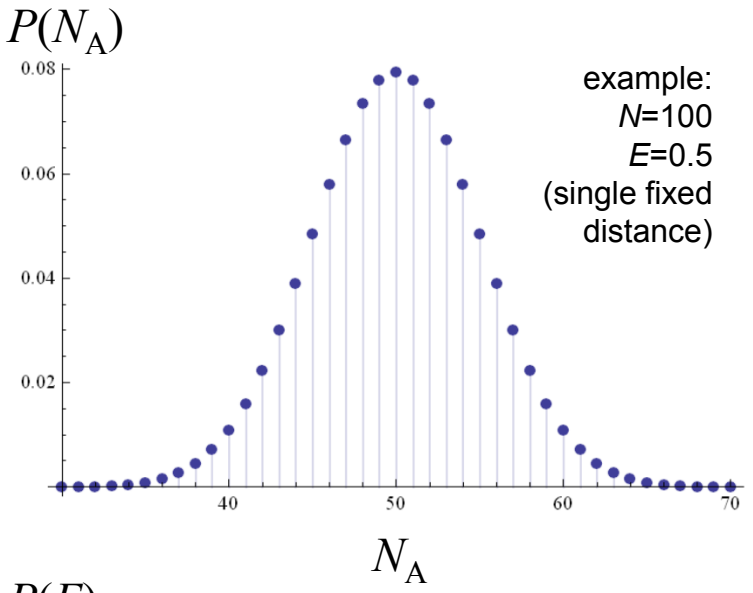
Probability of observing N_A acceptor photons in a fluorescence burst of N photons, given a fixed mean transfer efficiency $\langle E \rangle$ underlying the signal:

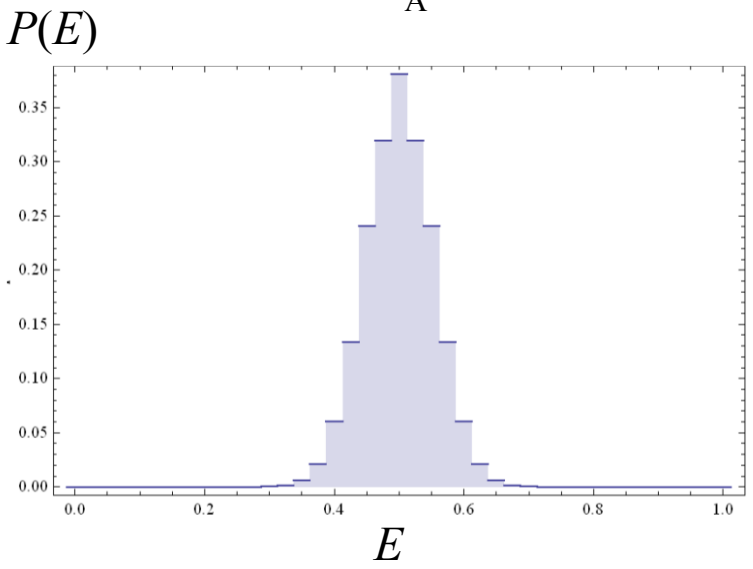
$$P(N_A) = \binom{N}{N_A} \langle E \rangle^{N_A} \left(1 - \langle E \rangle\right)^{N - N_A}$$

$$P(N_A, N_D) = \frac{N!}{N_A! N_D!} \langle E \rangle^{N_A} \left(1 - \langle E \rangle\right)^{N_D}$$

⇒ **Shot noise** broadens transfer efficiency distributions

⇒ *E* distributions cannot be converted directly to distance distributions!
→ but: the underlying true transfer efficiency distribution can be obtained by deconvolution of the shot noise contribution





Distances from FRET efficiencies

Important points to consider for quantitative distance measurements with FRET:

- **Precision** for distances and distance changes is greatest close to R_0 (where dE/dr is maximal)
- Förster theory is not **accurate** for very short distances (compared to the size of the fluorophores, i.e. < 1nm for typical dyes)
- **Shot noise** (see above)
- **Reorientation** of donor and acceptor must be fast relative to the donor fluorescence lifetime (κ^2 = 2/3), otherwise additional broadening and shift of $\langle E \rangle$
  → measure fluorescence anisotropy
- Instrument must be **calibrated** to correct for differences in quantum yields of the dyes and detection efficiencies to obtain accurate distances
- **Time scales**
  → slow distance dynamics (relative to the interphoton time) will lead to a broadening of the E distribution (underlying distance distribution can be obtained by shot noise deconvolution)
  → rapid distance dynamics (relative to the interphoton time) will lead to fast sampling of the distance distribution and will not result in broadening

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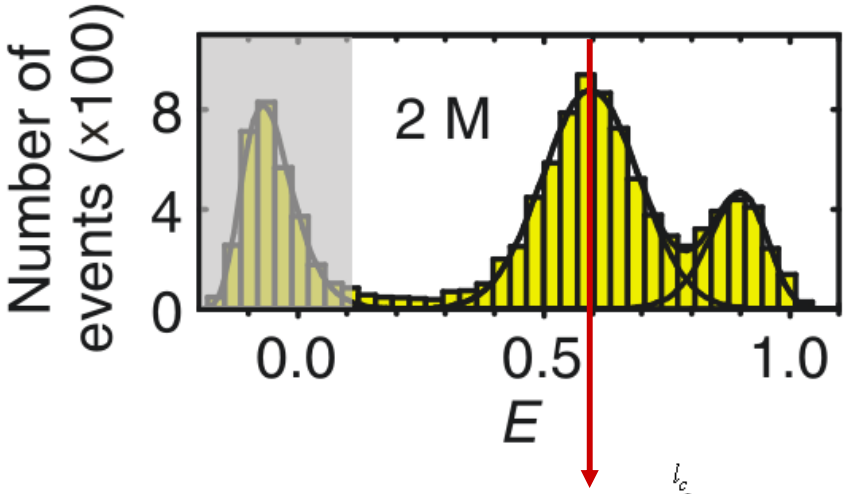
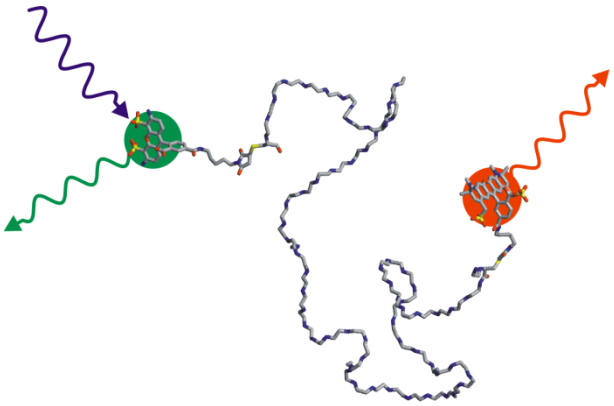
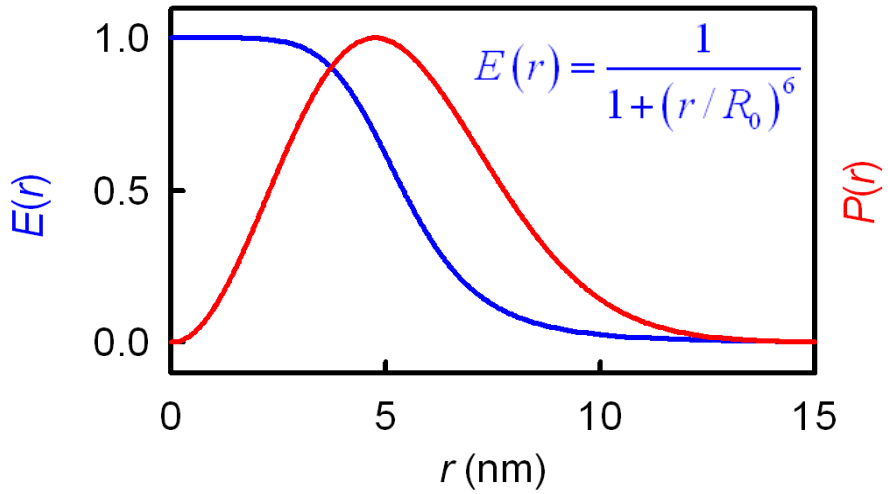
Distance distributions from FRET efficiencies





$$\langle E \rangle = \int_a^{l_c} P(r)\,E(r)\,dr$$

requires model for $P(r)$, e.g. Gaussian chain



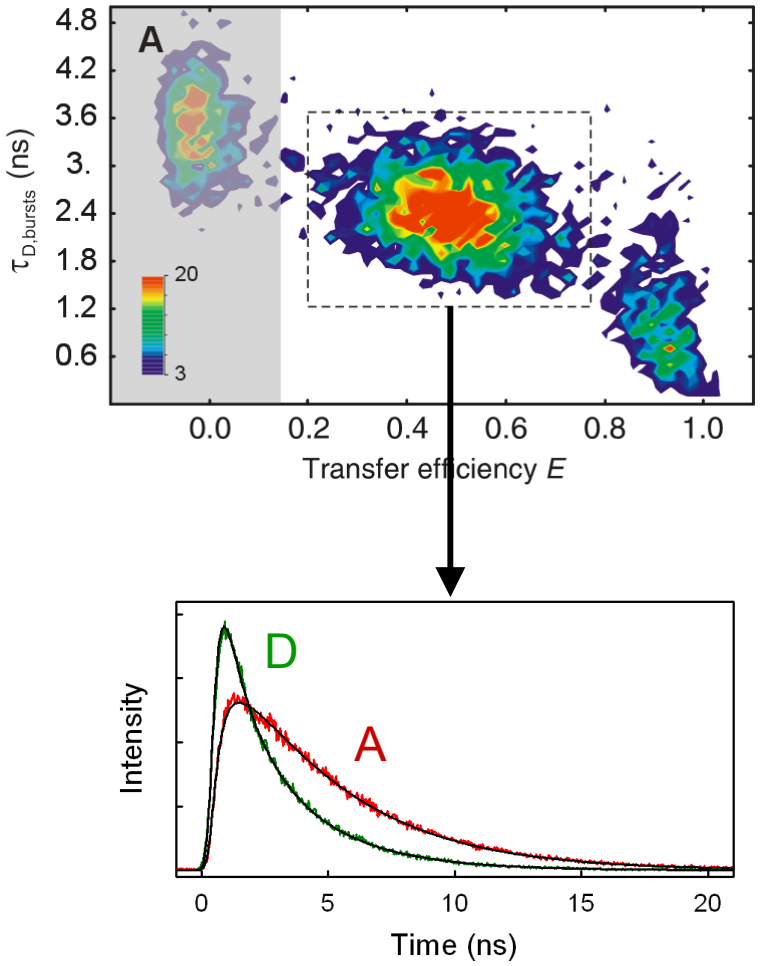
$$P(r) = \frac{4\pi r^2}{\left(\frac{2}{3}\pi\langle r^2\rangle\right)^{3/2}}\; e^{-\frac{3r^2}{2\langle r^2\rangle}}$$

where $\langle r^2 \rangle = 2 l_p n l$

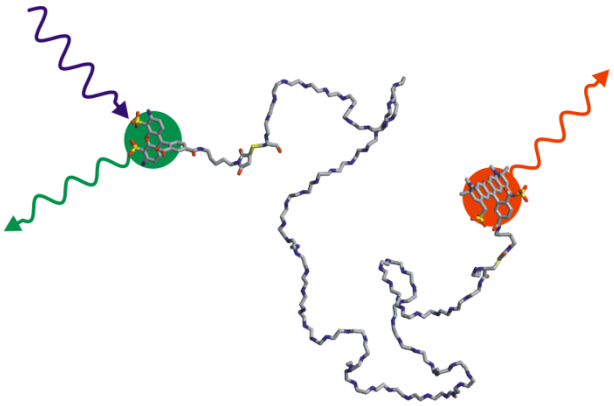
with l_p: persistence length
n: number of peptide segments
l: segment length (3.8 Å)

→ but: direct information about $P(r)$ lost due to ms-averaging over bursts

Distance distributions from fluorescence lifetimes

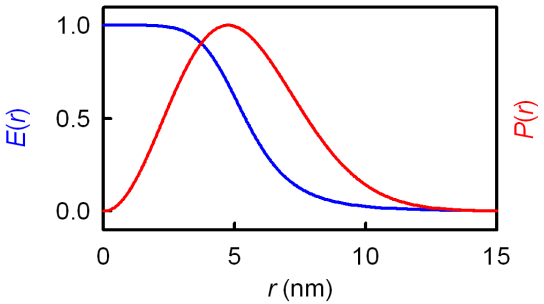


subpopulation-specific fluorescence intensity decays provide more direct test of $P(r)$



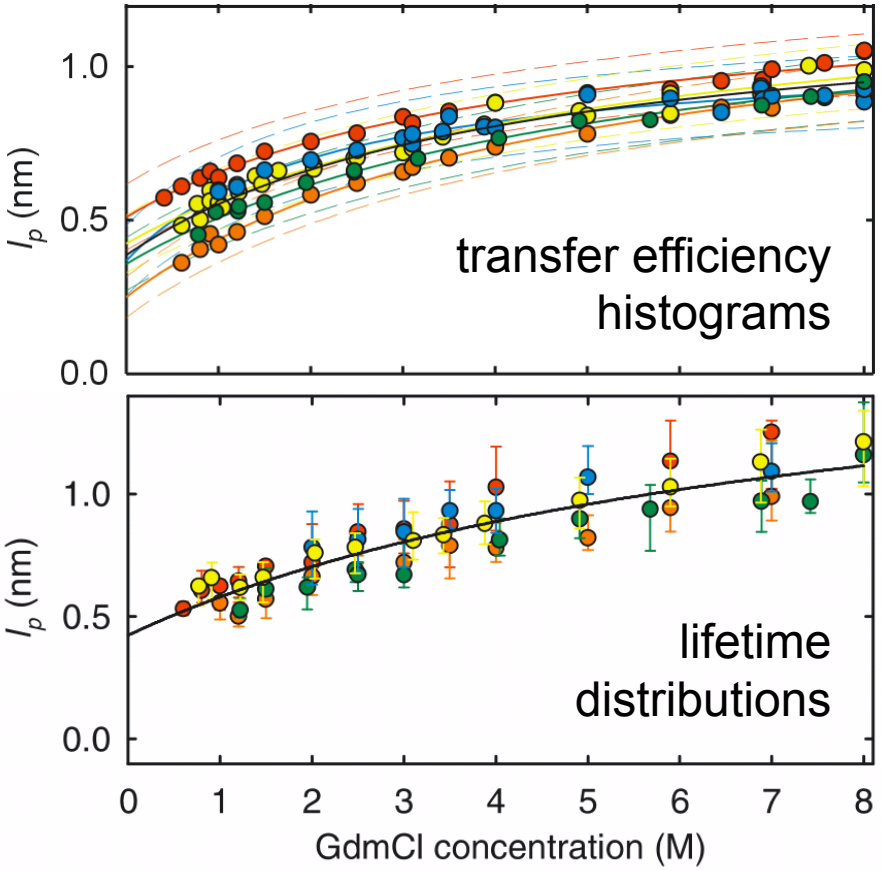
$$I(t) \propto \int_0^\infty P(r)\, e^{-\frac{t}{\tau_{D0}}\left(1+\left(\frac{R_0}{r}\right)^6\right)} dr$$

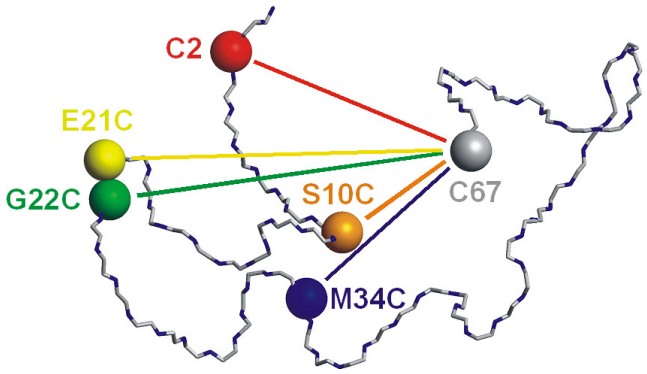
$$P(r) = \frac{4\pi r^2}{\left(\frac{2}{3}\pi\left\langle r^2\right\rangle\right)^{3/2}}\, e^{-\frac{3r^2}{2\left\langle r^2\right\rangle}}$$



Example: Distance distributions in the unfolded state

Example: mapping unfolded state dimensions





$$p_{eq}(r) = \frac{4\pi r^2}{\left(\frac{2}{3}\pi\left\langle r^2\right\rangle\right)^{3/2}} e^{-\frac{3r^2}{2\left\langle r^2\right\rangle}}$$

→ collapse is largely uniform

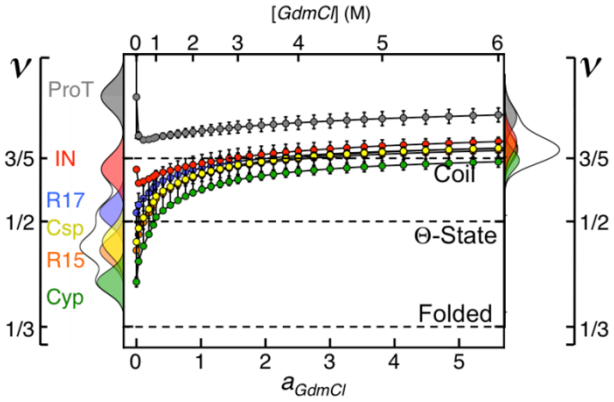
$$l_p = \frac{\left\langle r^2\right\rangle}{2nl}$$

Hoffmann *et al.* (2007) *PNAS 104*, 105-110.
Soranno *et al.* (2012) *PNAS 109,* 17800–17806

Polymer concepts quantify key properties of IDPs

Polymer scaling laws allow a classification of expansion and collapse

$$R_G = R_{G0} N_{bonds}^{\nu}$$



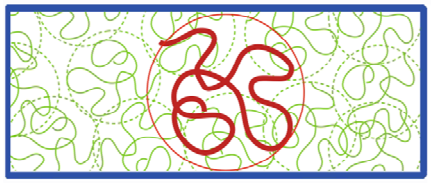
$$P(r_G, \varepsilon, R_{G\Theta}) = Z^{-1} r_G^6 \exp\left[-\frac{7r_G^2}{2R_{G\Theta}^2} + nq(\phi, \varepsilon)\right]$$

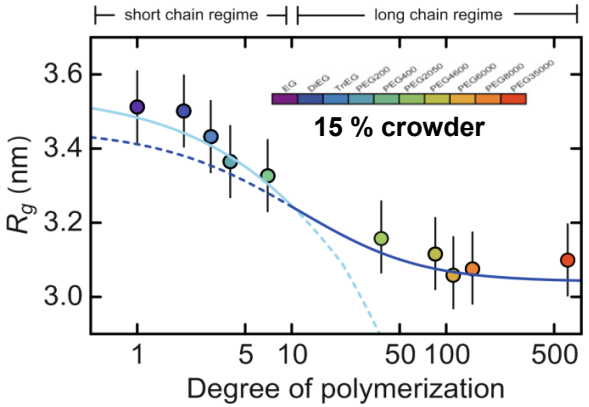
with $q = \frac{1}{2}\varepsilon\phi - \frac{1-\phi}{\phi}\ln(1-\phi)$

Sanchez Theory of Coil-Globule Transitions

Hofmann *et al.* (2012)
PNAS 109, 16155-16160

Polymer effects in molecular crowding





Joanny, Grant, Pincus, Turkevich, 1981:

$$R_g(N, P, \phi, a) = R_{g0}\left(\frac{1}{1 + a\phi/\phi^*(P)}\right)^{1/5} \quad \text{for} \quad P < N^{1/2}$$

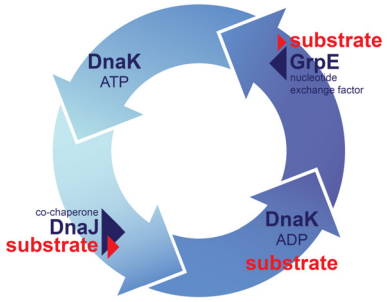
Schäfer, Kappeler, 1993:

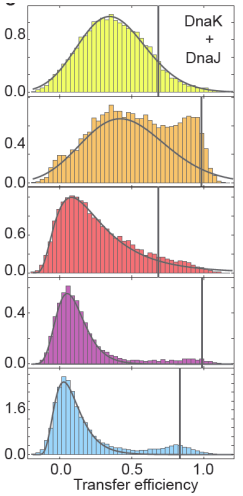
$$R_g^2(N_1, N_2, c_{p2}) = l_R^2 N_R^{(1)} \left\{0.636 + 0.165\left(N_R^{(1)}\right)^{1/2} - 0.292\left(N_R^{(1)}\right)^{1/2} f_{12}^2 G(W_2, y_N)\right\}$$

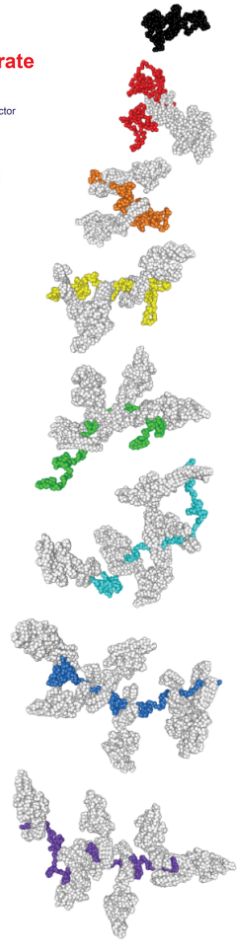
Excluded volume screening/ Flory-Huggins Theory

Soranno, König *et al.* (2014)
PNAS 109, 16155-16160

Expansion of denatured protein by molecular chaperones







Excluded Volume Effects

Kellner *et al.* (2014)
PNAS 111, 13355-13360

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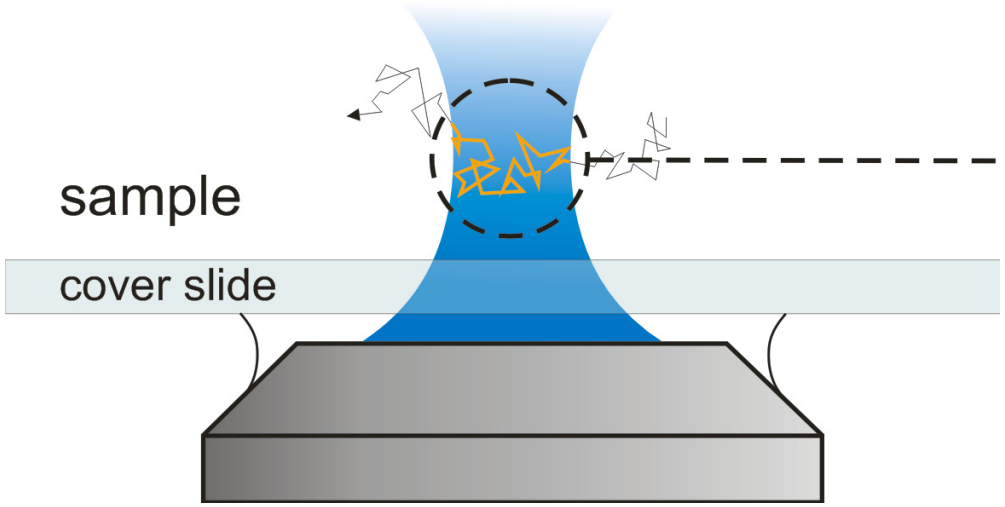
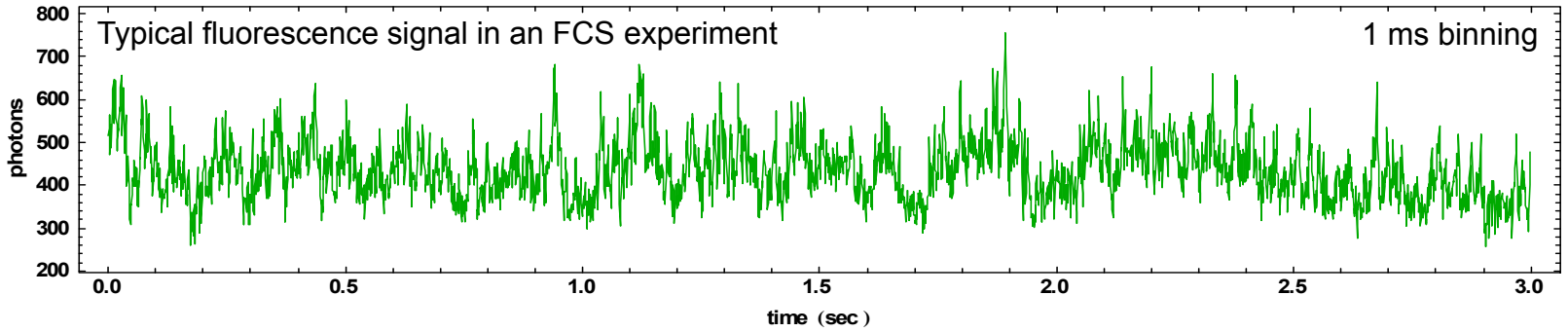
Fluorescence Correlation Spectroscopy (FCS)



Confocal fluorescence detection, concentrations of fluorescent molecules typically in the nM to μM range



→ Translational diffusion through the confocal volume leads to fluorescence intensity fluctuations

→ Can be analyzed in terms of correlation functions

FCS: Translational diffusion

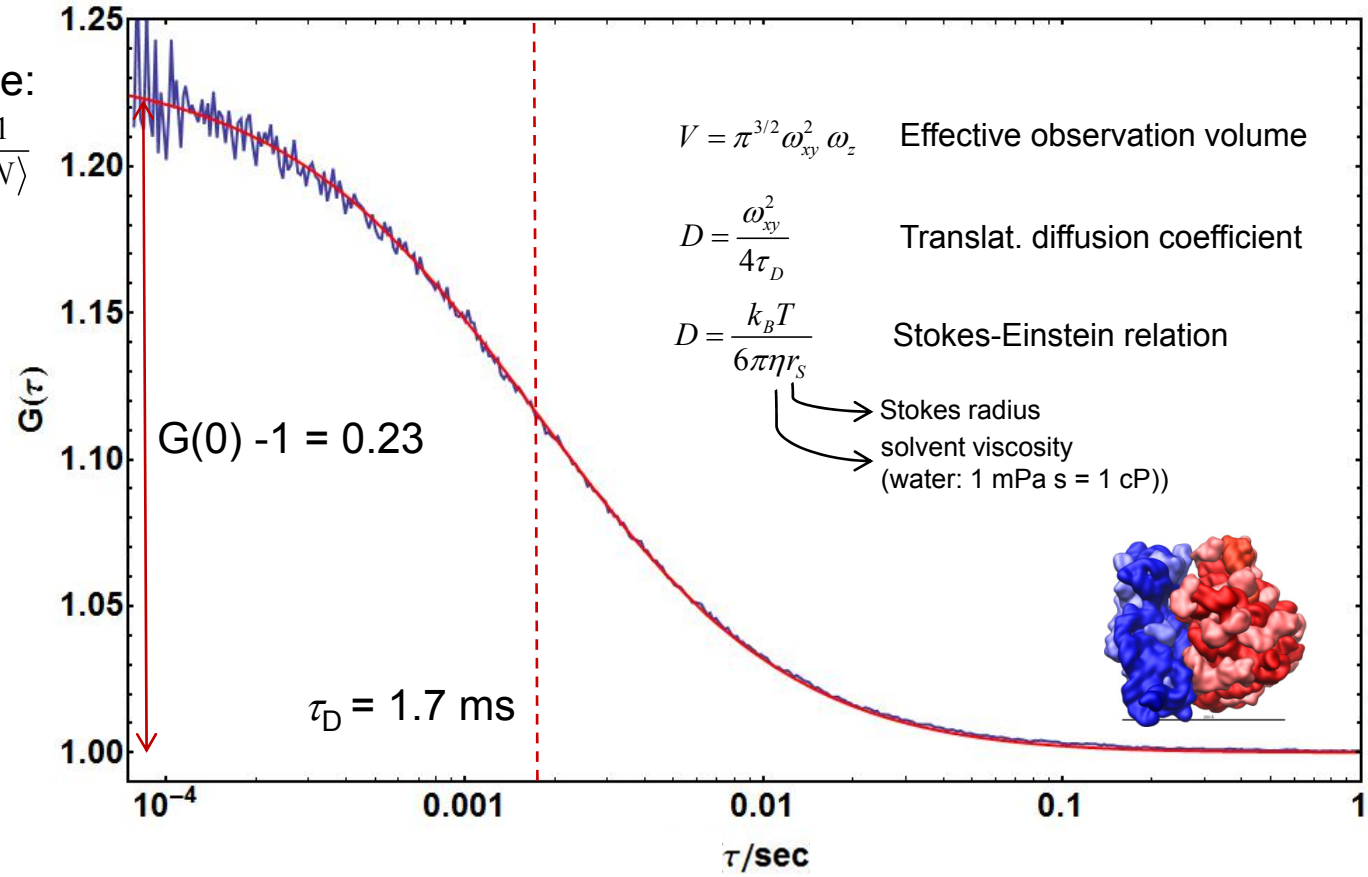
Correlation function for translational diffusion: $G(\tau) = 1 + \frac{1}{\langle N \rangle} \frac{1}{1+\tau/\tau_D} \frac{1}{\sqrt{1+(\tau/S^2\tau_D)}}$

$S = \frac{\omega_z}{\omega_{xy}}$ Ratio of axial to lateral radii of the observation volume

τ_D → Translational diffusion time

Amplitude:

$G(0) - 1 = \frac{1}{\langle N \rangle}$



$V = \pi^{3/2} \omega_{xy}^2 \omega_z$ Effective observation volume

$D = \frac{\omega_{xy}^2}{4\tau_D}$ Translat. diffusion coefficient

$D = \frac{k_B T}{6\pi\eta r_S}$ Stokes-Einstein relation

→ Stokes radius

→ solvent viscosity (water: 1 mPa s = 1 cP))

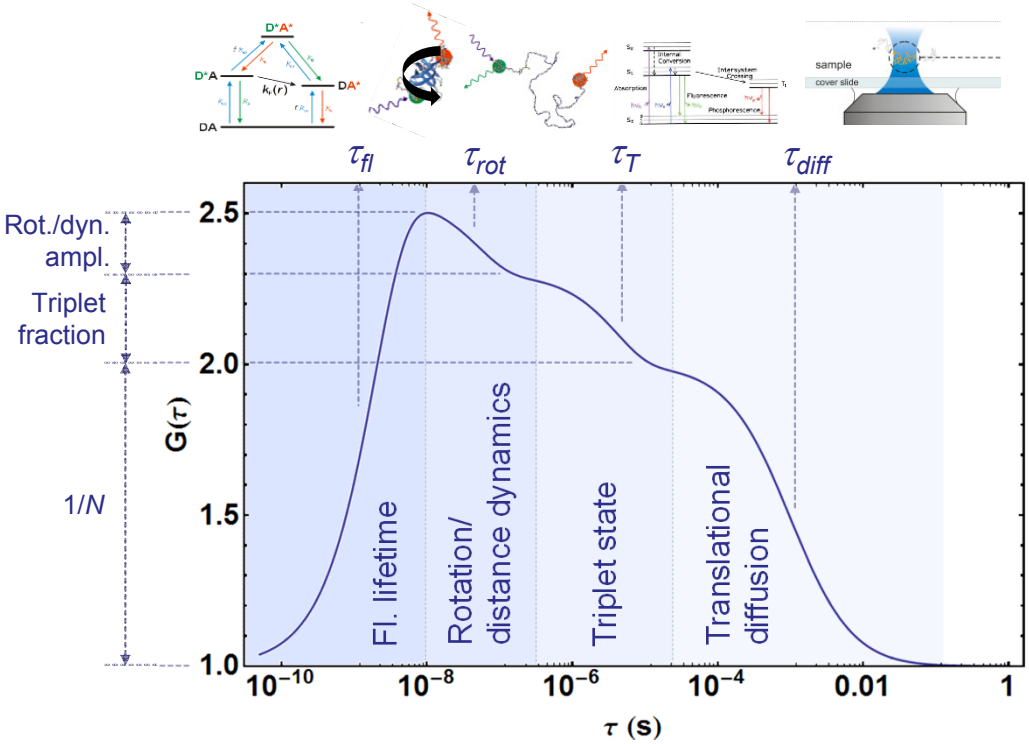
assume S = 5, ω_{xy} = 350 nm → calculate the Stokes radius and the concentration of the fluorescently labeled macromolecule

Processes detectable by FCS

Any process that leads to fluctuations of fluorescence intensity
on an accessible time scale will contribute to the correlation function.

Examples:

- *Translational diffusion* (~1 ms)
- *Photophysics*:
  triplet state blinking
  (intersystem crossing, ~1 μs),
  antibunching
  (fluorescence lifetime ~1 ns)
- *Rotational diffusion* (~1 to 100 ns)
- *Conformational dynamics*
  (FRET, quenching)
- *Molecular interactions*
  (binding → change in diffusion
  coefficient or crosscorrelation
  between different colors)



Monitoring processes on longer time scales than milliseconds typically requires immobilization → accessible time scales limited by photobleaching (>minutes for low excitation rates)

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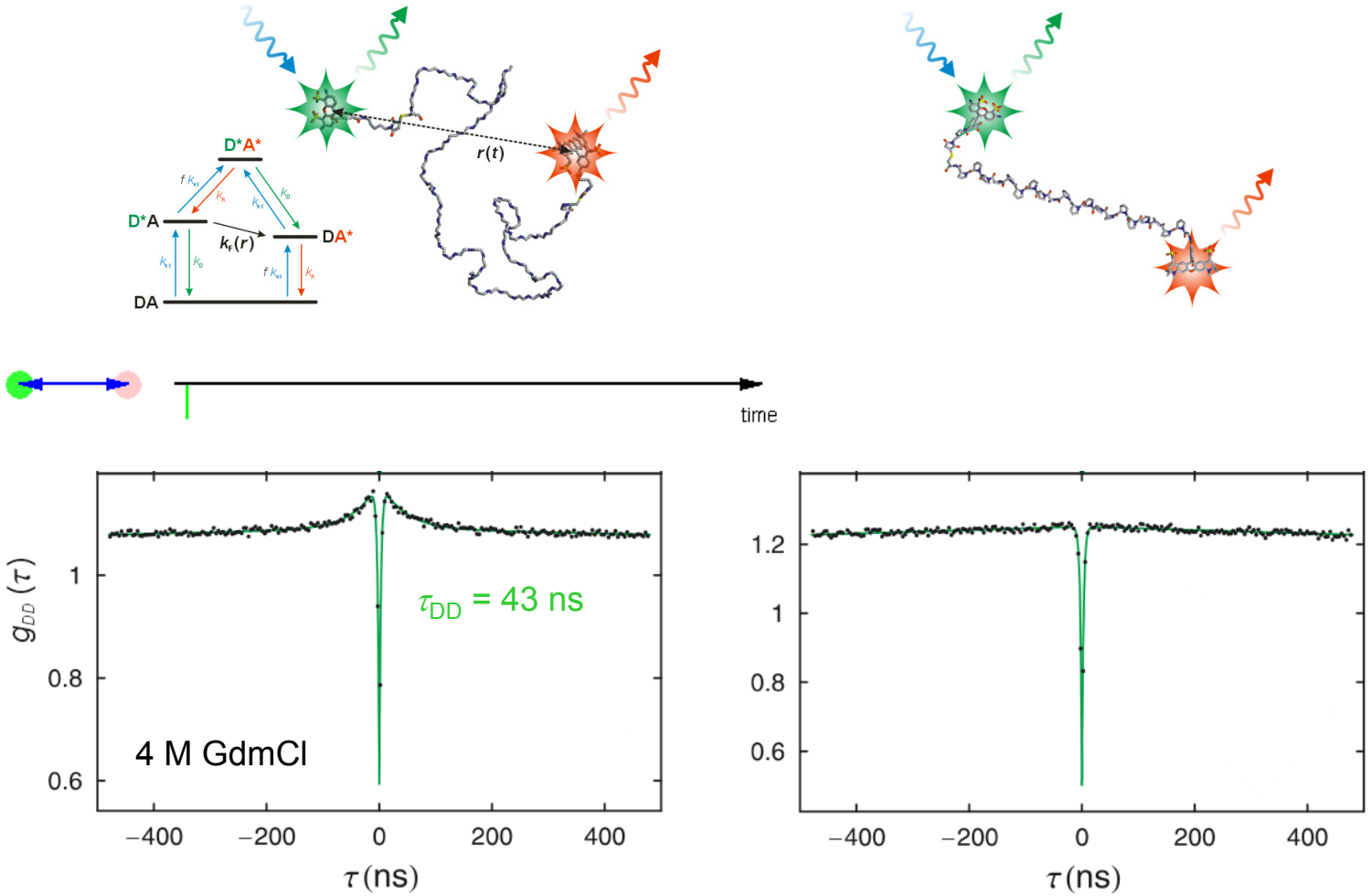
- Unfolded state dynamics from FCS

Dynamics from single molecule photon statistics

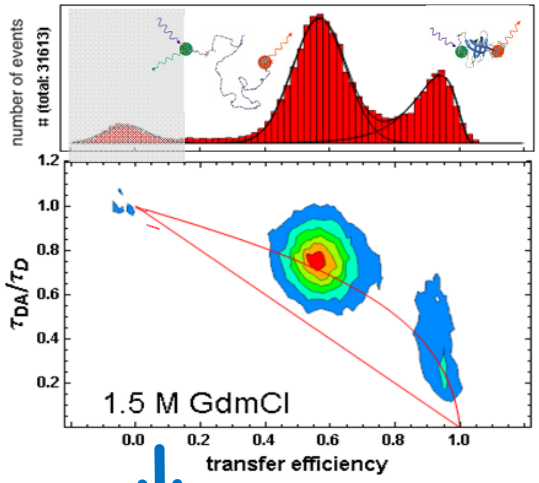


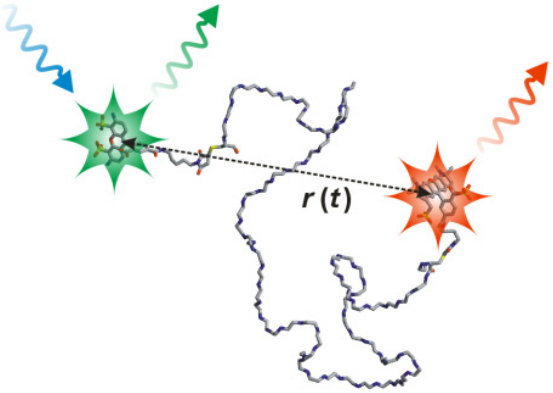
→ unfolded state dynamics are very rapid (close to Rouse/Zimm time)

→ how can the fluorescence intensity correlation functions be analyzed in terms of distance fluctuations (i.e. reconfiguration times or intramolecular diffusion coefficients)?

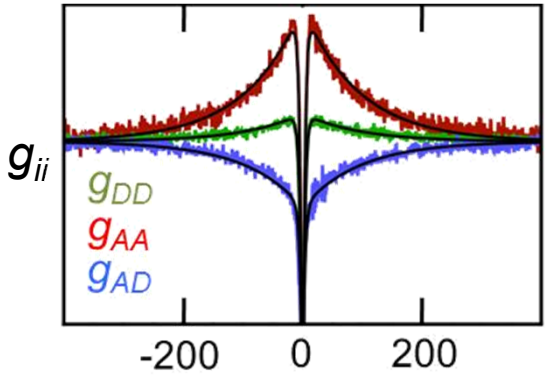
Distributions and dynamics from single-molecule FRET

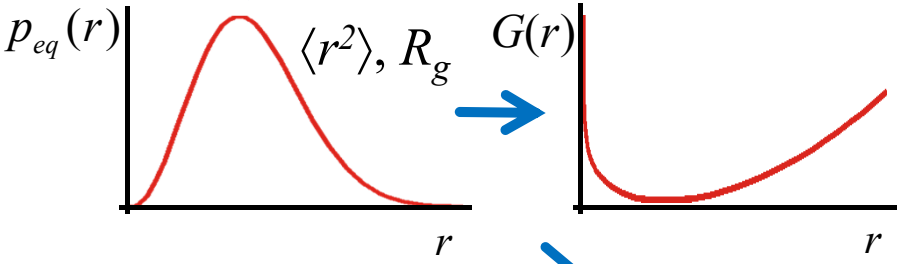
FRET efficiencies + lifetimes

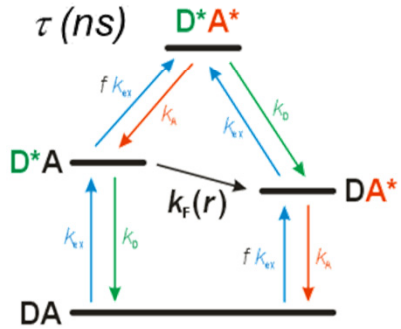




nanosecond fluorescence correlation spectroscopy (nsFCS)







$$\frac{\partial \mathbf{p}(r,t)}{\partial t} = \left[D \frac{\partial}{\partial r} p_{eq}(r) \frac{\partial}{\partial r} \frac{1}{p_{eq}(r)} \mathbf{I} + \mathbf{K_0}(r) \right] \mathbf{p}(r,t)$$

only free parameter

Nettels et al., PNAS 2007
Gopich et al., J Chem Phys 2009

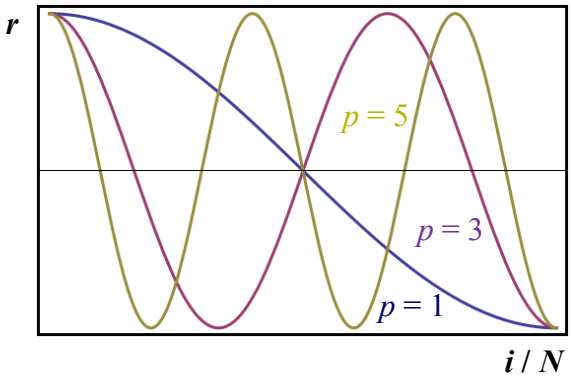
Unfolded state dynamics: the Rouse model

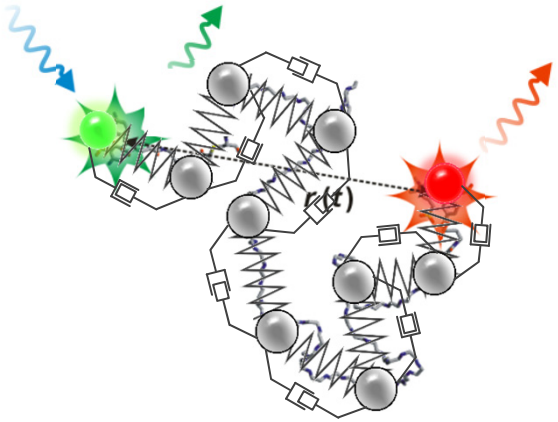
Rouse model with internal friction

$$-\zeta_i \boldsymbol{\kappa} \frac{d\mathbf{r}}{dt} - \zeta_s \frac{d\mathbf{r}}{dt} - \kappa_0 \boldsymbol{\kappa} \mathbf{r} + \mathbf{f}(t) = 0$$

Sum of relaxation modes

$$\langle \mathbf{r}(0)\mathbf{r}(t) \rangle \propto \sum_p \frac{1}{p^2} e^{-t/\tau_p}$$





Scaling of dynamics with chain dimensions

$$\tau_0 \propto \langle r^2 \rangle \eta$$

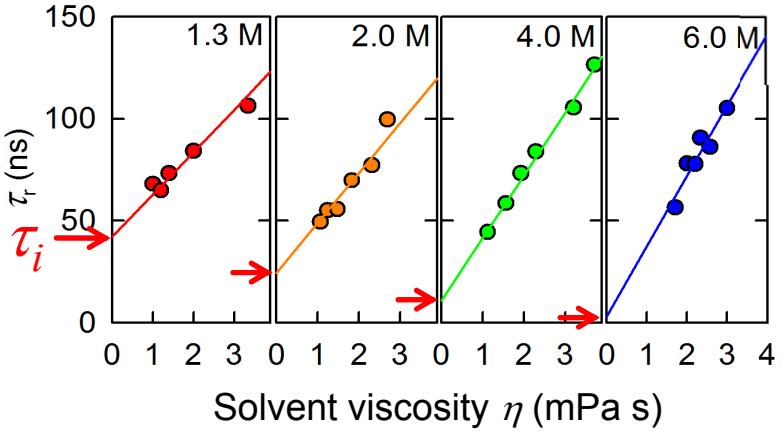
Internal friction time τ_i additive and independent of p

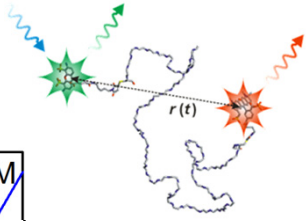
$$\tau_p = \frac{\tau_0}{p^2} + \tau_i$$

Cerf 1958
De Gennes 1979
Portman, Takada & Wolynes, 2001
Khatri & McLeish 2007

Quantifying internal friction in unfolded proteins

$$\tau_r = \tau_0(\eta) + \tau_i$$





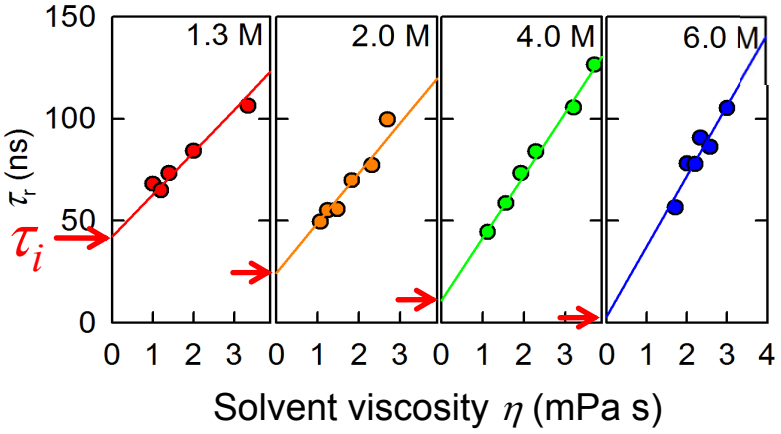
CspTm

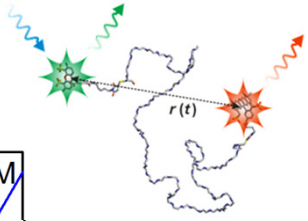
$$\langle \mathbf{r}(0)\mathbf{r}(t) \rangle \propto \sum_p \frac{1}{p^2} e^{-t/\tau_p}$$

Makarov, JCP 2010

Quantifying internal friction in unfolded proteins

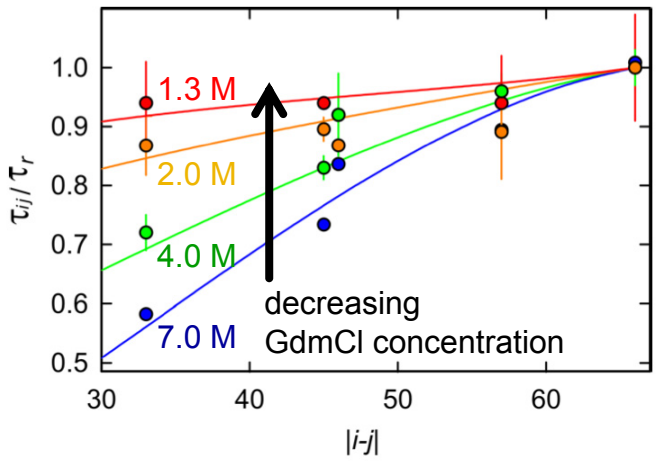
$$\tau_r = \tau_0(\eta) + \tau_i$$





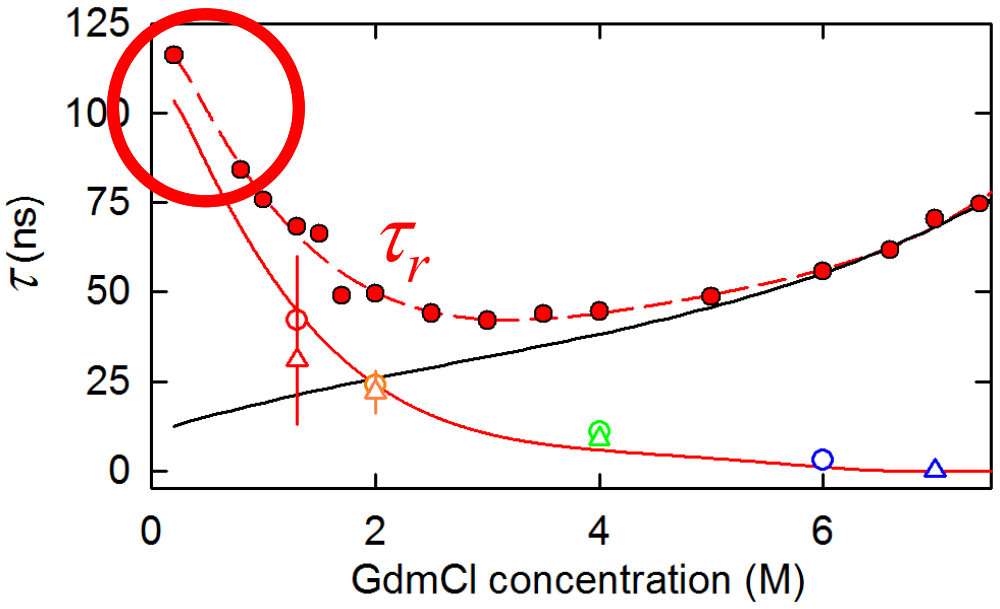
CspTm

$$\langle \mathbf{r}(0)\mathbf{r}(t) \rangle \propto \sum_p \frac{1}{p^2} e^{-t/\tau_p}$$



Internal friction dominates unfolded state dynamics under native conditions:

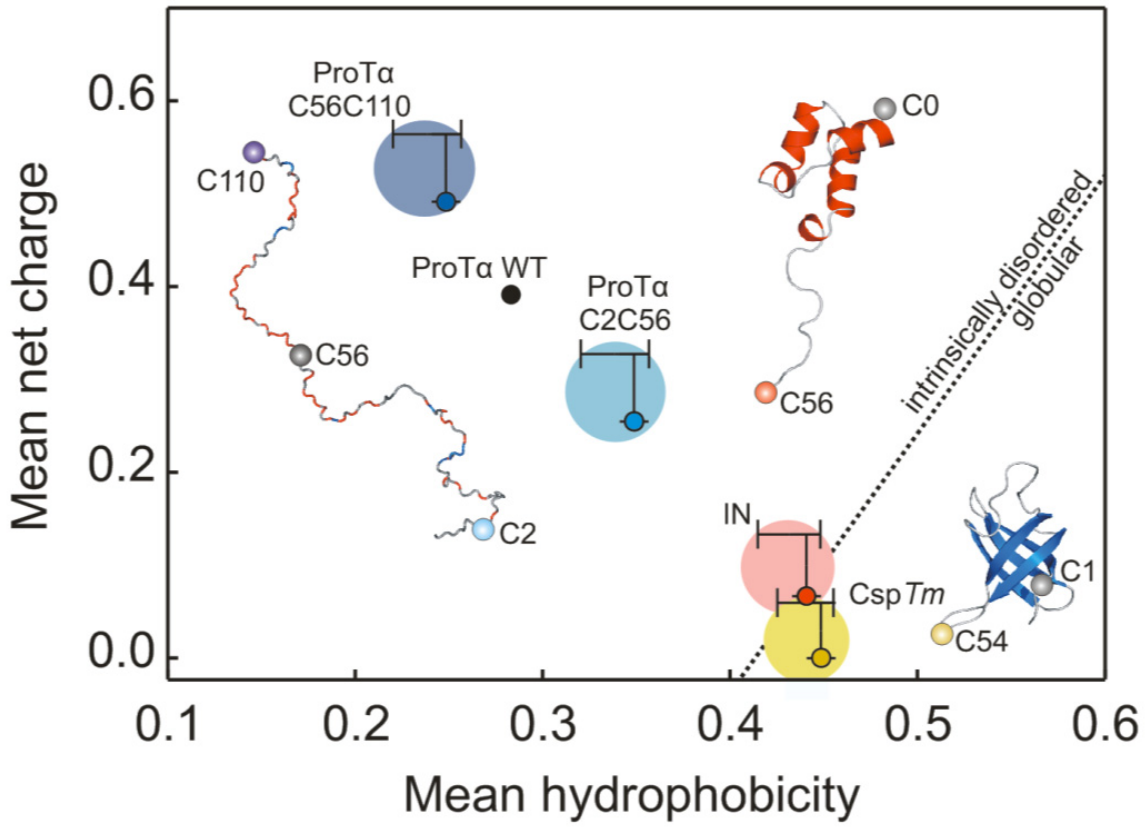
$$\tau_i \gg \tau_0$$



$$\tau_0 \propto \langle r^2 \rangle \eta$$

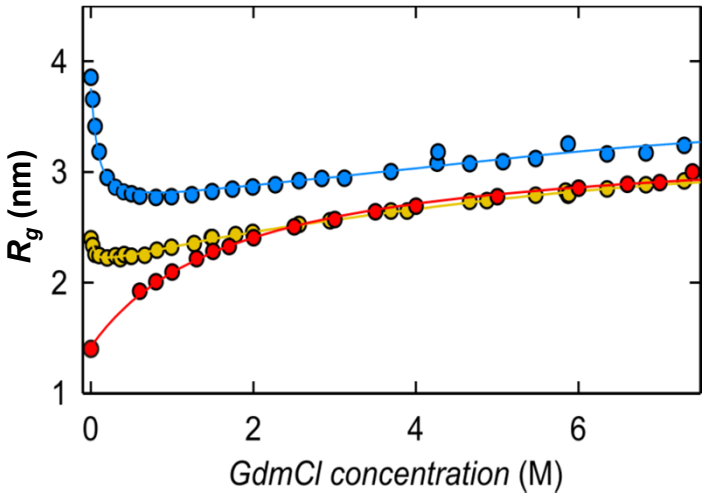
$$\tau_i = \tau_r - \tau_0(\eta)$$

Internal friction in intrinsically disordered proteins?



Internal friction in intrinsically disordered proteins?

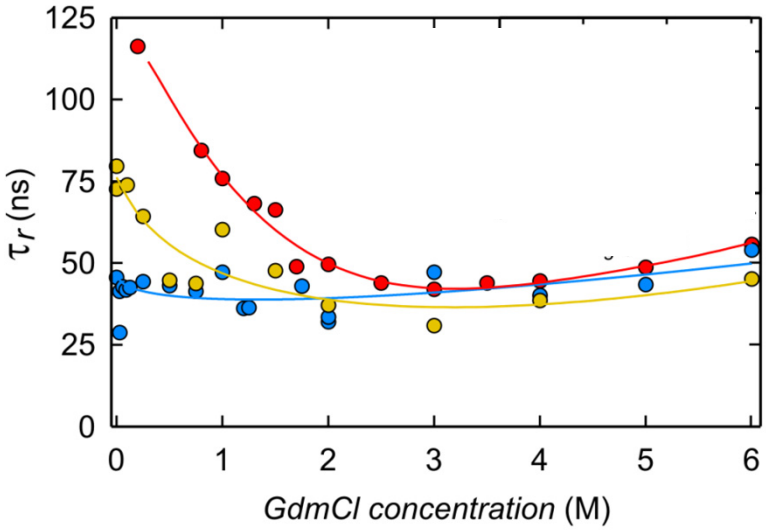
Dimensions

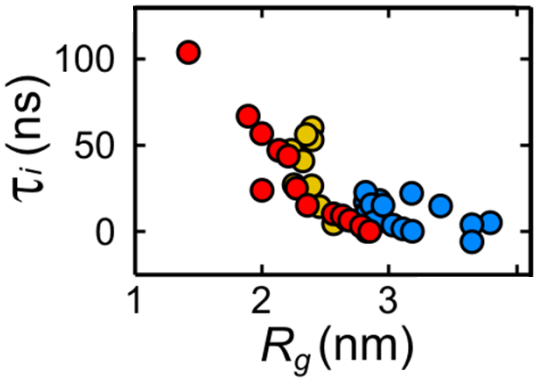


Müller-Späth *et al.* (2010) *PNAS 107,* 14609–14614

ProTα
Integrase
Csp

Dynamics





→ increased reconfiguration dynamics of charged IDPs

→ greater internal friction in more compact unfolded states

Soranno *et al.* (2012) *PNAS 109,* 17800–17806

Further Reading

Single-molecule FRET of protein structure and dynamics – a primer
Schuler B
J Nanobiotech 11(Suppl 1):S2 (2013)

Single-molecule detection and identification of multiple species by multiparameter fluorescence detection
Widengren J, Kudryavtsev V, Antonik M, Berger S, Gerken M, Seidel CAM
Anal Chem 78: 2039-2050 (2006)

Single-molecule spectroscopy of protein folding dynamics –
expanding scope and timescales
Schuler B, Hofmann H
Curr Opin Struct Biol, 23: 36-47 (2013)

Theory of Single-Molecule FRET efficiency histograms
Gopich I, Szabo A
Adv. Chem. Phys., 146: 245-297 (2012)