Single-Molecule FRET with Diffusion and Conformational Dynamics

Gopich and Szabo, 2007

Numerics IV – stochastic processes

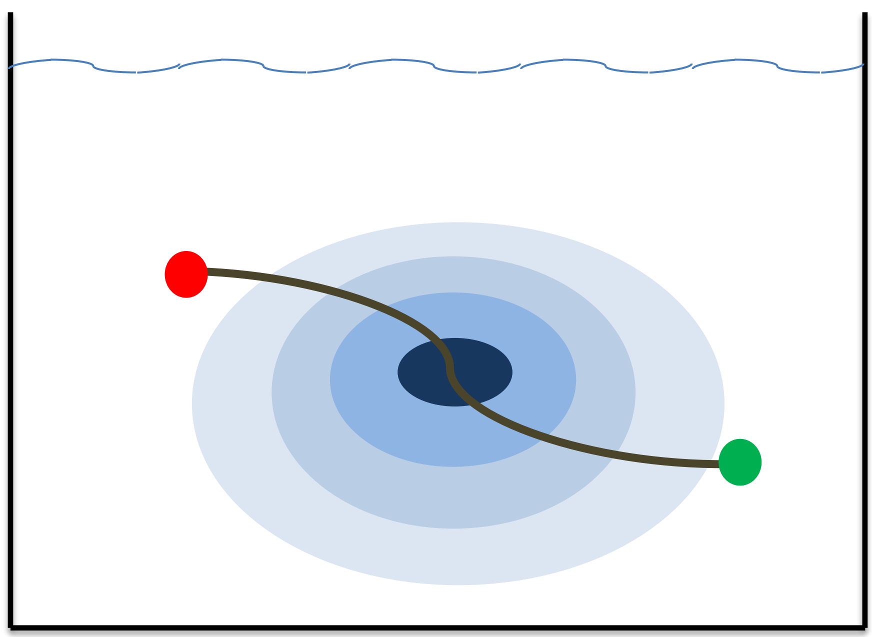
Franziska Kreuchwig

Outline

1. Conformational Dynamics and Diffusion
2. FRET
3. Rigorous Way
4. Approximative Way
   a) Diffusion without Dynamics
   b) Dynamics without Diffusion
   c) Dynamics AND Diffusion
5. FRET efficiency histograms

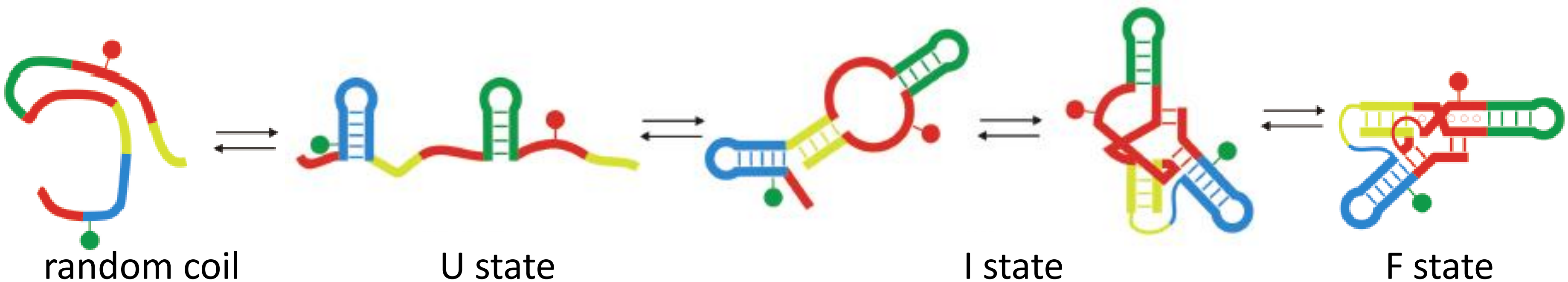
Aim

- Get information about conformational dynamics on diffusing molecules from FRET
- Without modelling diffusion



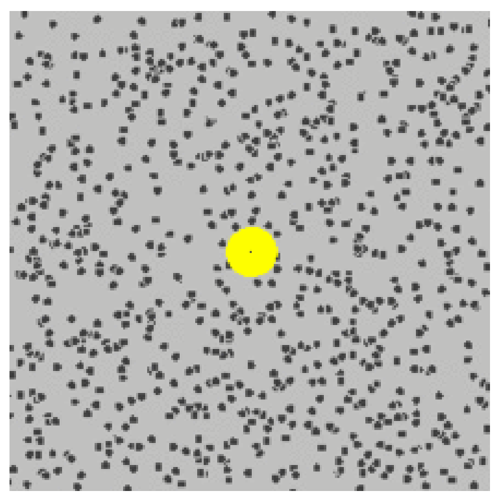
Conformational Dynamics & Diffusion

- Conformational Dynamics = change of shape



Kobitzki et al., 2007

- Diffusion = Brownian motion



wikipedia.org

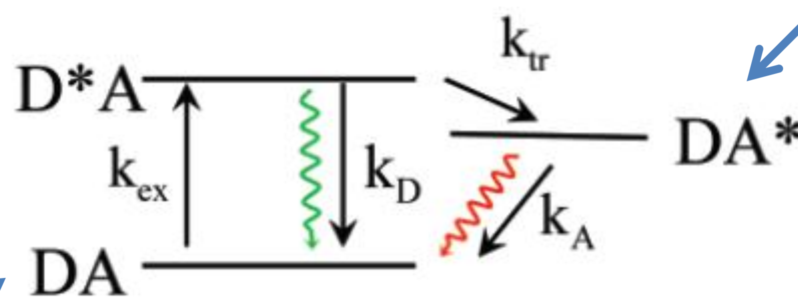
FRET

Förster (Fluorescence) resonance energy transfer

Sahoo et al., 2011

donor excited

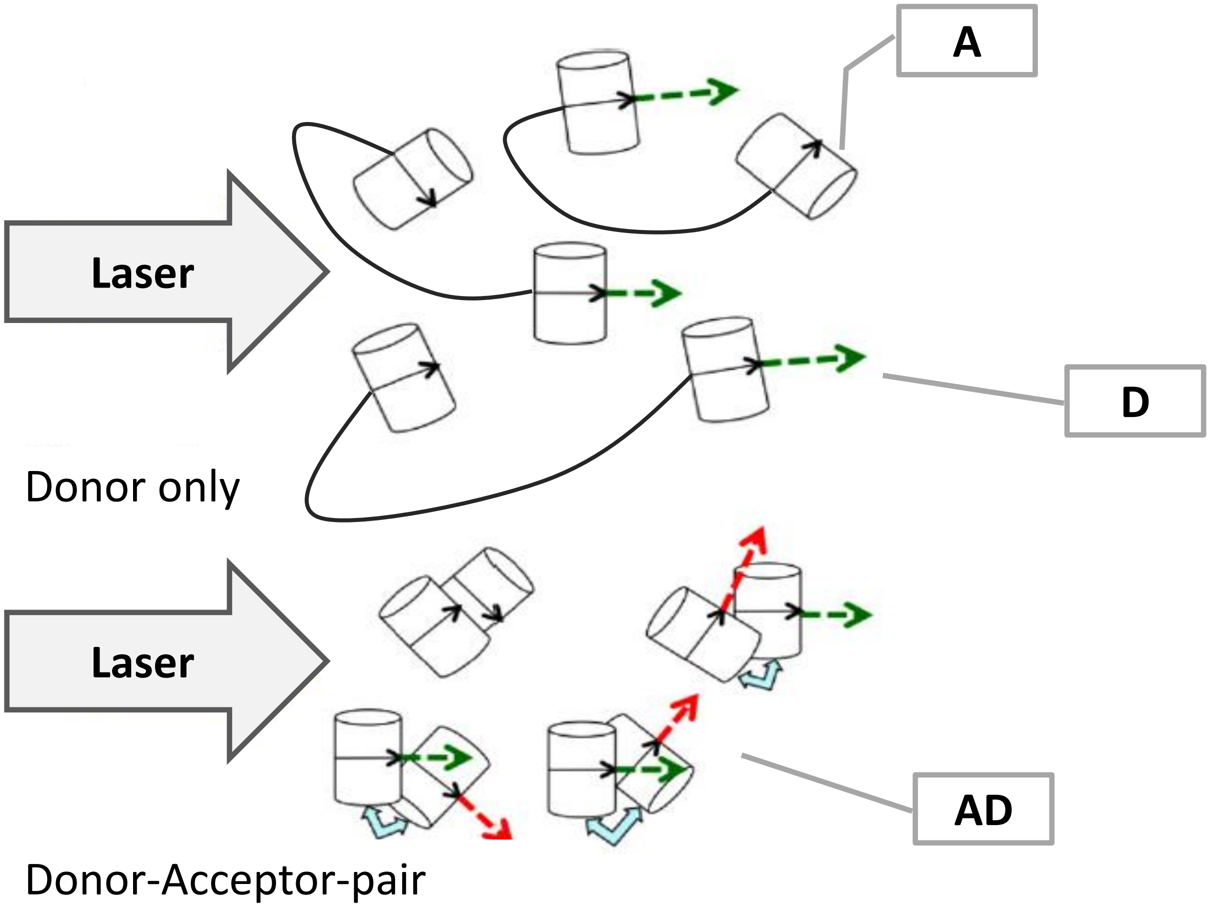
excitation transfer to acceptor



donor-acceptor-pair

Gopich et al., 2007

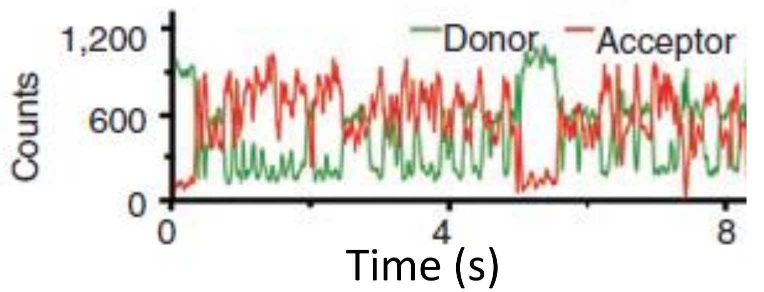
FRET – basic principle



Sahoo et al., 2011

Rate of transfer depends on donor-acceptor distance

Photon trajectory and FRET efficiency

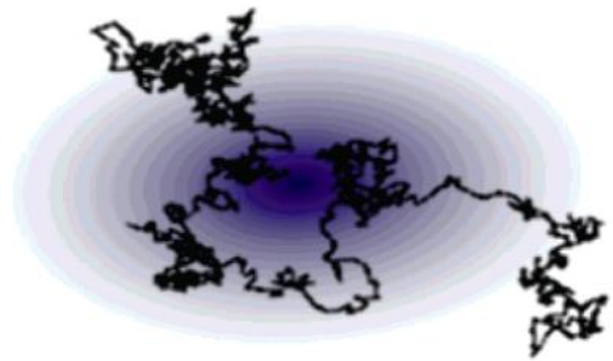


$$E = \frac{\#\, photons_A}{\#\, photons_{total}} \quad per\ time\ unit$$

$<E>$ mean FRET efficiency

Problems

- <E> influenced by
  - Photophysical processes
  - Energy transfer
  - Dye blinking



Gopich et al., 2007

- Fluorescence intensity influenced by diffusion through laser spot
- Random detection of emitted photons

Single-Molecule FRET with Diffusion and Conformational Dynamics

RIGOROUS WAY

Poisson-Process

- model countable, singular events in continuos time

$$P[(N(t+\Delta t)-N(t))=n]=\frac{k\Delta t^{n}}{n!}e^{-k\Delta t} \quad n\in \mathrm{N}$$

- k - expected # of events per unit time
- [t, t+Δt] - duration between two events

Joint Probability of detecting photons in time bin

- Poisson probability

$$P(N_A \mid T) = \frac{(n_A T)^{N_A}}{N_A!} e^{-n_A T} \qquad P(N_D \mid T) = \frac{(n_D T)^{N_D}}{N_D!} e^{-n_D T}$$

$$P(N_A, N_D \mid T) = \frac{(n_A T)^{N_A}}{N_A!} \frac{(n_D T)^{N_D}}{N_D!} e^{-(n_A + n_D)T}$$

- n_A / n_D – mean number of acceptor/donor photons per time unit

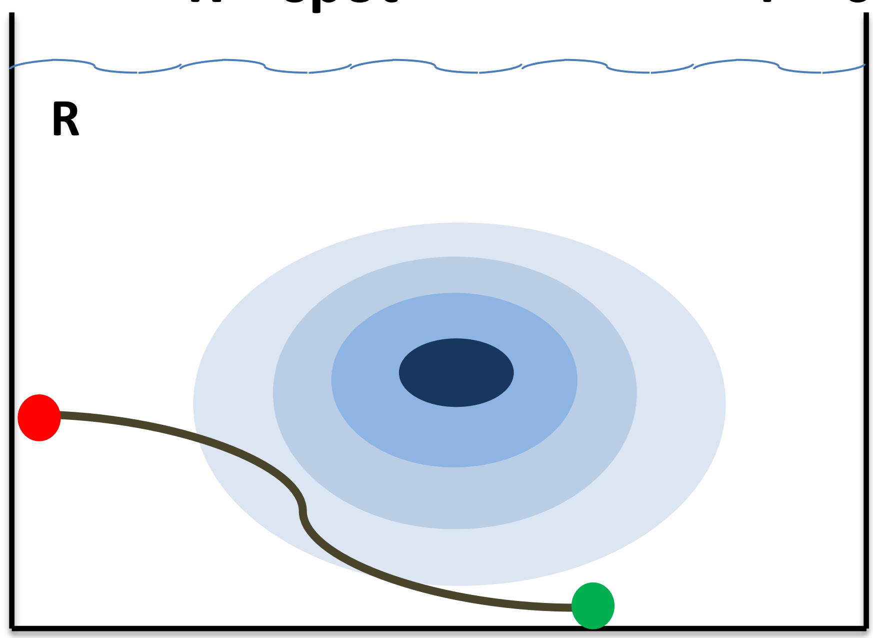
$$P(N_A, N_D \,|\, T) = \frac{(n_A T)^{N_A}}{N_A!} \frac{(n_D T)^{N_D}}{N_D!} e^{-(n_A+n_D)T}$$

$$P(N_A, N_D \,|\, T) = \frac{(nT)^{N_{AD}} N_{AD}!}{N_A! N_D! N_{AD}!} e^{-nT} \underbrace{\left(\frac{n_A}{n_A + n_D}\right)}_{<\mathrm{E}>}^{N_A} \left(1 - \underbrace{\left(\frac{n_A}{n_A + n_D}\right)}_{<\mathrm{E}>}\right)^{N_D}$$

$$P(N_A, N_D \,|\, T) = \frac{(nT)^{N_{AD}}}{N_{AD}!} e^{-nT} \frac{N_{AD}!}{N_A! N_D!} \langle E \rangle^{N_A} (1 - \langle E \rangle)^{N_D}$$

Diffusion and slow conformational dynamics

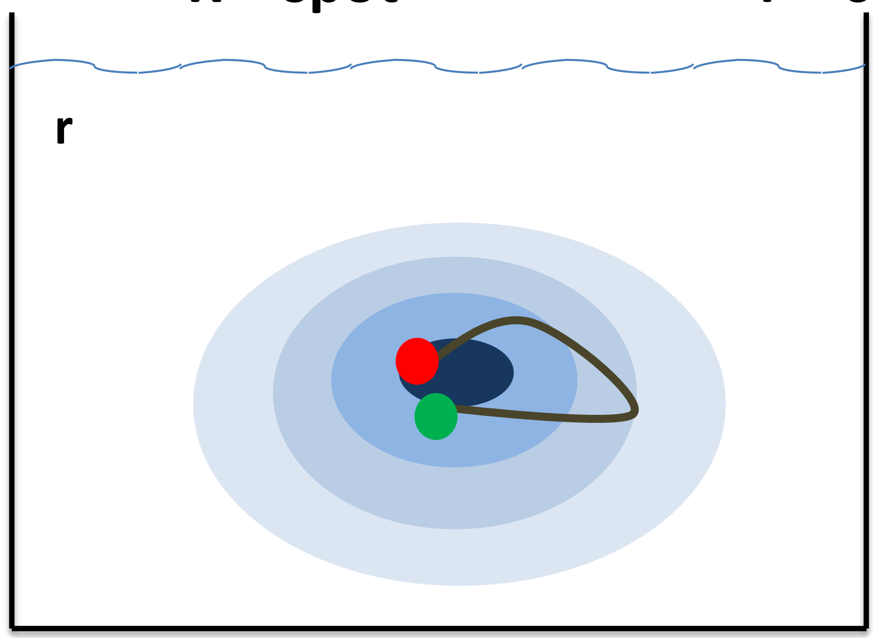
$$P(N_A, N_D \mid T) = \underbrace{\frac{(nT)^{N_{AD}}}{N_{AD}!} e^{-nT}}_{\text{R = spot}} \frac{N_{AD}!}{N_A! N_D!} \underbrace{\langle E \rangle^{N_A} (1 - \langle E \rangle)^{N_D}}_{\text{r = conformation}}$$



Solving the path integral too difficult!

Diffusion and slow conformational dynamics

$$P(N_A, N_D \mid T) = \underbrace{\frac{(nT)^{N_{AD}}}{N_{AD}!} e^{-nT}}_{\textbf{R = spot}} \frac{N_{AD}!}{N_A! N_D!} \underbrace{\langle E \rangle^{N_A} (1 - \langle E \rangle)^{N_D}}_{\textbf{r = conformation}}$$



Solving the path integral too difficult!

Single-Molecule FRET with Diffusion and Conformational Dynamics

STEPWISE APPROXIMATION

Conditions for approximation

$$P(N_A, N_D \mid T) = \underbrace{\frac{(nT)^{N_{AD}}}{N_{AD}!} e^{-nT}}_{\text{R}} \frac{N_{AD}!}{N_A! N_D!} \underbrace{\langle E \rangle^{N_A} (1 - \langle E \rangle)^{N_D}}_{\text{r}}$$

- Condition I

$$\langle E \rangle (r) = \frac{n_A(R,r)}{n_A(R,r) + n_D(R,r)}$$

- Condition II

$$n(R) = n_A(R,r) + n_D(R,r)$$

- Condition III

→ quasi-immobilized molecule during observation

time unit < diffusion time

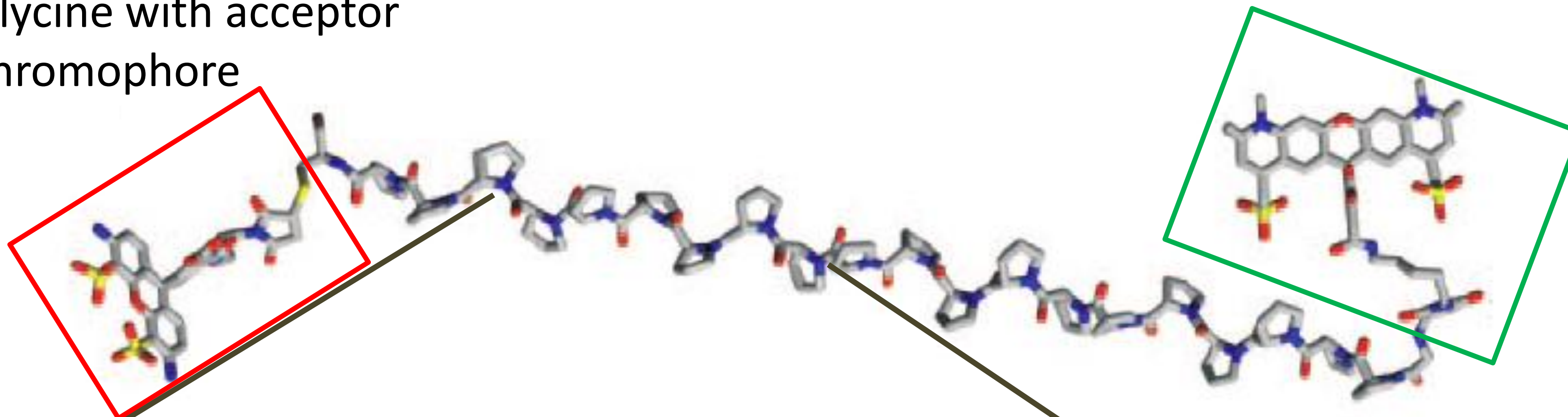
Single-Molecule Analysis

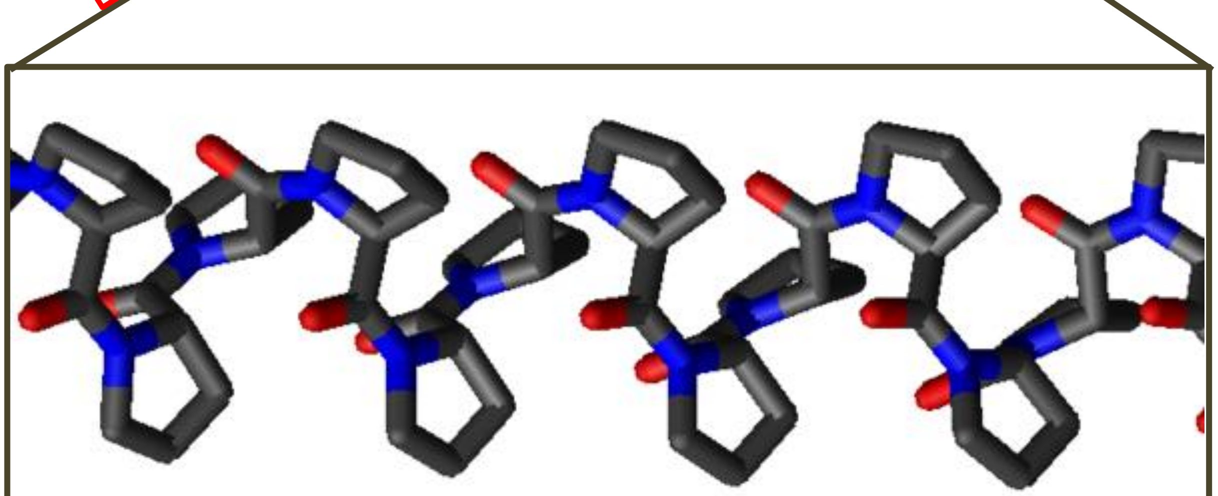
Only one conformation

Polyproline

Glycine with acceptor chromophore

Cysteine with donor chromophore





Schuler et al., 2005

- specific backbone dihedral angles

Single-Molecule Analysis

One conformation

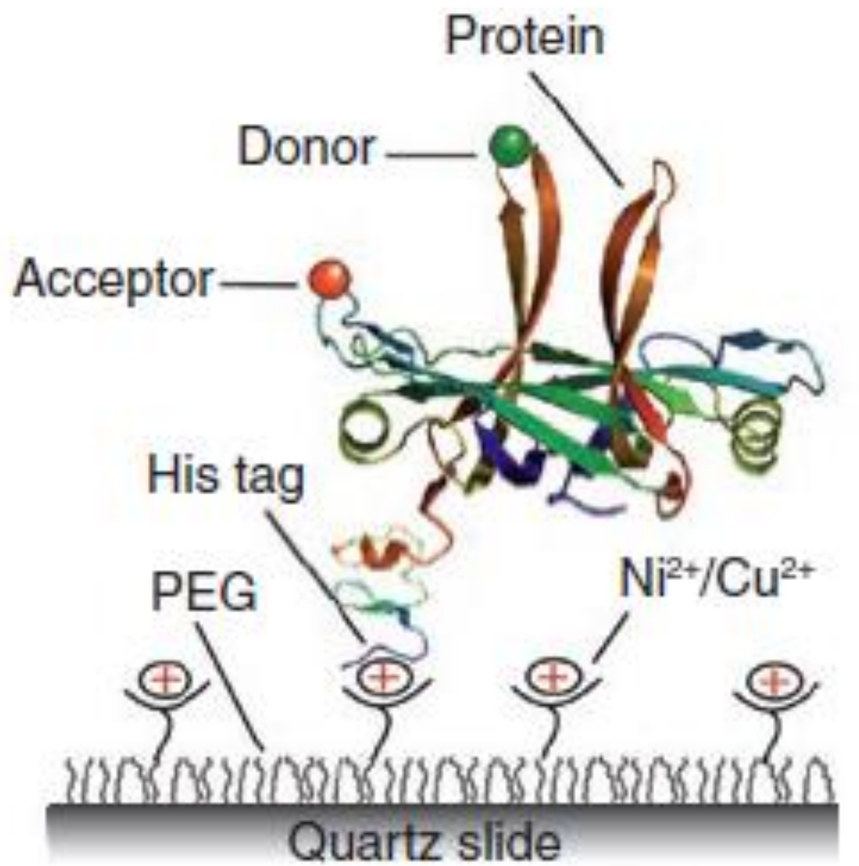
$$P(N_A, N_D \mid T) = \underbrace{\frac{(nT)^{N_{AD}}}{N_{AD}!} e^{-nT}}_{\text{R}} \frac{N_{AD}!}{N_A! N_D!} \underbrace{\langle E \rangle^{N_A} (1 - \langle E \rangle)^{N_D}}_{\text{r}}$$

$$P(N_A, N_D \mid T) = \underbrace{P(N_{AD} \mid T)}_{\textbf{variable}} \frac{N_{AD}!}{N_A! N_D!} \underbrace{\langle E \rangle^{N_A} (1 - \langle E \rangle)^{N_D}}_{\textbf{constant}}$$

$$P(N_{AD} \mid T) = \left\langle \frac{\left[\int_0^T n(t) dt \right]^{N_{AD}}}{N_{AD}!} e^{-\int_0^T n(t) dt} \right\rangle_R$$

Expected value of all paths

Single-Molecule Analysis
Immobilized protein – two-state system



Roy et al., 2008

Single-Molecule Analysis

Immobilized protein

$$P(N_A, N_D \mid T) = \underbrace{\frac{(nT)^{N_{AD}}}{N_{AD}!} e^{-nT}}_{\text{R}} \frac{N_{AD}!}{N_A! N_D!} \underbrace{\langle E \rangle^{N_A} (1 - \langle E \rangle)^{N_D}}_{\text{r}}$$

$$P(N_A, N_D \mid T) = \underbrace{\frac{(nT)^{N_{AD}}}{N_{AD}!}}_{\textbf{constant}} e^{-nT} \underbrace{B_c(N_A, N_D \mid T)}_{\textbf{variable}}$$

$$B_c(N_A, N_D \mid T) = \frac{N_{AD}!}{N_A! N_D!} \underbrace{\int_0^1 \langle E \rangle^{N_A} (1 - \langle E \rangle)^{N_D} P_C(\langle E \rangle \mid T) d\langle E \rangle}_{\textbf{Expected value of all conformations}}$$

Single-Molecule Analysis

Conformational Dynamics **and** Diffusion

$$P(N_A, N_D \,|\, T) = \boxed{P(N_{AD} \,|\, T)} \frac{N_{AD}!}{N_A! N_D!} \langle E \rangle^{N_A} (1 - \langle E \rangle)^{N_D}$$

Diffusion

$$P(N_A, N_D \,|\, T) = \frac{(nT)^{N_{AD}}}{N_{AD}!} e^{-nT} \boxed{B_c(N_A, N_D \,|\, T)}$$

Conformational Dynamcis

$$P(N_A, N_D \,|\, T) \approx P(N_{AD} \,|\, T) B_c(N_A, N_D | T)$$

Single-Molecule FRET with Diffusion and Conformational Dynamics

APPLICATION

FRET efficiency histograms

Sum over all photon counts > threshold N_T

Use one of the previously presented equations

$$FEH(E) = \mathcal{N}^{-1} \sum_{N_{AD}=N_T}^{\infty} \sum_{N_A=[(E-h/2)N_{AD}]+1}^{[(E+h/2)N_{AD}]} P(N_A, N_{AD}-N_A \mid T)$$

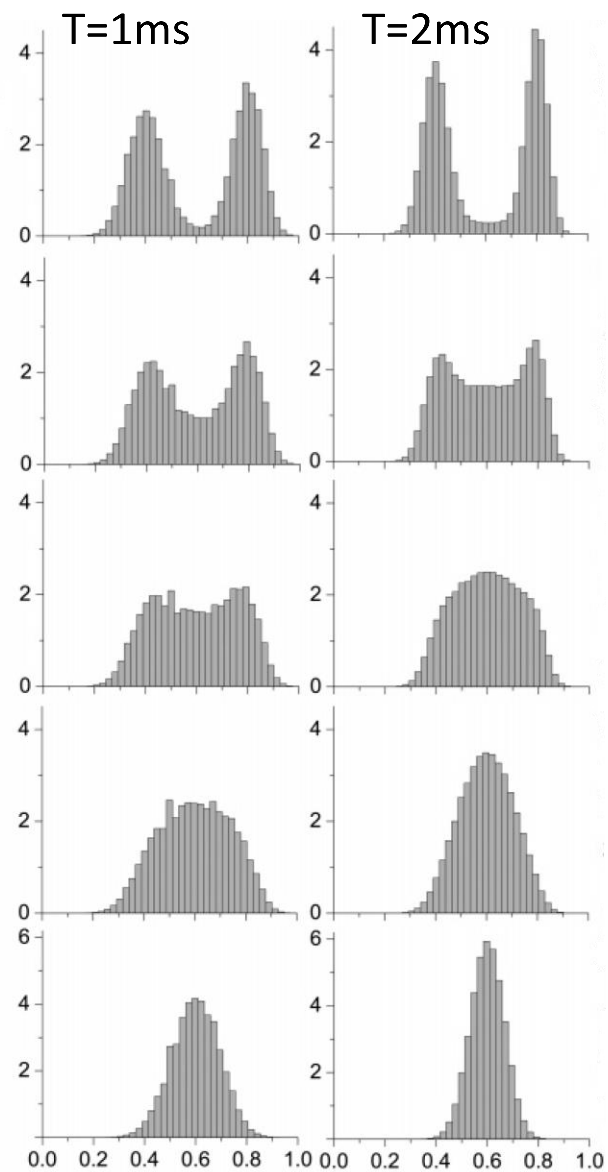
Normalization factor

Determination of step size h
all acceptor counts in interval
[(E- h/2),(E+ h/2)]

FRET efficiency histograms

Bin size

Relaxation rates



k = 0.1 ms^{-1}

kT << 1

k = 1 ms^{-1}

kT ≈ 1

k = 2 ms^{-1}

kT > 1

k = 4 ms^{-1}

kT >> 1

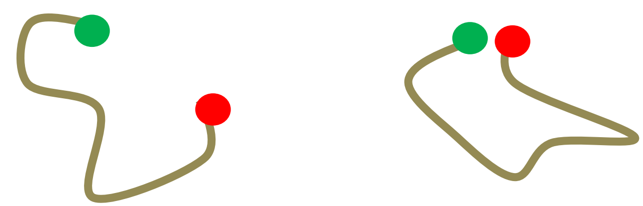
k = 20 ms^{-1}

kT >> 1

- 2 conformations

→different FRET efficiencies

$E_1 = 0.4$ and $E_2 = 0.8$



- Interconversion rates

$k = k_1 + k_2$ $(k_1 = k_2)$

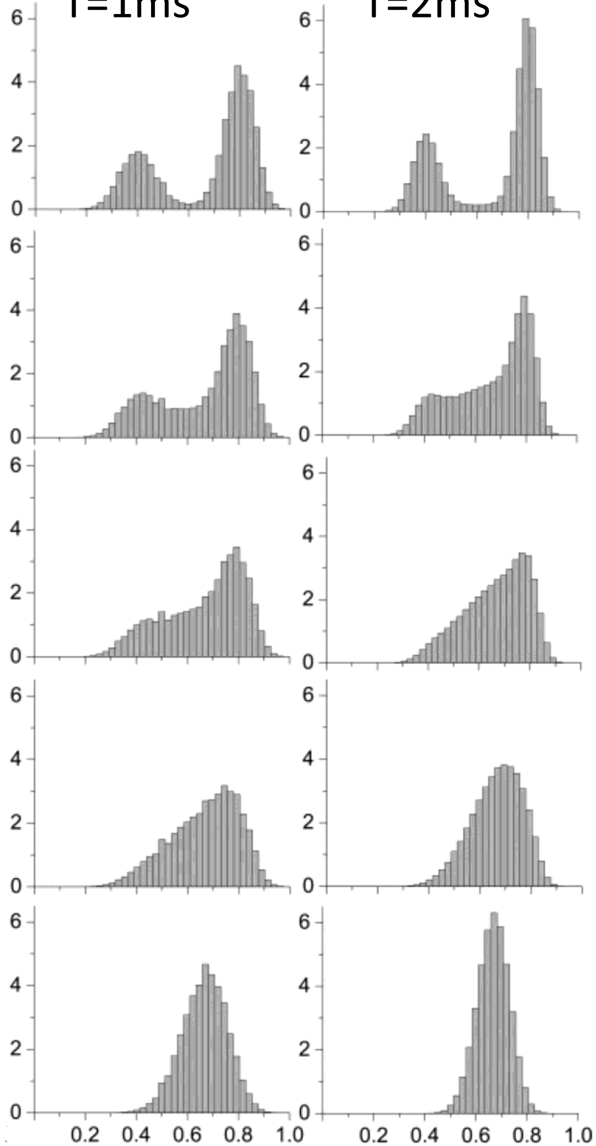
Gopich et al., 2007

FRET efficiency histograms

Bin size

T=1ms T=2ms

Relaxation rates



$k = 0.1\ ms^{-1}$

$k = 1\ ms^{-1}$

$k = 2\ ms^{-1}$

$k = 4\ ms^{-1}$

$k = 20\ ms^{-1}$

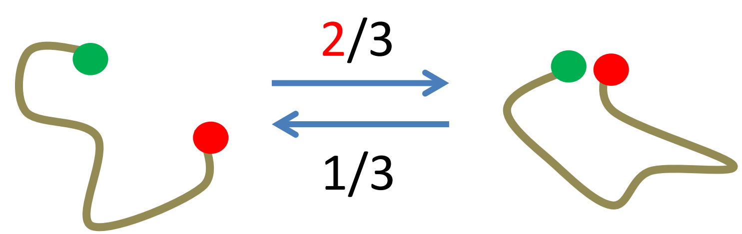
- 2 conformations

→different FRET efficiencies

$E_1 = 0.4$ & $E_2 = 0.8$

- Interconversion rates

$k = k_1 + k_2 \qquad (k_1 = 2k_2)$



Gopich et al., 2007

Summary

Rigorous theory

→ Not easy to solve

→ Path integral

→ More than two-states

Approximative theory

→ 3 conditions must hold

→ very simplified (only two-states)

→ often used

Further work

→ Decoding photon color patterns by using a maximum likelihood method (more than two-states)

References

- Gopich et al., J. Phys. Chem. 2007 *„Single-Molecule FRET with Diffusion and Conformational Dynamics*“
- Schuler et al., PNAS 2005 *„Polyproline and the ‘‘spectroscopic ruler’’ revisited with single-molecule fluorescence*“
- Roy et al., Nature Methods 2008 *„A practical guide to single-molecule FRET*“
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