Application of the Maximum Entropy Method in time-resolved FRET measurements to reconstruct distance distributions for donor-acceptor pairs

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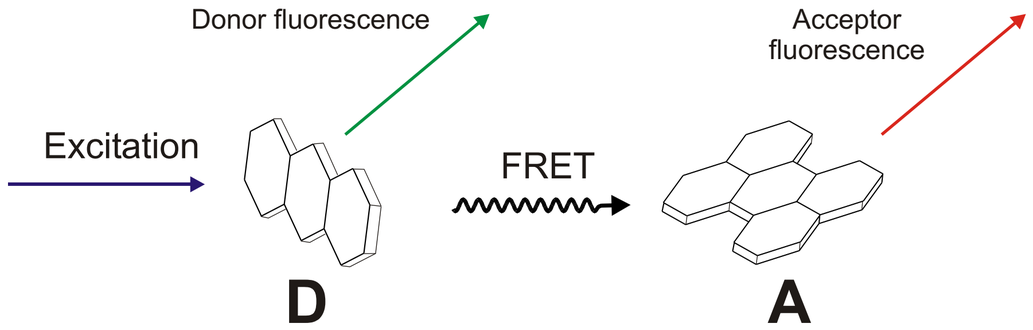
Forster resonance energy transfer (FRET) method is widely used in fluorescence spectroscopy. As a result of energy transfer both intensity and kinetics of fluorescence for donor and acceptor molecules change significantly. Therefore measurements of fluorescence decay can provide important information about distance between donor and acceptor and their mutual orientation.

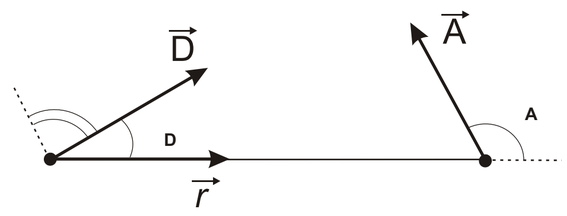


$$D^* + A \xrightarrow{FRET} D + A^* \qquad k_{FRET} \quad \frac{1}{\tau_D} \left(\frac{R_0}{r}\right)^6$$

τ_D - fluorescence decay lifetime of donor in the absence of acceptor,
R_0 - Forster radius.

$$R_0 \quad 0.2108\left[\kappa^2 n^{-4} \int_0^{\infty} I_D(\lambda)\varepsilon_A(\lambda)\lambda^4 d\lambda\right]^{1/6}$$

$$\kappa^2 \quad (\cos\theta \quad 3\cos\theta_D \cos\theta_A)^2$$



The case, when donor and acceptor are connected by flexible covalent linkage, is considered in this work.
Donor-acceptor distance for the ensemble of molecules can be characterized by $P(r)$ distribution and fluorescence decay law for the donor molecule in the presence of acceptor is following

$$F_{DA}(t) \quad F_0 \int_0^{\infty} P(r) \exp\left(-\frac{t}{\tau_{DA}}\right) dr \qquad \tau_{DA} \quad \frac{\tau_D}{1 \quad (R_0/r)^6}$$

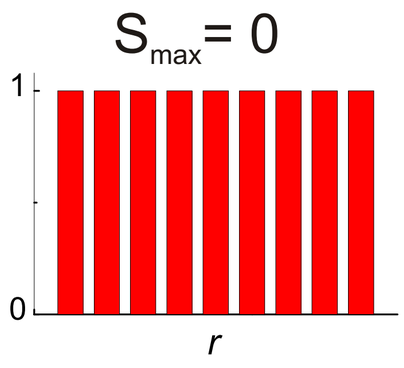
Decay law functions for the fluorescence of donor and acceptor molecules have complicated character and their reconstruction without *a priori* assumptions represents ill-posed mathematical problem and requires regularization methods.
Additional difficulty is the fact that experimentally observed decay curve $I_{exp}(t)$ represents convolution of decay law function $F(t)$ and instrument response function $L(t)$

$$I_{\exp}(t) \quad L(t) \quad F(t) \quad \int_0^t L(t')F(t \quad t')dt'$$

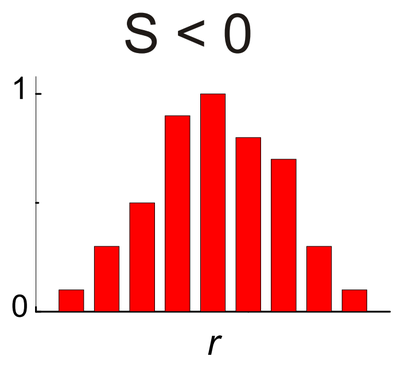
Maximum Entropy Method (MEM) was used to determine $P(r)$ distribution without *a priori* assumptions. According to MEM ideology [1] such a function $P(r)$ must be selected among possible distributions, that equally good describe decay law $F(t)$, which maximizes the entropy function S

$$S= \quad \sum_i P(r_i) - m(r_i) - P(r_i)\log\frac{P(r_i)}{m(r_i)}$$

where m(r)- initial model.



Equiprobable distribution of donor-acceptors pairs in 1D-space



Structured distribution of donor-acceptors pairs in 1D-space

This requirement can be fulfilled by maximizing the following functional

$$S \quad \chi^2 \quad 1$$

where $\chi^2 \quad \sum_i W(t_i)\left[I_{\exp}(t_i) \quad I_{calc}(t_i)\right]^2$ - Pearson parameter,

$W(t)$ - statistical weight, α - regularization parameter.

Capability of the elaborated method to reconstruct $P(r)$ function was tested in model calculations, where $P(r)$ was represented by mono- and bimodal Gaussian distributions (Fig.1).

$$P(r) \quad \sum_{i \ 1}^{2} \alpha_i \exp\frac{(r \quad \bar{r}_i)^2}{2\sigma_i^2}$$

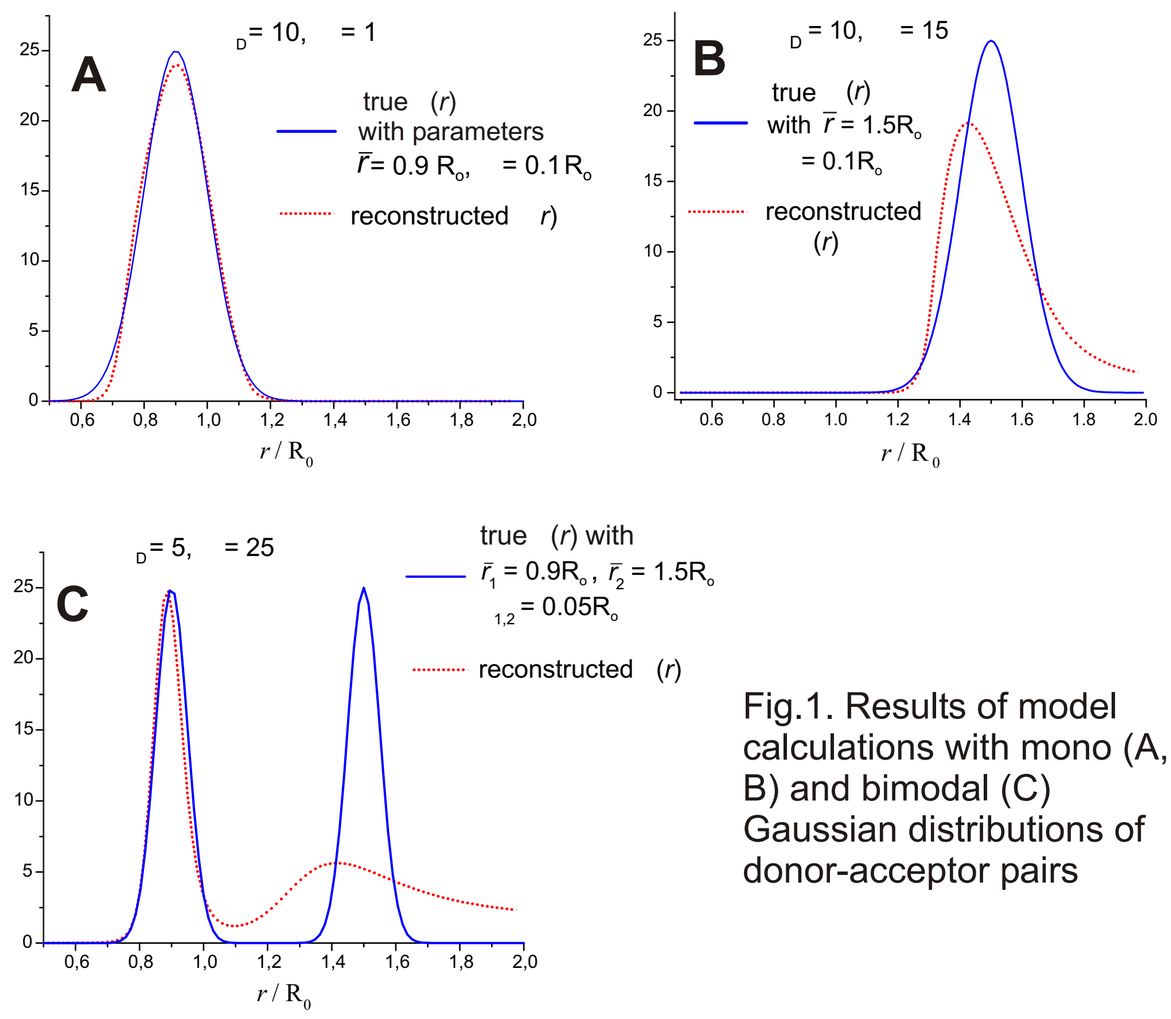


Fig.1. Results of model calculations with mono (A, B) and bimodal (C) Gaussian distributions of donor-acceptor pairs

One can see that recovered parameters of peaks (position, area under the peak) do not differ from the true ones by more than 2-5%. Application of the developed method to study protein structure and dynamics was demonstrated (Fig.2,3) for human serum albumin labeled with pyridoxal-5'-phoshate (PLP).

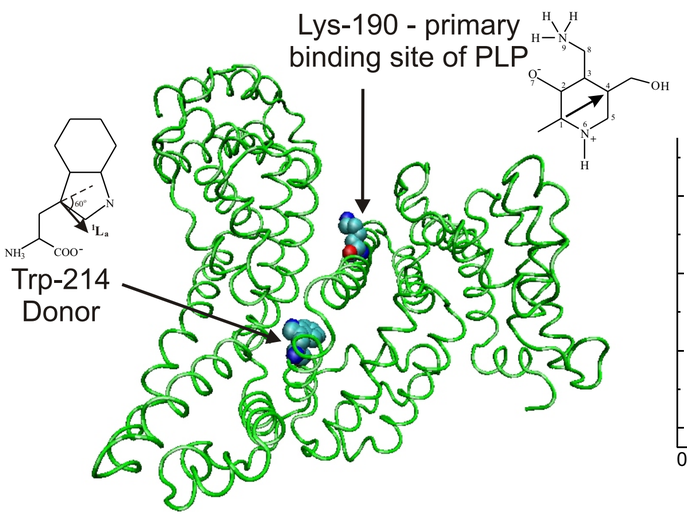


Fig.2. Spatial structure of human serum albumin (1ao6.pdb). Energy donor (Trp-214) and primary site for acceptor (PLP) binding are indicated.

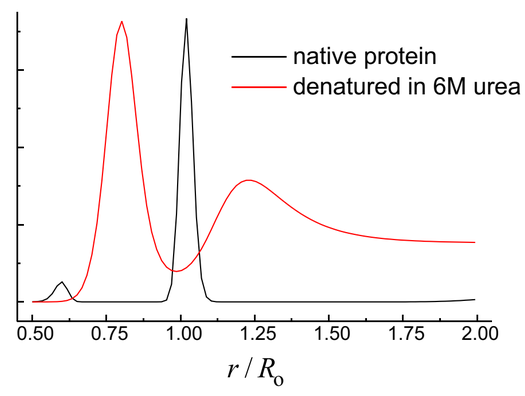


Fig.3. Recovered distance distributions between donor and acceptor for native (black) and denatured (red) protein.

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[1]. J.-C. Brochon // Methods Enzymol.- 1994.- V.240.- P. 262-311.