

Time-resolved Fluorescence Resonance Energy Transfer on DNA duplexes



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Abstract

Fluorescence resonance energy transfer (FRET) is an established technique to determine distances within biopolymers. FRET is based on the radiationless long-range dipolar coupling of two fluorescent dyes, the so-called donor and acceptor fluorophores. The FRET efficiency depends on the inter-fluorophore distance; distances accessible via FRET range between 10 and 100 Å. In time-resolved FRET experiments, the distance information is retrieved from the analysis of fluorescence emission decays of the donor fluorophore in the absence and presence of the acceptor fluorophore.

Here we report distance measurements using our home-built time-resolved FRET setup. Excitation is performed using the frequency-doubled output of a pulsed titanium:sapphire laser, and the nanosecond decay profile of the donor is measured via time-correlated single photon counting. Calibration with a series of donor-acceptor labelled DNA molecules yields inter-fluorophore distances in good agreement with calculated values based on standard DNA B-form geometry and the chemistry of fluorophore attachment. Furthermore, multiple distance distributions for various mixtures of DNA molecules of different lengths can be extracted correctly from the donor decays.

References

- D. Klostermeier & D. P. Millar *Time-resolved fluorescence resonance energy transfer: A versatile tool for the analysis of nucleic acids* Biopolymers 61, 159 (2002)
- A. A. Deniz, M. Dahan, J. R. Grunwell, T. Ha, A. E. Faulhaber, D. S. Chemla, S. Weiss & P. G. Schultz *Single-pair fluorescence resonance energy transfer on freely diffusing molecules: Observation of Förster distance dependence and subpopulations* PNAS 96, 3670 (1999)

Experimental setup

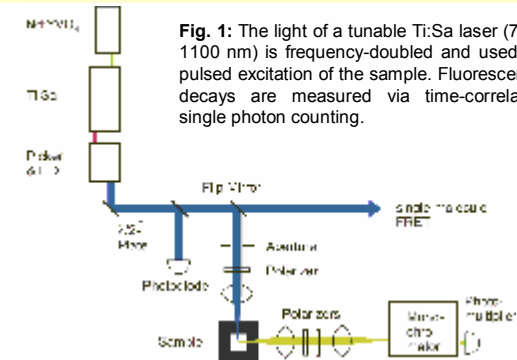
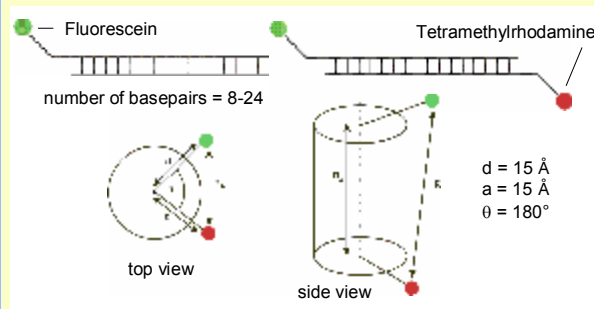


Fig. 1: The light of a tunable Ti:Sa laser (700-1100 nm) is frequency-doubled and used for pulsed excitation of the sample. Fluorescence decays are measured via time-correlated single photon counting.

DNA duplexes



Donor decays of a 8bp DNA duplex

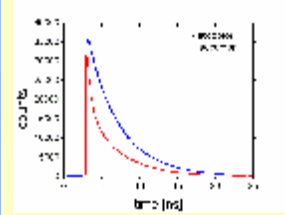


Fig. 2: The donor fluorescence decays more rapidly in the presence of the acceptor fluorophore.

$$I_D(t) = I_0 \sum \alpha_i \cdot \exp\{-t/\tau_{D_i}\}$$

$$I_{DA}(t) = I_0 \int P(R) \sum \alpha_i \cdot \exp\{-t/\tau_{D_i}(1+R^6/R_0^6)\} dR$$

R : distance between fluorophores
 R_0 : Förster distance
 τ_{D_i} : individual donor lifetimes
 α_i : decay amplitudes
 P(R) : donor-acceptor distance distribution
 $P(R) = 4\pi R^2 \cdot \exp\{-(R-\mu)^2/2\sigma^2\}$

Inter-dye distances and transfer efficiency

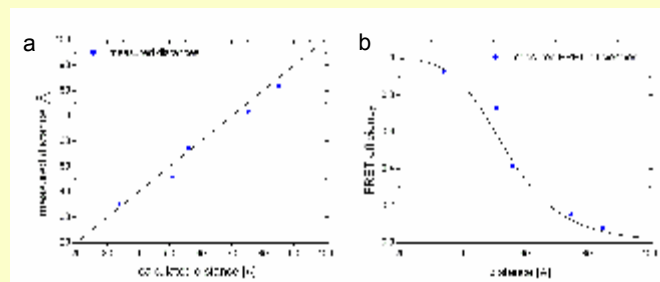


Fig. 3a: The measured distances (closed circles) are displayed in comparison to the calculated distances of the two fluorophores.

Fig. 3b shows the distance dependence of the FRET efficiency for the fluorophores fluorescein and tetramethylrhodamine (solid line). The closed circles display the efficiencies measured from the DNA duplexes.

Mixtures of DNA duplexes of different lengths

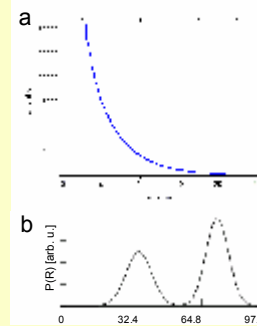


Fig. 4a: Donor decay in the presence of the acceptor for a mixture (50% 8bp duplex, 50% 20bp duplex).

Fig. 4b: For this mixture, the fit yields one distribution centered at 37Å which represents the 8bp DNA duplex, and a second one centered at 72.6Å for the 20bp DNA.

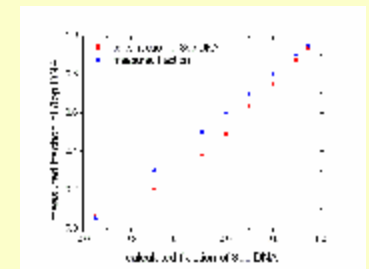


Fig. 5: Results for different mixtures of 8bp and 20bp DNA duplexes.

Anisotropy decays of dsRNA

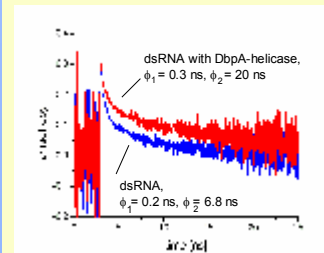


Fig. 6: Anisotropy decays of labelled dsRNA show an increase in the global rotational correlation time (ϕ_2) upon binding of the helicase DbpA.

Outlook

Having established the possibilities and limitations of our time-resolved FRET set-up we will now employ this technique to identify functional conformers of proteins that are involved in the modulation of nucleic acid structures, such as helicase and topoisomerases.

Acknowledgements

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