Coulombic Coupling in FRET Experiments

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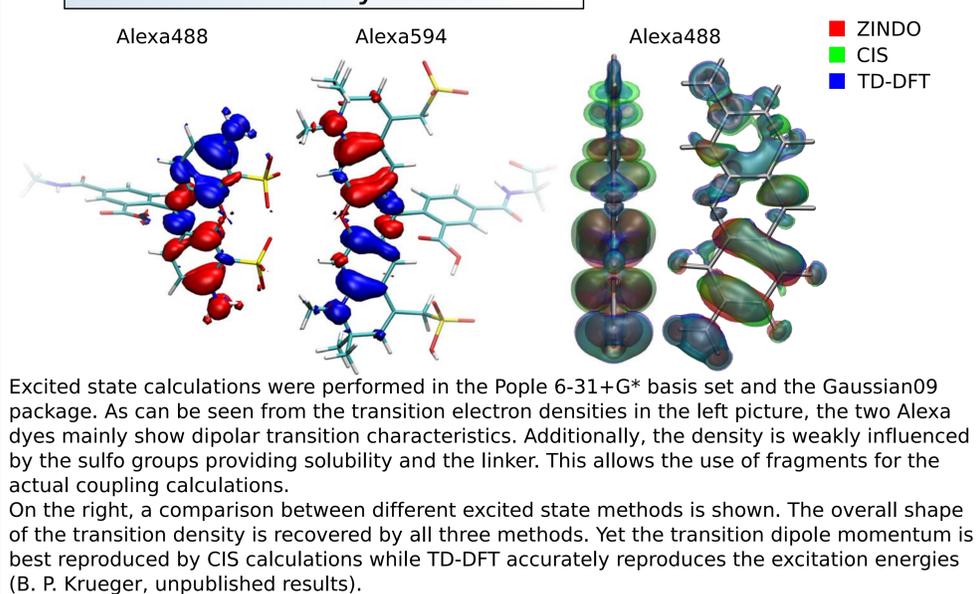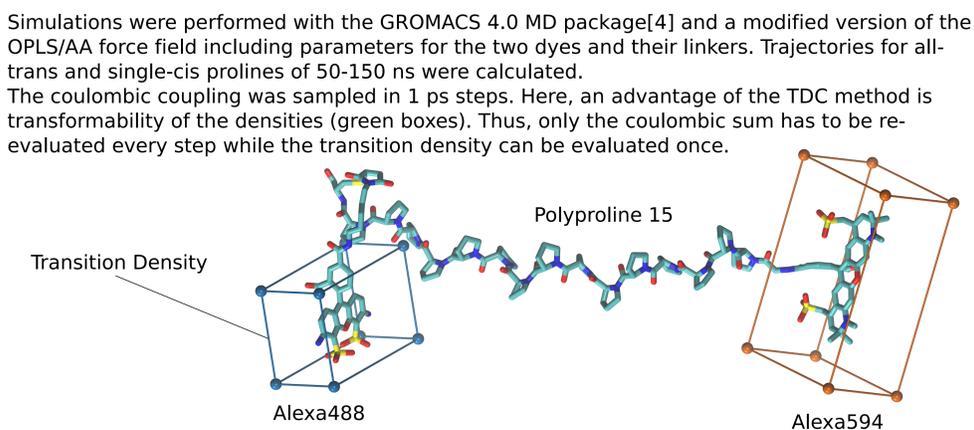### - MPI for Biophysical Chemistry, Göttingen -

Introduction

The possibility to attach dyes at distinct positions in biomolecules led to a rebirth of Förster / Fluorescence Resonance Energy Transfer (FRET) Experiments. The successful use in experiments is a consequence of the RET theory developed by Theodor Förster in the late 40s [1]. Försters assumptions resulted into a simple formula, in principle allowing direct measurement of the dye-to-dye distance and thus the retrieval of information about the underlying system [2].
Although widely used in experiments, the simple formula of T. Förster includes assumptions which can lead to a significant loss of accuracy[3]. In this context, theoretical consideration of the underlying process and structures can aid in the interpretation of experiments and provide insight into the biological processes.
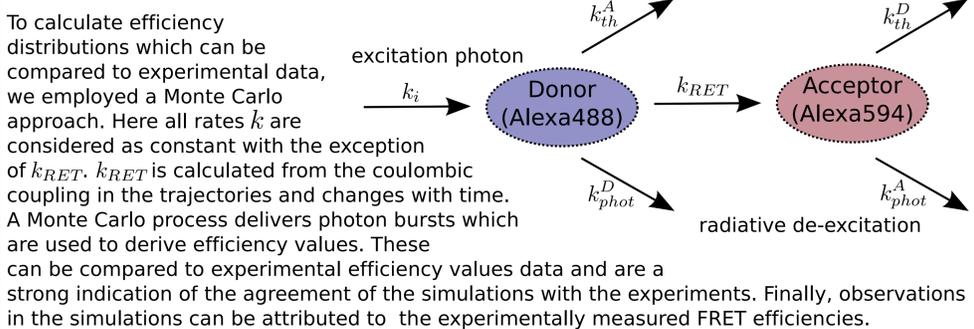
Transition Density Calculation

Alexa488   Alexa594   Alexa488

ZINDO (red)
CIS (green)
TD-DFT (blue)

Excited state calculations were performed in the Pople 6-31+G* basis set and the Gaussian09 package. As can be seen from the transition electron densities in the left picture, the two Alexa dyes mainly show dipolar transition characteristics. Additionally, the density is weakly influenced by the sulfo groups providing solubility and the linker. This allows the use of fragments for the actual coupling calculations.
On the right, a comparison between different excited state methods is shown. The overall shape of the transition density is recovered by all three methods. Yet the transition dipole momentum is best reproduced by CIS calculations while TD-DFT accurately reproduces the excitation energies (B. P. Krueger, unpublished results).

Simulations & Coupling Calculations

Simulations were performed with the GROMACS 4.0 MD package[4] and a modified version of the OPLS/AA force field including parameters for the two dyes and their linkers. Trajectories for all-trans and single-cis prolines of 50-150 ns were calculated.
The coulombic coupling was sampled in 1 ps steps. Here, an advantage of the TDC method is transformability of the densities (green boxes). Thus, only the coulombic sum has to be re-evaluated every step while the transition density can be evaluated once.

Transition Density

Polyproline 15

Alexa488   Alexa594

Monte Carlo Approach

To calculate efficiency distributions which can be compared to experimental data, we employed a Monte Carlo approach. Here all rates k are considered as constant with the exception of k_{RET}. k_{RET} is calculated from the coulombic coupling in the trajectories and changes with time.
A Monte Carlo process delivers photon bursts which are used to derive efficiency values. These can be compared to experimental efficiency values data and are a strong indication of the agreement of the simulations with the experiments. Finally, observations in the simulations can be attributed to the experimentally measured FRET efficiencies.

excitation photon

k_i → Donor (Alexa488) → k_{RET} → Acceptor (Acc-594)

k_{th}^A thermal de-excitation k_{th}^D
k_{phot}^D radiative de-excitation k_{phot}^A

Open Questions

- How does the dye environment (e.g. protein) influence the resonance energy transfer process?
- Can multiple expansion centers partially recover the spatial extension of the density?
- How can we efficiently model the short range (Dexter) energy transfer process?
- Can we make suggestions about specific dye and linker usage in experiments?

References

[1] T. Förster, *Zwischenmolekulare Energiewanderung und Fluoreszenz*, Annalen der Physik 2 (1948) 55--75.
[2] L. Stryer and R. P. Haughland, Energy Transfer - A Spectroscopic Ruler, PNAS 58 (1967) 719.
[3] D. B. VanBeek, M. C. Zwier, J. M. Shorb, and B. P. Krueger, Fretting about FRET: correlation between kappa and R, Biophys J 92 (2007) 4168-78.
[4] B. Hess, C. Kutzner, D. van der Spoel, and E. Lindahl, *GROMACS 4: Algorithms for highly efficient, load-balanced, and scalable molecular simulation*, JCTC 4 (2008) 435--447.
[5] W.W. Schulz, Note on the Computation of the multipole tensor in spherical harmonics, Chem. Phys. Lett. 254 (1996), 337--340
[6] B. P. Krueger, G. D. Scholes, and G. R. Fleming, *Calculation of Couplings and Energy-Transfer Pathways between the Pigments of LH2 by the ab Initio Transition Density Cube Method*, JCP B 102 (1998) 9603--9604.
[7] R. B. Best, K. A. Merchant, I. V. Gopich, B. Schuler, A. Bax, and W. A. Eaton, Effect of flexibility and cis residues in single-molecule FRET studies of polyproline, PNAS 104 (2007) 18964--18969.
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Acknowledgments

Förster Resonance Energy Transfer

$$E(R) = \frac{1}{1 + \frac{R}{R_0}^6}$$

The radiationless energy transfer process, described by Förster is transmitting energy from an excited donor via virtual photon exchange to an acceptor molecule in the ground state. Usually the measured quantity is the efficiency of the RET, E(R), defined e.g. by the ratio of photons collected from the donor and the acceptor. Försters formula then allows a direct calculation of the distance R, assuming that the Förster Radius R_0 is known:

$$R_0 = \frac{c\kappa^2\phi_D}{n^4} \int d\omega f_D(\omega)\epsilon_A(\omega)\omega^4$$

Here, κ^2 contains the averaged orientation while the transition dipole momentums are contained in c. ϕ_D is the excited state lifetime of the donor in absence of the acceptor molecule.
The integral over ω accounts for the spectral overlap and n is the dielectricity of the surrounding media. In order to obtain the above formula and constant, several assumptions are made:

$\hbar\omega_a$ (F)RET $\hbar\omega_i$
Alexa488   Alexa594

- The transition densities are approximated as dipoles (Ideal Dipole Approximation).
- The (dipole - dipole) orientation is isotropically sampled and thus the orientation factor κ^2 is 2/3.
- The surrounding medium is isotropic and homogeneous.
- The electronic structures of donor and acceptor can be treated separately (Weak coupling limit).
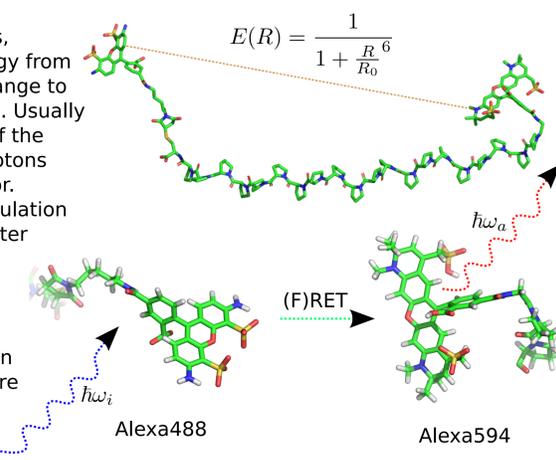
Coulombic Coupling

The rate of the RET process follows a "Golden Rule" like scheme: $k_{ret} = \frac{2\pi}{\hbar}|V^2| \cdot J$
V is the coupling potential (Transition matrix element) while the Franck-Condon overlap J accounts for the density of final states. We tested several methods to determine the coulombic coupling potential from simulated dye conformations.

Dipole - Dipole: cartesian dipole interaction

The dipole-dipole interaction energy, as used by T. Förster to derive the RET formula can be seen as a special case of the multipole expansion. In cartesian coordinates, it takes the simple form:

$$V(\vec{r}) = \frac{\vec{p}_1\vec{p}_2 - 3(\hat{r}\vec{p}_1 \cdot \hat{r}\vec{p}_2)}{r^3}$$

$\vec{r}$ $\vec{p}_2$ $\vec{p}_1$
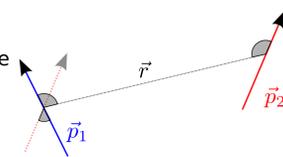
Multipole - Multipole: expansion of the densities to the order n

In general, the interaction of two charge distributions can be evaluated by multipole expansion. The 1/r potential is evaluated in a Laplace expansion. From each charge distribution, multipole momentums are calculated. Below, the convoluted multipole momentum according to Ref. [5] and the corresponding potential calculation are shown. The order L in the convoluted form denotes the total momentum: 0 for monopole-monopole, 1 for monopole-dipole, 2 for dipole dipole, ...

$$Q_{LM}^{CP} = \sum_{l+l'=L}(-1)^l\frac{4\pi(2L+1)}{(2l+1)(2l'+1)}\sum_{m+m'=M}\frac{\xi_{lm}\xi_{l'm'}}{\xi_{LM}}q_{lm}^C q_{l'm'}^P$$

$$V_{mp} = \sum_{L,M}\frac{4\pi}{2L+1}Q_{LM}^{CP}\frac{Y_{LM}(\hat{d})}{|d|^{L+1}}$$
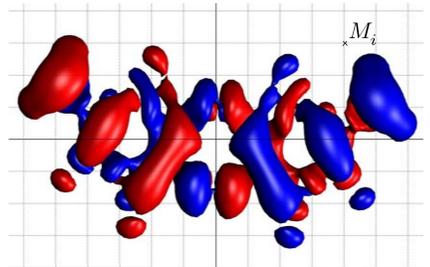
C   d   P   Expansion Centers
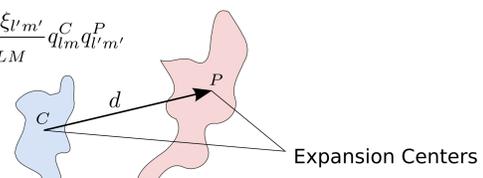
Transition Density Cube: Direct coulombic sum of the densities
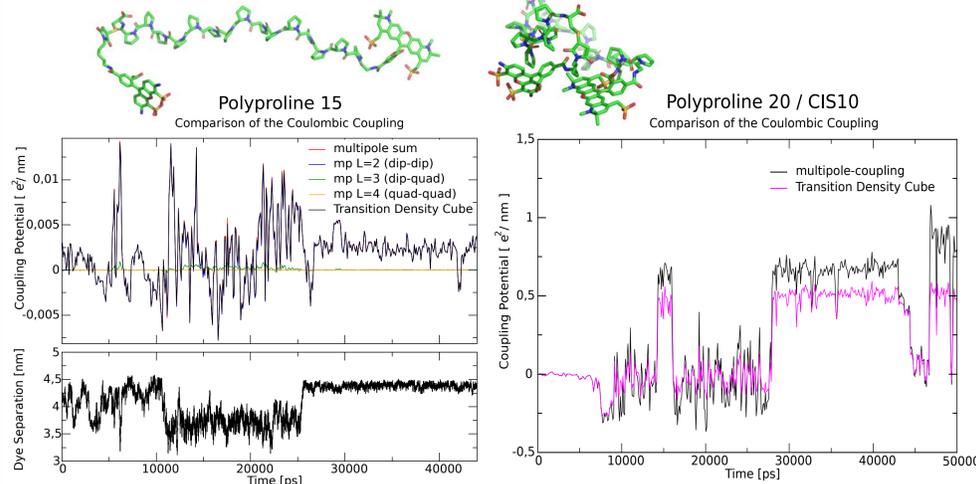
At small dye separations or in large resonating structures, the size of the system cannot be neglected. B. P. Krueger et al. [6] developed a method which allows the direct coupling of transition densities via discrete mapping on a grid:

$$M(r) = \int_s \Psi_g\Psi_e^* ds dr$$

$$V_{coul} = \sum_{i,j}\frac{M_i \cdot M_j}{d_{ij}}$$

M_i

Results and Comparison:

Polyproline 15
Comparison of the Coulombic Coupling

multipole sum
mp L=2 (dip-dip)
mp L=3 (dip-quad)
mp L=4 (quad-quad)
Transition Density Cube

Coupling Potential [e² / nm]
Dye Separation [nm]
Time [ps]

Polyproline 20 / CIS10
Comparison of the Coulombic Coupling

multipole-coupling
Transition Density Cube

Coupling Potential [e² / nm]
Time [ps]

The coulombic coupling of the dyes in polyproline 15 is dominated by the dipole - dipole term. At separations of 3 nm, TDC shows no improvement over a multipole expansion. Yet, a significant difference between the coupling potentials is visible in the case of polyproline 20 with a *cis* bond located at residue 10. Here the multipole coupling overestimates the coulombic coupling between the two dyes. In this case, the Transition Density Cube method provides an improvement of the coupling treatment, assuming that the two electronic systems can still be considered as separated (weak coupling limit).