single-molecule fluorescence resonance energy transfer II

(8) multi-color FRET

michael börsch

18/03/2005

Energy transfer between several fluorophores

Examples

1. three color FRET

Schwille group, Ha group, Deniz group

2. photonic wires

Sauer group

Energy transfer between three fluorophores

Single-Molecule Three-Color FRET

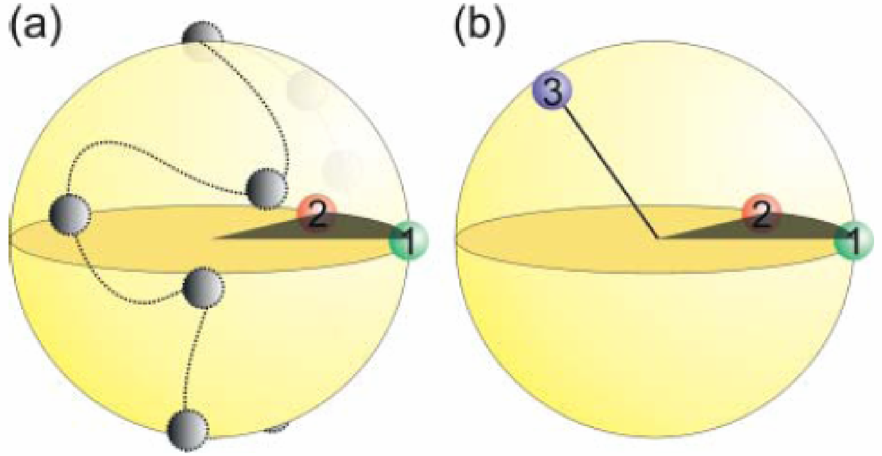


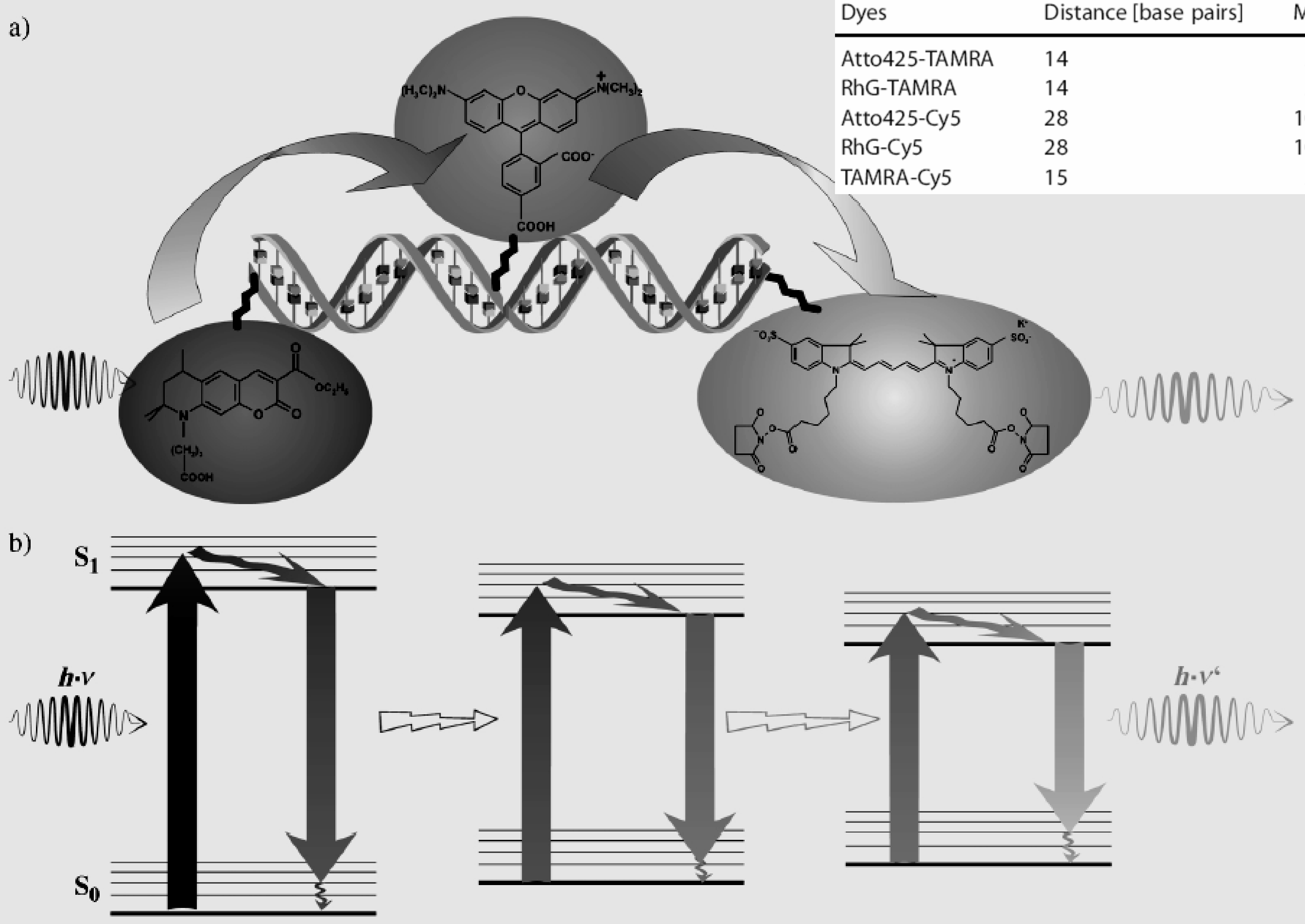
FIGURE 1 A need for three-color FRET. (*a*) A FRET pair attached to the points 1 and 2 reports on the distance between 1 and 2 but no information can be obtained on the position of the point 3. (*b*) By attaching another spectrally distinguishable fluorophore to the point 3 which has appreciable FRET with fluorophores 1 and 2, we can deduce the location of point 3.

1. two donors / one acceptor: excitating both donors with one laser
2. two-step FRET: middle-energy fluorophors acts as a bridge
3. one donor / two acceptors: donor is quenched by either of the acceptor dyes

or: expanding the FRET distance (by two-step FRET)

Energy transfer between three fluorophores

1. ensemble



Dyes	Distance [base pairs]	Mean distance [Å]
Atto425-TAMRA	14	50
RhG-TAMRA	14	50
Atto425-Cy5	28	100
RhG-Cy5	28	100
TAMRA-Cy5	15	55

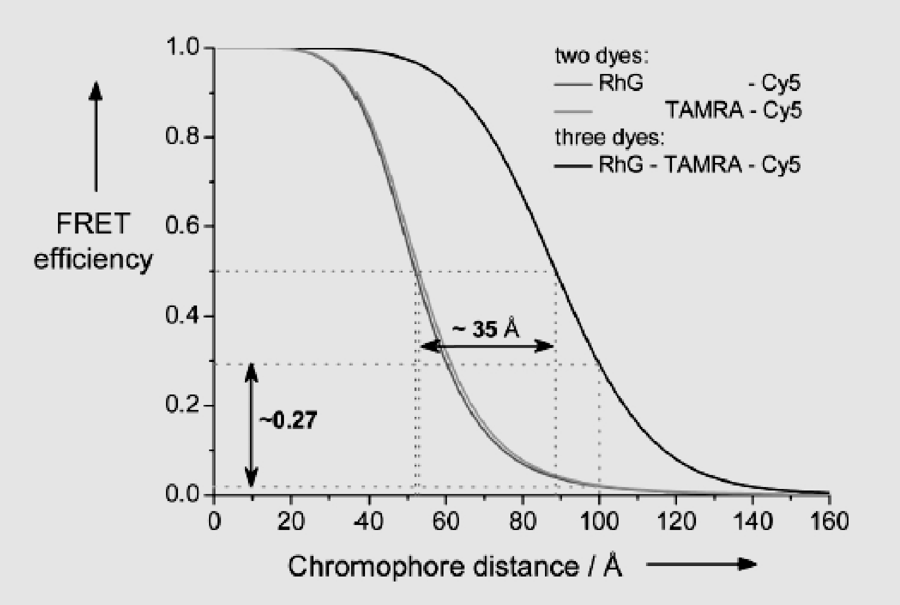
Figure 1. *a) Positions of the three dyes (Atto425 (shown) or Rhodamine Green (RhG), respectively, TAMRA and Cy5) attached to the 28 mer dsDNA. b) Interaction of two*

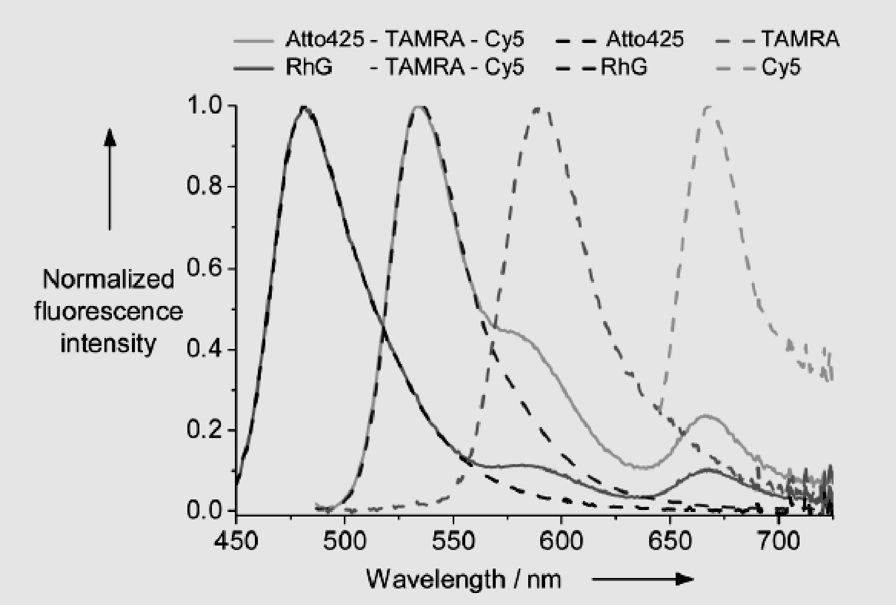
Elke Haustein,[a] Michael Jahnz,[a] and Petra Schwille*[a, b]

CHEMPHYSCHEM ***2003**, 4, 745*

Energy transfer between three fluorophores

two-step FRET: increase in FRET efficiency at 100 Å about 30 %, results in an increased R_0 ~ 35 Å





Elke Haustein,[a] Michael Jahnz,[a] and Petra Schwille*[a, b]

CHEMPHYSCHEM **2003**, *4*, 745

Energy transfer between three fluorophores

Dyes	Calc. FRET efficiency	FRET Ratio $[I_A/(I_D+I_A)]$
RhG-TAMRA	-	0.26
TAMRA-Cy5	0.42	0.57
RhG-Cy5	0.02	0.03
RhG-(TAMRA-)Cy5	0.29	0.17
Atto425-TAMRA	-	0.13
TAMRA-Cy5	0.42	0.60
Atto425-Cy5	-	0.01
Atto425-(TAMRA-)Cy5	-	0.08

Elke Haustein,[a] Michael Jahnz,[a] and Petra Schwille*[a, b]

addition of the central TMR in the 'bridge' increases Cy5 intensity through FRET

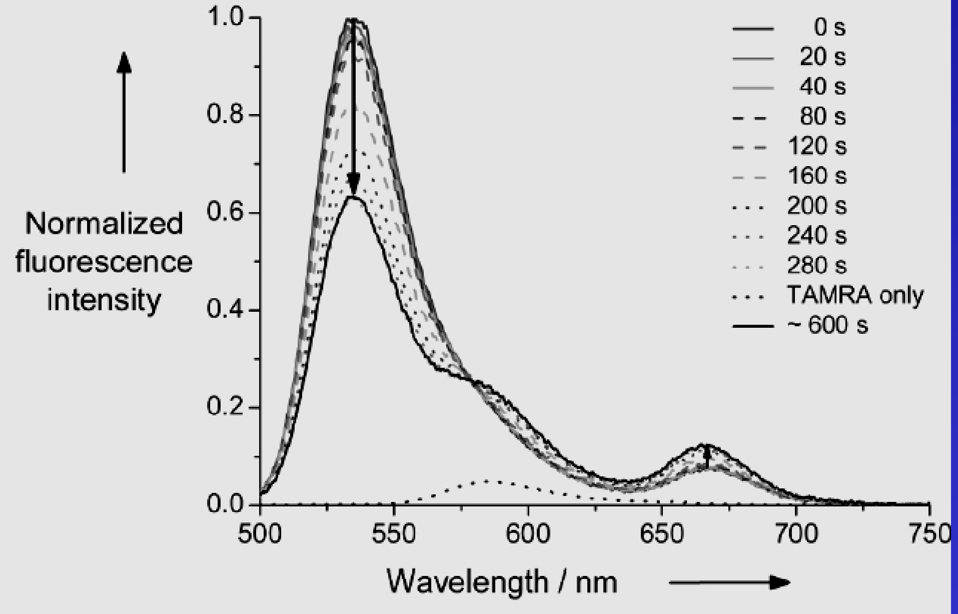


Figure 5. *Annealing kinetics of the TAMRA-labeled ss-14 mer and the corresponding RhG-Cy5 labeled oligonucleotide at room temperature. During the annealing of the "centre dye" a distinct increase both in orange and in red fluorescence can be observed.*

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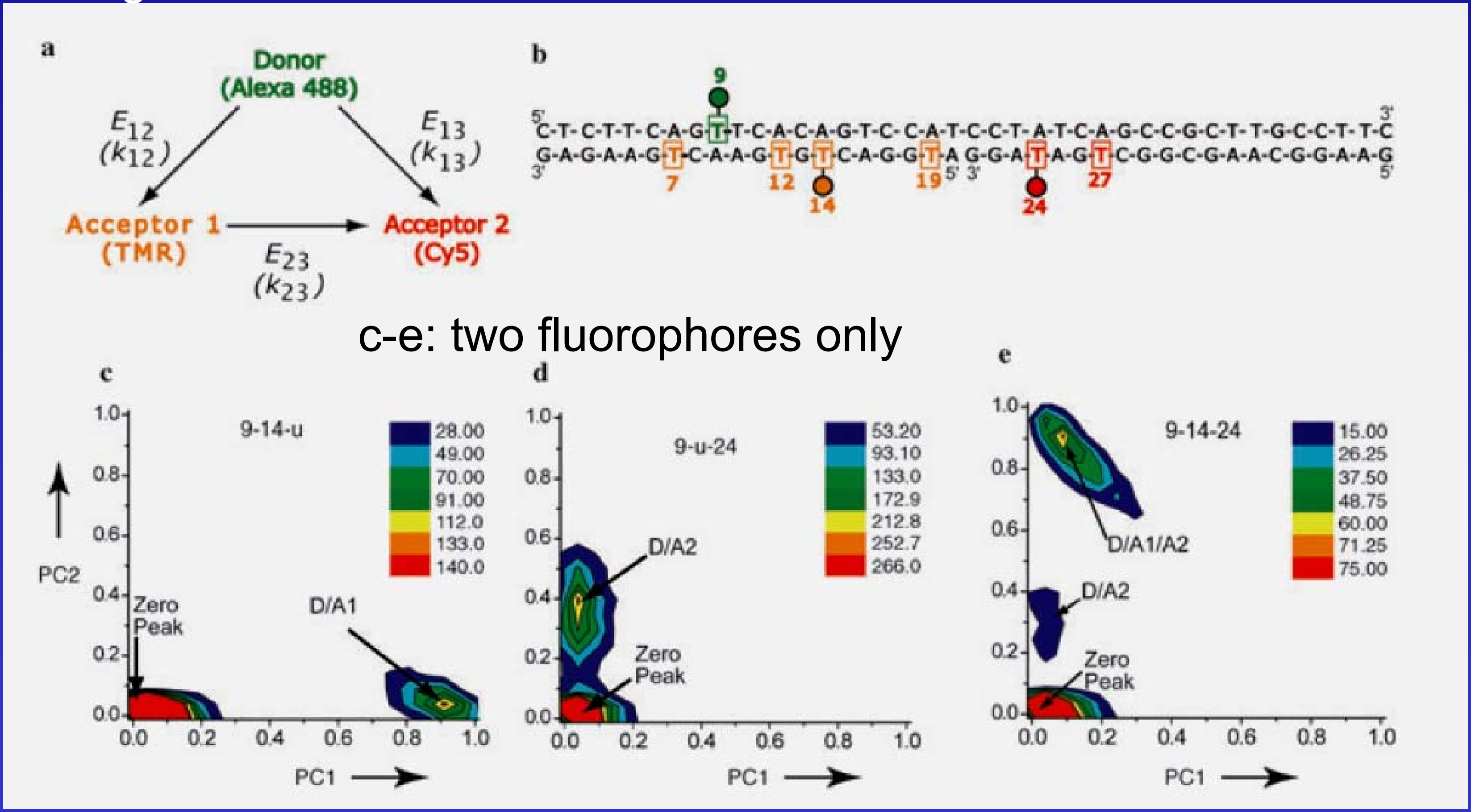
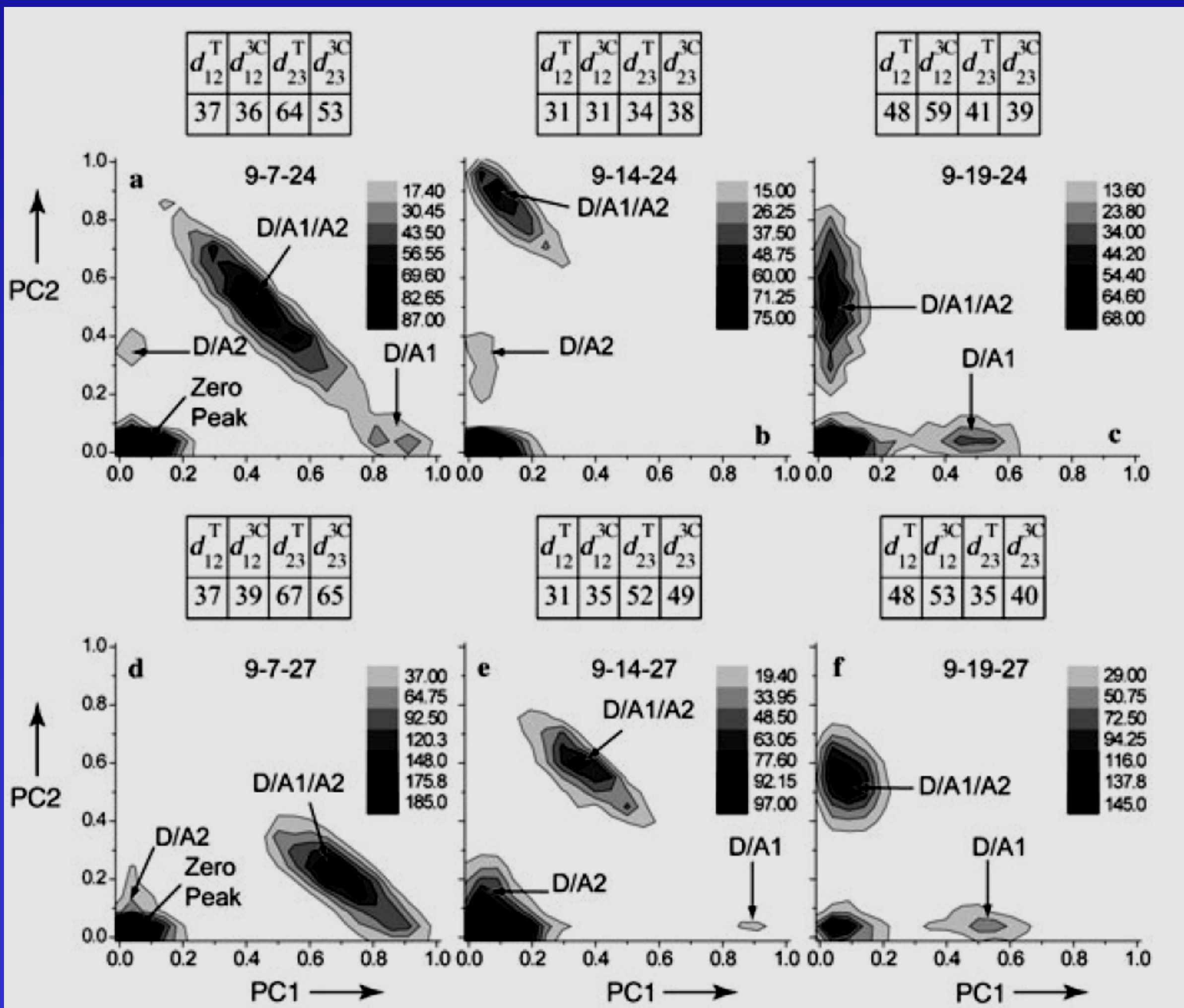
Energy transfer between three fluorophores

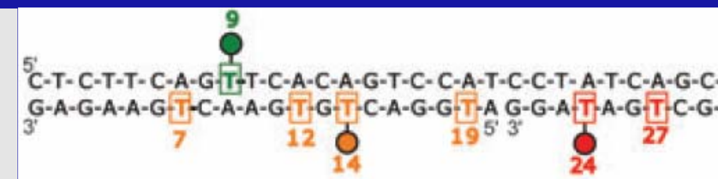
2. single molecules in solution



Jean-Pierre Clamme and Ashok A. Deniz*[a]   ChemPhysChem **2005**, 6, 74–77

Energy transfer between three fluorophores





$$\mathrm{PC1} = \frac{I_2}{I_1 + I_2 + I_3} \qquad \mathrm{PC2} = \frac{I_3}{I_1 + I_2 + I_3}$$

$$E_{23} = \frac{\mathrm{PC1} \cdot E_{13} - E_{13} + \mathrm{PC2}}{\mathrm{PC1} + \mathrm{PC2} - E_{13}}$$

$$E_{12} = \frac{\mathrm{PC1} + \mathrm{PC2} - E_{13}}{\mathrm{PC1} \cdot E_{13} - 2E_{13} + \mathrm{PC2} \cdot E_{13} + 1}$$

E_{13} from PC2 values of two-label constructs

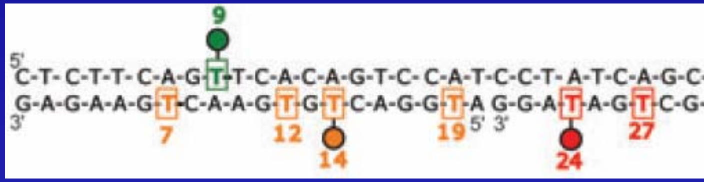
Jean-Pierre Clamme and Ashok A. Deniz*[a]

ChemPhysChem **2005**, *6*, 74–77

Energy transfer between three fluorophores

- with constant D-A2, the different intermediary A1 positions can be discriminated (9-7-24, 9-14-24, 9-19-27)
- with constant D-A1, the different A2 positions can be discriminated (with limitations: 9-19-24 = 9-19-27)
- distance measurements in triple-FRET with 5 Å resolution (as stated in the paper)
- subpopulation analysis in 2D-plot (500000 points)

- improvements : cyclic multicolor excitation, fluorescence lifetimes



$$PC1 = \frac{I_2}{I_1 + I_2 + I_3} \qquad PC2 = \frac{I_3}{I_1 + I_2 + I_3}$$

$$E_{23} = \frac{PC1 \cdot E_{13} - E_{13} + PC2}{PC1 + PC2 - E_{13}}$$

$$E_{12} = \frac{PC1 + PC2 - E_{13}}{PC1 \cdot E_{13} - 2E_{13} + PC2 \cdot E_{13} + 1}$$

E_{13} from PC2 values of two-label constructs

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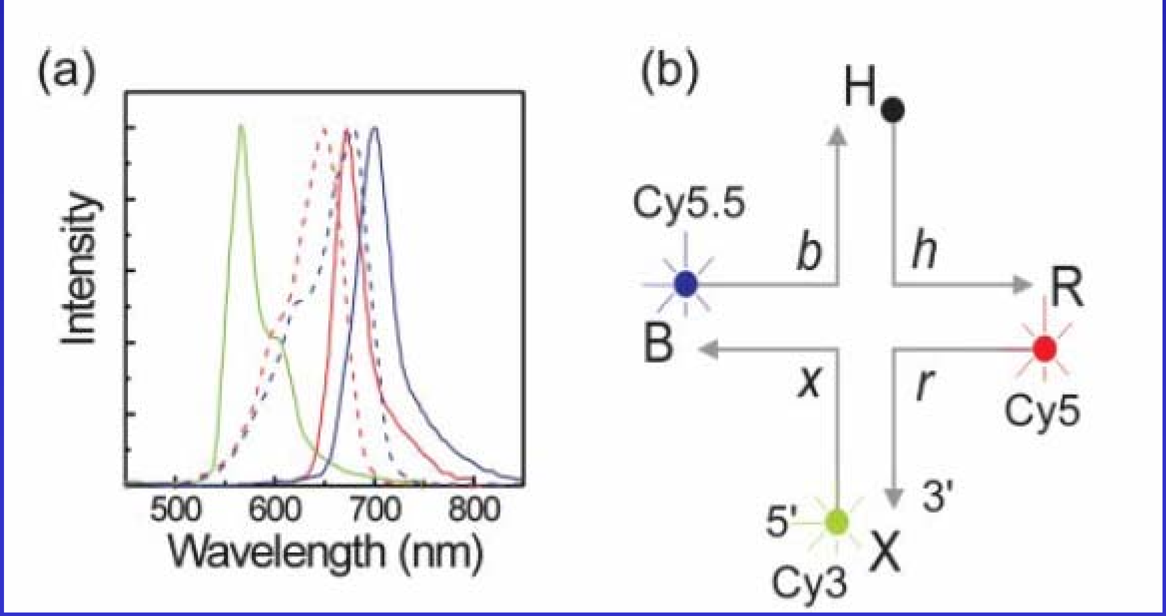
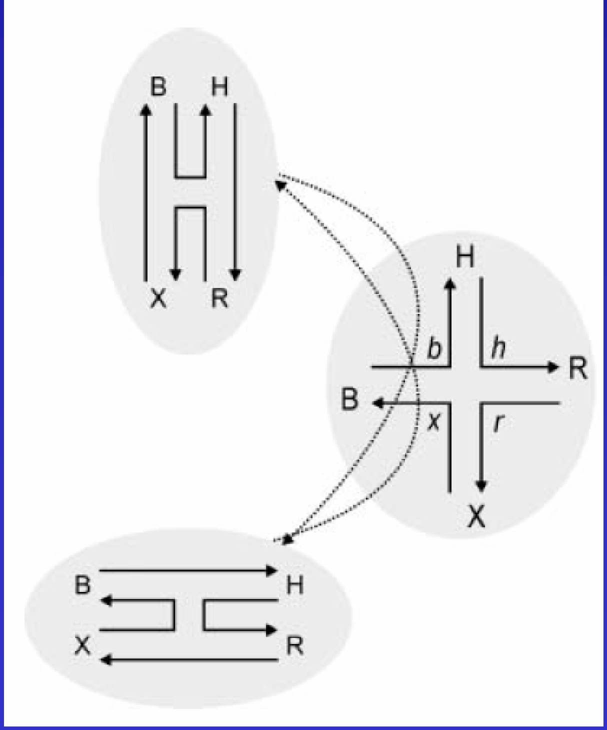
ChemPhysChem **2005**, 6, 74–77

Energy transfer between three fluorophores

3. time trajectories of single molcules (Taekjip Ha group)
one donor / two acceptors for Holliday junction dynamics,
question: correlated / noncorrelated switching, intermediate ?
→ exploring the position of a third point



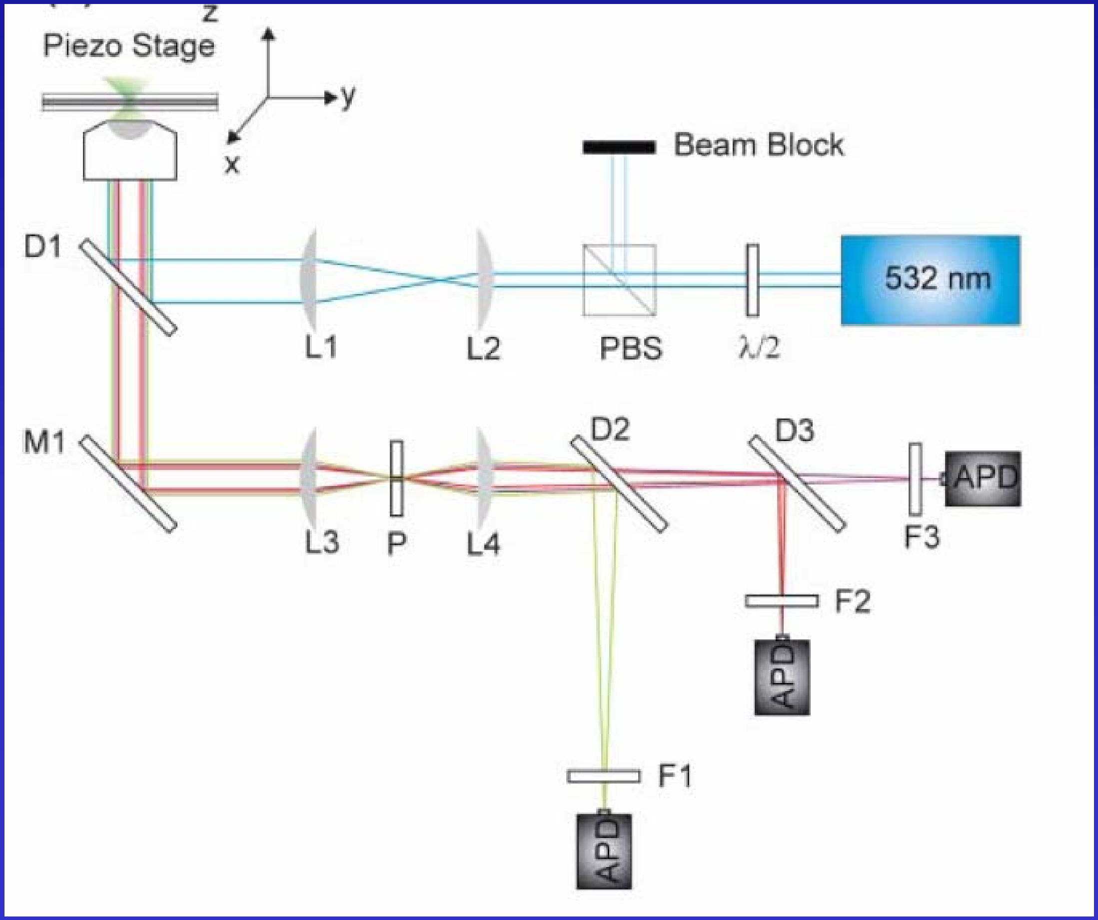


Energy transfer between three fluorophores

confocal 3-APD setup

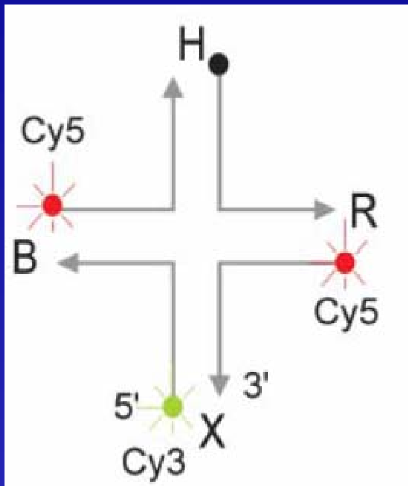
surface immobolized Holliday junctions, only 5% with 3 labels

(slow) surface scanning integration times 6-20 ms



Sungchul Hohng, Chirlmin Joo, and Taekjip Ha
Biophysical Journal 87(2) 1328–1337

Energy transfer between (three) fluorophores



evaluation 1: one donor, two identical acceptors
different dwell times after photobleaching of one acceptor

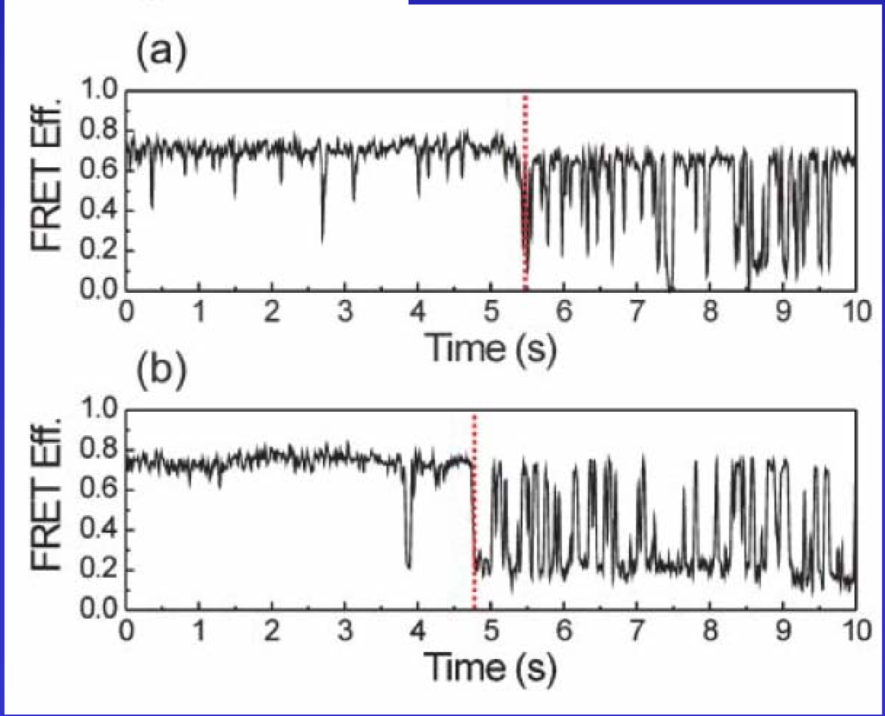


FIGURE 4 Single Holliday junction dynamics using one donor and two identical acceptors. (*a* and *b*). Two-color FRET experiment on $X^3R^5B^5$. When both of the two acceptors are active, the acceptor signals are almost constant, which supports the model depicted in Fig. 3. After one of the two acceptors bleaches (*dotted red lines*), time-traces show kinetics similar to X^3B^5 (*a*) or X^3R^5 (*b*), depending on which acceptor bleaches.

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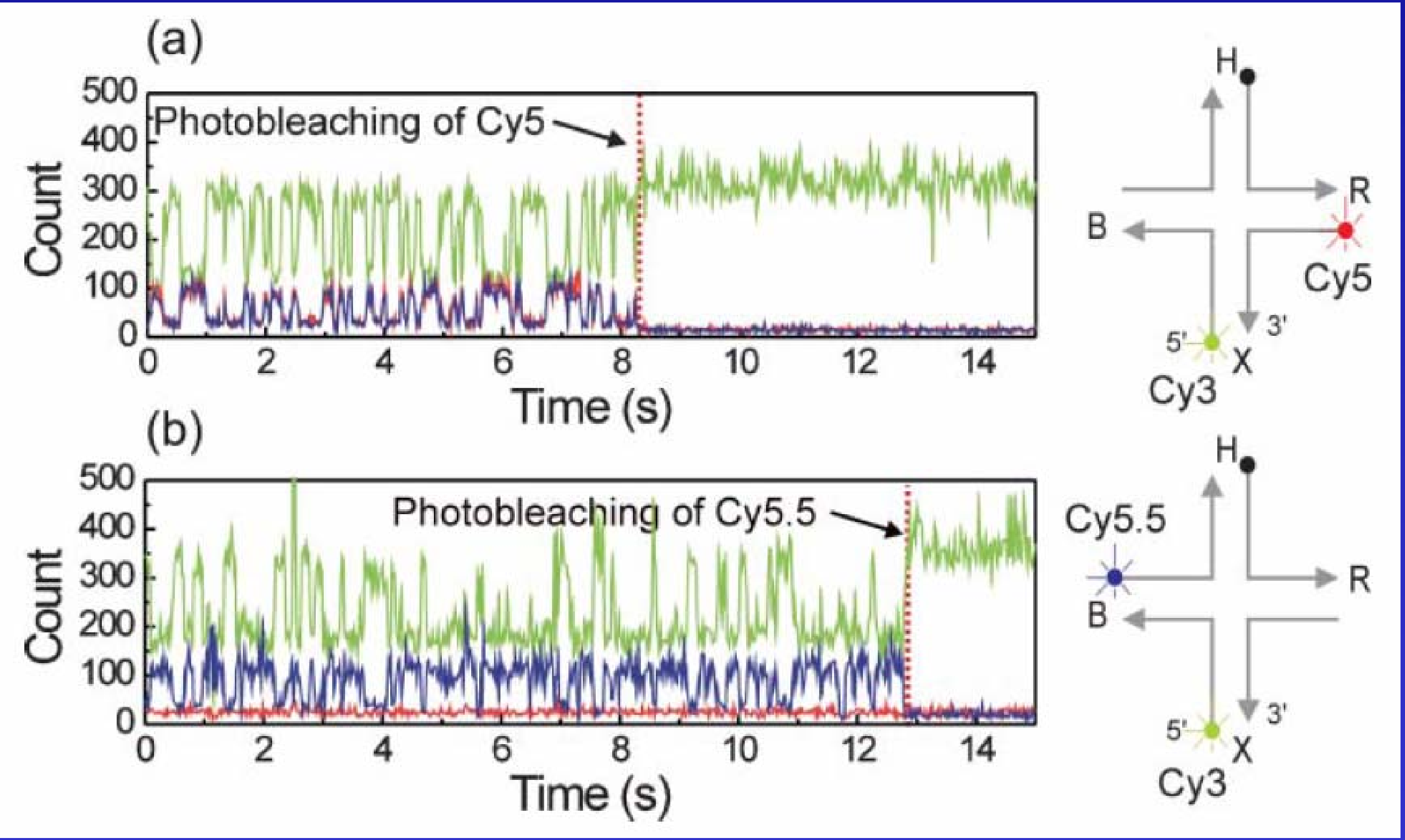
Energy transfer between (three) fluorophores

cy3: green
cy5: red
cy5.5: blue

X^3R^5
red-blue parallel

$X^3B^{5.5}$



evaluation 2: one donor, one acceptor
cross-talk determination

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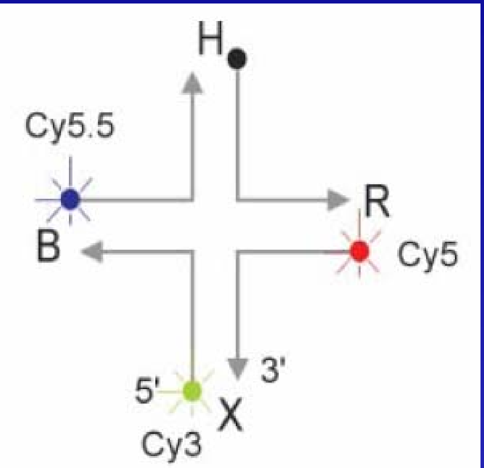
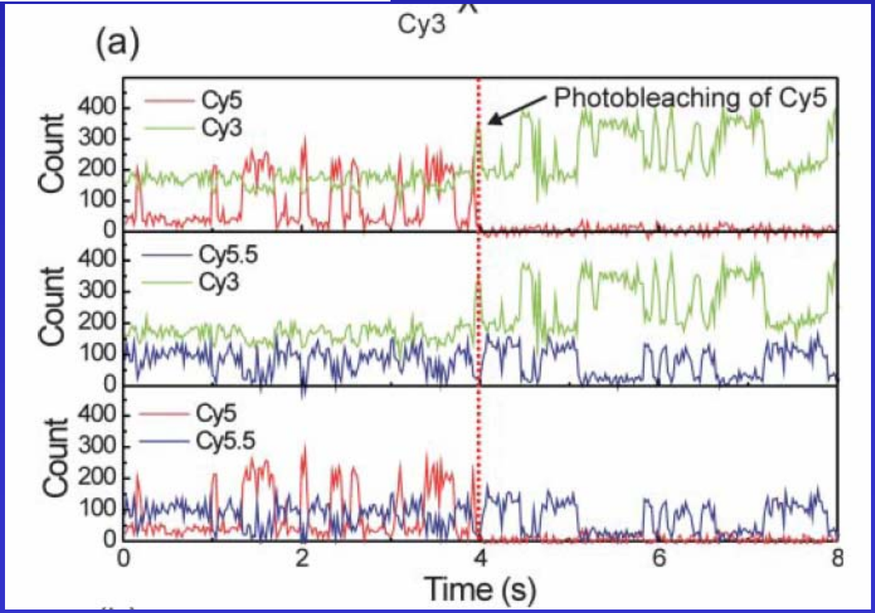
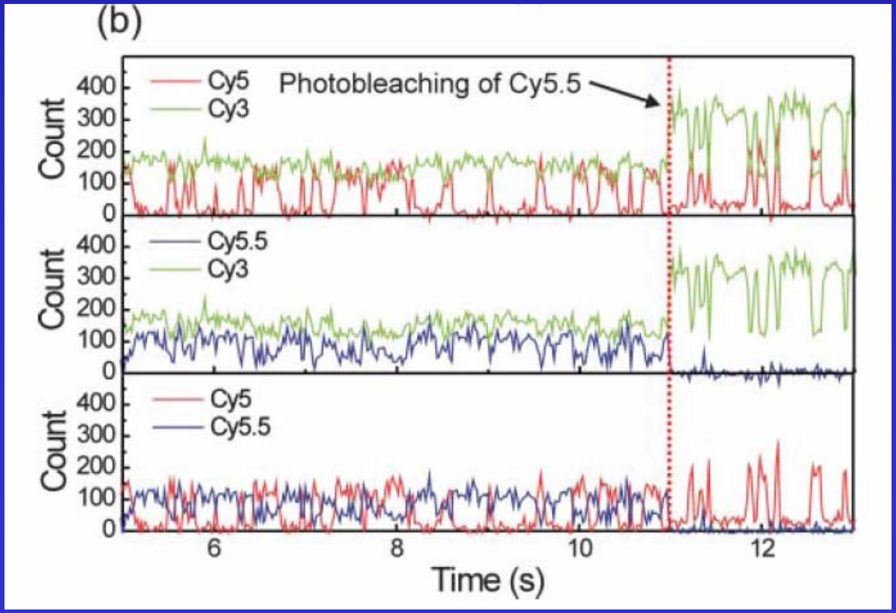
Energy transfer between three fluorophores



red-blue intensity traces antiparallel, nearly constant green intensity (Cy3)





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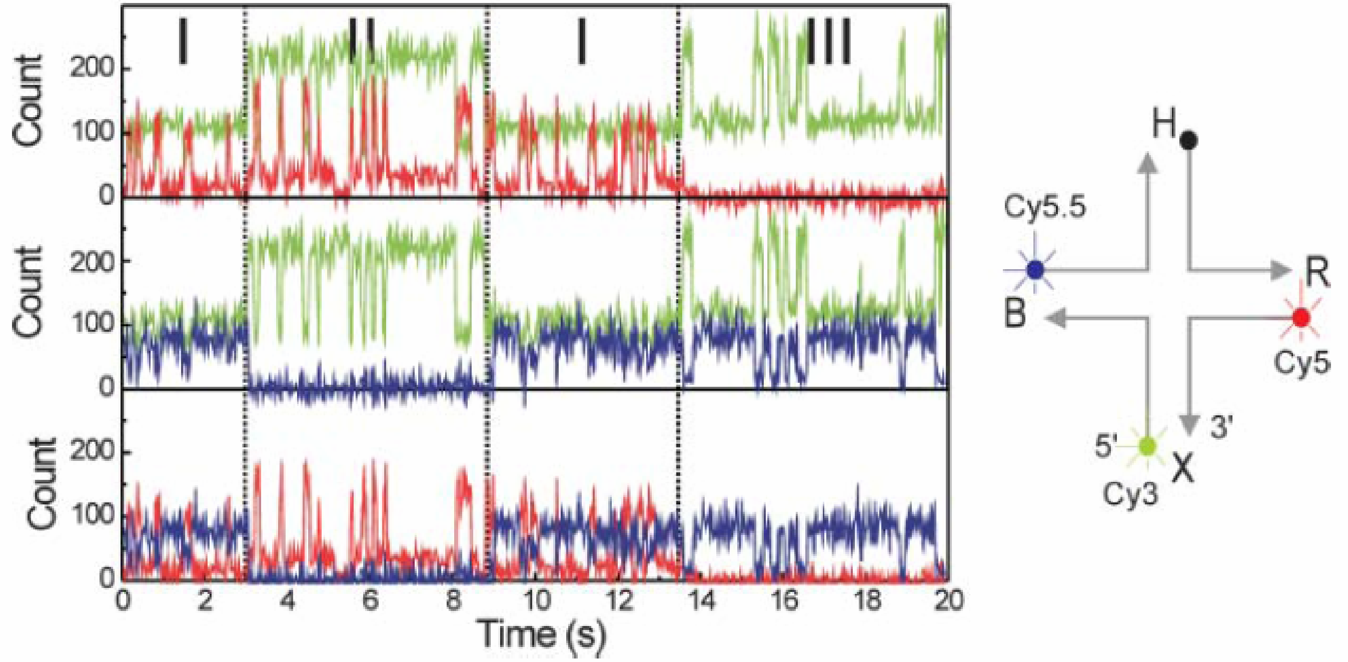
Energy transfer between three fluorophores

Three-color FRET time-traces (Cy3 in *green*, Cy5 in *red*, and Cy5.5 in *dark blue*) which were used to derive distances between three dyes. In the region I, the three dyes are all active. In the region II, Cy5.5 is inactive and data from this region is used for calculating E_{12}. Using data in region III where Cy5 is bleached, E_{13} is calculated. As explained in the text, E_{23} is calculated by comparing Cy5 intensities in regions I and II or in regions I and III. Distances between the three dyes are readily obtained from E_{12}, E_{13}, and E_{23} using the calculated values for R_0 for each FRET pair.

Cy5-Cy5.5:
~ 8.7 nm
calc: 7.9 nm



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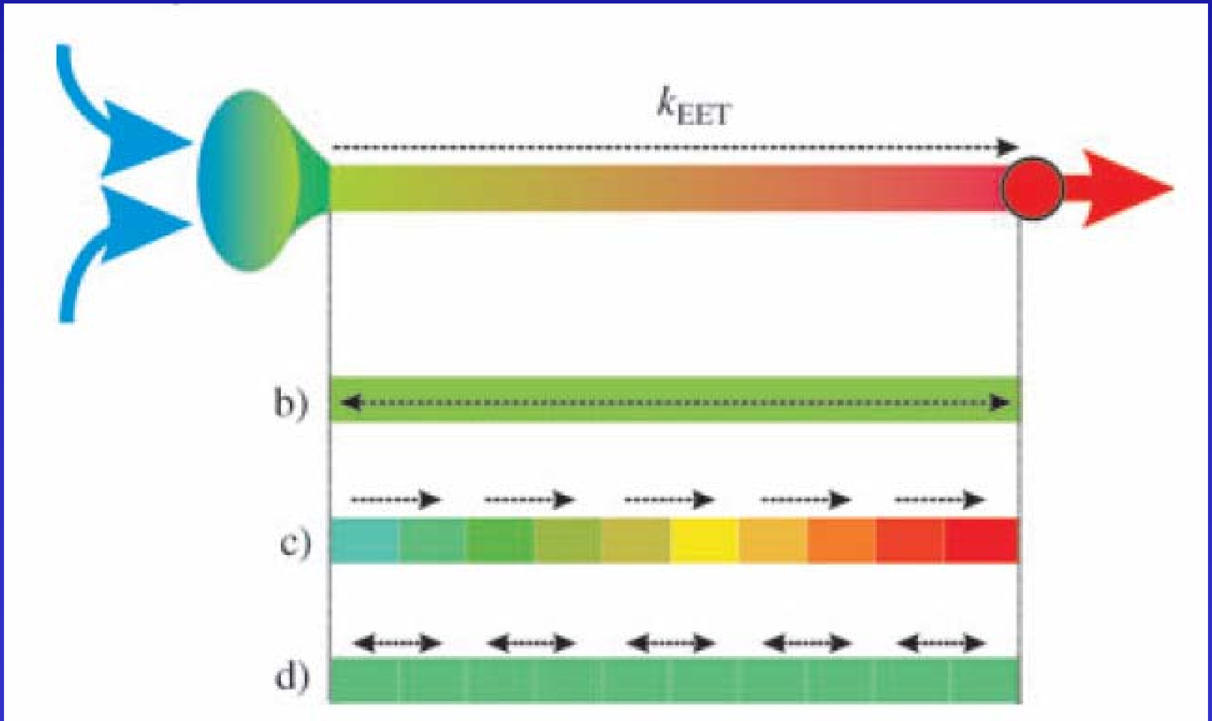
Energy transfer between several fluorophores



directed electronic excitation transfer:

b) strong coupling

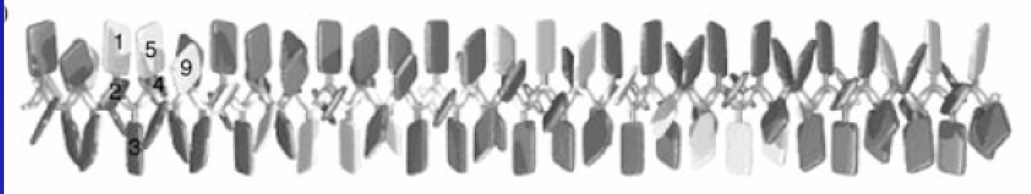
c) weak coupling with stepwise EET

d) energy hopping

ChemPhysChem **2005**, 6, 217 – 222

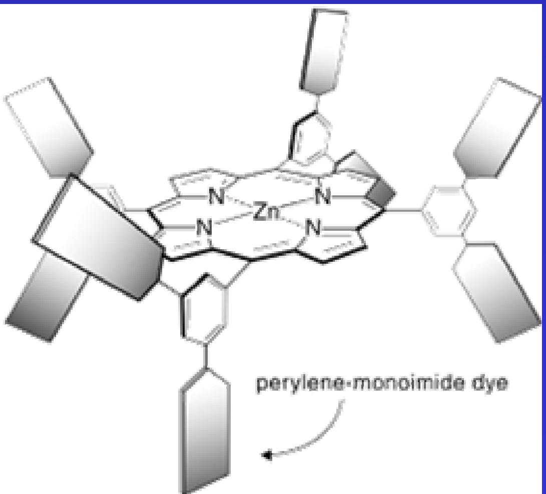
Energy transfer between several fluorophores

directed electronic excitation transfer:



J. Hernando, P. A. J. de Witte, E. M. H. P. van Dijk, J. Korterik, R. J. M. Nolte, A. E. Rowan, M. F. Garcia-Parajo, N. F. van Hulst, *Angew. Chem.* **2004**, *116*, 4137–4141; *Angew. Chem. Int. Ed.* **2004**, *43*, 4045–4049.

strong coupling: poly-perylene, coherent energy transfer



artificial light harvesting complexes with perylene and porphyrine

A. Ambroise, C. Kirmaier, R. W. Wagner, R. S. Loewe, D. F. Bocian, D. Holten, J. S. Lindsey, *J. Org. Chem.* **2002**, *67*, 3811–3826.

Energy transfer between several fluorophores

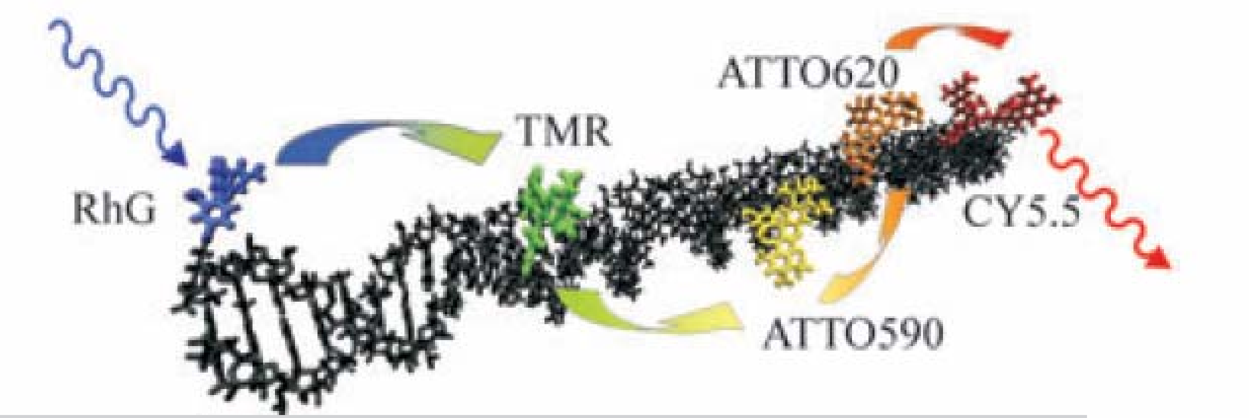


Table 1. *Spectroscopic parameters and R_0 values of the chromophores used for the construction of DNA-based photonic wires.*

Philip Tinnefeld,* Mike Heilemann, and Markus Sauer*[a]

	λ_{abs} [nm]	λ_{em} [nm]	ε/l [10^5 mol^{-1} cm^{-1}]	Φ_f	R_0 [Å]
RhodGreen	508	534	0.74	0.9	
					65.0
TMR	560	582	0.95	0.9	
					71.7
ATTO590	603	625	1.20	0.8	
					72.3
LCR	622	638	1.20	0.8	
					74.5
ATTO680	689	703	1.25	0.3	

Ex @ 488 nm

$$E_{end\text{-}to\text{-}end} = E_{1,2} * E_{2,3} * E_{3,4} * E_{4,5}$$

ChemPhysChem ***2005***, *6*, *217 – 222*

Energy transfer between several fluorophores

Multistep Energy Transfer in Single Molecular Photonic Wires

Mike Heilemann,† Philip Tinnefeld,† Gabriel Sanchez Mosteiro,‡ Maria Garcia Parajo,‡ Niek F. Van Hulst,‡ and Markus Sauer*,†

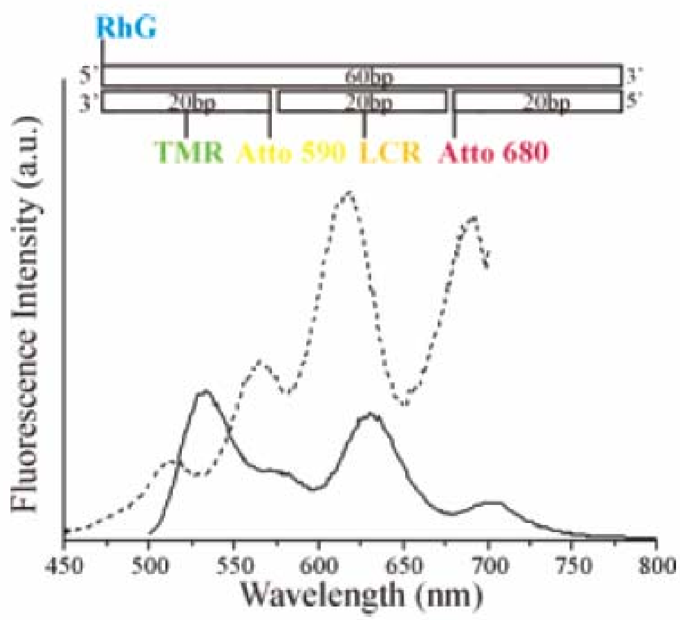
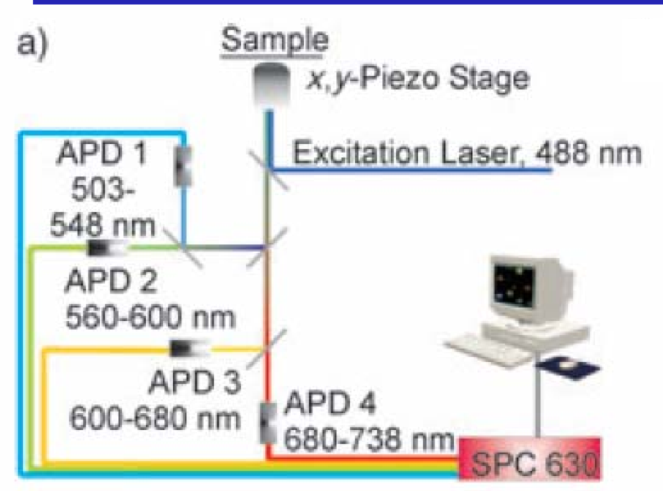
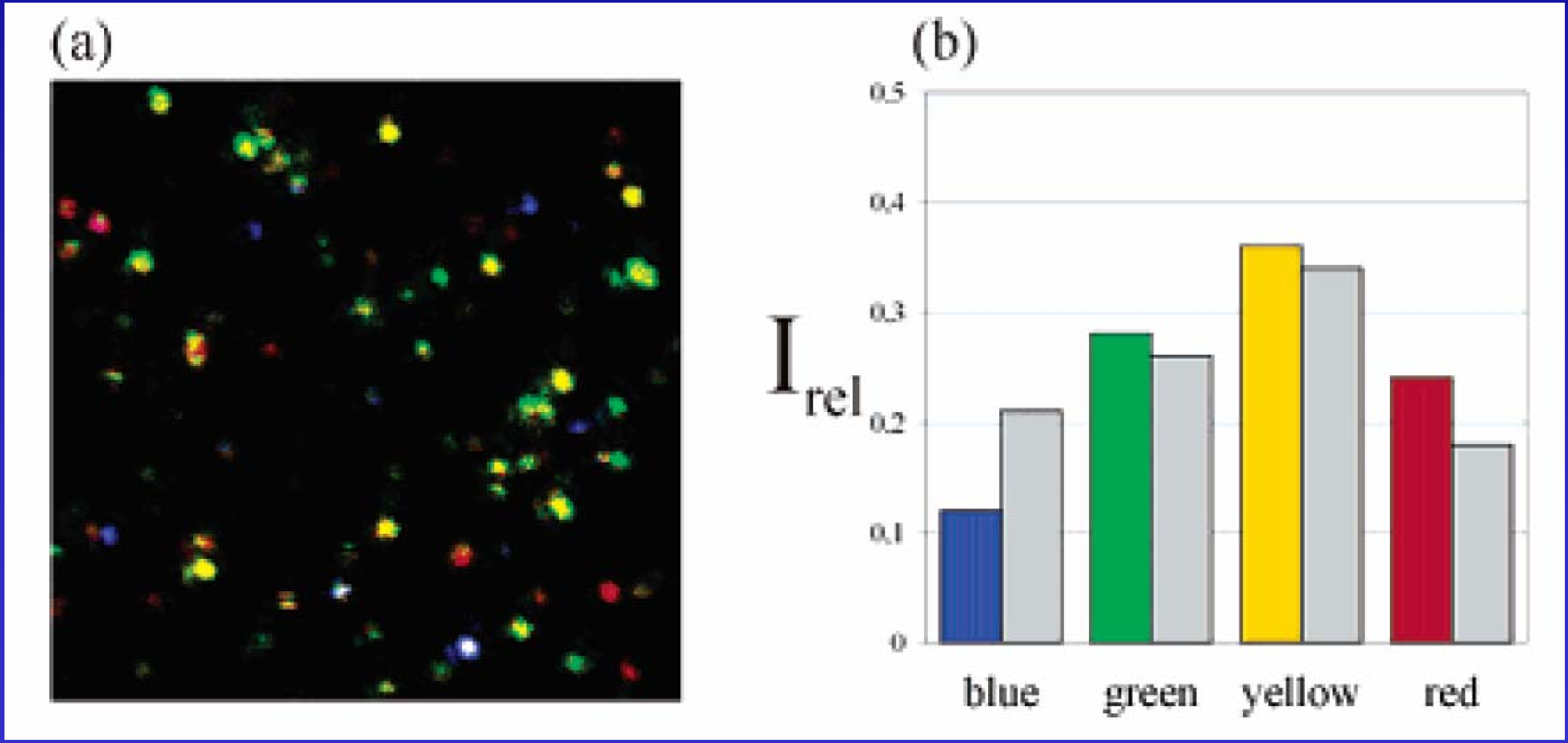


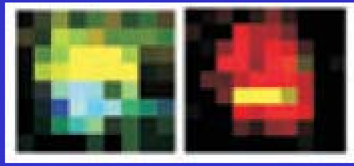
Figure 1. Fluorescence emission (488 nm excitation, straight line) and excitation spectrum (705 nm fluorescence, dotted line) of a DNA-based photonic wire containing five fluorophores measured in PBS, pH 7.4. The



J. AM. CHEM. SOC. 2004, *126*, 6514−6515

Energy transfer between several fluorophores

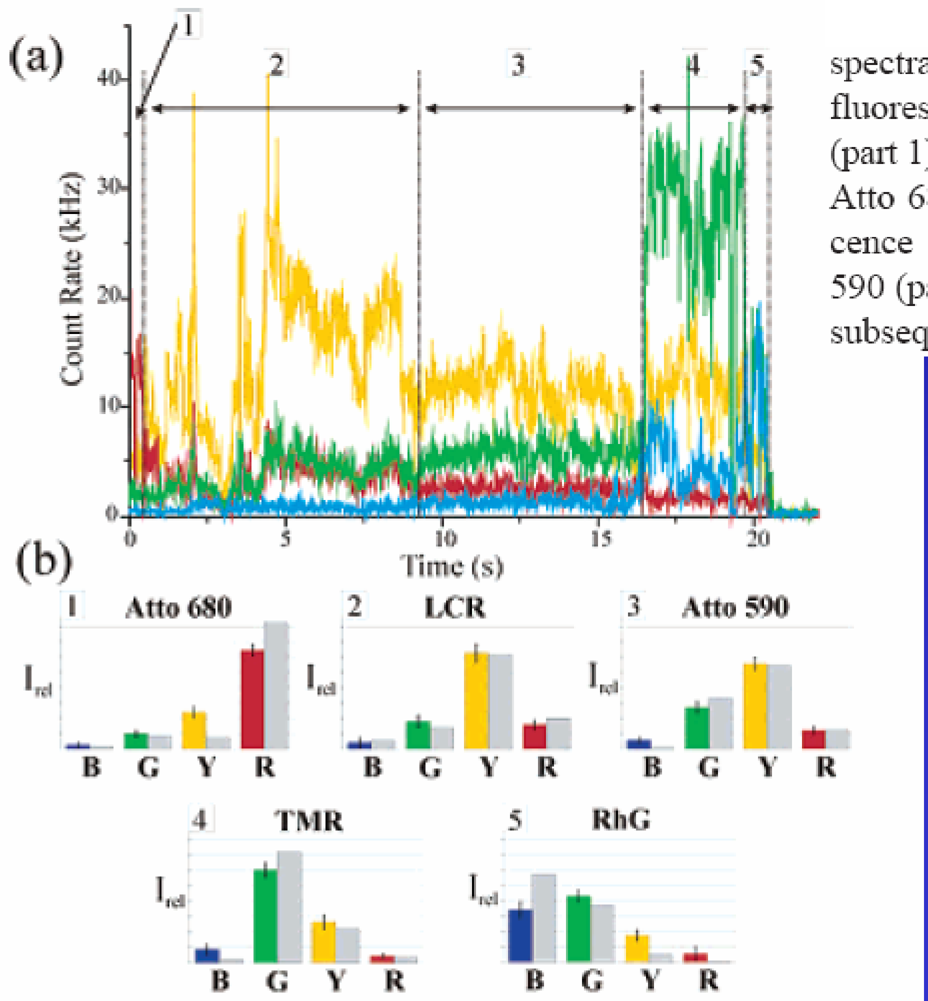




temporal FRET efficiency fluctuations (false colored) in single photonic wires revealed during scanning

J. AM. CHEM. SOC. **2004**, *126*, 6514−6515

Energy transfer between several fluorophores



According to these patterns, five regions with different spectral characteristics can be retrieved from the four different fluorescence intensity trajectories. The first 0.4 s of the trajectory (part 1) can be attributed to the emission of the far red chromophore Atto 680. After photobleaching of the final acceptor, the fluorescence emission is dominated by LCR (part 2) followed by Atto 590 (part 3), TMR (part 4), and finally RhG (part 5). Overall, five subsequent photobleaching events can be uncovered.

- overal E_{FRET} up ~ 0.9
- across 13.4 nm
- ~ 200 nm spectral shift

emission pattern ot the 4 FRET acceptors and FRET donor R6G in 4 detection channels

Energy transfer between several fluorophores

photonic wires

- overal E_{FRET} up to ~ 0.9 for 10% of the photonic wires
- across 13.4 nm
- ~ 200 nm spectral shift

- complex behaviour due to competing EET processes
- nonfluorescent energy traps

to be continued:

single-molecule
FRET
imaging
methods