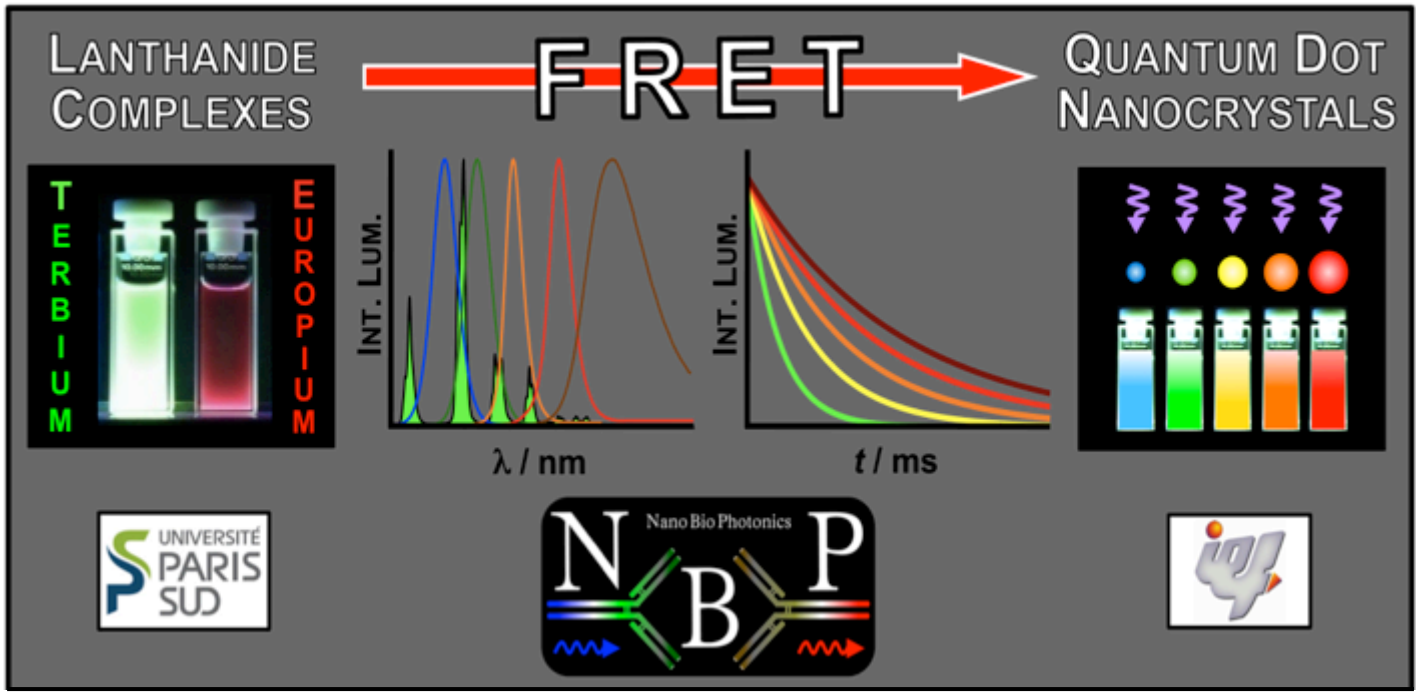
LUMINESCENCE DANS LA NANODIMENSION

FRET POUR UNE BIOANALYSE ULTRASENSIBLE ET MULTIPLEXÉE



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W. Russ Algar - *University of British Columbia, Canada*

Igor L. Medintz - *NRL Washington, DC, USA*

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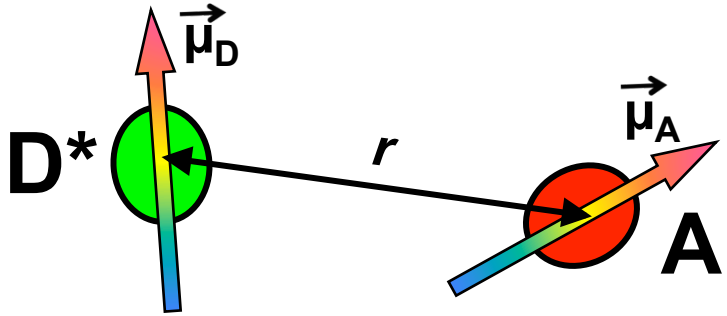
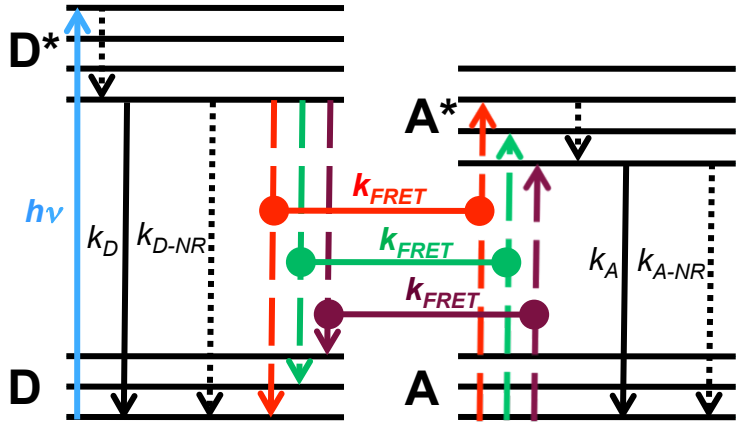
ENS Cachan, France

Non-radiative resonant energy transfer

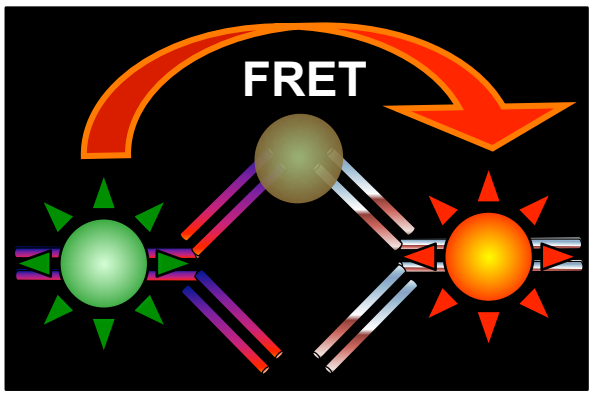
from an excited donor molecule (D*) to a ground-state acceptor molecule (A)



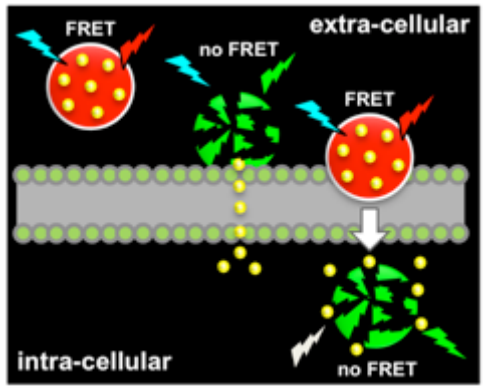
Strong distance dependence ($1/r^6$) over ca. 1 to 20 nm



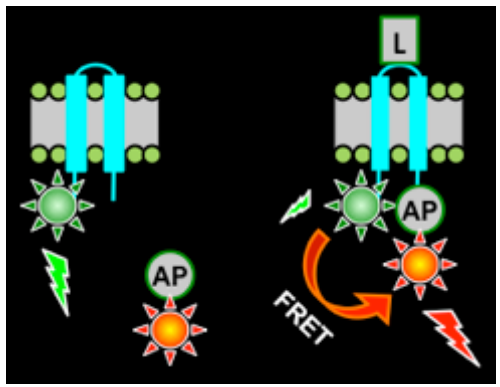
Biomolecular detection and characterization in complex environments. Analysis of structures and networks.



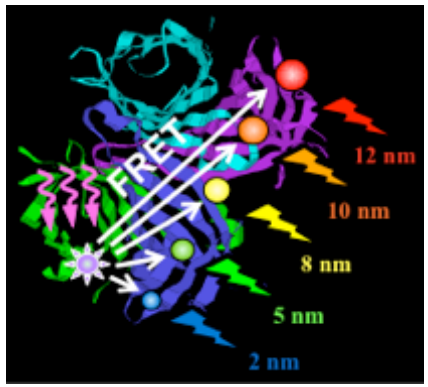
INTERACTIONS



TRANSPORT



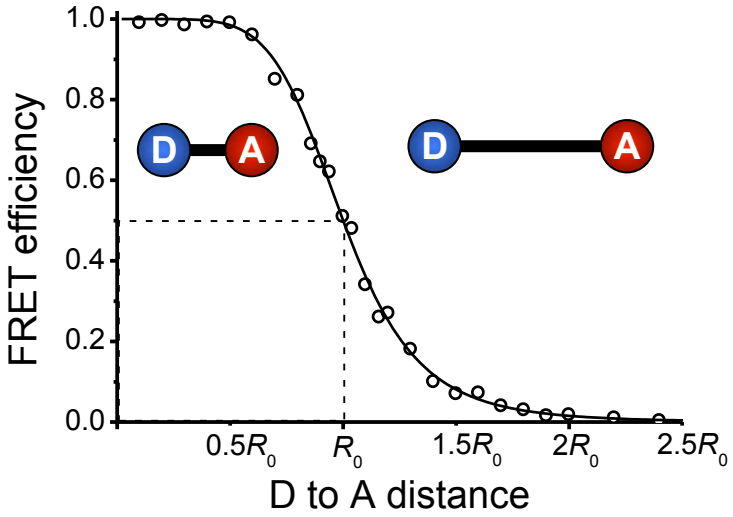
SIGNALING



STRUCTURE

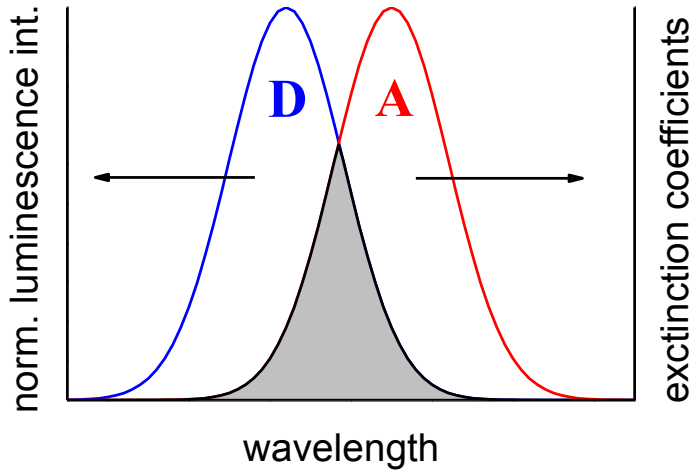
Donor-acceptor distance $r = R_0$ ⟶ **FRET efficiency $\eta_{FRET} = 50\ \%$**

$$R_0^{\,6} = 8.79 \cdot 10^{-5}\, n_r^{-4} \Phi_D \kappa^2 \int F_D(\lambda)\varepsilon(\lambda)\lambda^4 d\lambda \qquad \text{(in Å}^{-6}\text{)}$$



FRET efficiency

$$\eta_{FRET} = \frac{R_0^6}{R_0^6 + r^6}$$



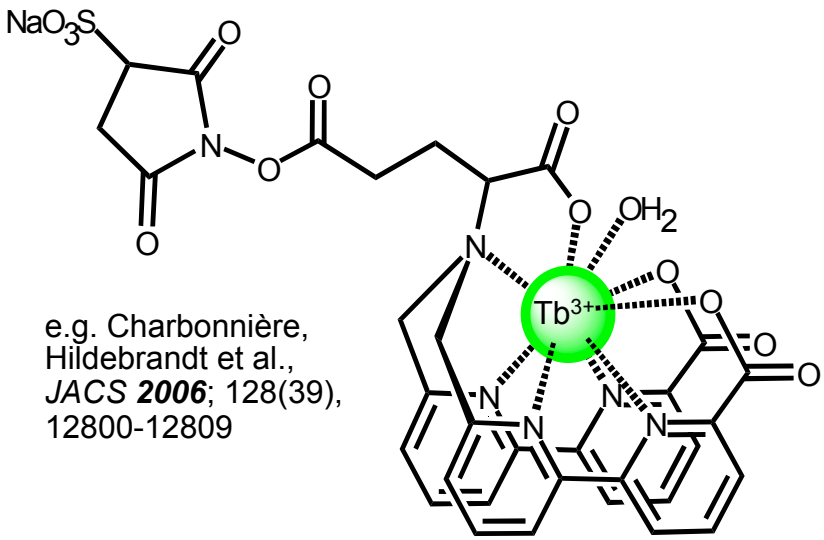
LUMINESCENCE

FRET efficiency ⟹

$$\eta_{FRET} = \frac{k_{FRET}}{k_{FRET} + k_D + k_{D-NR}} = 1 - \frac{\Phi_{DA}}{\Phi_D} = 1 - \frac{\tau_{DA}}{\tau_D} = 1 - \frac{I_{DA}}{I_D}$$

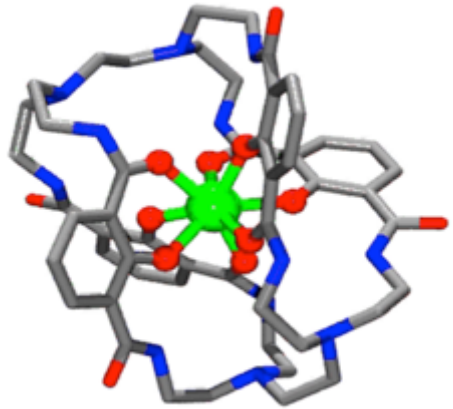
quantum yield — decaytime — intensity

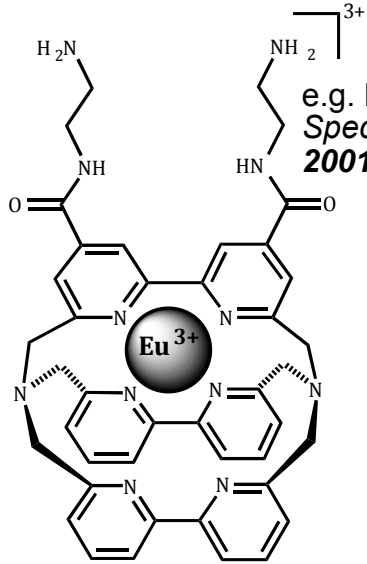
TR-FRET Donors



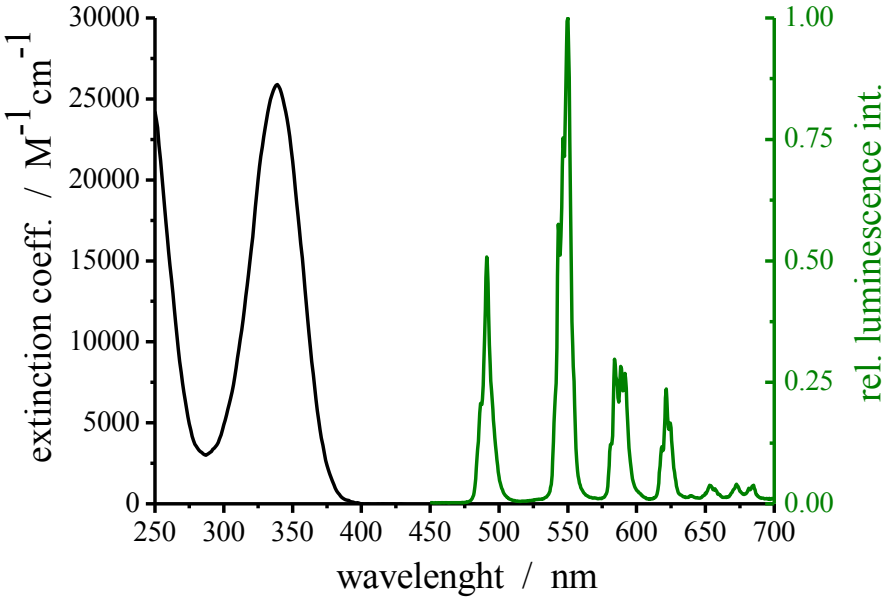
e.g. Charbonnière, Hildebrandt et al., *JACS* ***2006***; 128(39), 12800-12809

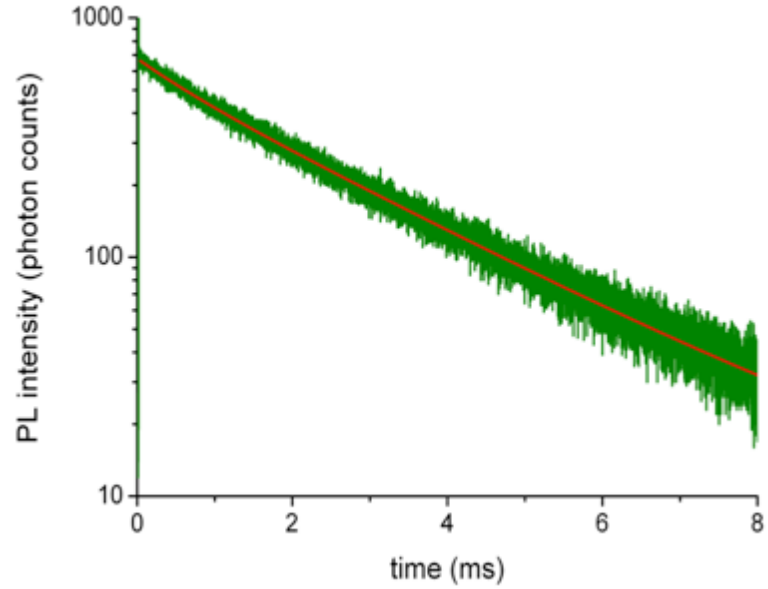
lumiphore
www.lumiphore.com





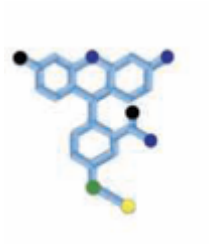
e.g. Bazin et al., *Spectrochim.Act.* ***2001,*** *A 57.*



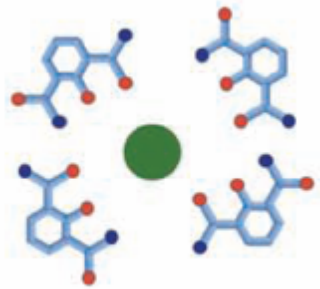


TR-FRET Donor-acceptor combinations

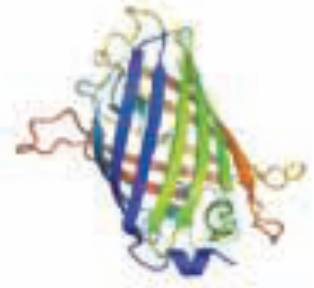
Dyes
(5x magn.)

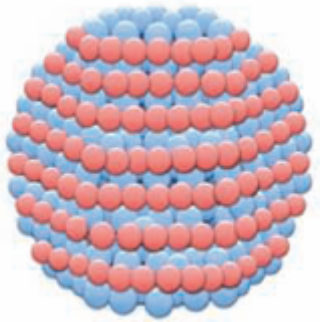


Tb-complexes
(5x magn.)



FPs
(2x magn.)



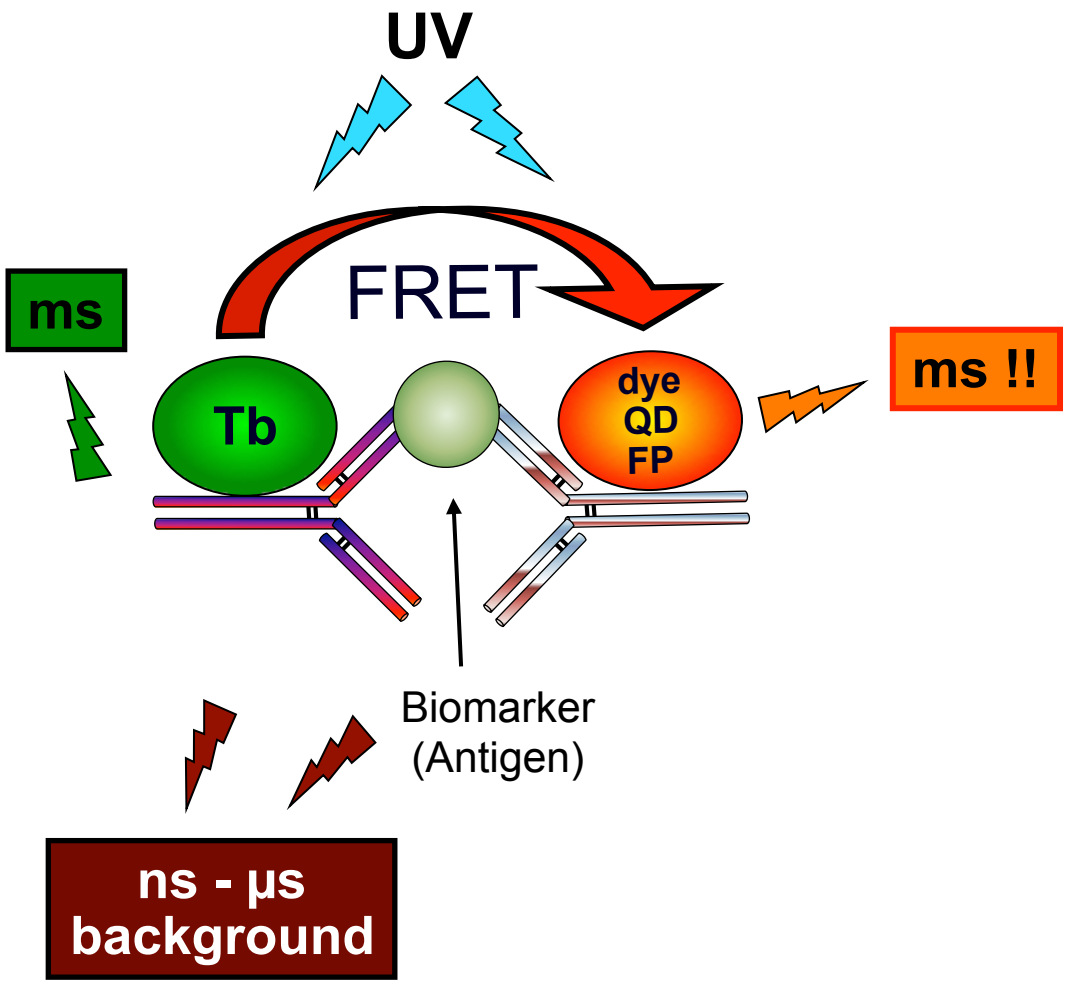


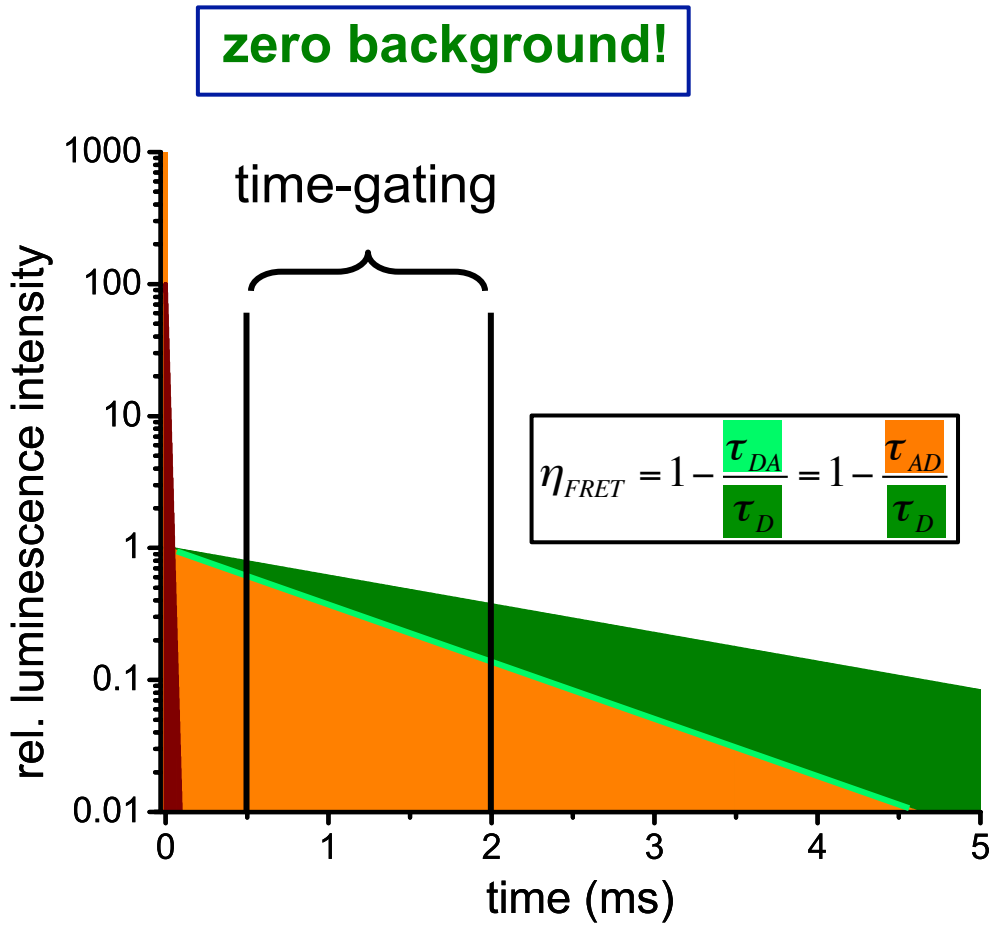
QDs

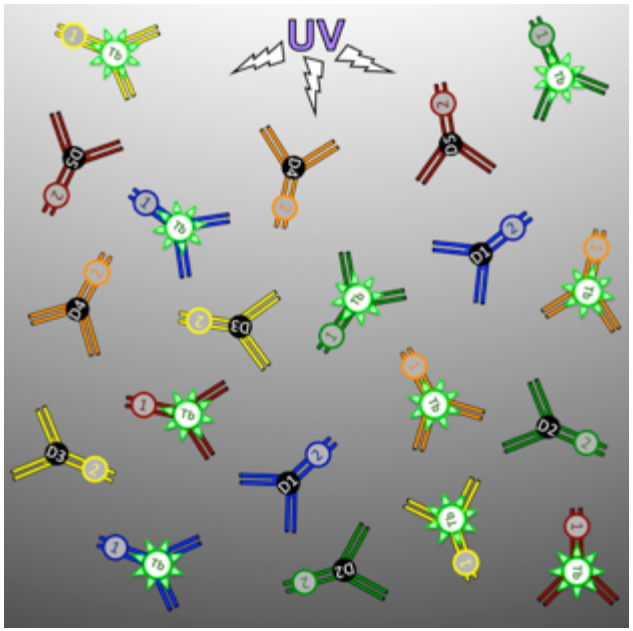
5 nm

FRET

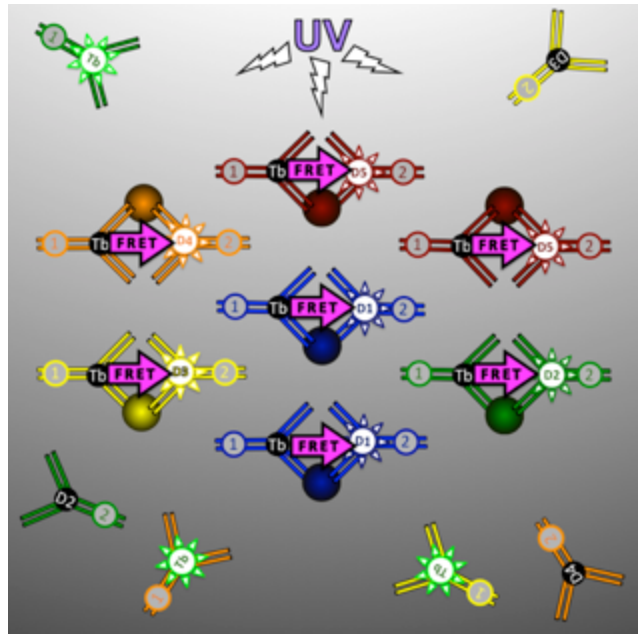
HOMOGENEOUS LIQUID-PHASE LUMINESCENCE ASSAYS FOR FAST AND BACKGROUND-FREE MEASUREMENTS



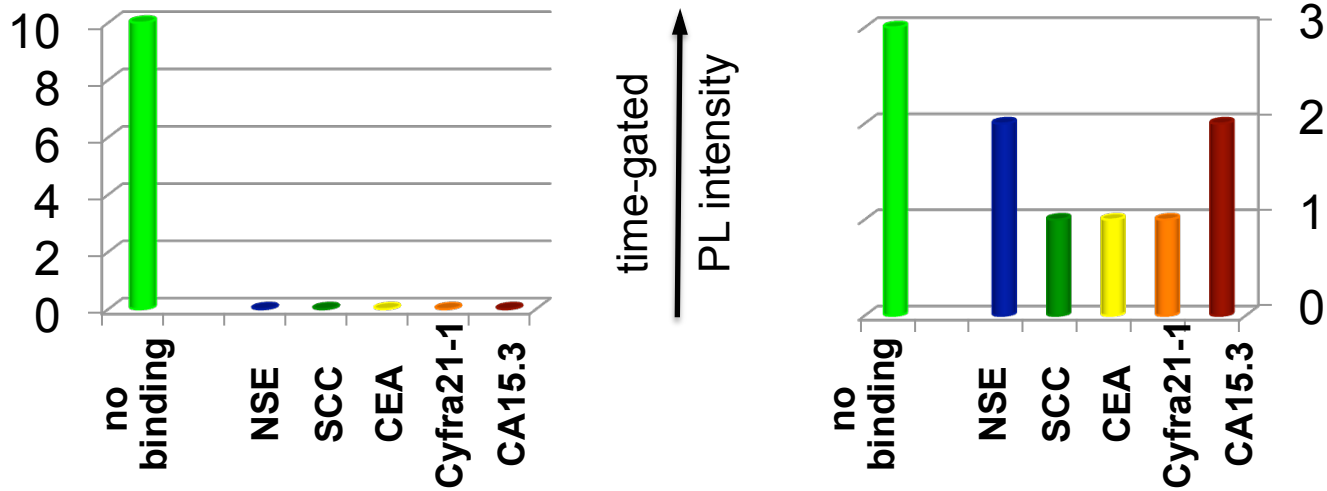




assay without tumor markers



addition of tumor markers

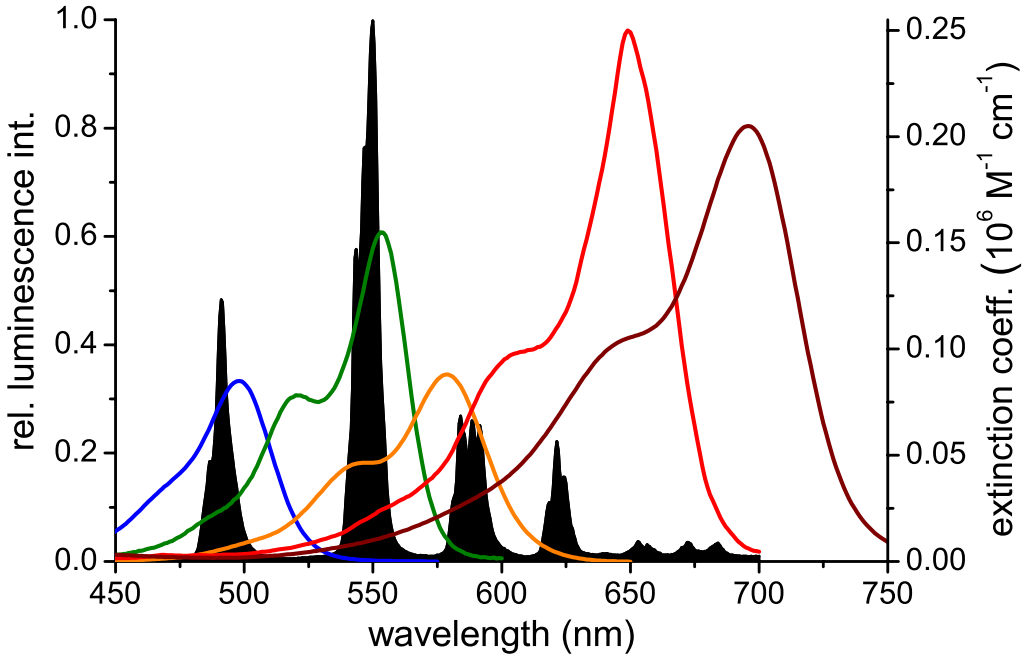


- Simultaneous measurement of several different biomarkers in one single sample.
- More qualitative information per measurement (which disease)
- More quantitative information per measurement (which state of the disease)

D. Geißler et al., *J. Am. Chem. Soc.* **2013**, 135, 1102-1109

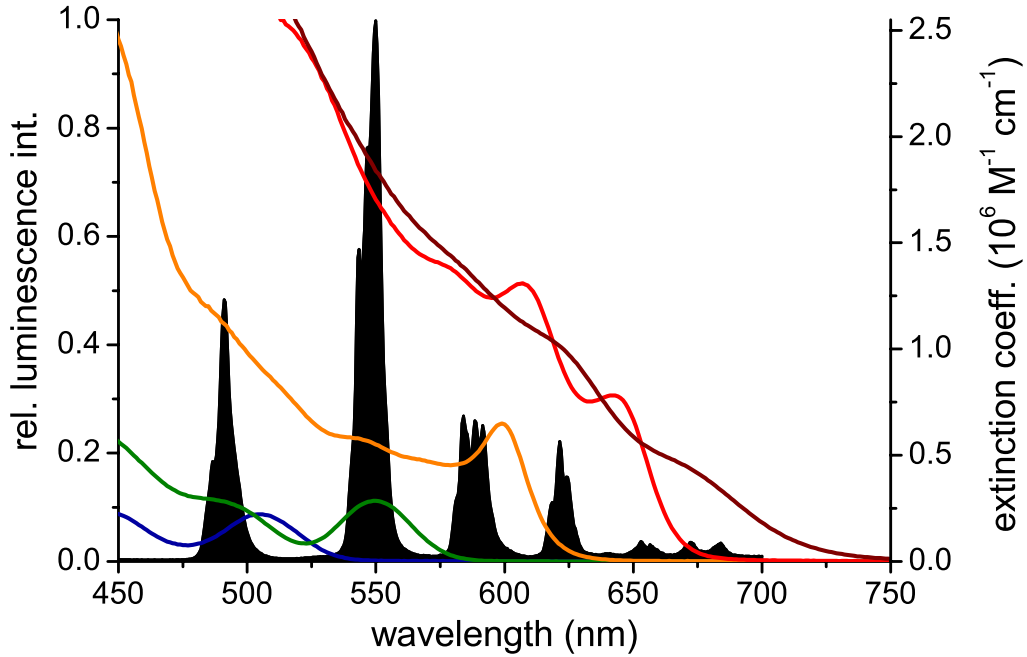
LTC to dye (or FP)

10-fold lower than QD



LTC to QD

10-fold higher than dye



Förster distances

$4\text{nm} < R_0 < 6\text{nm}$

$$J = \int \bar{I}_{\mathrm{D}}(\lambda)\ \varepsilon_{\mathrm{A}}(\lambda)\ \lambda^4\,\mathrm{d}\lambda = \int \bar{I}_{\mathrm{D}}(\tilde{\nu})\ \varepsilon_{\mathrm{A}}(\tilde{\nu})\ \frac{\mathrm{d}\tilde{\nu}}{\tilde{\nu}^4}$$

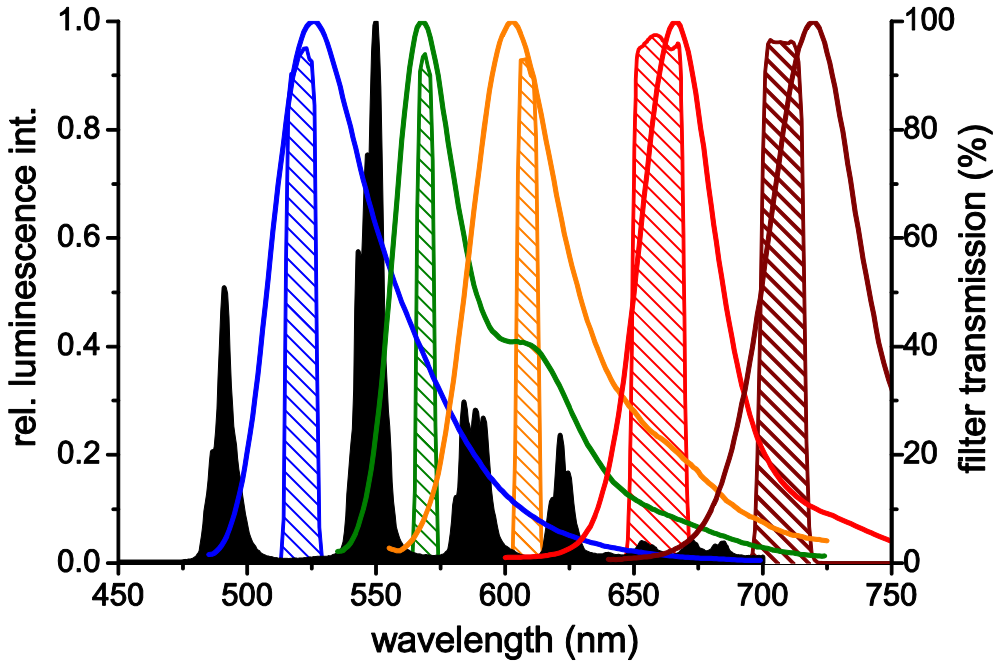
$$R_0 = \left(\frac{9(\ln 10)\kappa^2\Phi_{\mathrm{D}}}{128\pi^5 N_{\mathrm{A}} n^4} J\right)^{1/6}$$

Förster distances

$6\text{nm} < R_0 < 11\text{nm}$

N. Hildebrandt et al. *Coordination Chemistry Reviews* **2014**, 273–274, 125–138.

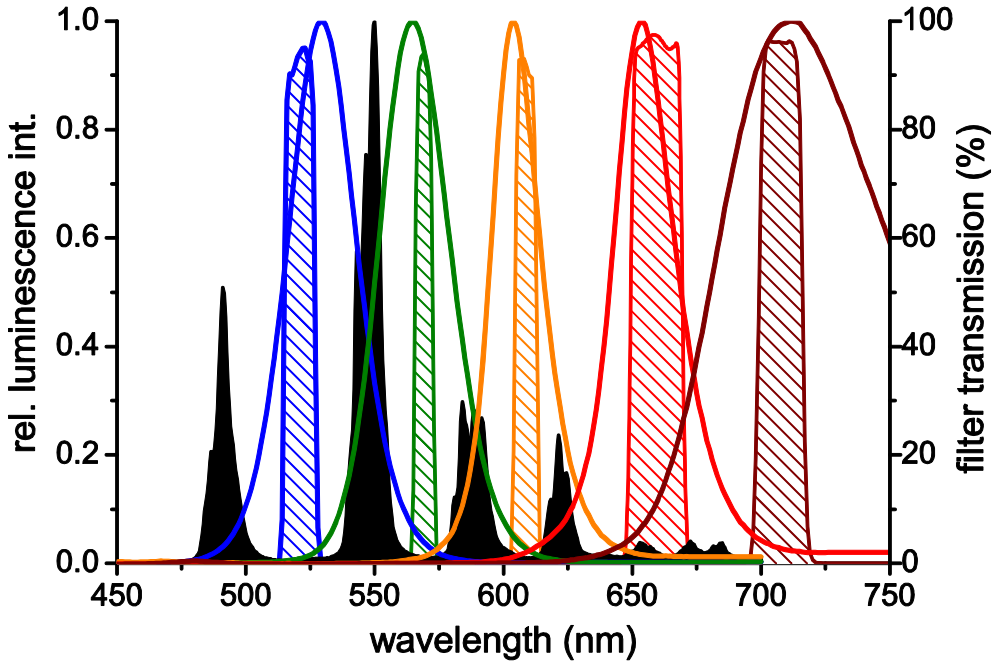
LTC to dye (or FP)



Spectral crosstalk

requires correction

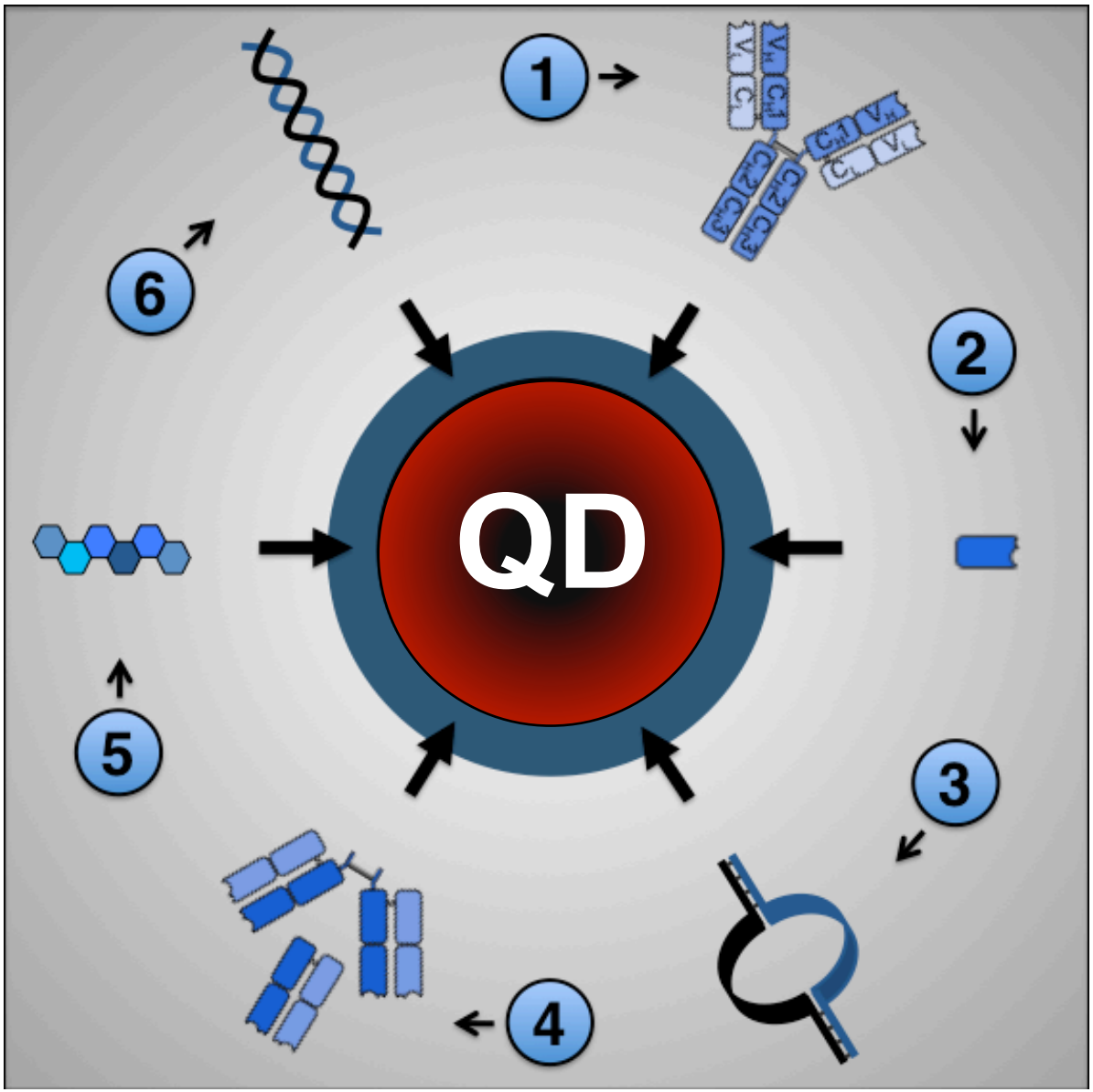
LTC to QD



Very few spectral crosstalk

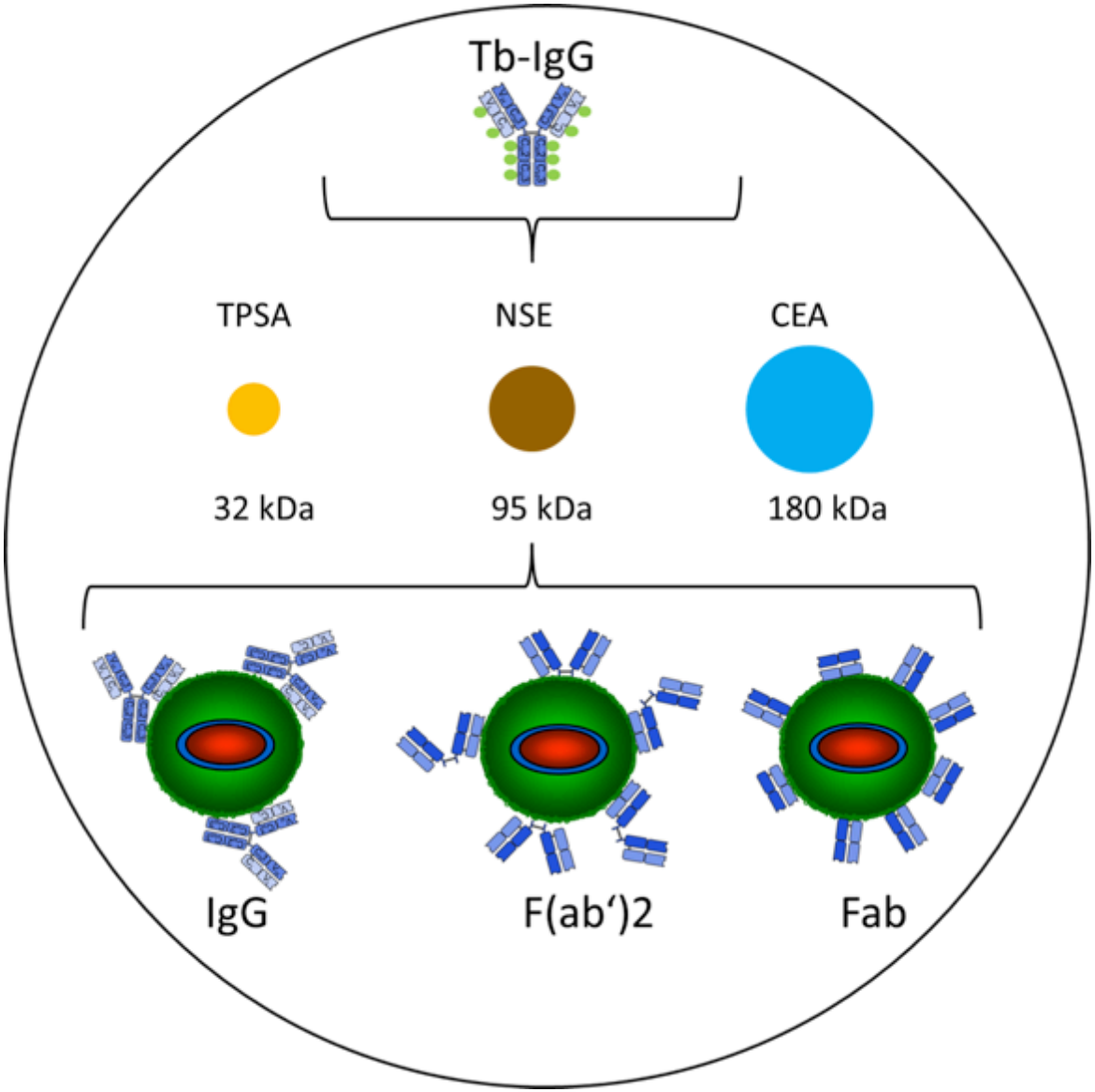
no correction required

N. Hildebrandt et al. *Coordination Chemistry Reviews* **2014**, 273–274, 125–138.



Diagnostic applications require specific biological recognition in serum samples

1. IgG antibodies
2. V_HH antibodies
3. Aptamers
4. F(ab) and F(ab')$_2$ antibodies
5. Peptides
6. Oligonucleotides



Many Tb donors

Different biomarkers

Versatile QD-antibody conjugates

Wegner et al.: *ACS Nano* **2013**, 7 (8), 7411–7419.
Wegner et al.: *Small* **2014**, 10 (4), 734-740.

- **Homogeneous** assay format
- **50 µL serum samples**
- **5 s per read**
- **Wavelength separation** by dichroic mirrors and bandpass filters:

  Parallel detection in 2 channels
  (Tb-donor - **ChD** and QD-acceptor – **ChA**)

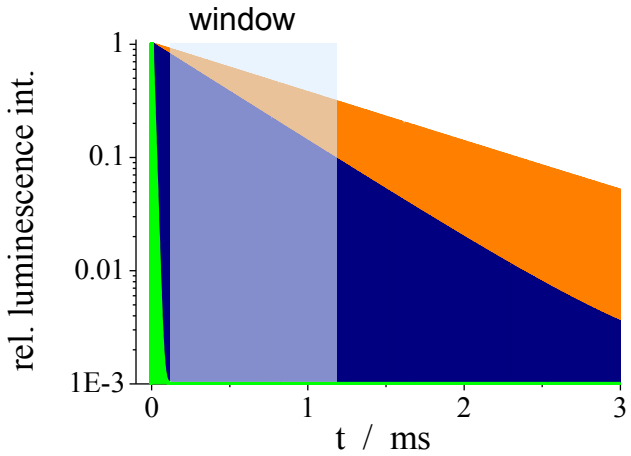
- **Time-gated detection** of luminescence intensities:

  I(**ChA**) and *I*(**ChD**) measured in a time window from 0.1 ms to 0.9 ms after excitation pulse

KRYPTOR compact plus

Intensity ratio:

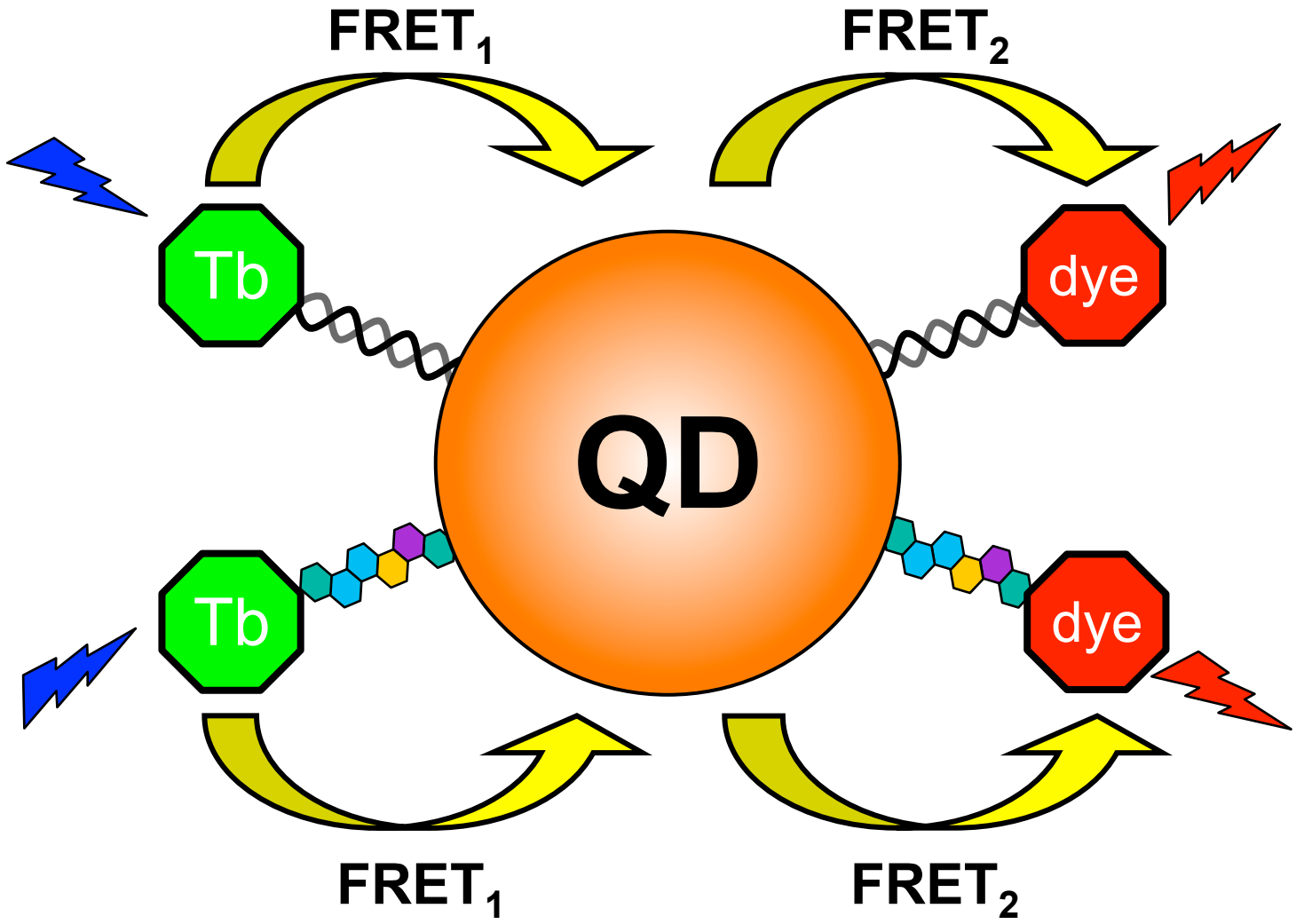
$$R = \frac{I(\mathbf{ChA})}{I(\mathbf{ChD})}$$



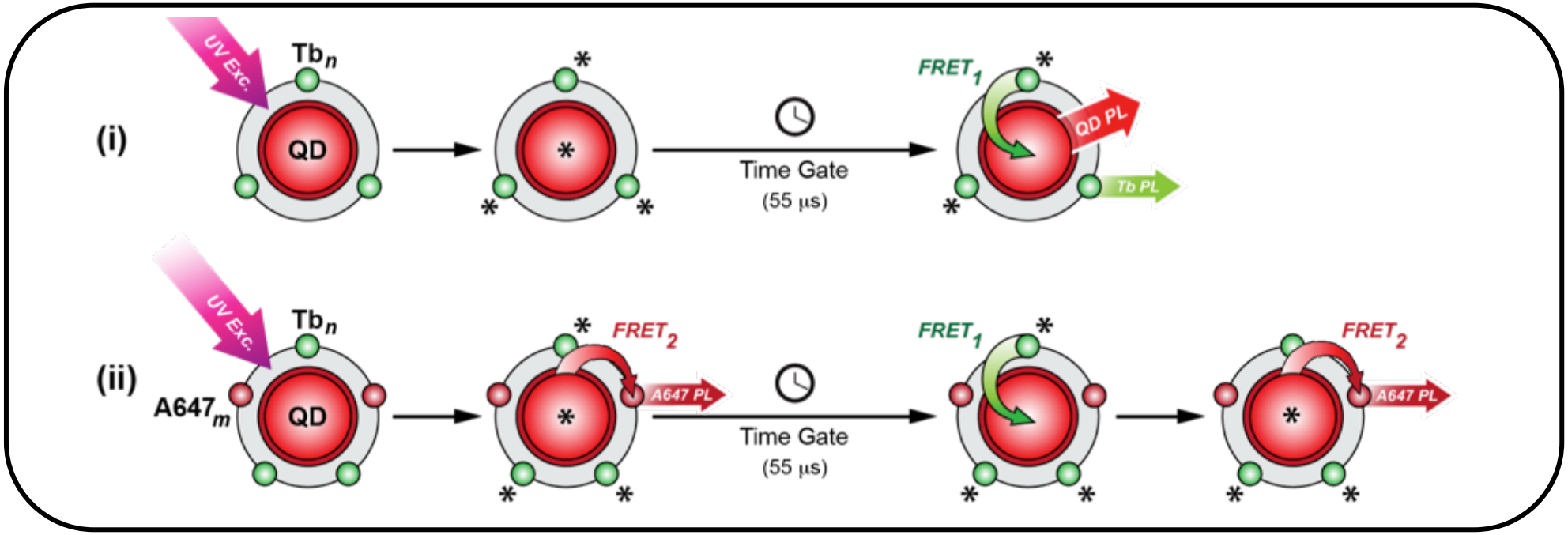
CEZANNE B·R·A·H·M·S

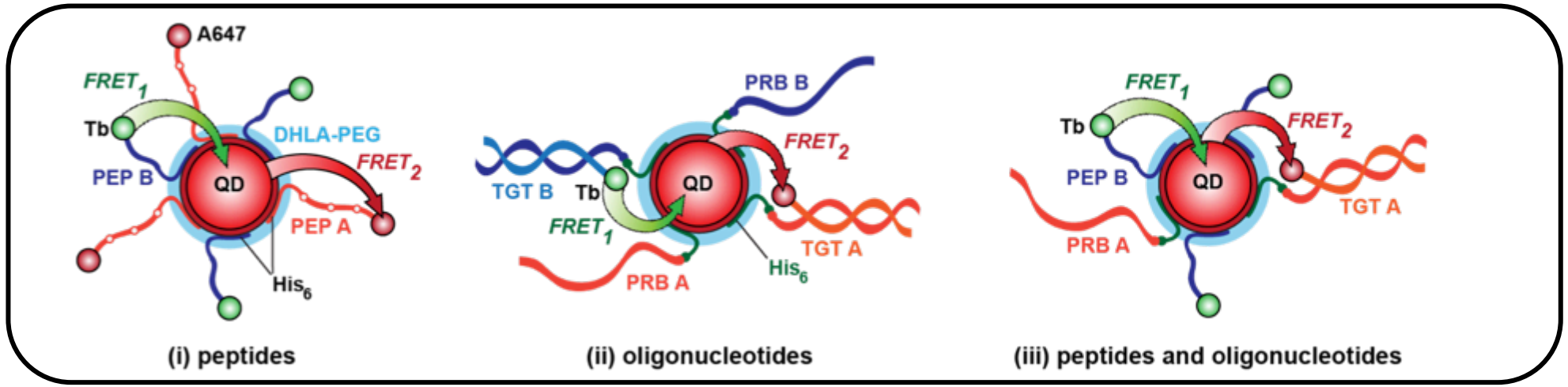
Parts of ThermoFisher Scientific

Multiplexed TR-FRET relays

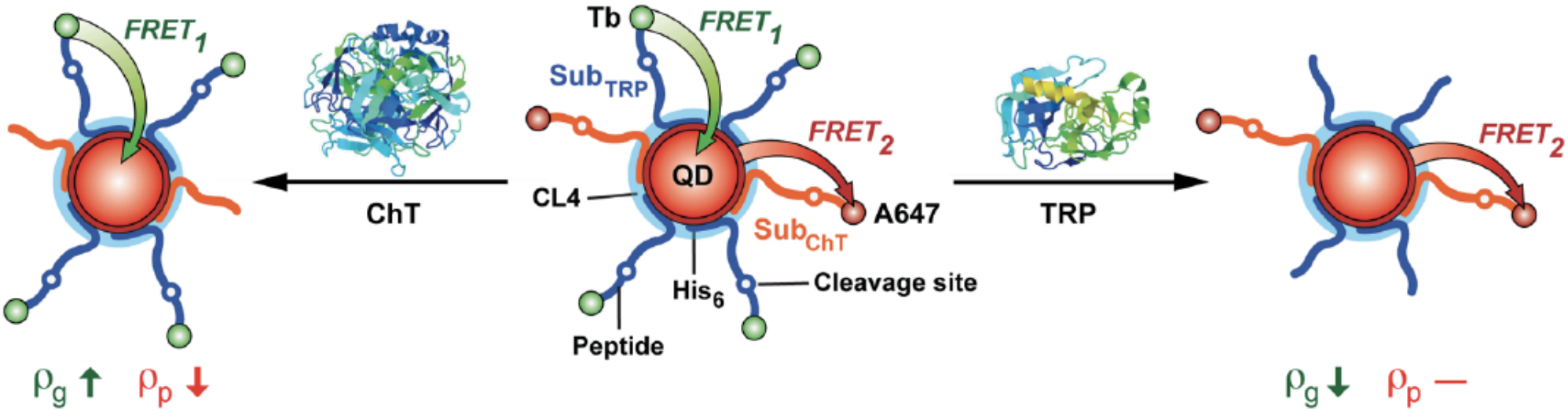


FRET RELAYS (QDs AS SIMULTANEOUS DONOR AND ACCEPTOR)



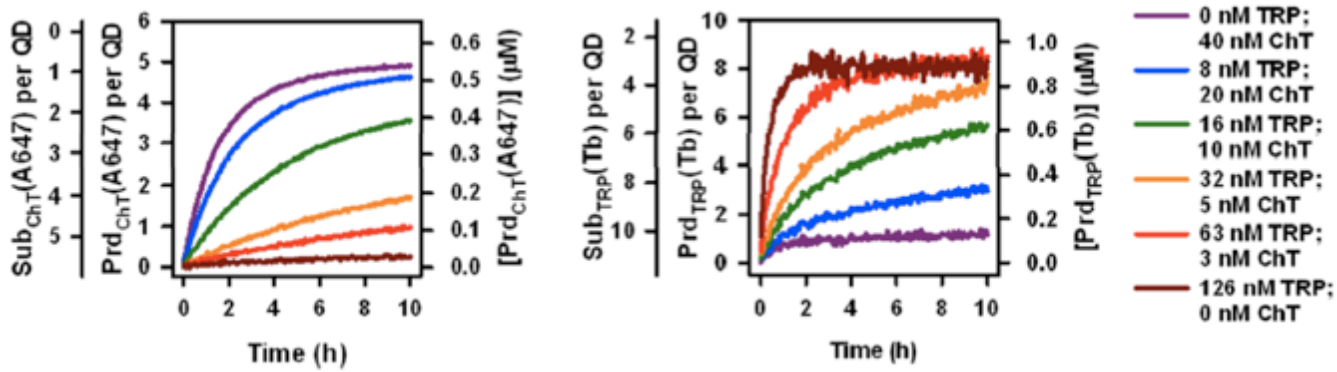


Multiplexed enzyme kinetics

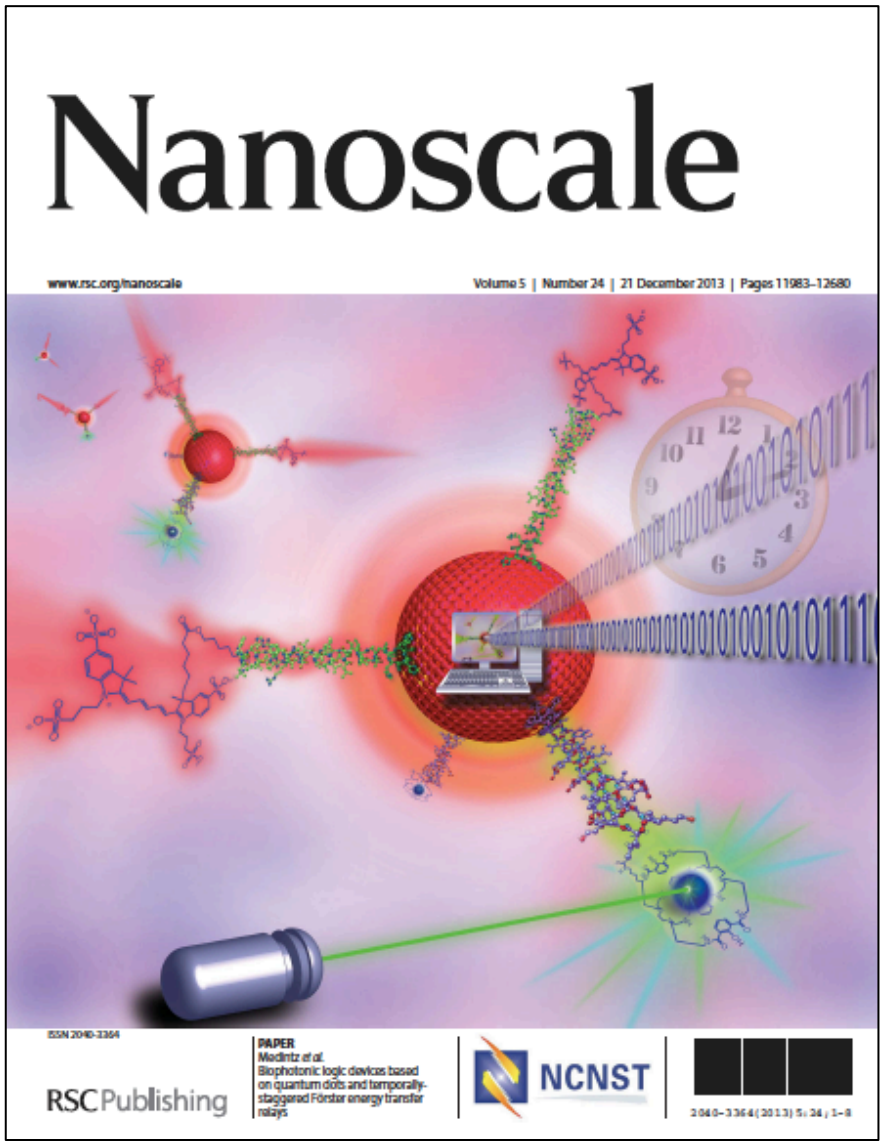


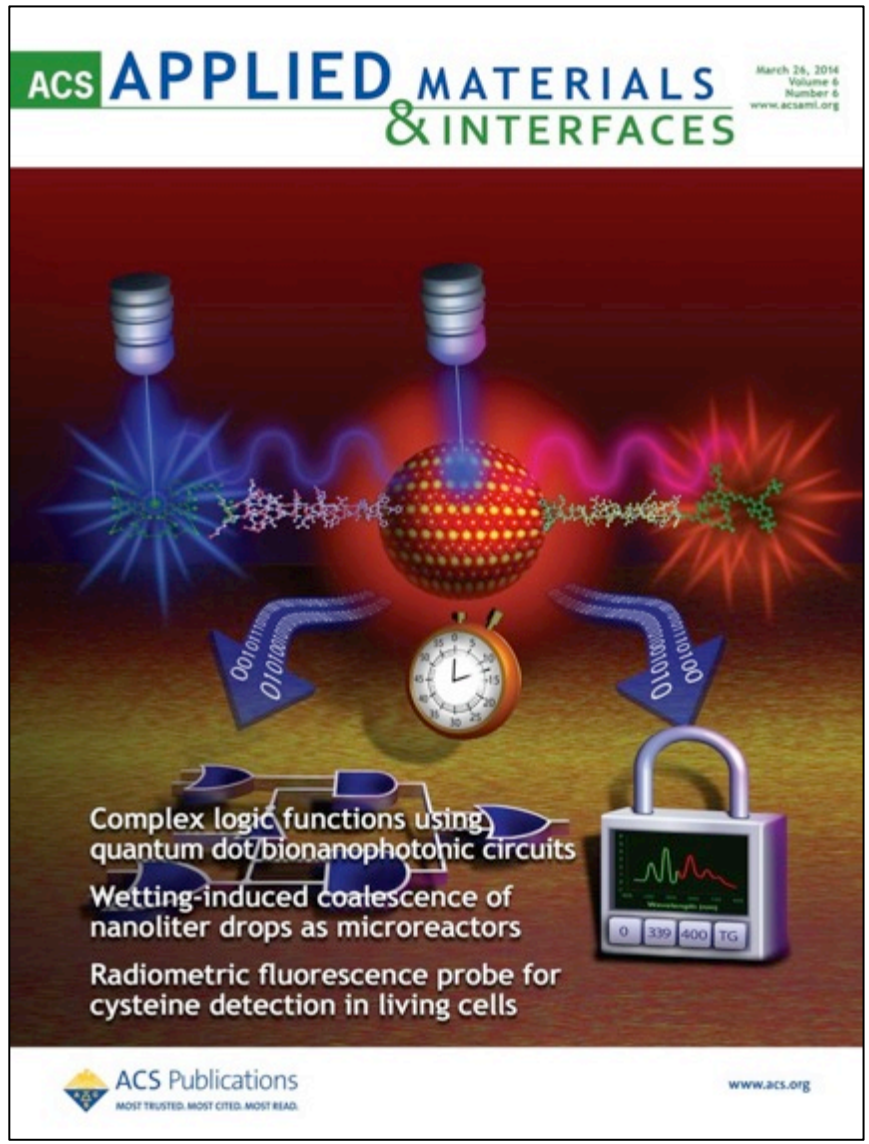
Sub_{TRP}(Tb) ●–GST**R**TDEGNQGGTSSPPPPPPPSHHHHHH

Sub_{ChT}(A647) ●–CSA**Y**AATDEGNQGTSPPPPPPPSHHHHHH

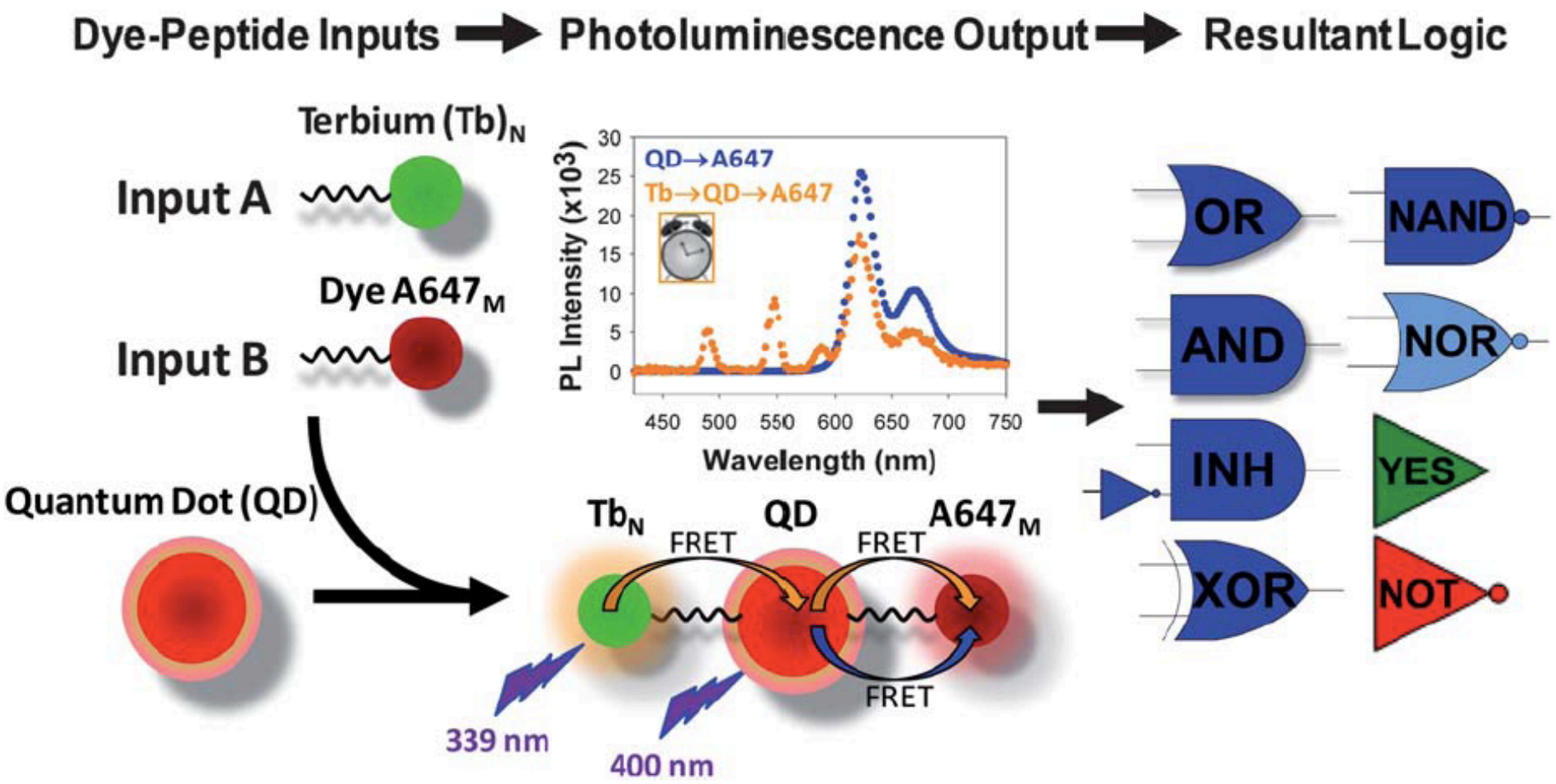


Algar et al., Anal.Chem. **2012**, 84 (22), 10136–10146.





J. C. Claussen et al.: *Nanoscale* **2013**, 5, 12156-12170 and *ACS Applied Materials & Interfaces* **2014**, 6, 3771-3778.

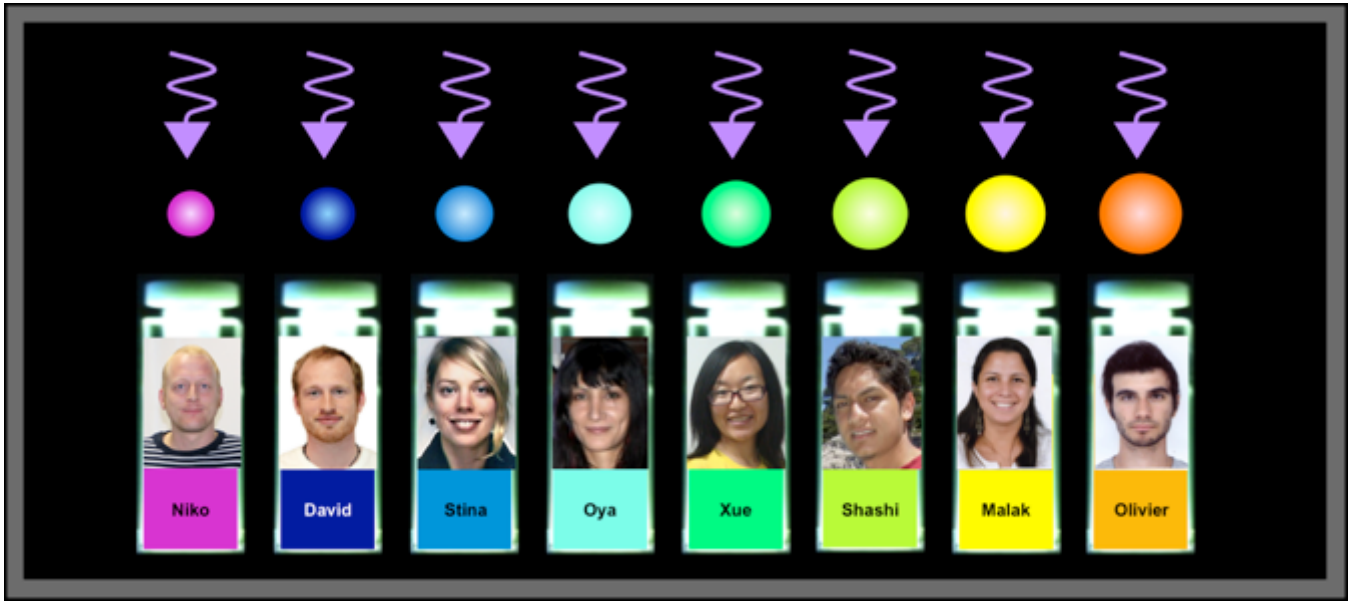


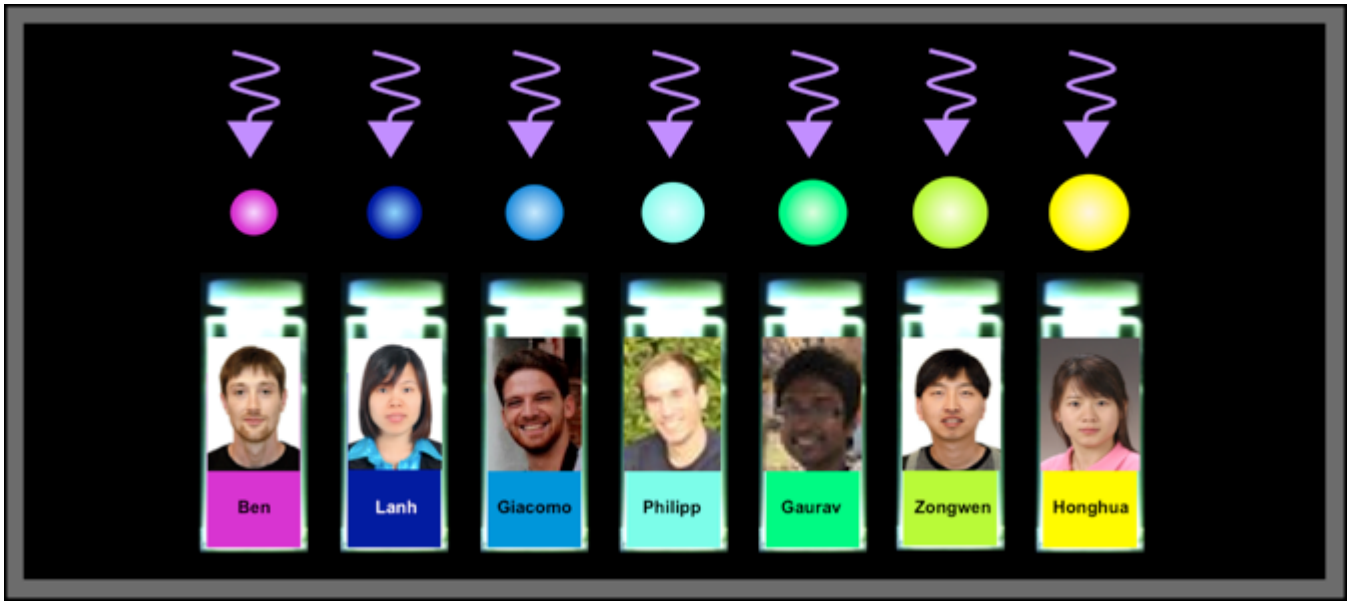
Combination of peptide self-assembly and time-gated $FRET_1$/$FRET_2$ allows for single-output boolean logic and set-reset function.

Acknowledgments

- Paul M. P. van Bergen en Henegouwen (Utrecht University, Netherlands)
- Travis Jennings (eBioscience, Inc., San Diego, USA)
- QVQ (Utrecht, Netherlands)
- Ken Raymond, Darren Magda, Nate Butlin (Lumiphore Inc., Richmond, USA)
- Cezanne SA – Thermo Fisher Scientific (Nîmes, France)

Funding


Niko
David
Stina
Oya
Xue
Shashi
Malak
Olivier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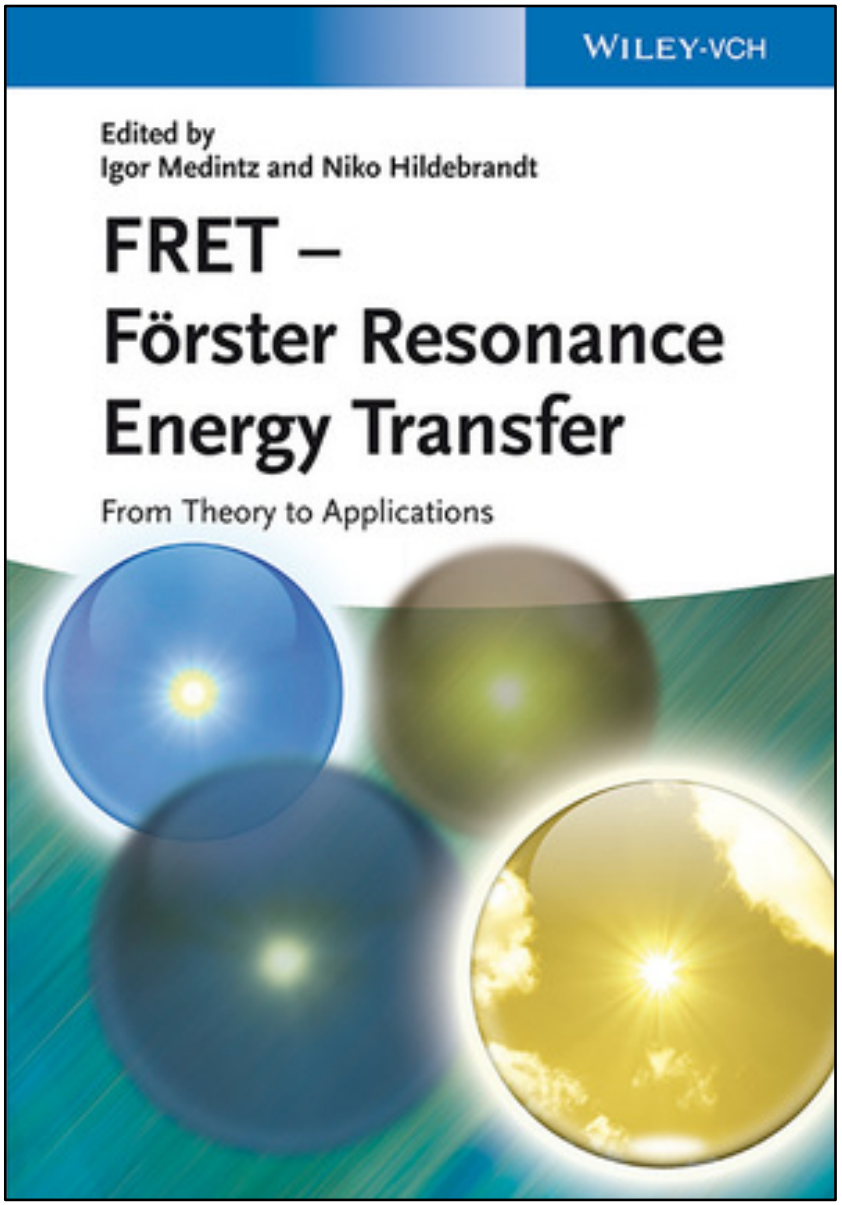
Lanh
Giacomo
Philipp
Gaurav
Zongwen
Honghua



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