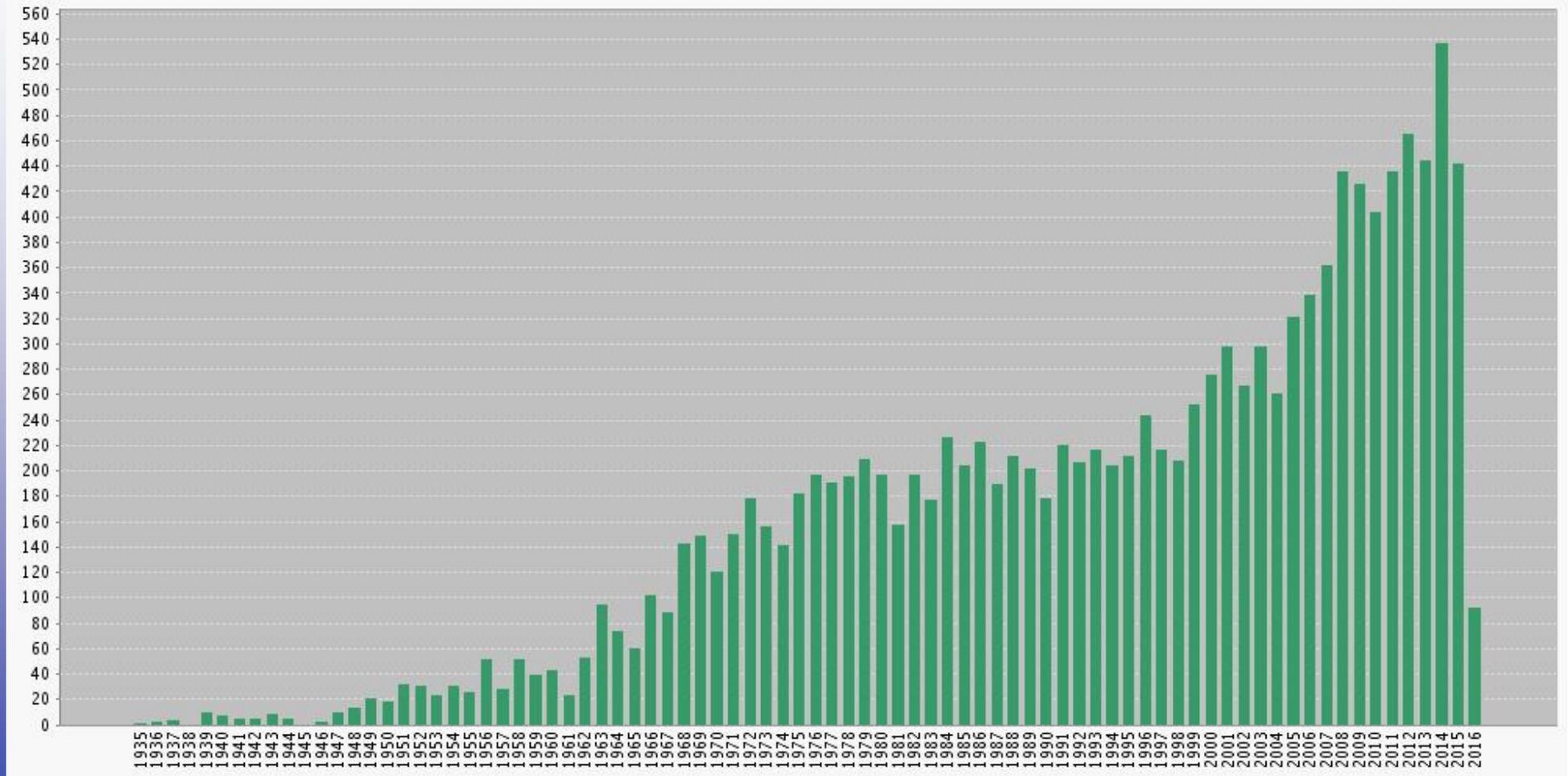


# Theodor Förster, 15.5.1910 – 20.5.1974

- 1933: PhD thesis: „Zur Polarisation von Elektronen durch Reflexion“ University of Frankfurt (Prof. E. Madelung)
- 1934: Assistant of Prof. K.-F. Bonhoeffer, Leipzig (Debye, Heisenberg, Kautzky)
- 1942: o. Prof. in Posen (Poznań)
- 1945: Department head MPI for Phys. Chem., Göttingen
- 1951: University of Stuttgart
  - ~80 Publications
  - Förster Cycle
  - FRET (Dipole-Dipole)
  - Excimers (Pyrene)
  - Diabatic and adiabatic reactions,  $k_f$ ,  $k_{nr}$

# Citation Index Theodor Förster





# Fluoreszenz Organischer Verbindungen

Von

**Theodor Förster**

Professor für physikalische Chemie  
Max Planck-Institut für physikalische Chemie  
Göttingen

Mit 81 Abbildungen



---

Göttingen · Vandenhoeck & Ruprecht · 1951

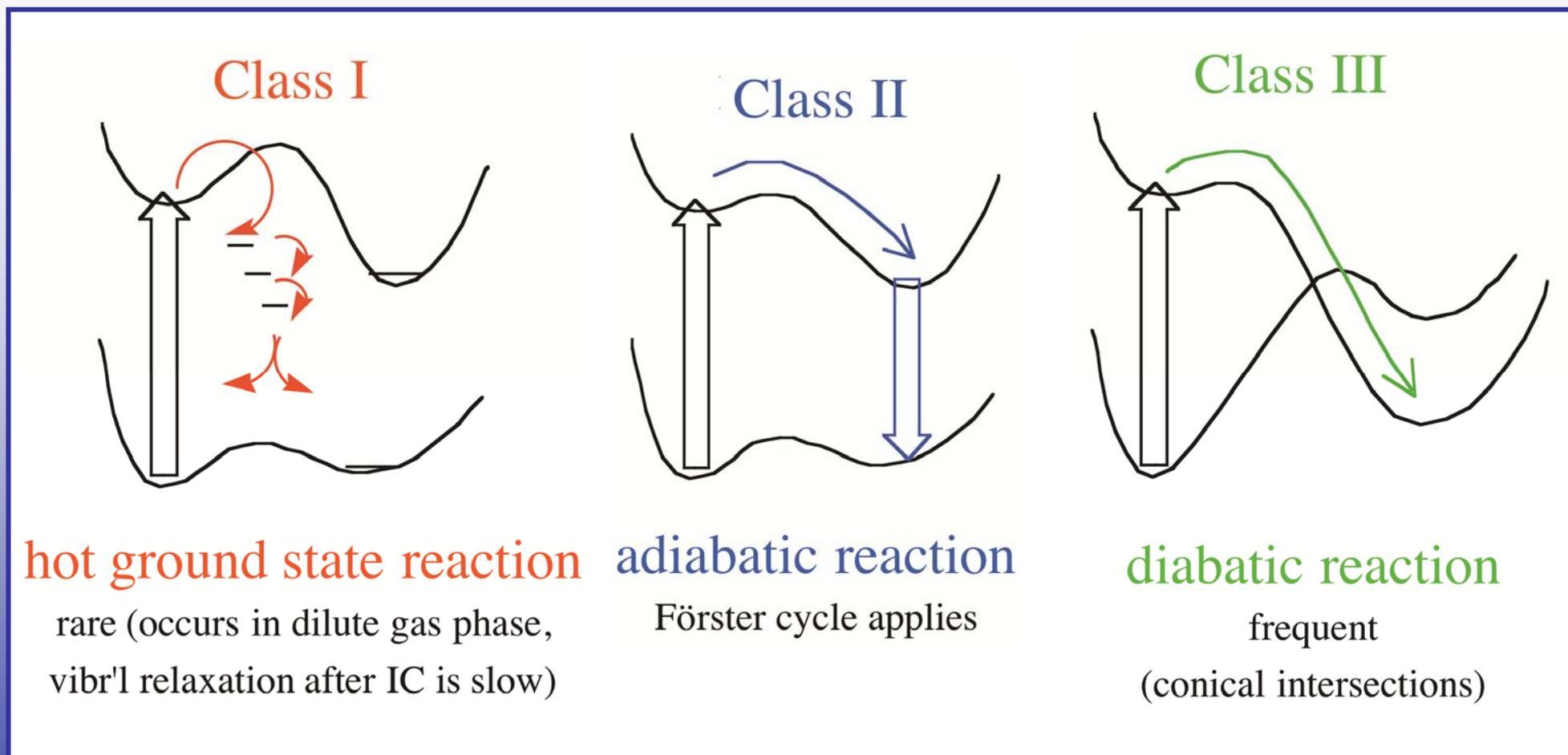
# Albert Weller's obituary (1974)

Albert Weller, referring to *Die Fluoreszenz organischer Verbindungen*: “the Hausbibel – a very appropriate name because in the first place this book could open new pathways to knowledge and secondly its concise formulations, in which every word was important, required two or more readings or still better the interpretation of an enlightened mediator before one could comprehend its precious contents. For the non-German speaking people it must have remained a book with seven seals; and since it has not been translated into English it has secured, as many American colleagues more or less have asserted, the German lead in fluorescence spectroscopy for years.”

# George Porter at the 1<sup>st</sup> “Theodor-Förster Gedächtnisvorlesung” 1975

„Photochemistry, which previously was concerned mainly with the final products or the dark reactions of intermediates such as free radicals, has become a new science of the excited state. No single person contributed more to this progress than Theodor Förster.“

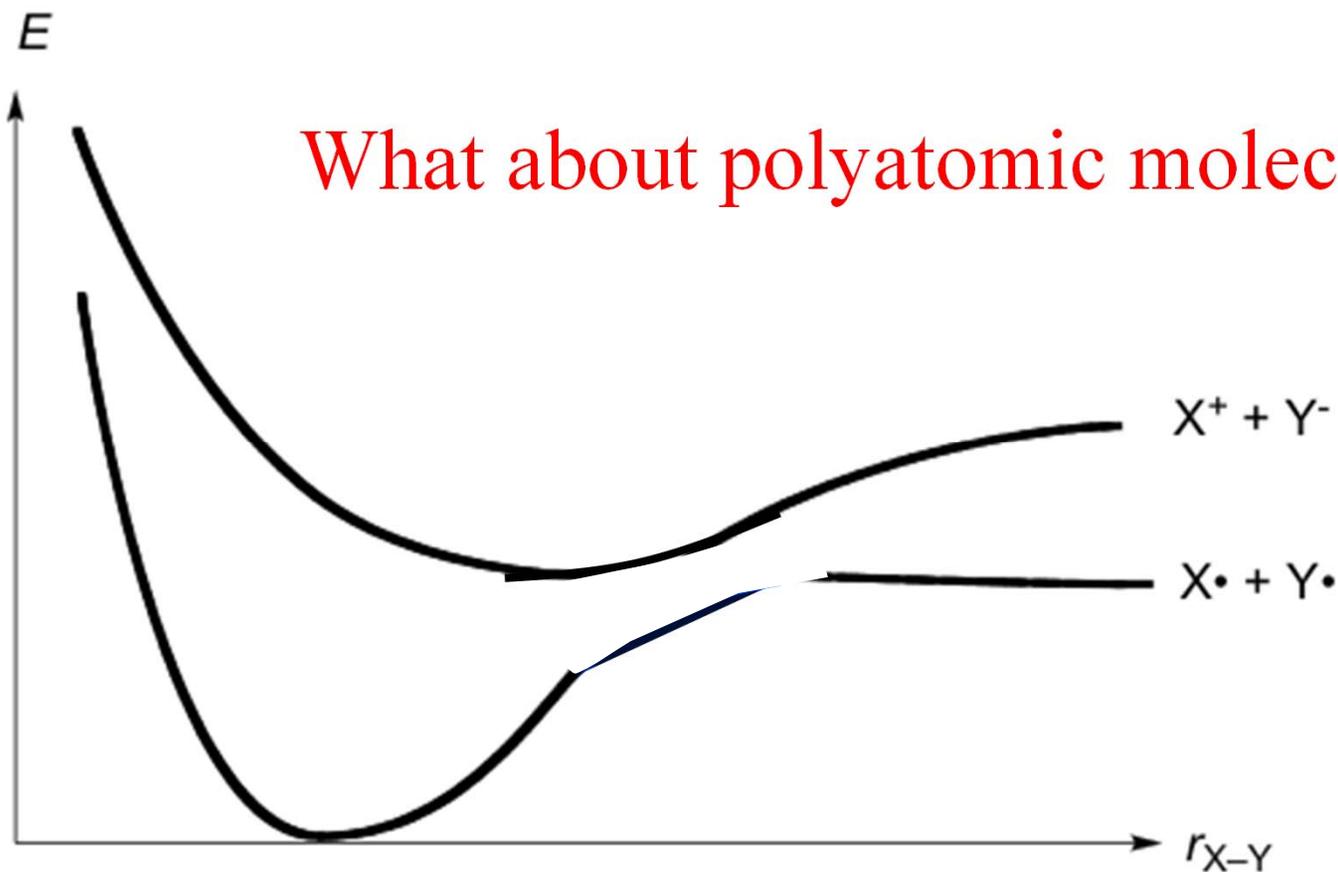
## Three classes of photoreactions (1970)



3<sup>rd</sup> IUPAC Symposium on Photochemistry 1970  
Pure and Applied Chemistry, 34, 1973, 225.

# Noncrossing rule for diatomic molecules (von Neumann, Wigner)

If there is only one degree of freedom ( $r_{X-Y}$ ): Two conditions ( $E_1 = E_2$  and  $V_{12} = 0$ ) cannot be met at the same time



*Proc. R. Soc. Lond. A.* **344**, 147–156 (1975)

*Printed in Great Britain*

## The intersection of potential energy surfaces in polyatomic molecules

BY H. C. LONGUET-HIGGINS, F.R.S.

*Centre for Research on Perception and Cognition,  
Laboratory of Experimental Psychology, University of Sussex*

*(Received 5 December 1974 – Revised 30 January 1975)*

### INTRODUCTION

A question of some importance in molecular spectroscopy and photochemistry is whether it is quite exceptional, or relatively common, for two electronic states of the same symmetry to have intersecting potential energy surfaces. For many years it was generally agreed that such crossings are exceptional in diatomic molecules (von Neumann & Wigner 1929), but not in polyatomic molecules (Teller 1937; Herzberg & Longuet-Higgins 1963). Recently, however, a new basis has been proposed for the ‘non-crossing rule’ (Naqvi & Byers Brown 1972), from which it has been inferred (Naqvi 1972) that this rule applies not only to diatomic but also to polyatomic molecules. The purpose of the present paper is to establish where the truth lies. The argument falls into three parts.



# Elektrolytische Dissoziation angeregter Moleküle

Von TH. FÖRSTER

Aus dem Max-Planck-Institut für physikalische Chemie, Göttingen.

(Vortrag, gehalten auf der 48. Hauptversammlung der Bunsengesellschaft in Wiesbaden am 15. Oktober 1949.)

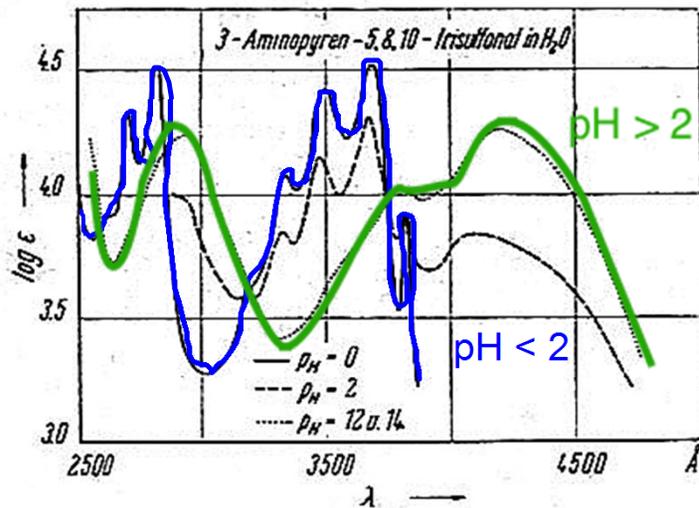


Abb. 3

Absorptionsspektrum von 3-Aminopyren-5, 8, 10-trisulfonat

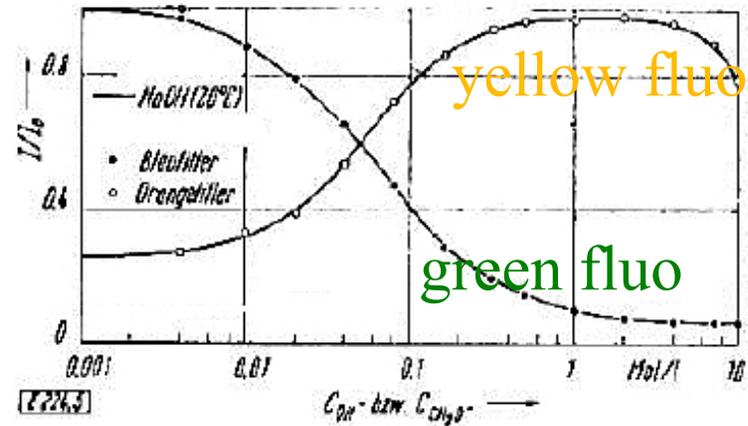
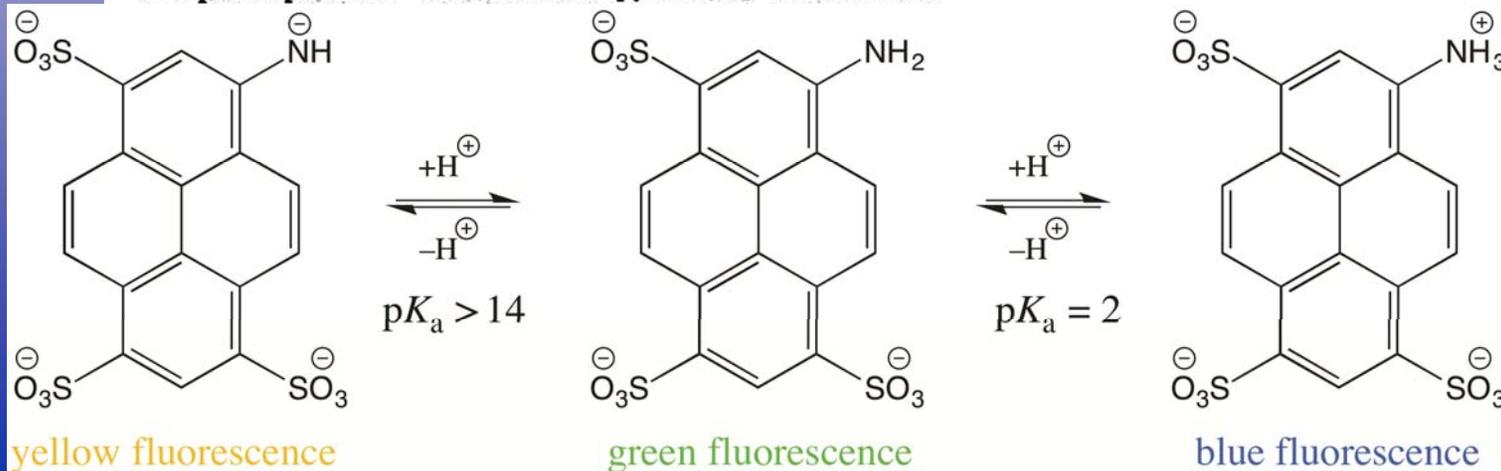


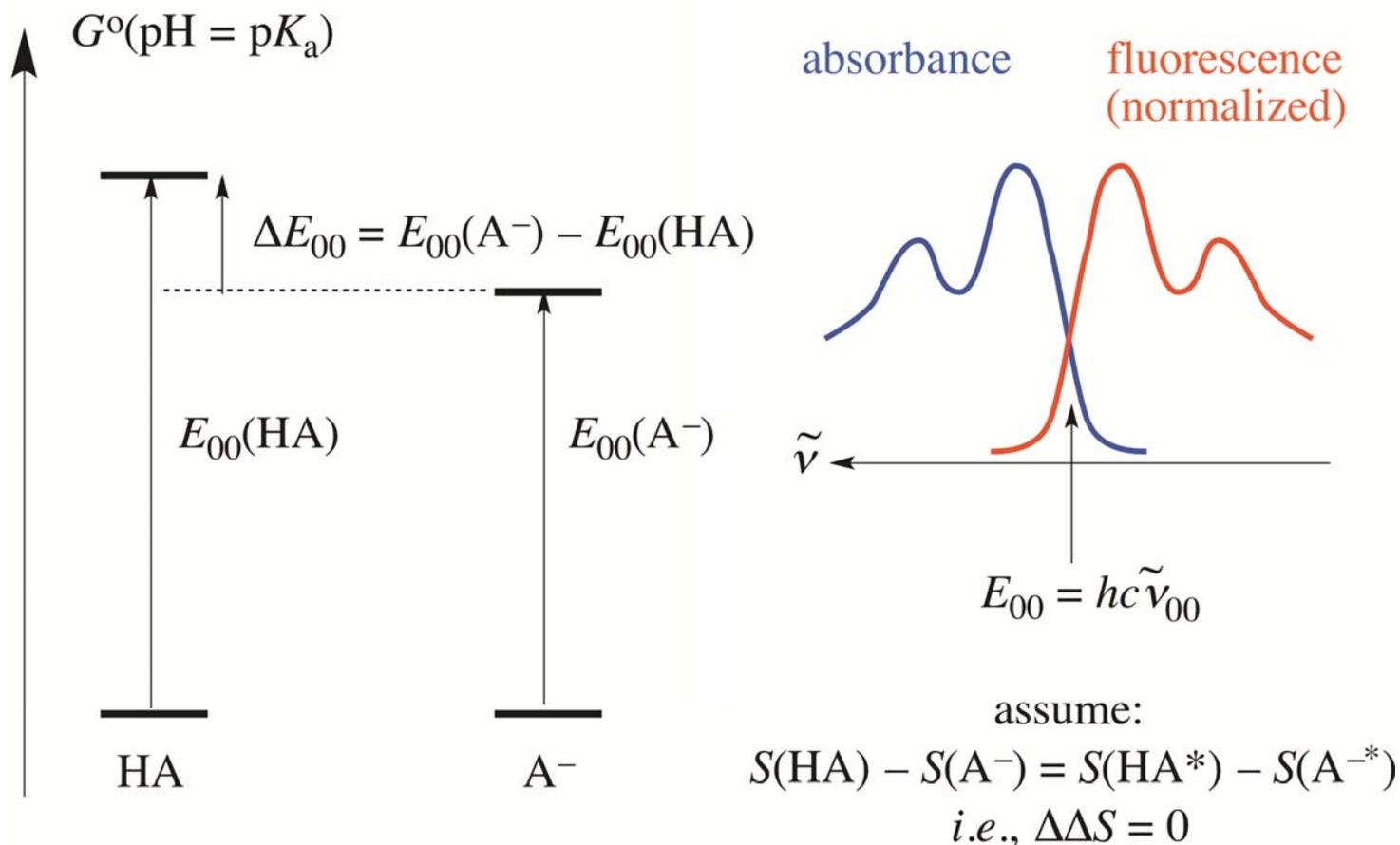
Abb. 5

Fluoreszenzumschlag des 3-Aminopyren-5, 8, 10-trisulfonat in alkalischen Lösungen. Grüne Fluoreszenz hinter BG 1, gelbe Fluoreszenz hinter OG 2 gemessen.



$pK_a^*(NH) = 12.7!$

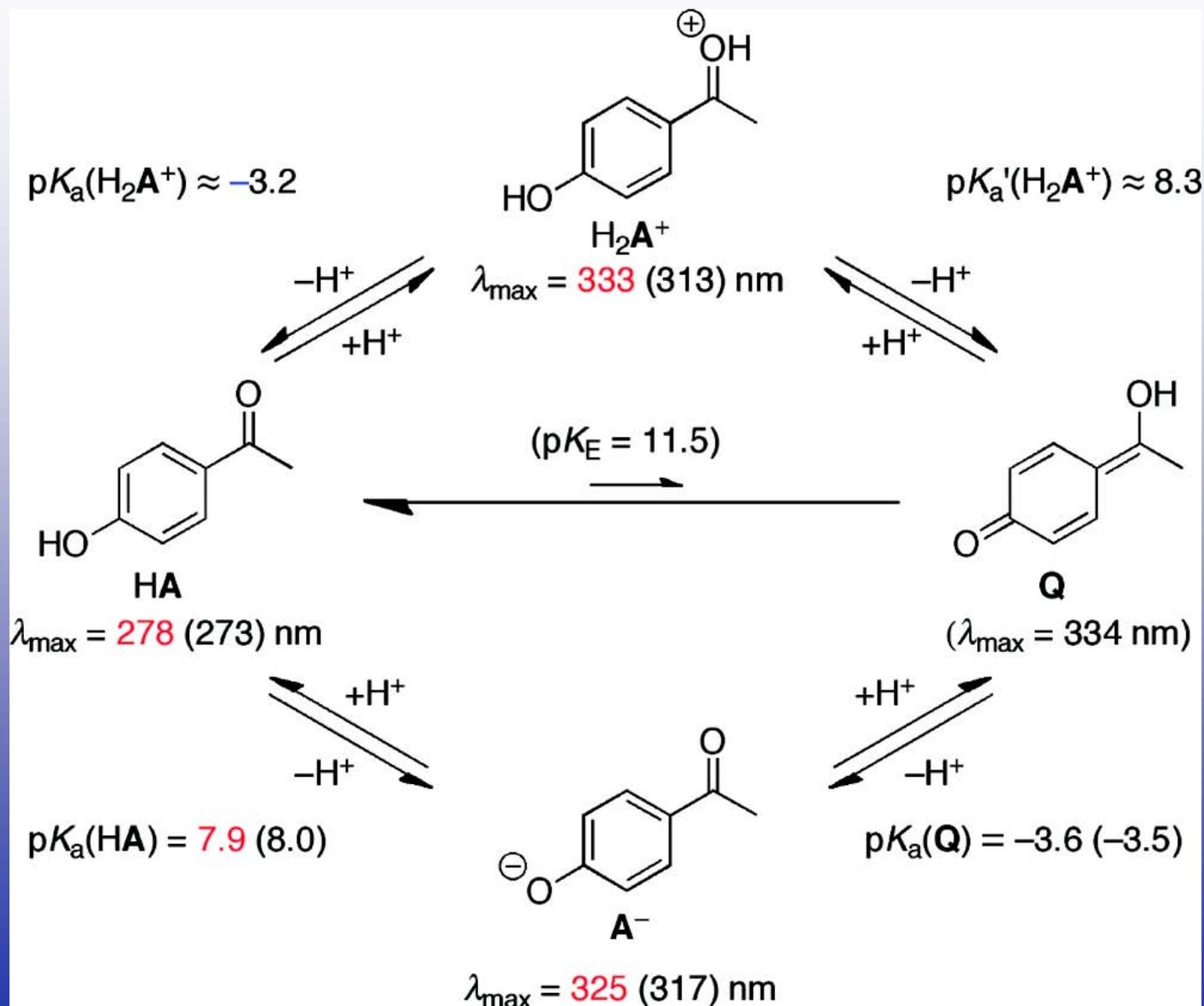
# The Förster Cycle



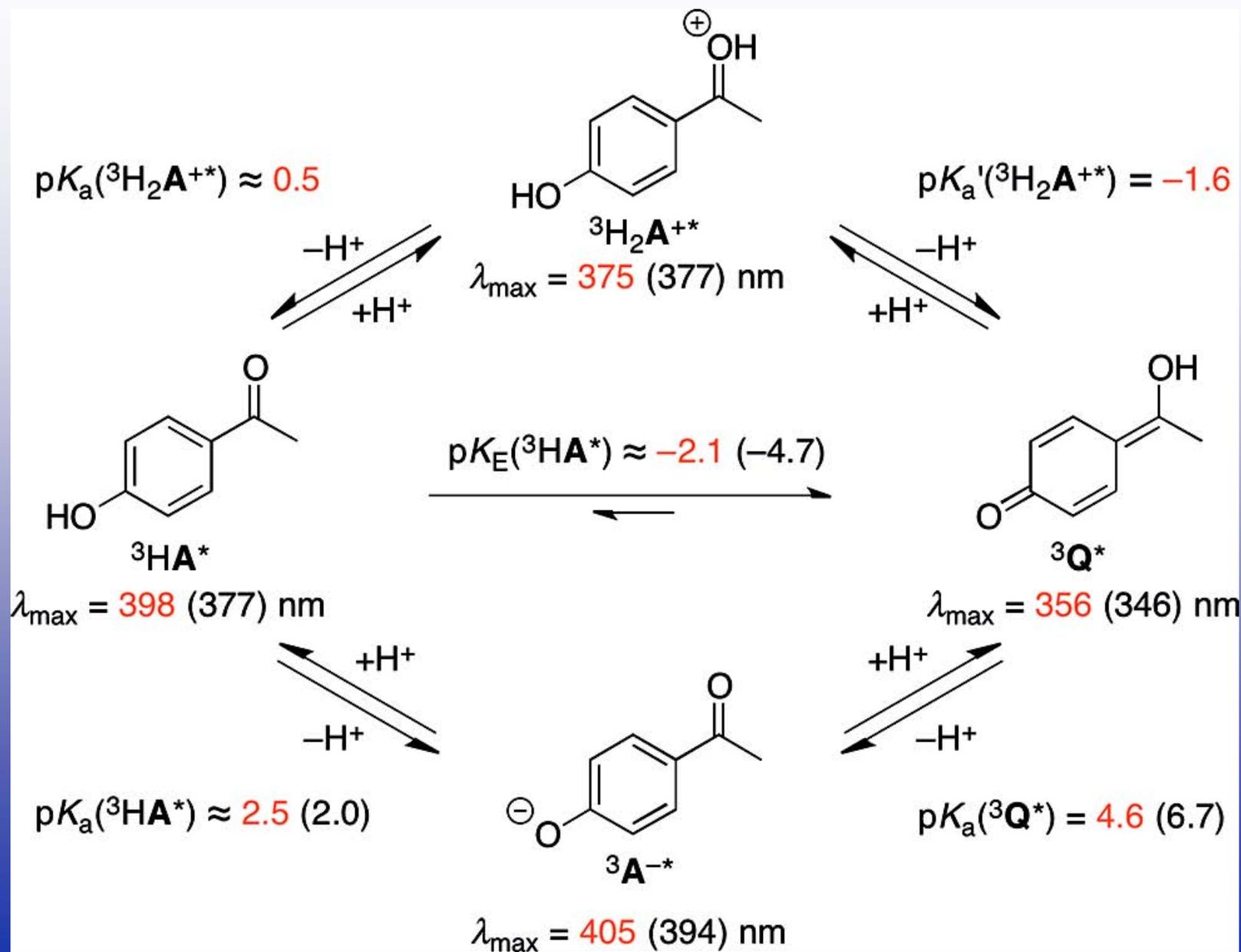
$$\Delta E_{00} = hc\Delta\tilde{\nu}_{00} \quad E \quad \Delta G^\circ = -RT \ln(K) = 2.3RT\text{p}K$$

$$\text{p}K_a^* = \text{p}K_a + 21.0(\Delta\tilde{\nu}_{00}/\mu\text{m}^{-1}) \text{ for } T = 298 \text{ K}$$

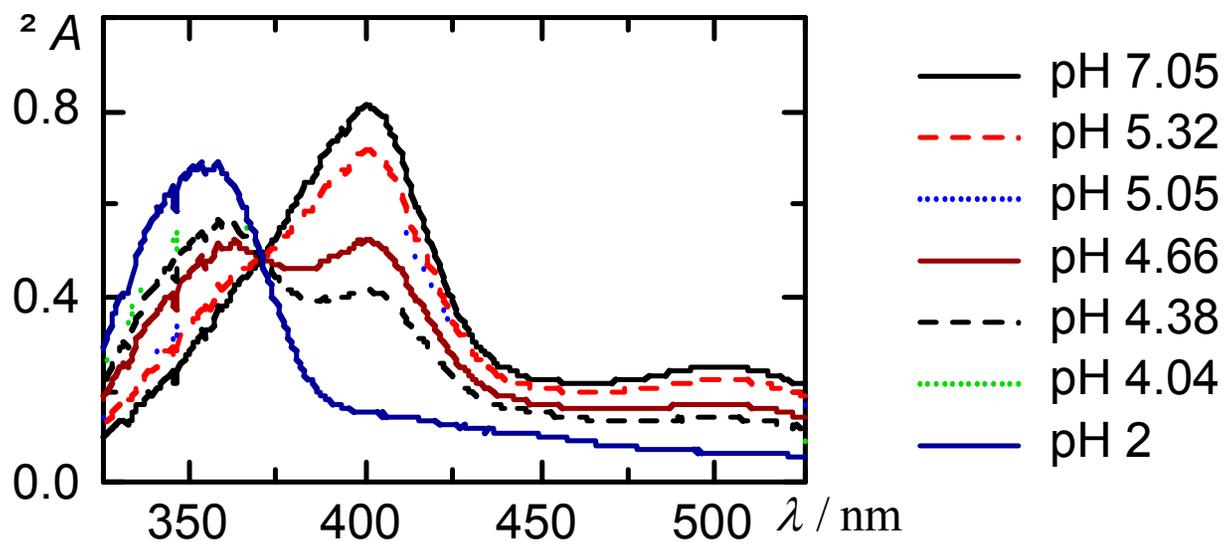
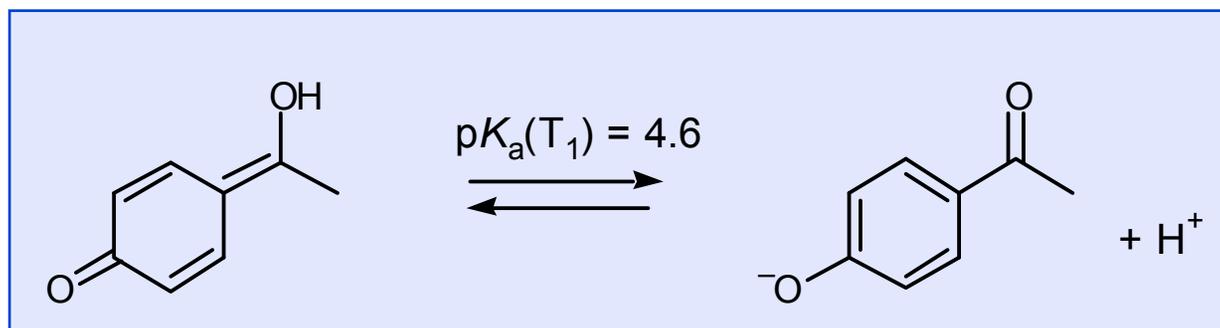
# *p*-Hydroxyacetophenone: Ground state protonation equilibria



# Triplet state equilibria: A proton shuttle

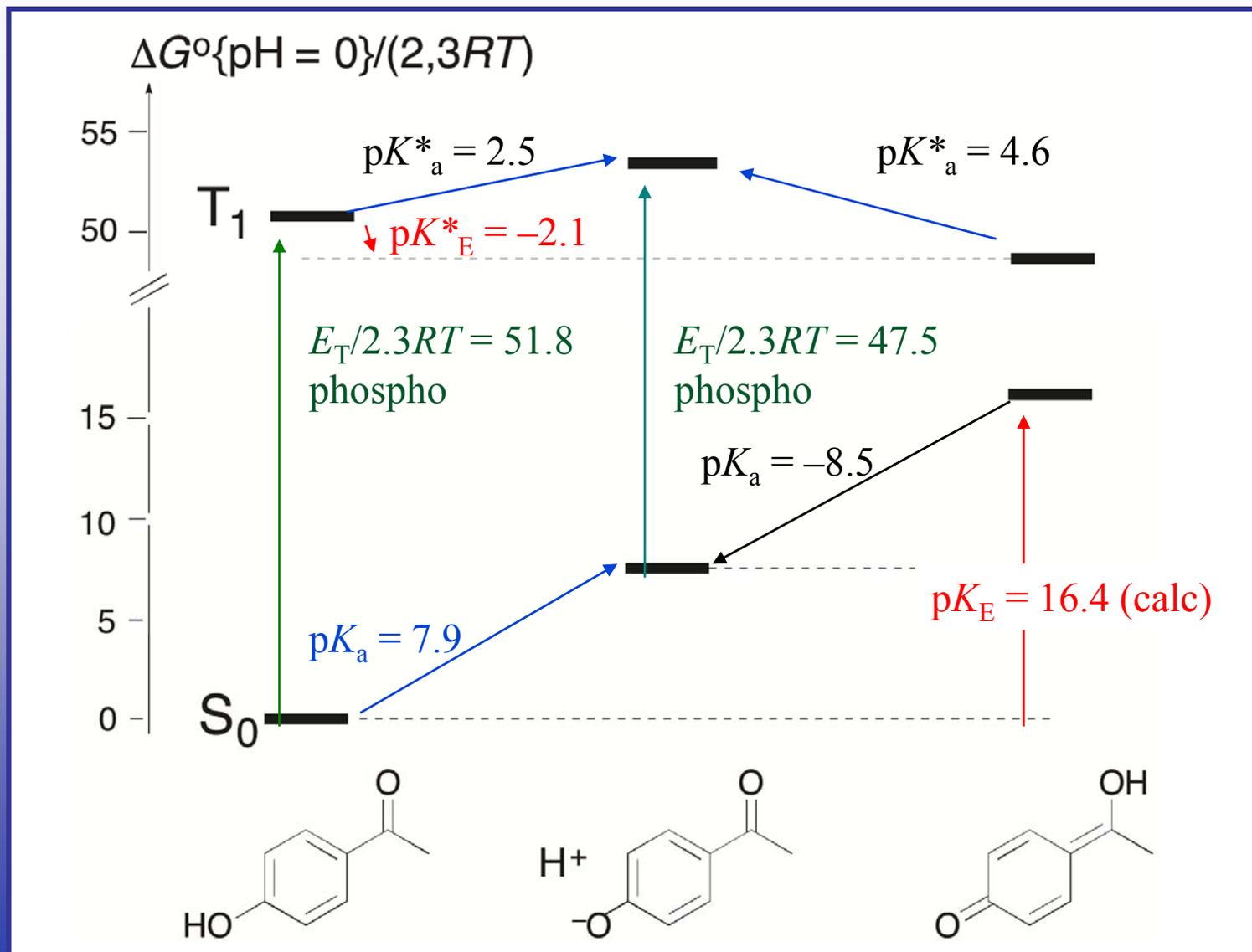


# Titration of the enol-triplet

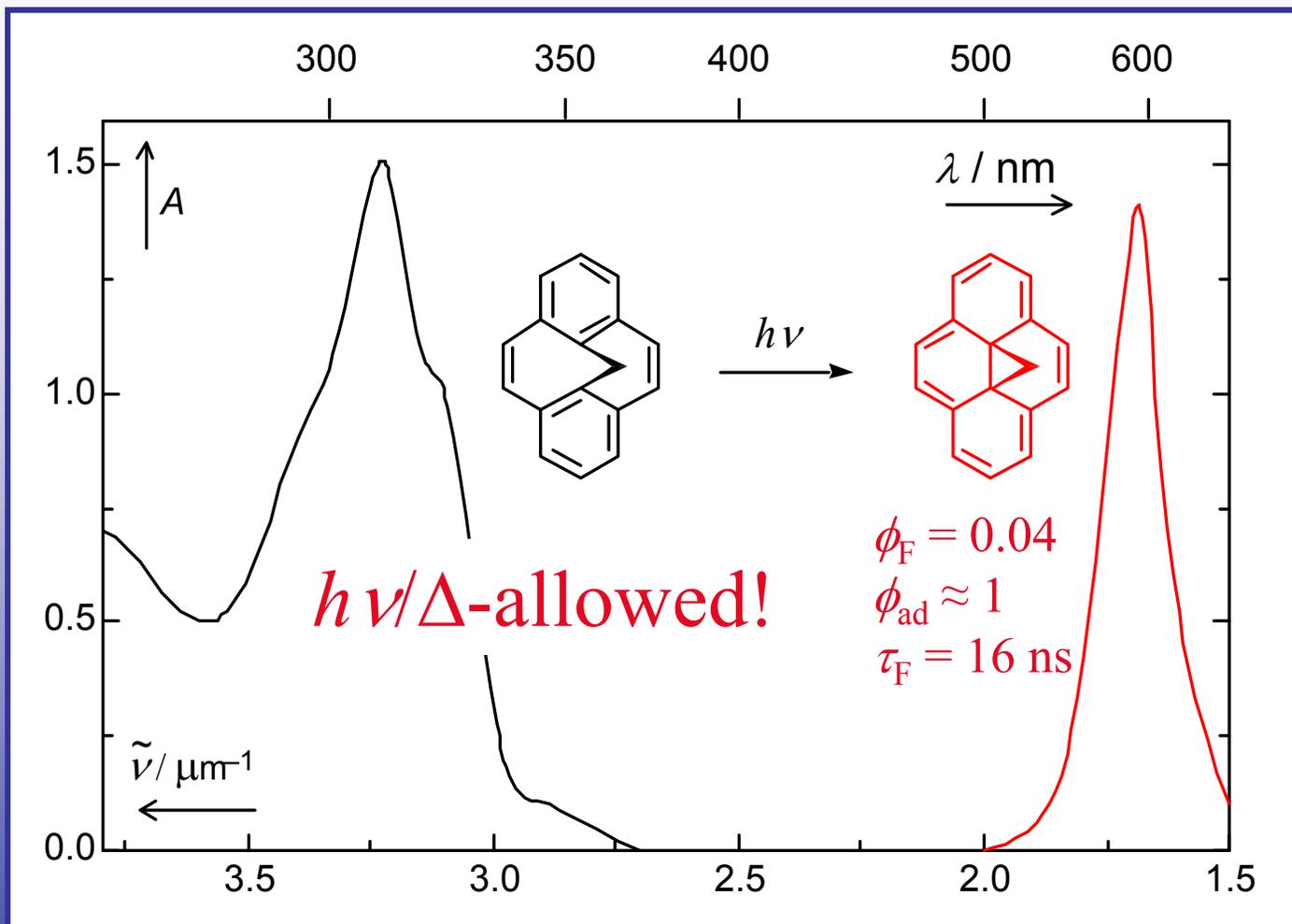


*JACS* 2000, 122, 9346

# Förster cycle



# An adiabatic electrocyclic reaction



*Helv. Chim. Acta* **1984**, *67*, 305.

$$\epsilon_j/\beta$$

- 1.25

- 1.07

- 0.59

- 0.45

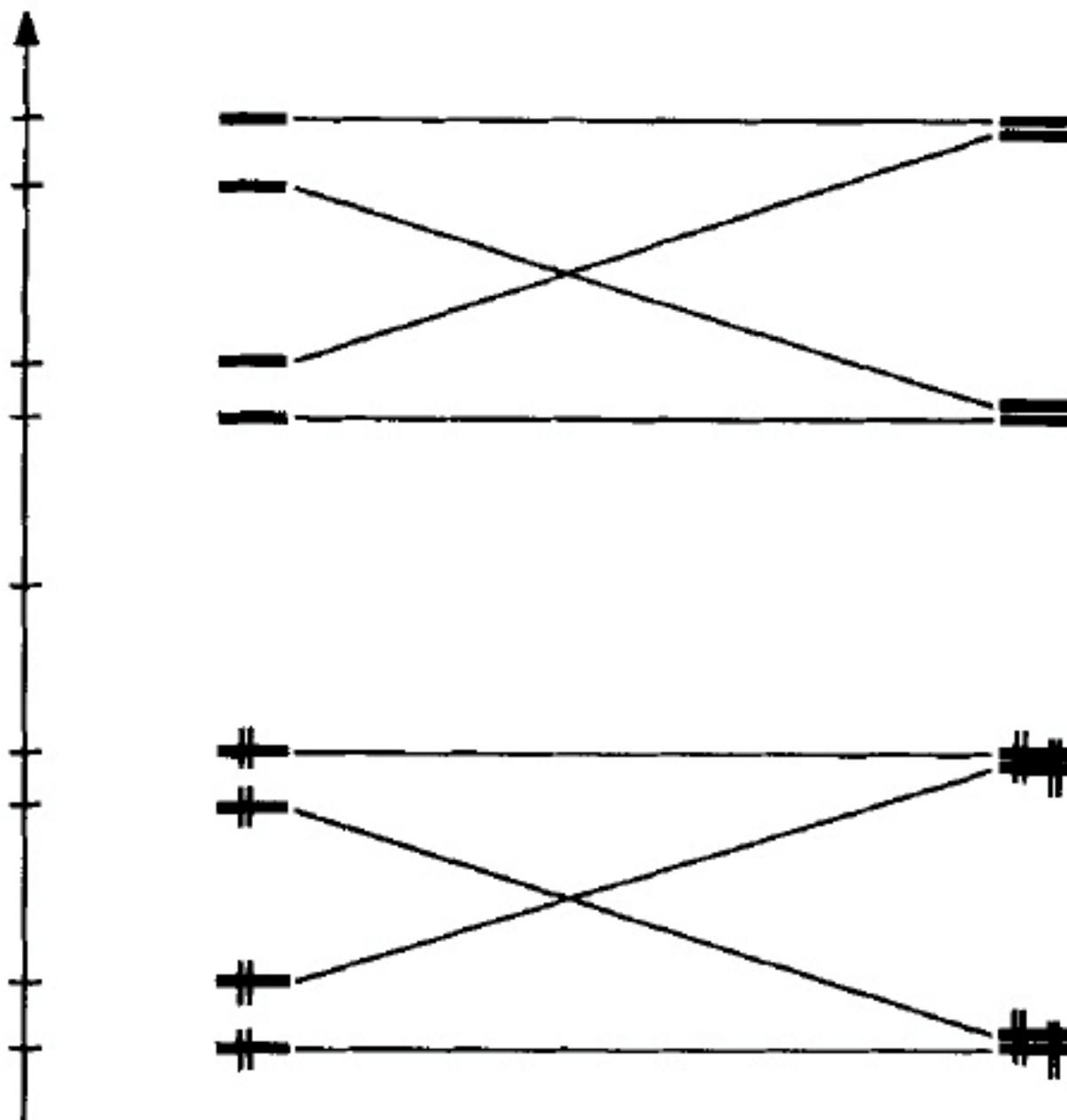
0

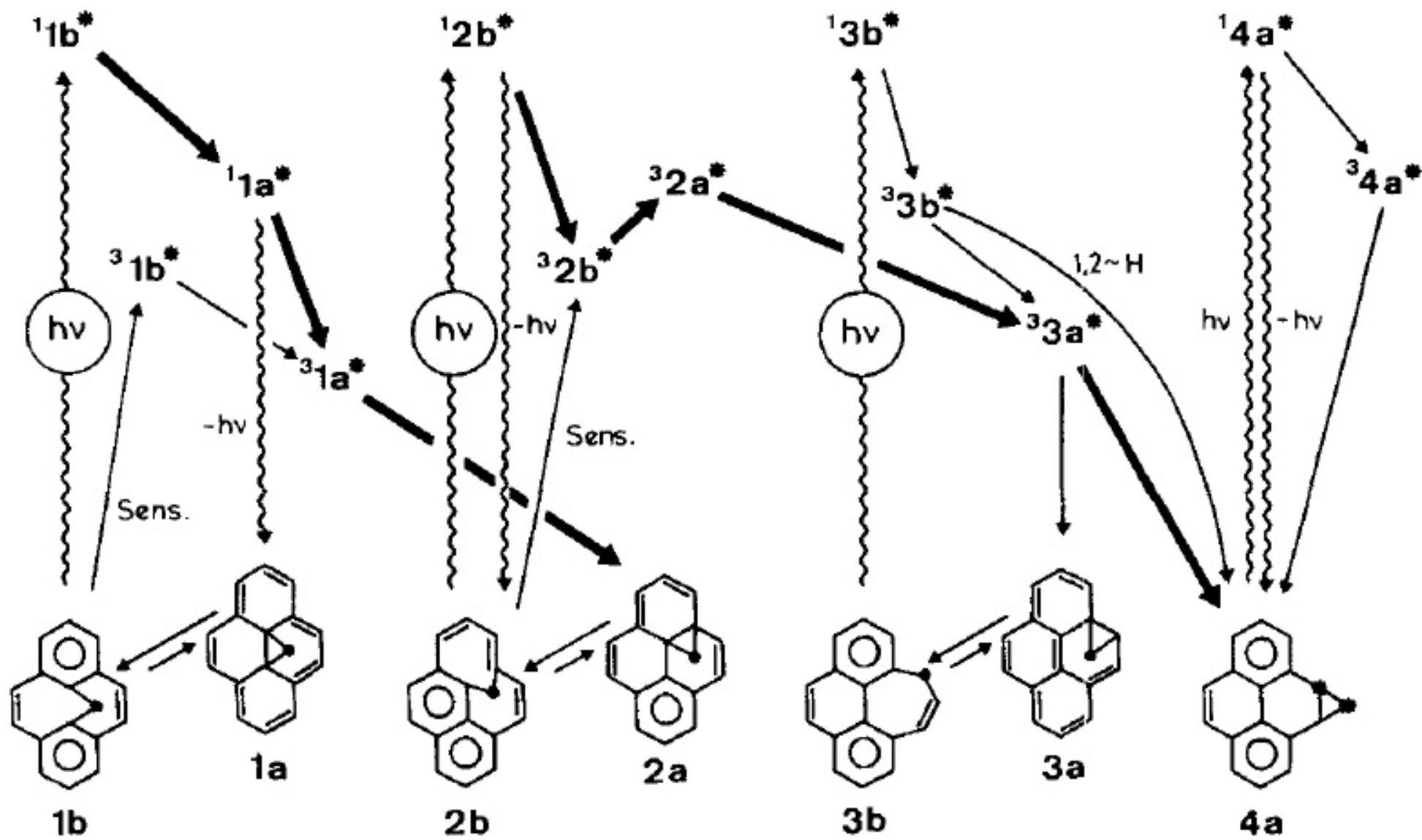
0.45

0.59

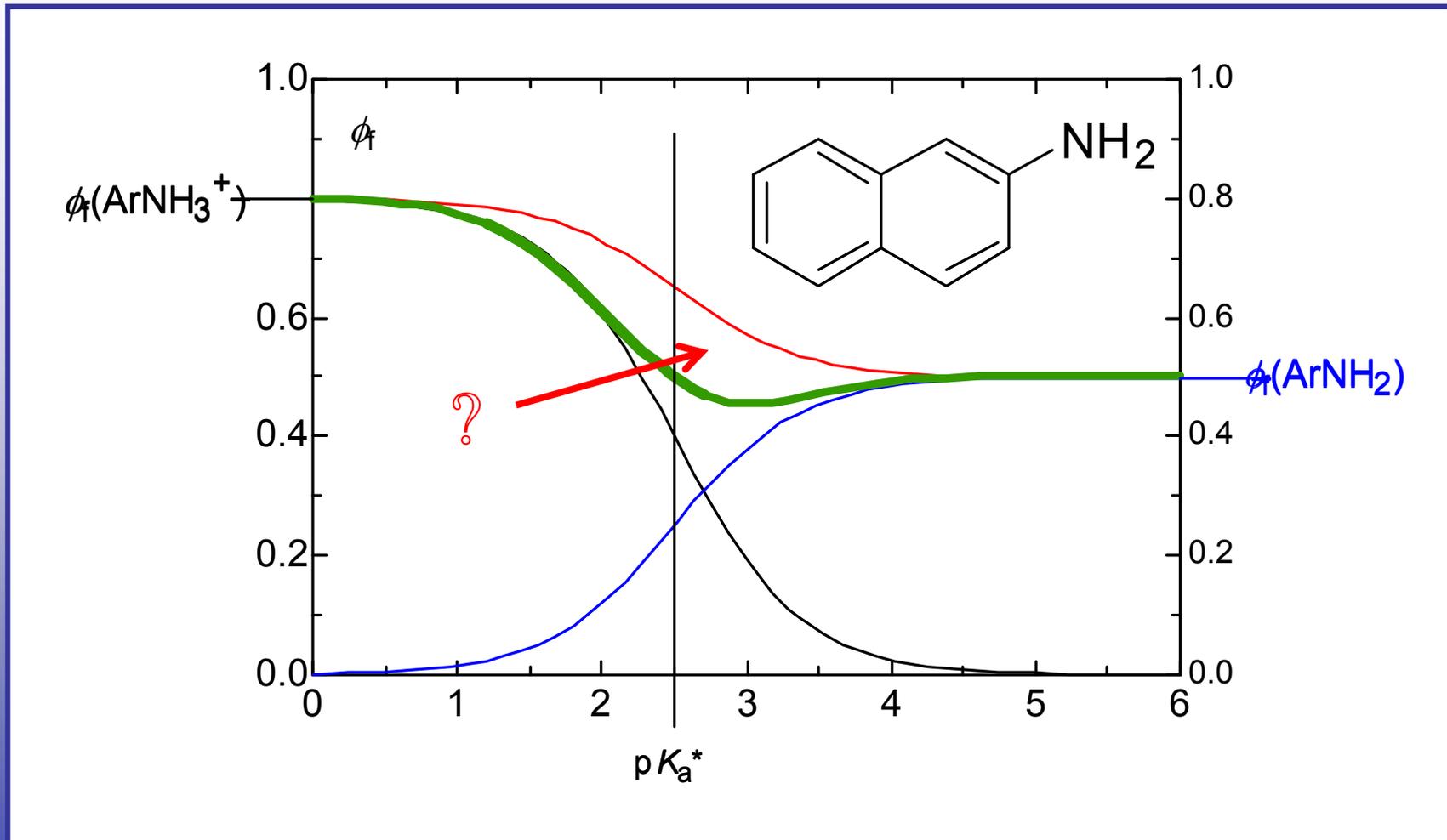
1.07

1.25





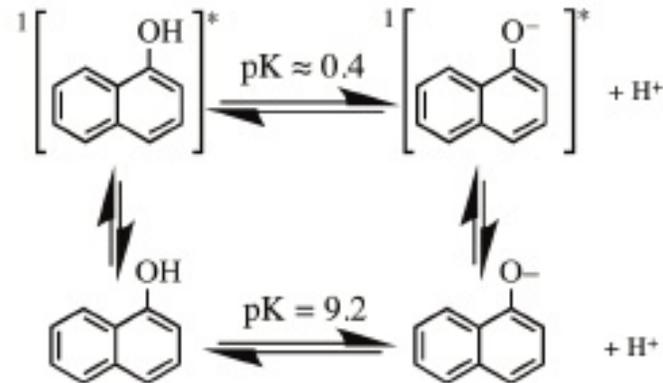
# Förster's paradox



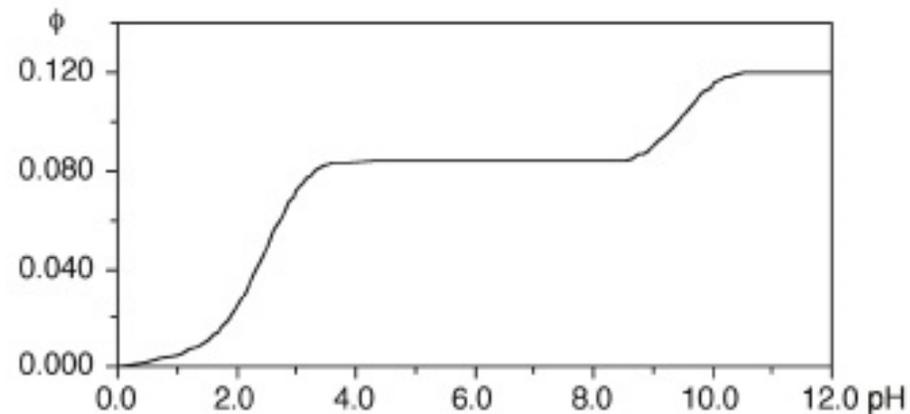
*Th. Förster, Chem. Phys. Lett. 1972, 17, 309.*

# 1-Naphthol in Aqueous Solution

with A. Hörmann and P. Schünemann



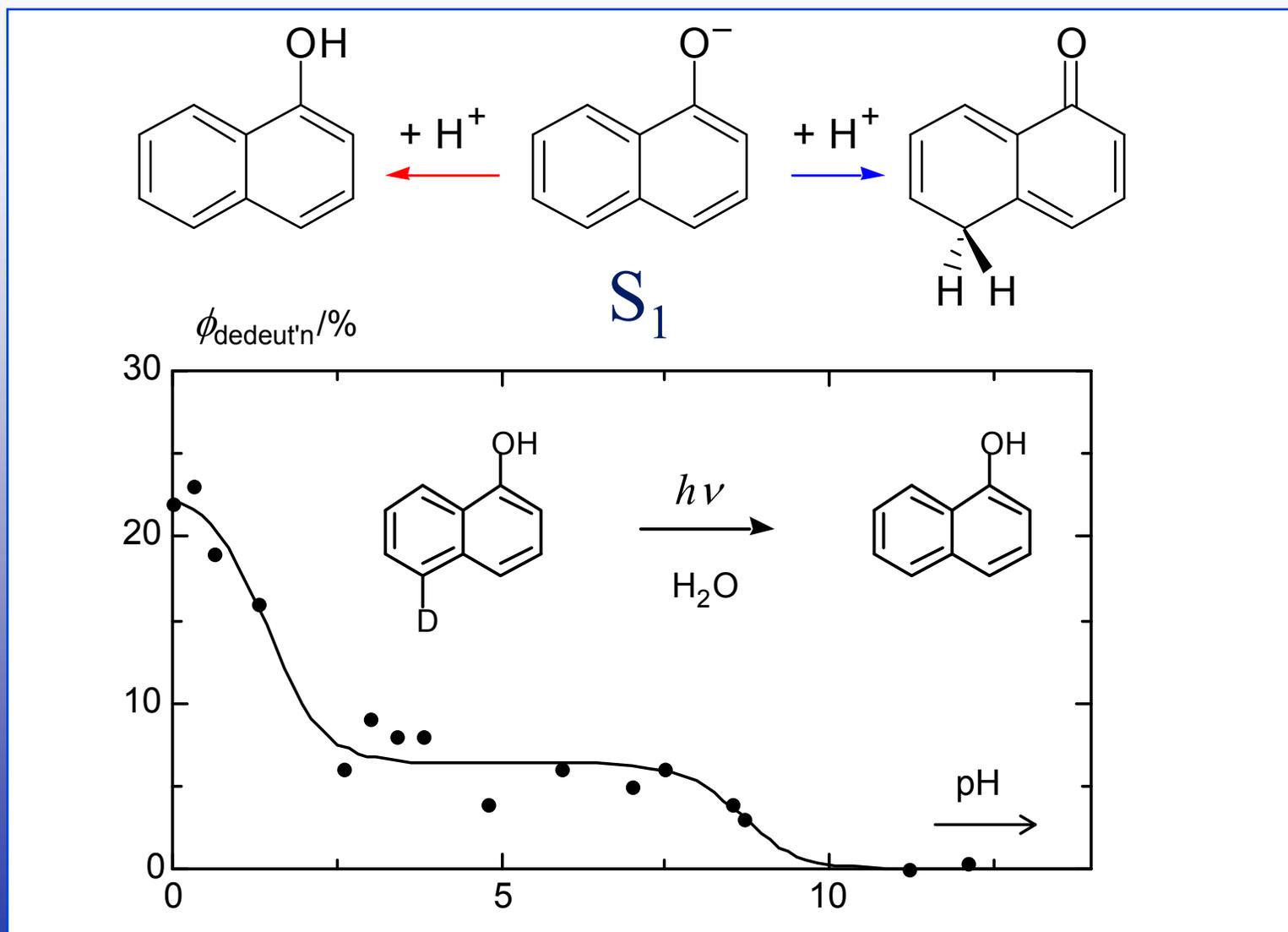
Fluorescence quantum yield of 1-naphtholate



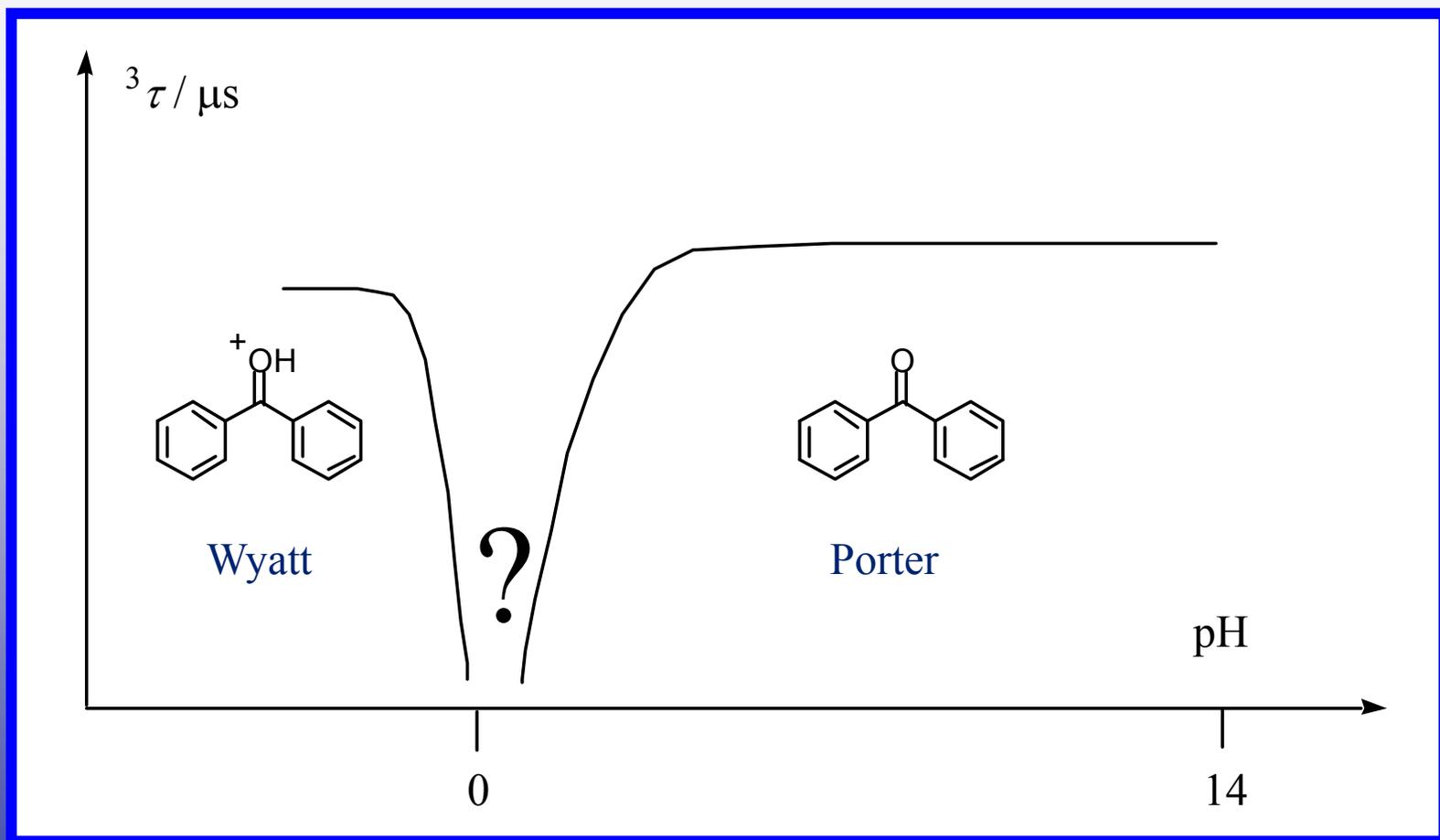
**Förster's Paradox:** Why is the adiabatic deprotonation to  $\text{RO}^-^*$  only 75% efficient, yet "no" fluorescence by  $\text{ROH}^*$ ?

--> There must be a quenching process competing with deprotonation (water)

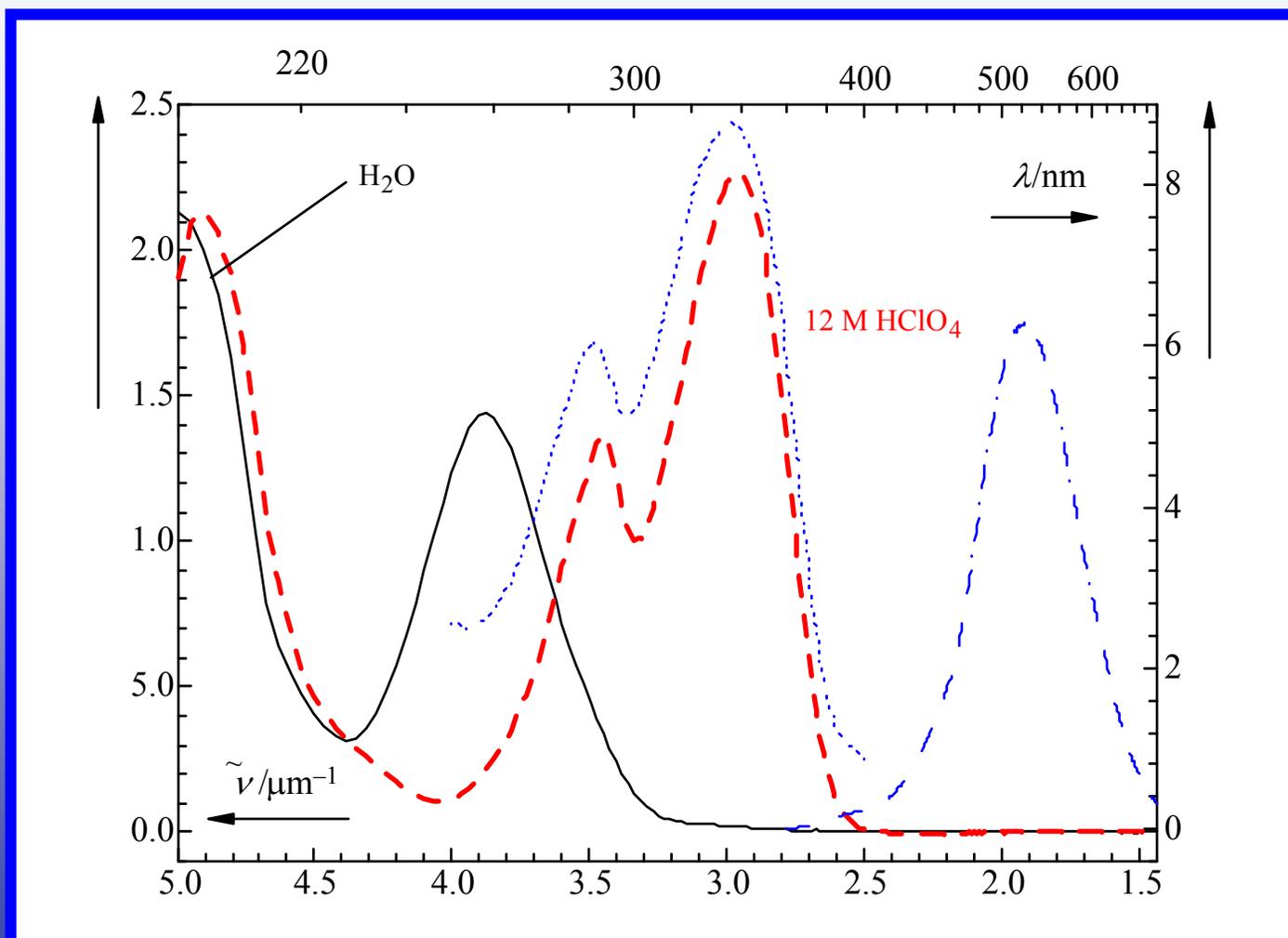
# The competing process: C-protonation



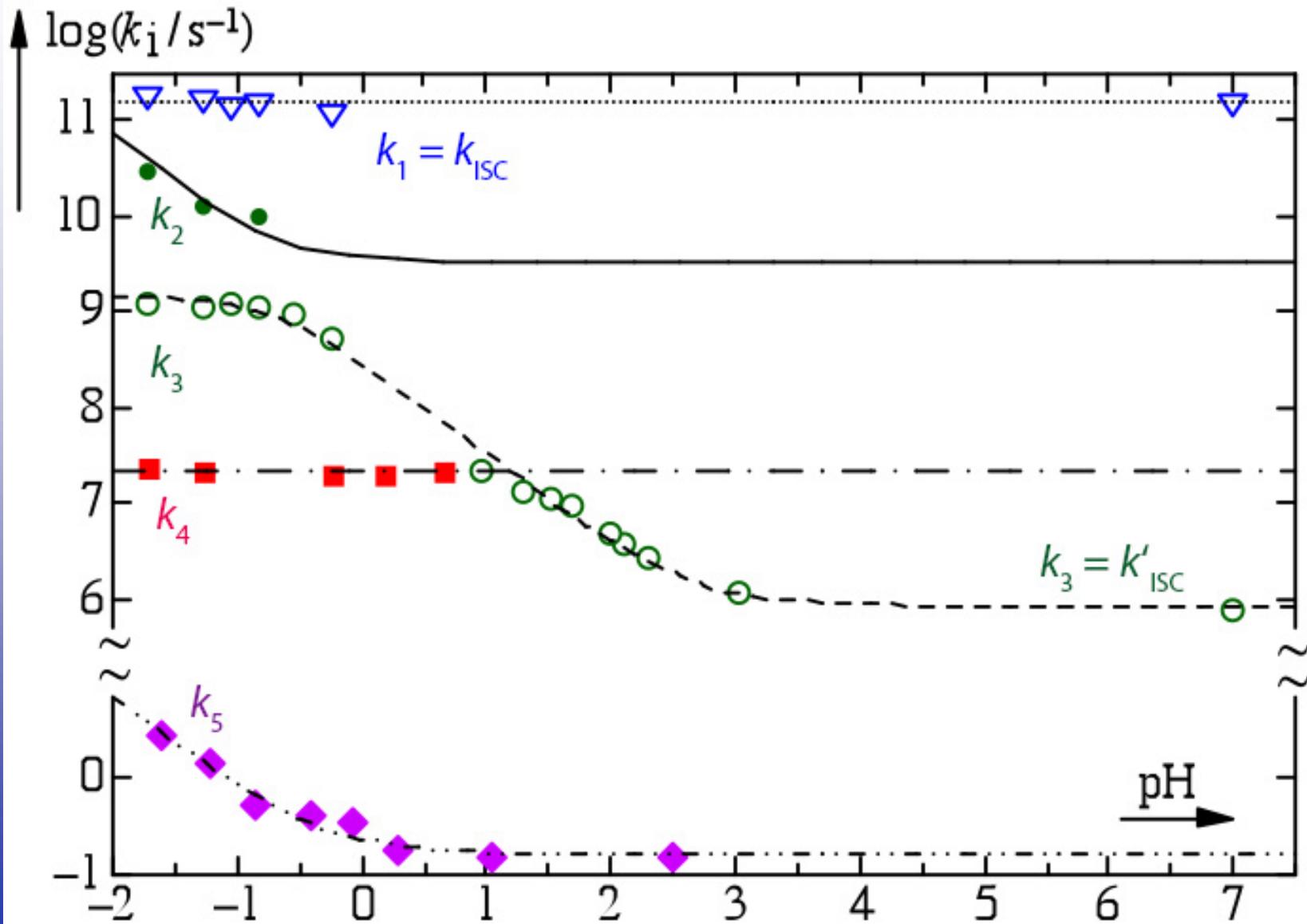
# pH-Dependence of the triplet-lifetime of benzophenone



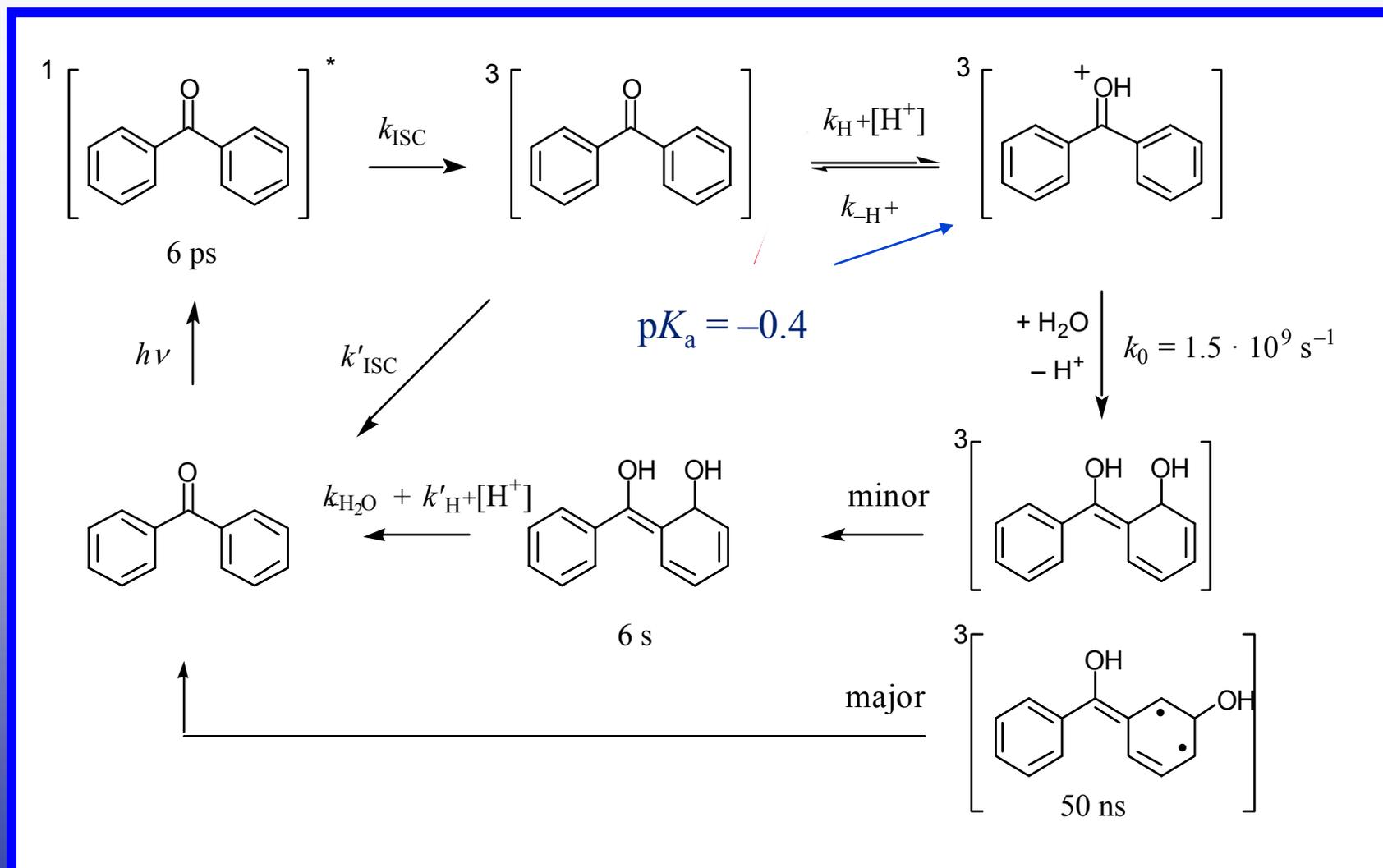
# Benzophenone in aqueous acid



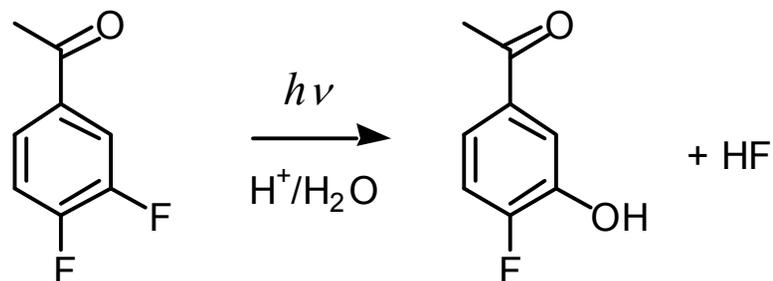
# Kinetic analysis



# Photohydration of benzophenone in aqueous acid



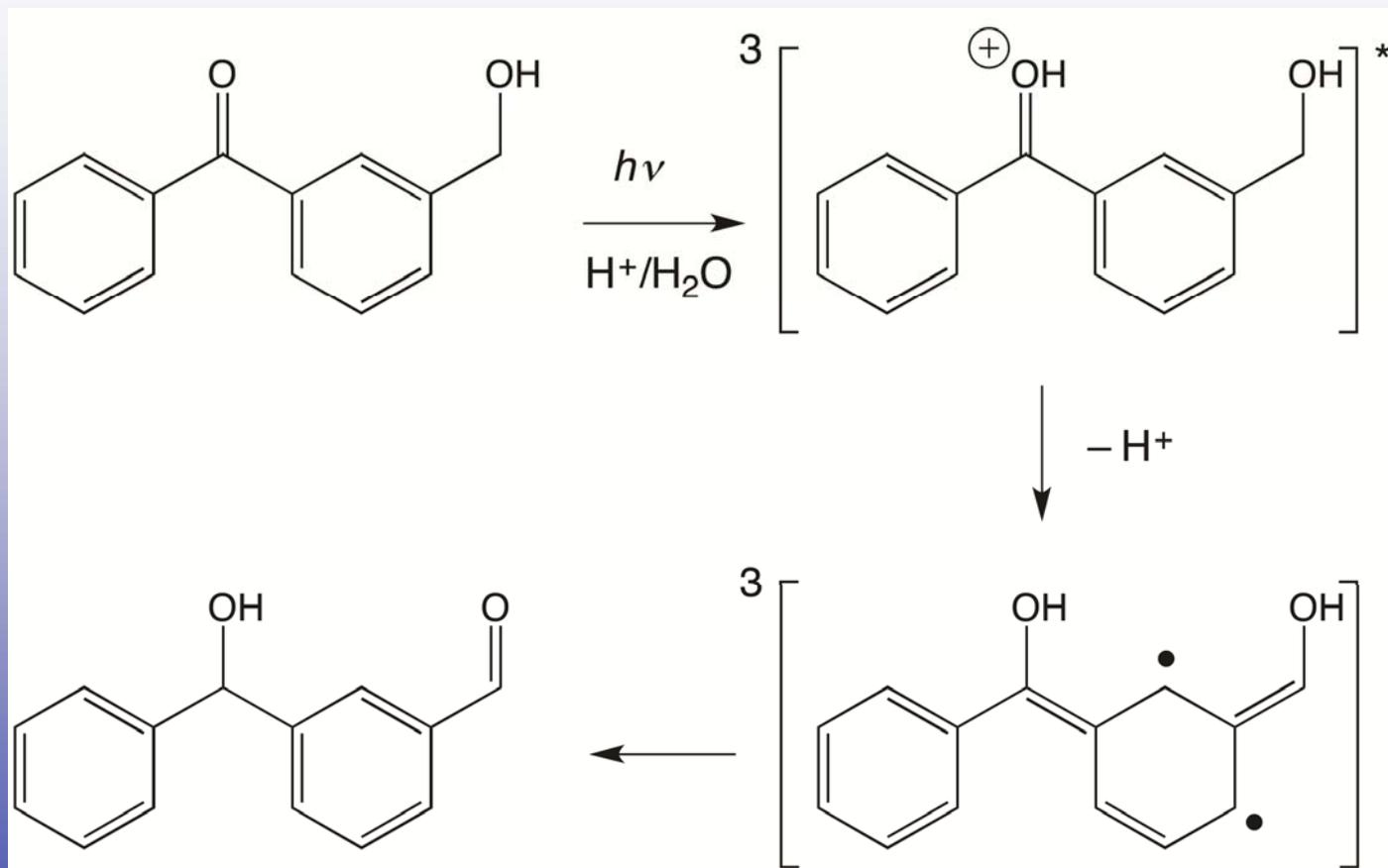
# Photosubstitution of aromatic ketones in aqueous acid



## Quantum yields

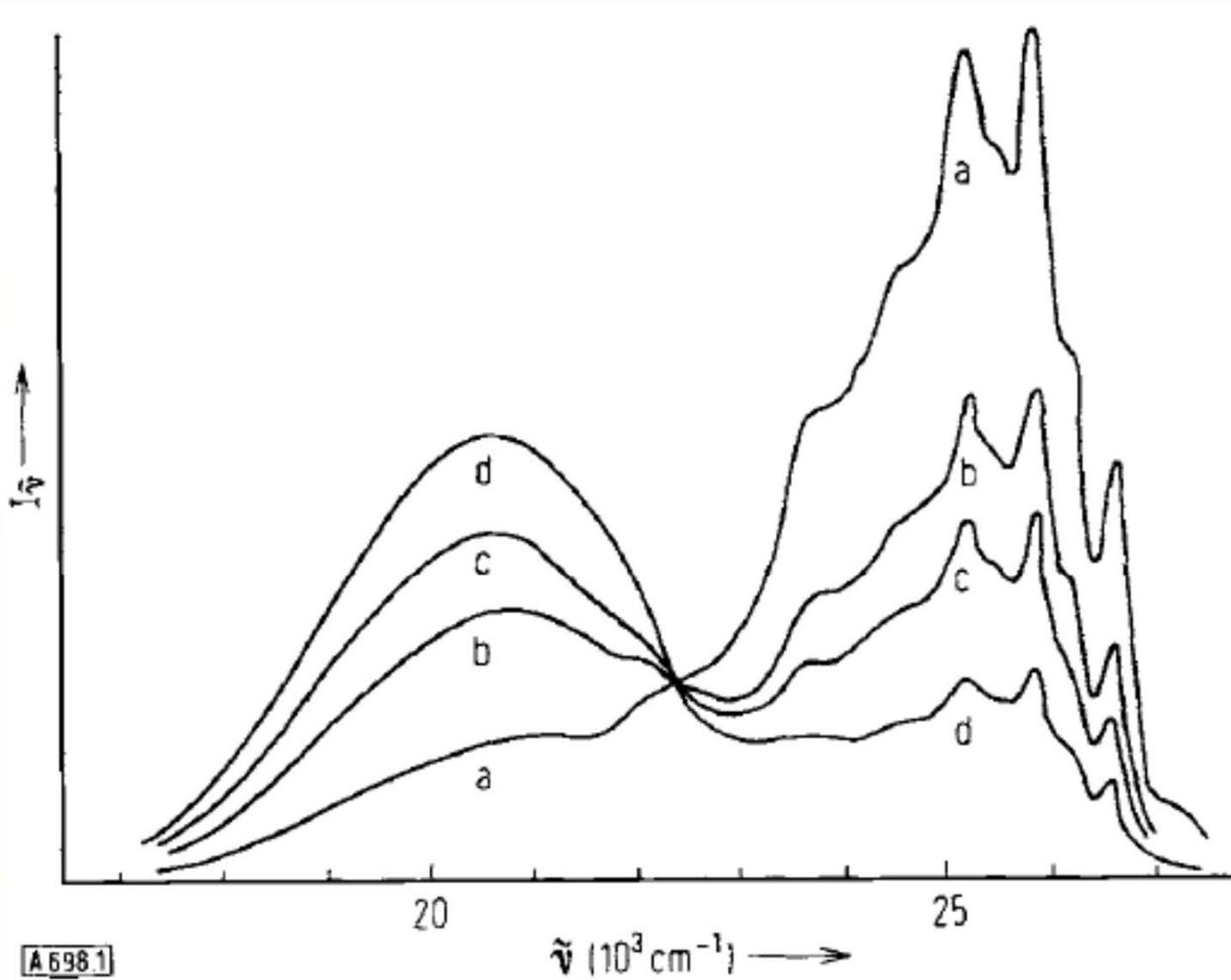
|                           |     |
|---------------------------|-----|
| 3-Fluoroacetophenon:      | 0.5 |
| 3,4-Difluoroacetophenon:  | 0.6 |
| 3,3'-Difluorobenzophenon: | 0.7 |

## Intramolecular photoredox reactions



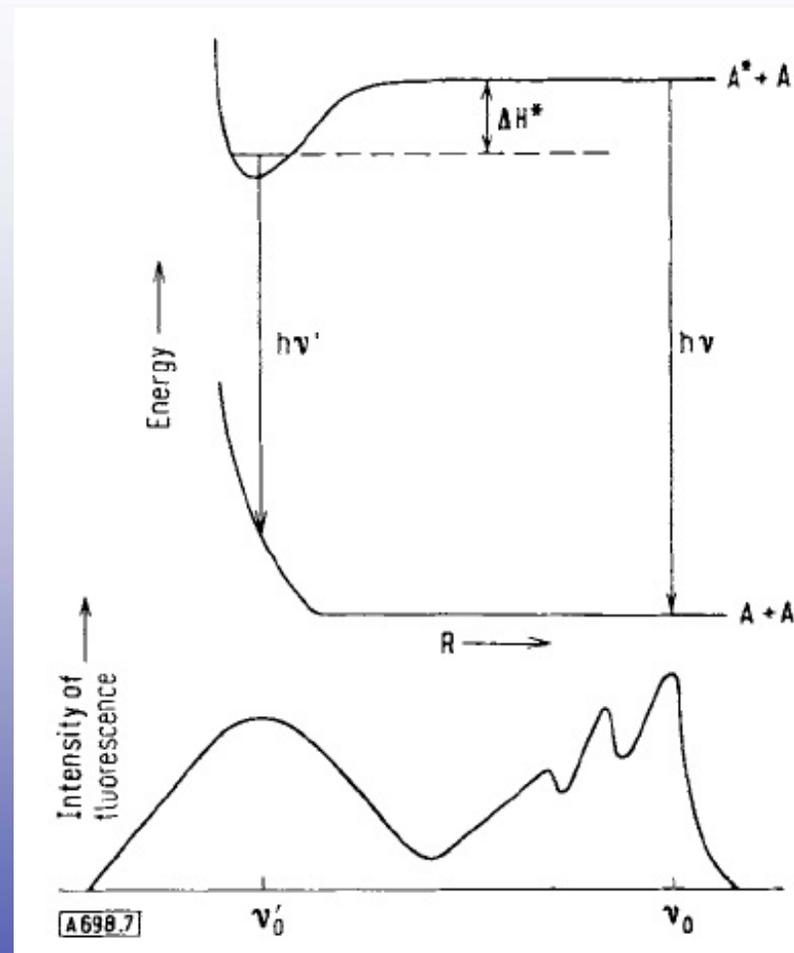
P. Wan, *Org. Lett.*, 7, 2005, 3387. *Photochem. Photobiol. Sci.*, 2008, 7, 588.

# Pyrene excimer



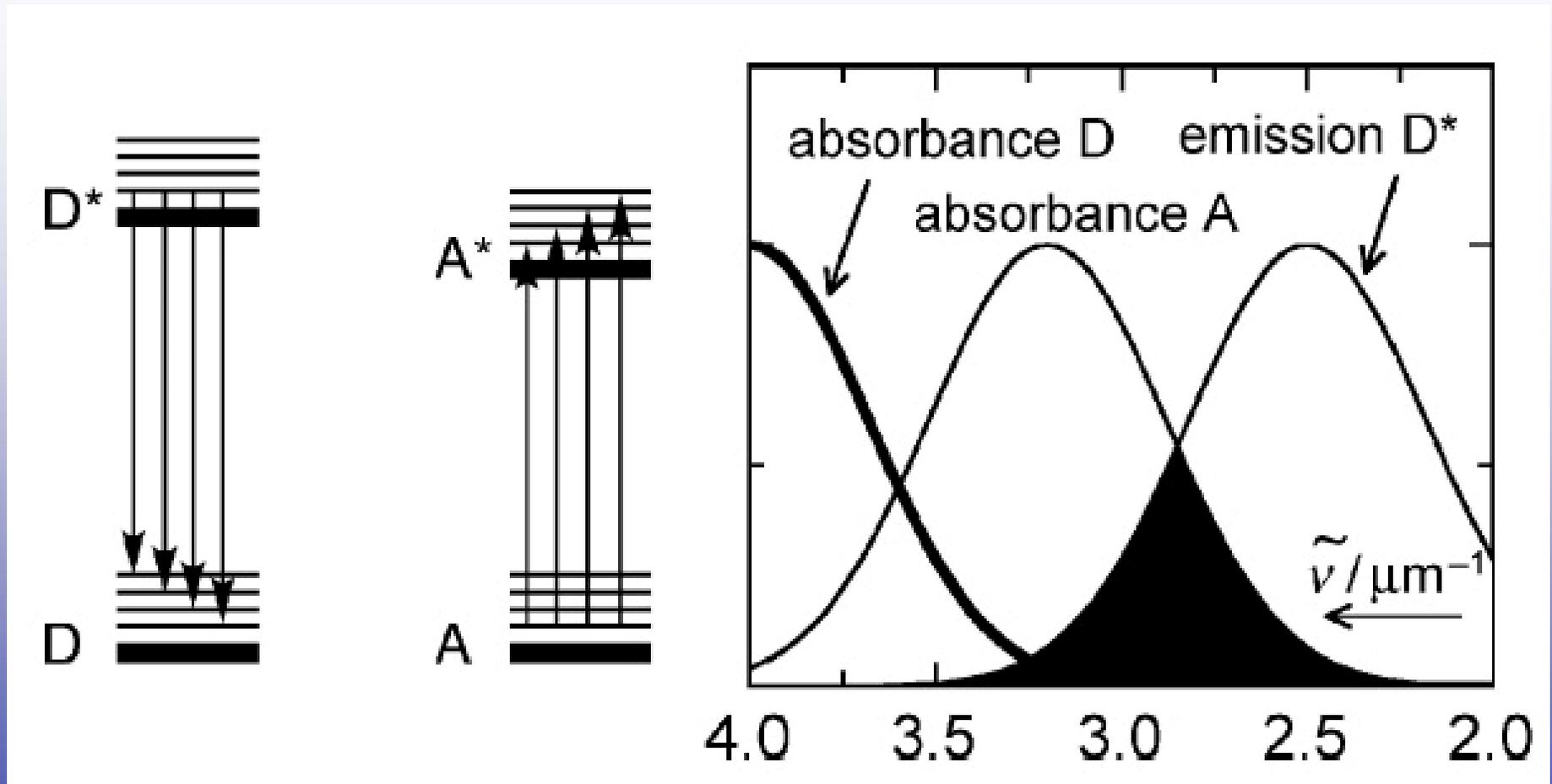
A 698.1

Fig. 1. Fluorescence spectra of pyrene in *n*-heptane.  
 $t = 20^\circ \text{C}$ ,  $c$  (mole/l):  $5 \times 10^{-5}$  (a),  $1.8 \times 10^{-4}$  (b),  $3.1 \times 10^{-4}$  (c),  
 $7.0 \times 10^{-4}$  (d).



A 698.7

Energy transfer processes are isoenergetic



Dark area: Spectral overlap integral  $J$

## Radiative energy transfer

- Donor  $D^*$  fluoresces, acceptor A absorbs
- Like a radio transmission
- The concentration of A does **not** affect the lifetime of the donor  $D^*$ ,  $\tau(D^*)$
- The probability  $p$  for absorption by A of a photon emitted from  $D^*$  for low absorbance of the acceptor A is:

$$p \approx 2.303c_A \ell \int_{\tilde{\nu}} \bar{I}_{\tilde{\nu}}^{D^*} \epsilon_A(\tilde{\nu}) d\tilde{\nu}$$

where  $\bar{I}_{\tilde{\nu}}^{D^*}$  is the normalized spectral radiant intensity:

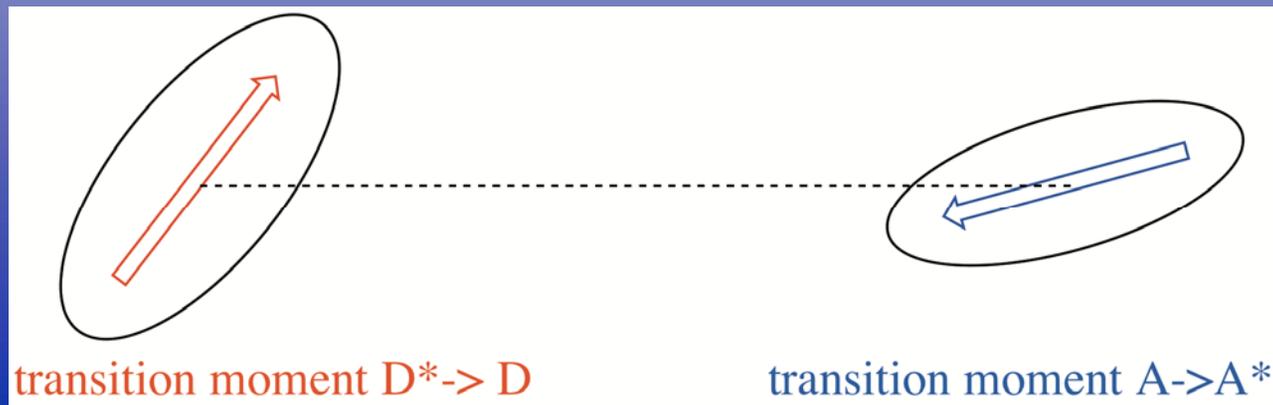
$$\bar{I}_{\tilde{\nu}}^{D^*} = I_{\tilde{\nu}}^{D^*} / \int_{\tilde{\nu}} I_{\tilde{\nu}}^{D^*} d\tilde{\nu}$$

$c_A$  is the concentration of acceptor A,  $\ell$  is the average path-length of the emitted photons.

- Note: This is the correct form of Eq. 2.33 in the book (which is wrong)

# Resonance energy transfer

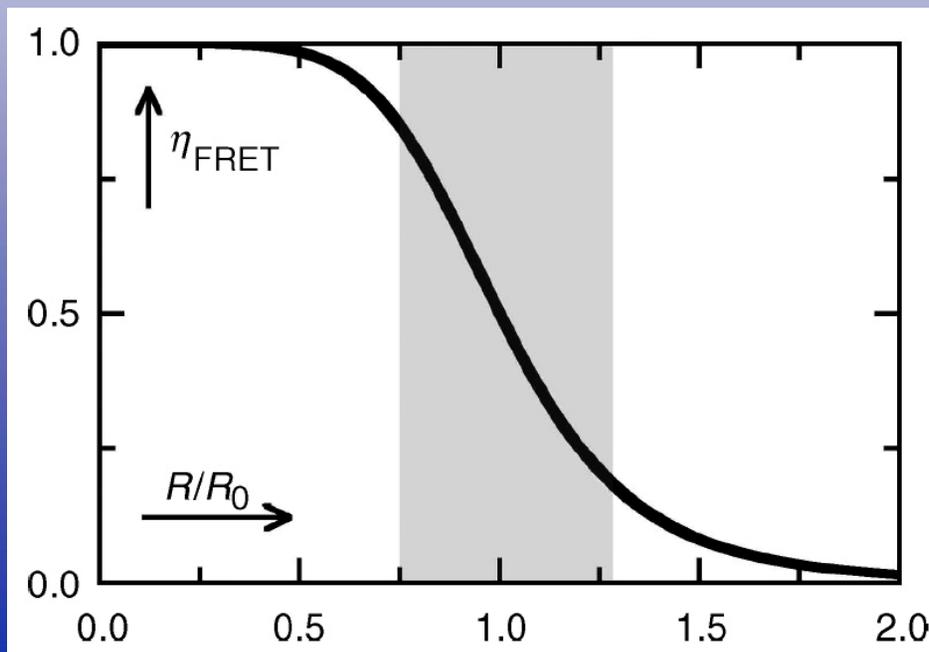
- In practice, the lifetime of the donor,  $\tau(D^*)$ , and its quantum yield of emission usually **decrease** upon addition of an acceptor A (concentration quenching).
- Some interaction between  $D^*$  and A, **not** like a radio transmission.
- The deactivation of  $D^*$  is **stimulated** by the acceptor A.
- Quantum description: an interaction term  $V_{if} = \langle \Psi_i | h^{op} | \Psi_f \rangle$  couples the initial wavefunction  $\Psi_i = \Psi_{D^*} \Psi_A$  to the final wavefunction  $\Psi_f = \Psi_D \Psi_{A^*}$ .
- Multipole expansion of the Coulombic interaction  $V_{if}$  and retain only the dipole–dipole term (for distances  $\gg$  molecular sizes).



## Förster resonance energy transfer (FRET) (1946, 1948)

- FRET was first used as an acronym in biological sciences for “fluorescence resonance energy transfer”, a **misnomer**; hence “F” for Förster.
- The rate constant  $k_{\text{FRET}}$  is proportional to  $V_{\text{if}}^2$ , and the dipole–dipole interaction falls off with the third power of the distance  $R$ , hence  $k_{\text{FRET}} \sim 1/R^6$ .
- The distance  $R$  at which  $k_{\text{FRET}} = 1/\tau_{\text{D}}^0$  is called the **critical distance**  $R_0$ .
- At  $R = R_0$ , the efficiency of energy transfer is 0.5:

$$\eta_{\text{FRET}} = k_{\text{FRET}} / (k_{\text{FRET}} + 1/\tau_{\text{D}}^0) = R_0^6 / (R_0^6 + R^6) = 0.5$$



“Molecular ruler”

Search for “FRET”  
gives 77’000 refs

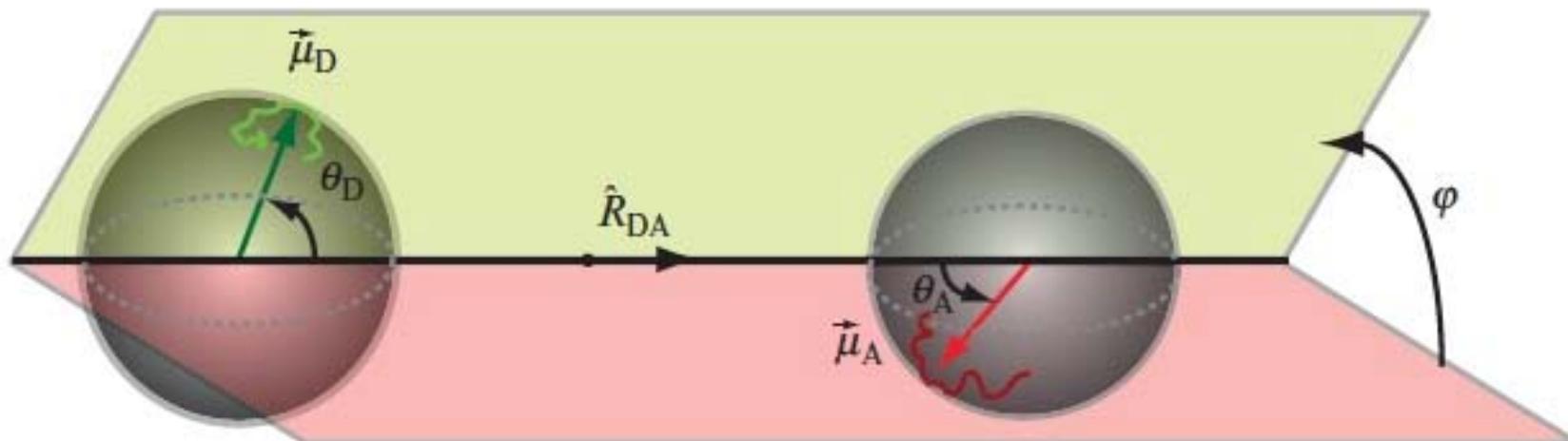
Can we predict  $R_0$  for a given pair  $D^* \dots A$ ?

- Yes! The Förster equation gives  $R_0$  as a function of experimentally accessible quantities:

$$R_0^6 = \frac{9 \ln(10)}{128 \pi^5 N_A} \frac{\kappa^2 \Phi_D^0}{n^4} J$$

- $\Phi_D^0$  is the fluorescence quantum yield of D in the absence of A
- $J$  is the **spectral overlap integral**
- $N_A$  is the Avogadro constant
- $n$  is the refractive index of the medium
- $\kappa$  is the orientation factor depending on the relative orientation of the transition moments of  $D^*$  and A,  $0$  [perp.]  $\leq \kappa^2 \leq 4$  [parallel];  **$\langle \kappa^2 \rangle = 2/3$**

# The orientation factor $\kappa^2$



| Relative orientation of dipoles             | $\kappa^2$ |
|---|------------|
|   | 1          |
|   | 4          |
|   | 0          |
| Dynamic averaging<br>(rotational diffusion) | 2/3        |
| Static averaging                            | 0.476      |

# The spectral overlap integral

It can be expressed in wavelengths or wavenumbers:

$$J^\lambda = \int_{\lambda} \bar{I}_\lambda^{\text{D}^*}(\lambda) \varepsilon_A(\lambda) \lambda^4 d\lambda,$$

where  $\bar{I}_\lambda^{\text{D}^*}$  is the normalized spectral radiant intensity

$$\bar{I}_\lambda^{\text{D}^*}(\lambda) = I_\lambda^{\text{D}^*}(\lambda) / \int_{\lambda} I_\lambda^{\text{D}^*}(\lambda) d\lambda$$

$$J^{\tilde{\nu}} = \int_{\tilde{\nu}} \bar{I}_{\tilde{\nu}}^{\text{D}^*}(\tilde{\nu}) \varepsilon_A(\tilde{\nu}) \frac{d\tilde{\nu}}{\tilde{\nu}^4},$$

where  $\bar{I}_{\tilde{\nu}}^{\text{D}^*}$  is the normalized spectral radiant intensity

$$\bar{I}_{\tilde{\nu}}^{\text{D}^*}(\tilde{\nu}) = I_{\tilde{\nu}}^{\text{D}^*}(\tilde{\nu}) / \int_{\tilde{\nu}} I_{\tilde{\nu}}^{\text{D}^*}(\tilde{\nu}) d\tilde{\nu}$$

## Conversion to a practical equation: Quantity calculus

- **Quantity calculus**, the manipulation of numerical values, physical quantities and units, **obeys the ordinary rules of algebra!**
- Use scaled quantities  $Q/[Q]$ :  $Q = \text{number} * \text{unit}$ ;  $[Q] = \text{unit}$
- With  $[\varepsilon] = \text{mol}^{-1} \text{ dm}^3 \text{ cm}^{-1}$ ,  $[\lambda] = \text{nm}$ ,  $[N_A] = \text{mol}^{-1}$  one obtains the practical equation:

$$R_0^6 = \frac{9 \ln(10)}{128 \pi^5 N_A} \frac{\kappa^2 \Phi_D^0}{n^4} J, \quad J^\lambda = \int_{\lambda} \bar{I}_{\lambda}^{\text{D}^*}(\lambda) \varepsilon_A(\lambda) \lambda^4 d\lambda,$$

$$\frac{R_0^6}{\text{nm}} = \frac{9 \ln(10)}{128 \pi^5} \frac{N_A}{\text{mol}^{-1}} \frac{\kappa^2 \Phi_D^0}{n^4} \frac{J^\lambda}{\text{mol}^{-1} \text{ dm}^3 \text{ cm}^{-1} \text{ nm}^5}$$

$$\frac{R_0}{\text{nm}} = 0.02108 \left( \frac{\kappa^2 \Phi_D^0}{n^4} \frac{J^\lambda}{\text{mol}^{-1} \text{ dm}^3 \text{ cm}^{-1} \text{ nm}^5} \right)^{1/6}$$

A take-home lesson for calculations with physical quantities:

In most papers and textbooks the Förster equation is given as:

$$R_0^6 = \frac{9000 \ln(10)}{128\pi^5 N_A} \frac{\kappa^2 \Phi_D^0}{n^4} J$$

This would give:

$$\frac{R_0}{\text{nm}} = \cancel{0.0667} \left( \frac{\kappa^2 \Phi_D^0}{n^4} \frac{J^\lambda}{\text{mol}^{-1} \text{dm}^3 \text{cm}^{-1} \text{nm}^5} \right)^{1/6}$$

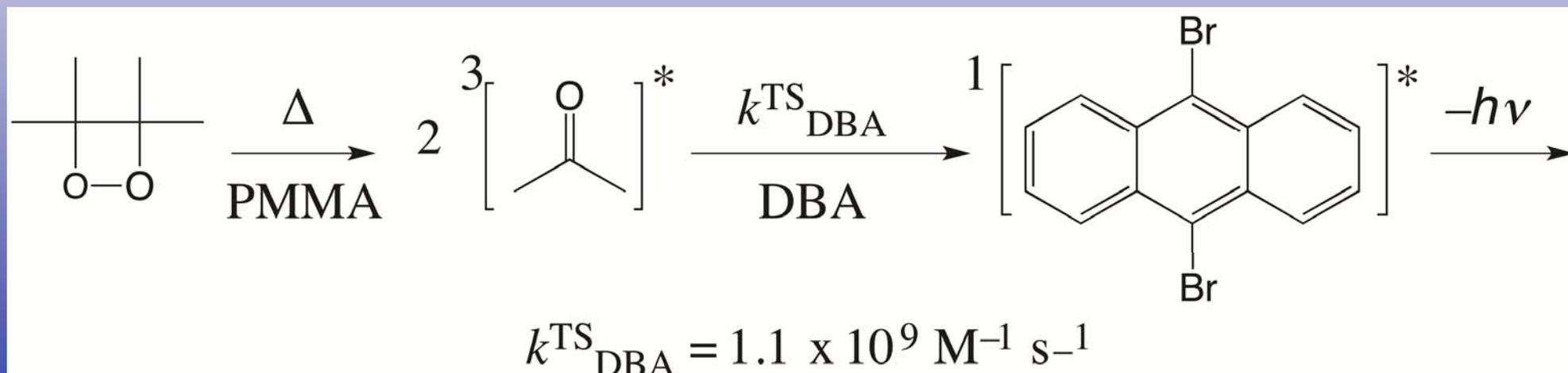
The first number in the numerator is **9**, not 9000!  
 $1000^{1/6} = 3.16$ ; the correct factor for  $R_0$  is 0.02108

*Photochem. Photobiol. Sci.*, 7, 2008, 1444

# Is triplet to singlet FRET possible?

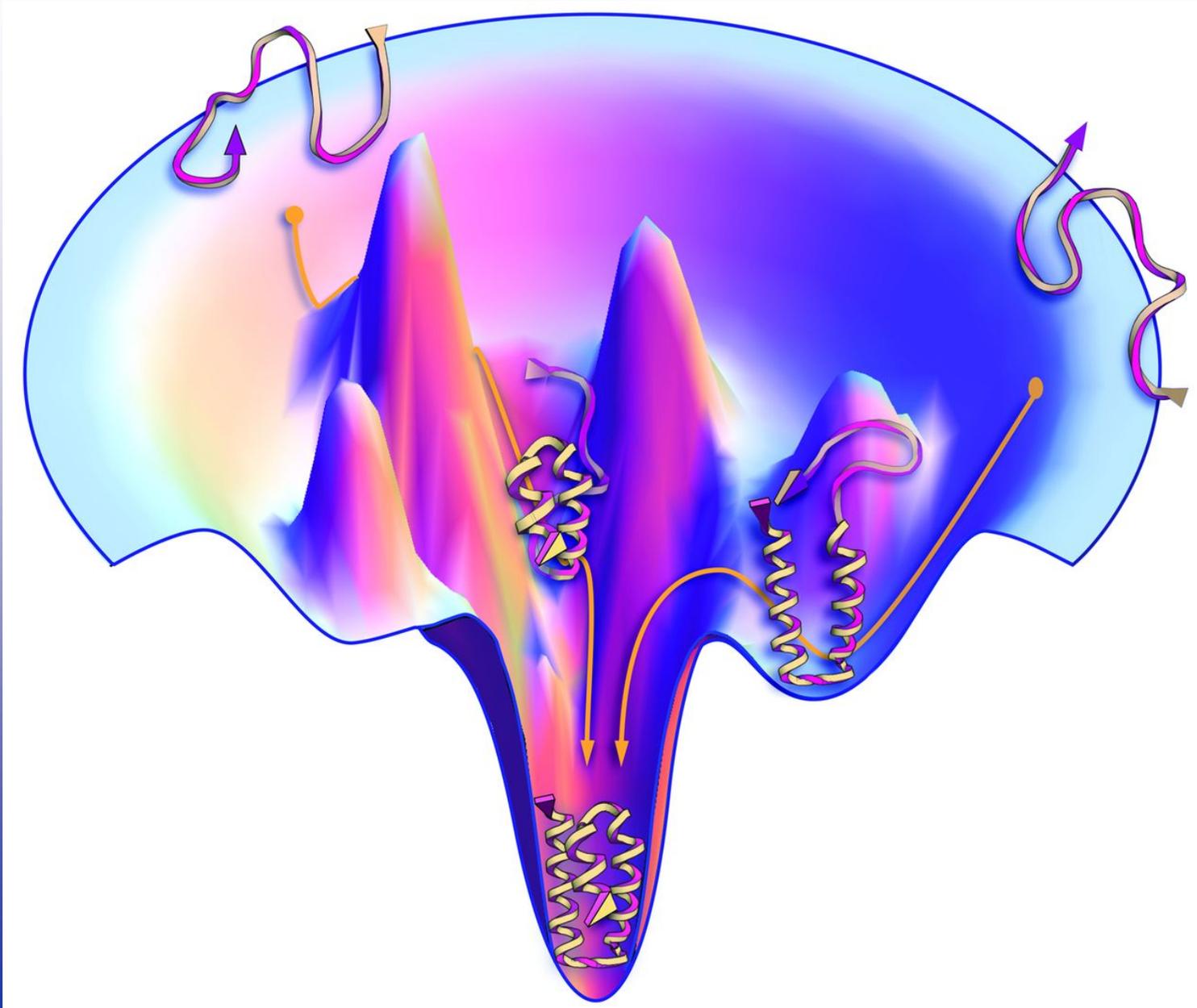
$$J^\lambda = \int \bar{I}_\lambda^{D^*} \epsilon_A(\lambda) \lambda^4 d\lambda; \quad \int \bar{I}_\lambda^{D^*} d\lambda = 1$$

Yes: Ermolaev 1963, Kellogg, 1964

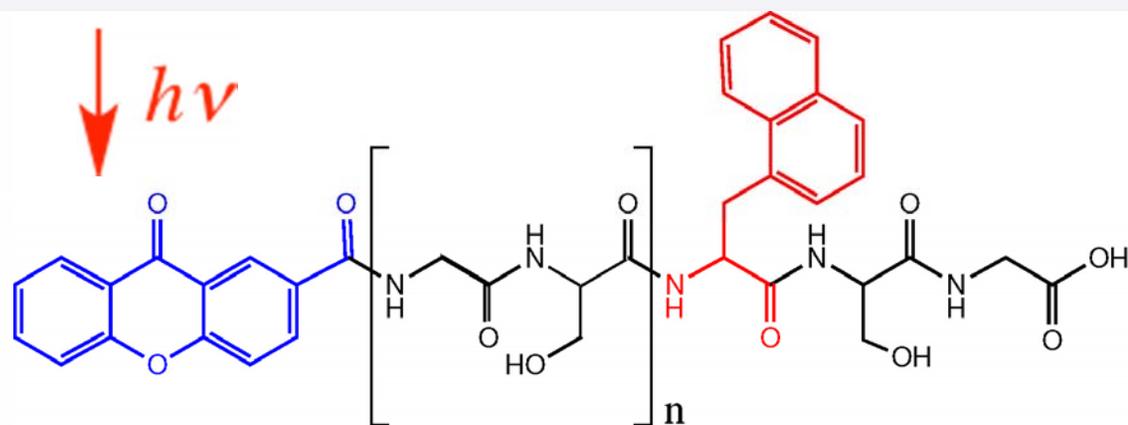
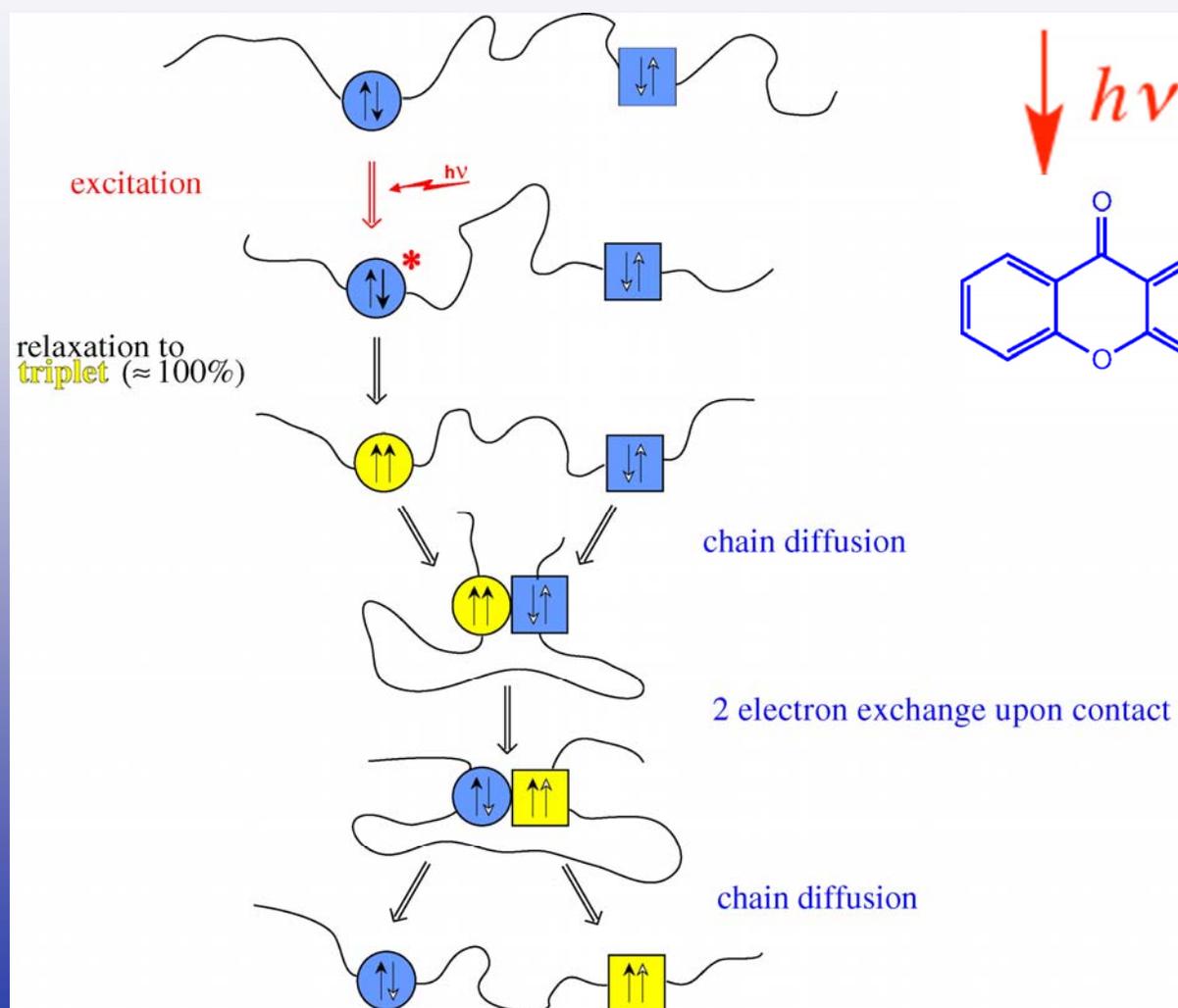


Adam, *JOC*, 43, 1978, 4495

# Protein folding

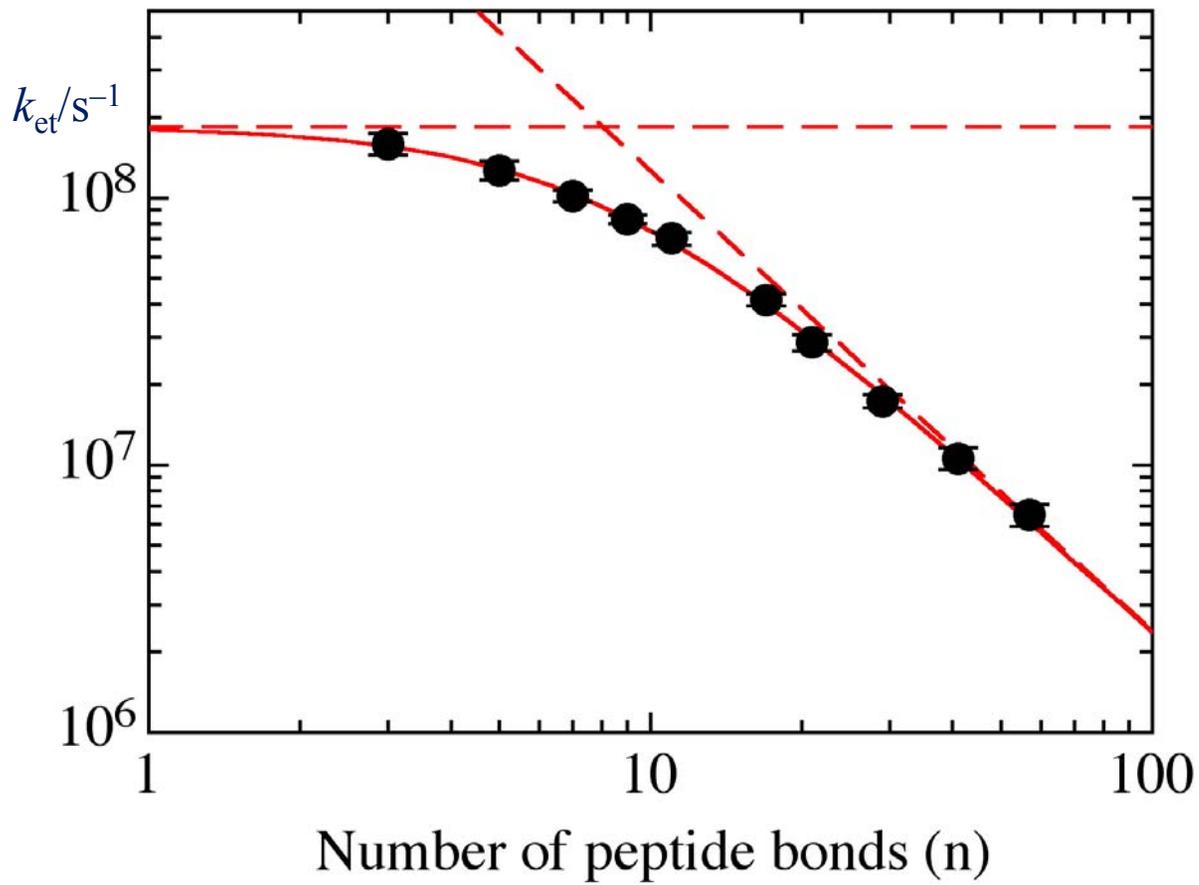


# The speed limit for protein folding measured by triplet-triplet energy transfer (requires contact)



*PNAS*, 96, 1999, 9597

Wagner, Klan, *JACS*,  
121, 1999, 9626

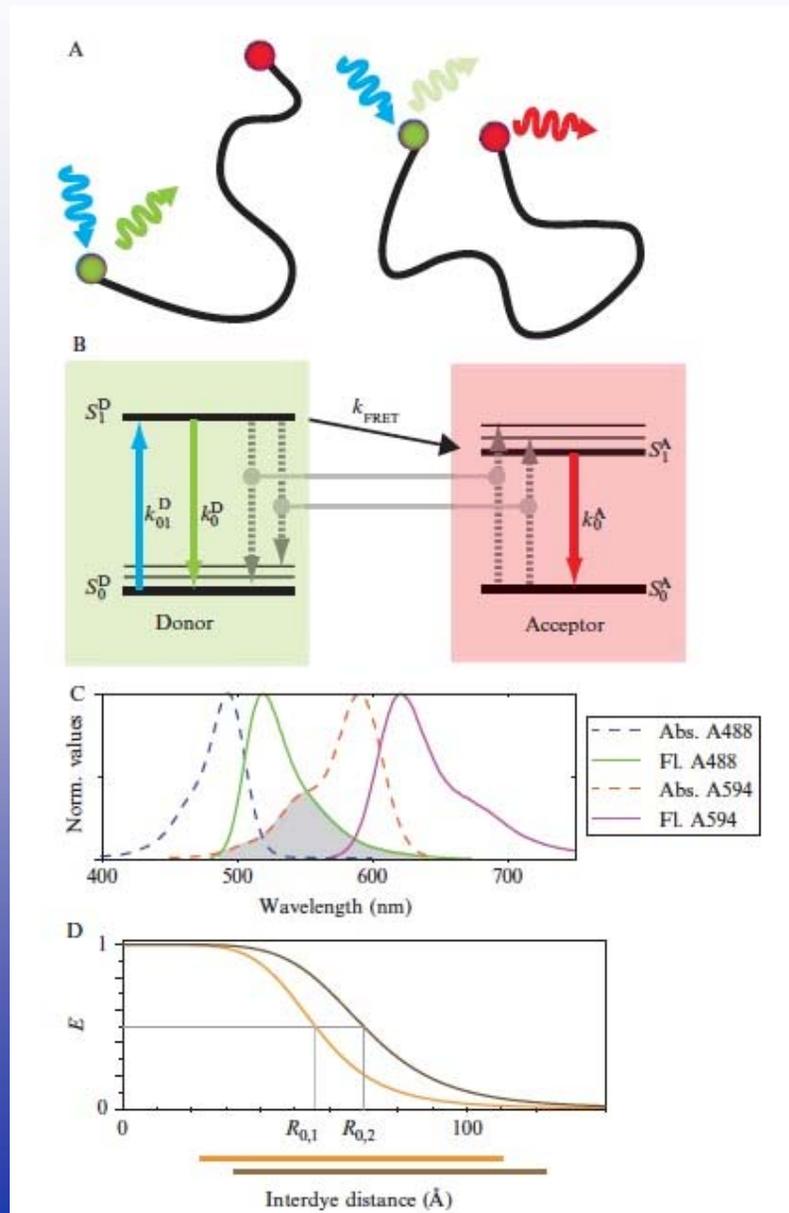


*PNAS, 96, 1999, 9597*



# Single molecule FRET: A tool to study protein folding

Single molecule detection  
avoids ensemble averaging

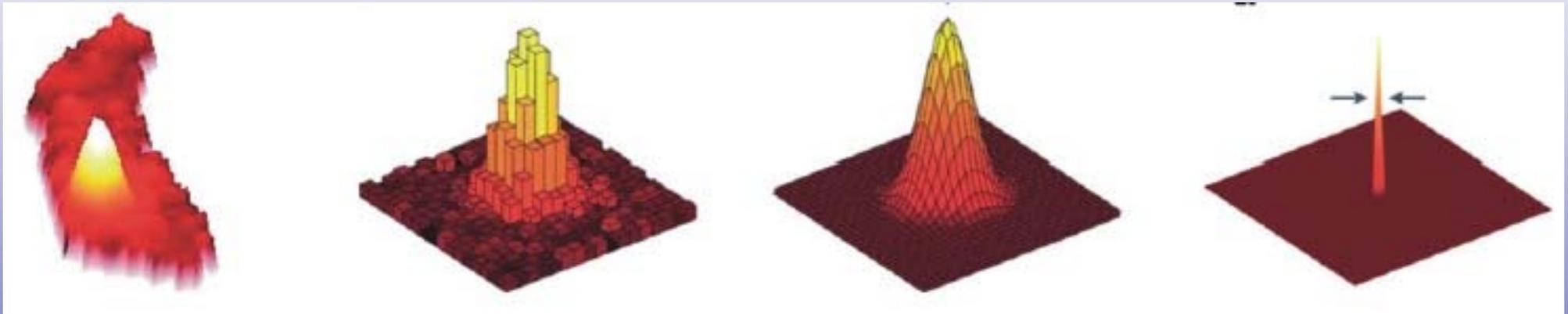


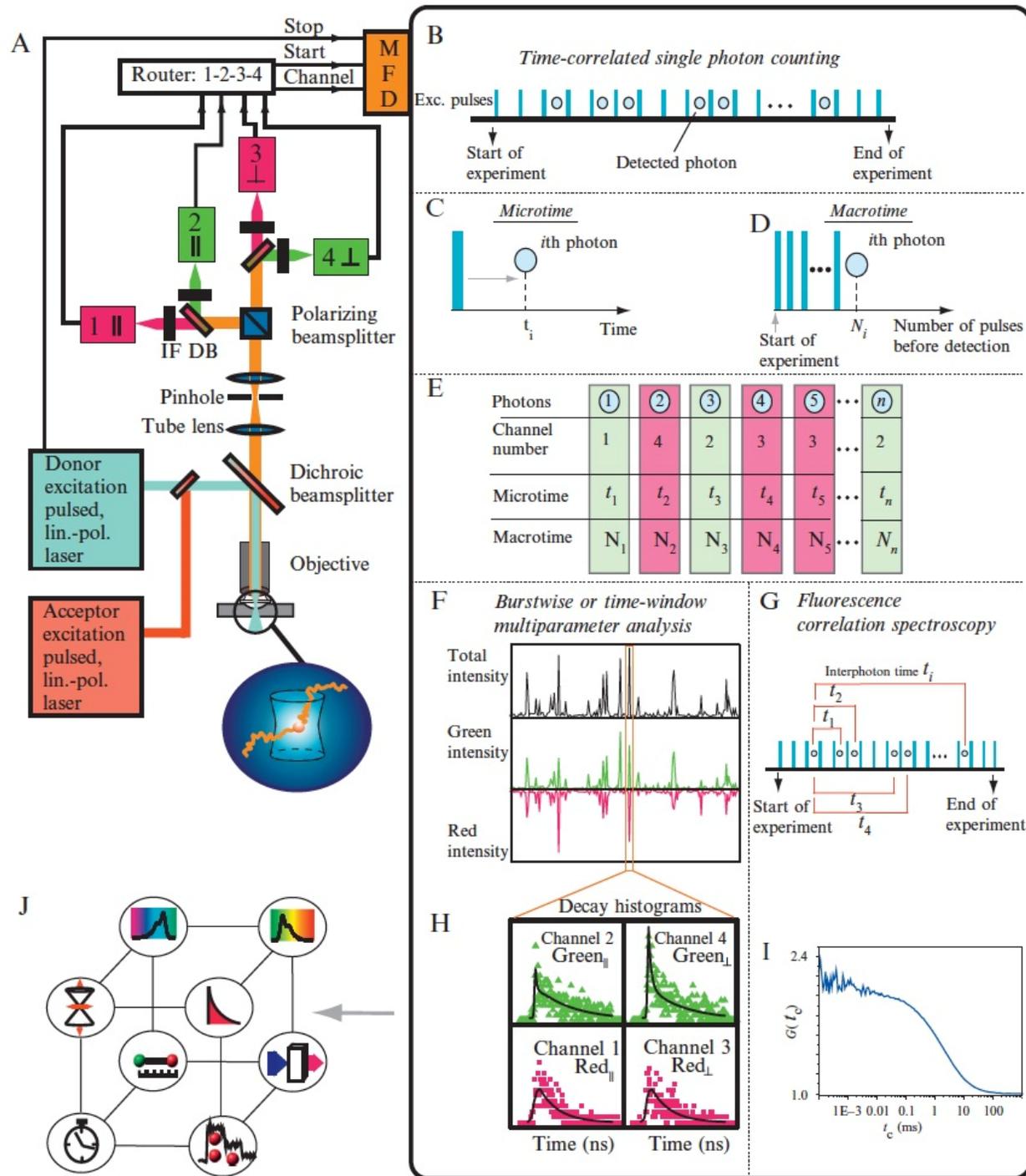
# Single molecule fluorescence spectroscopy at sub-diffraction resolution



W.E. Moerner, Stefan Hell, Eric Betzig,  
Nobel prize in Chemistry, 2014

Mathematical analyses allow localization of single, luminescent molecules to within a few nm



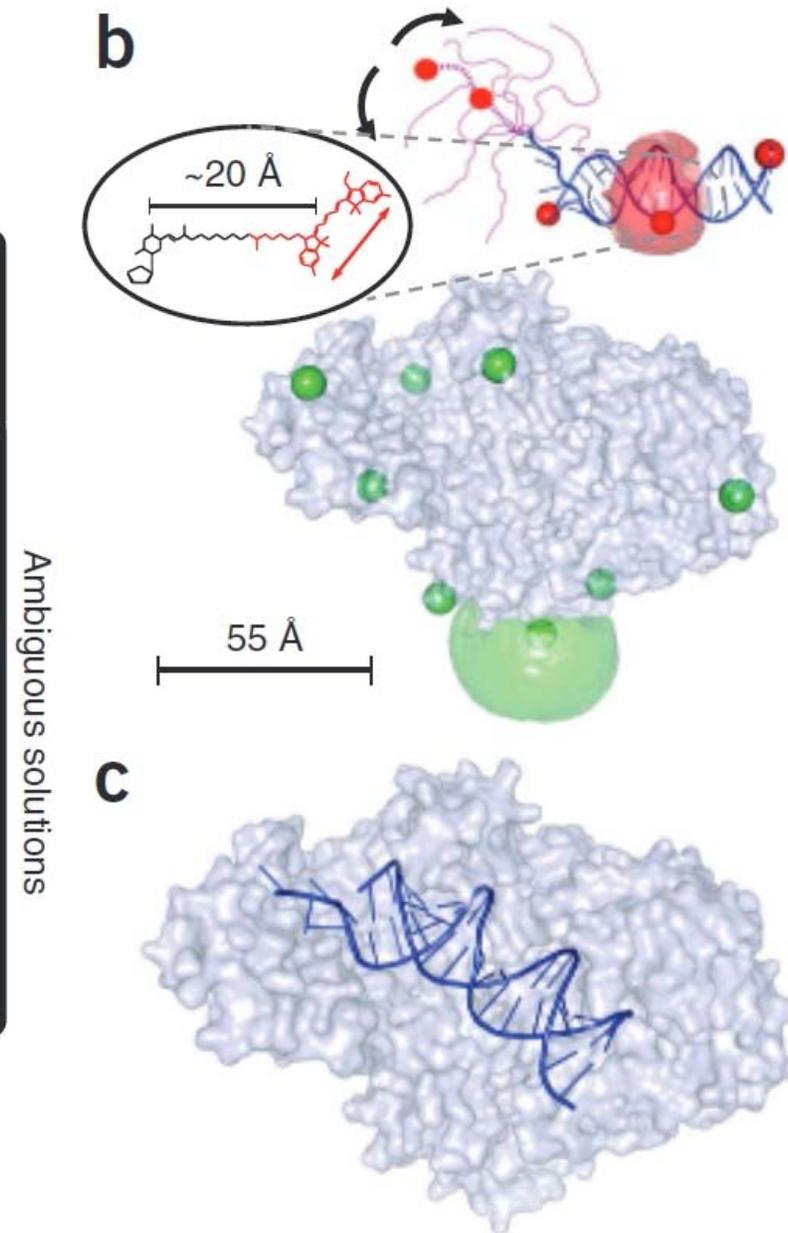
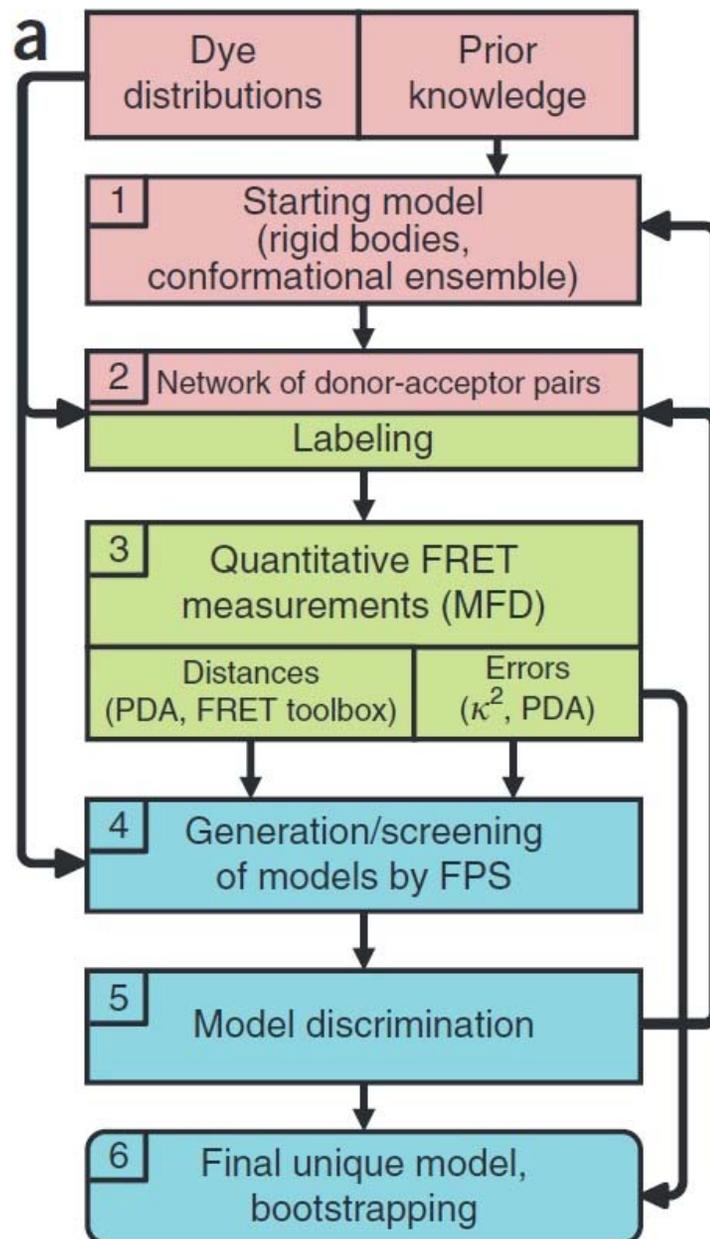


pulses define the macrotime (D)  
photon arrival times microtime (C)

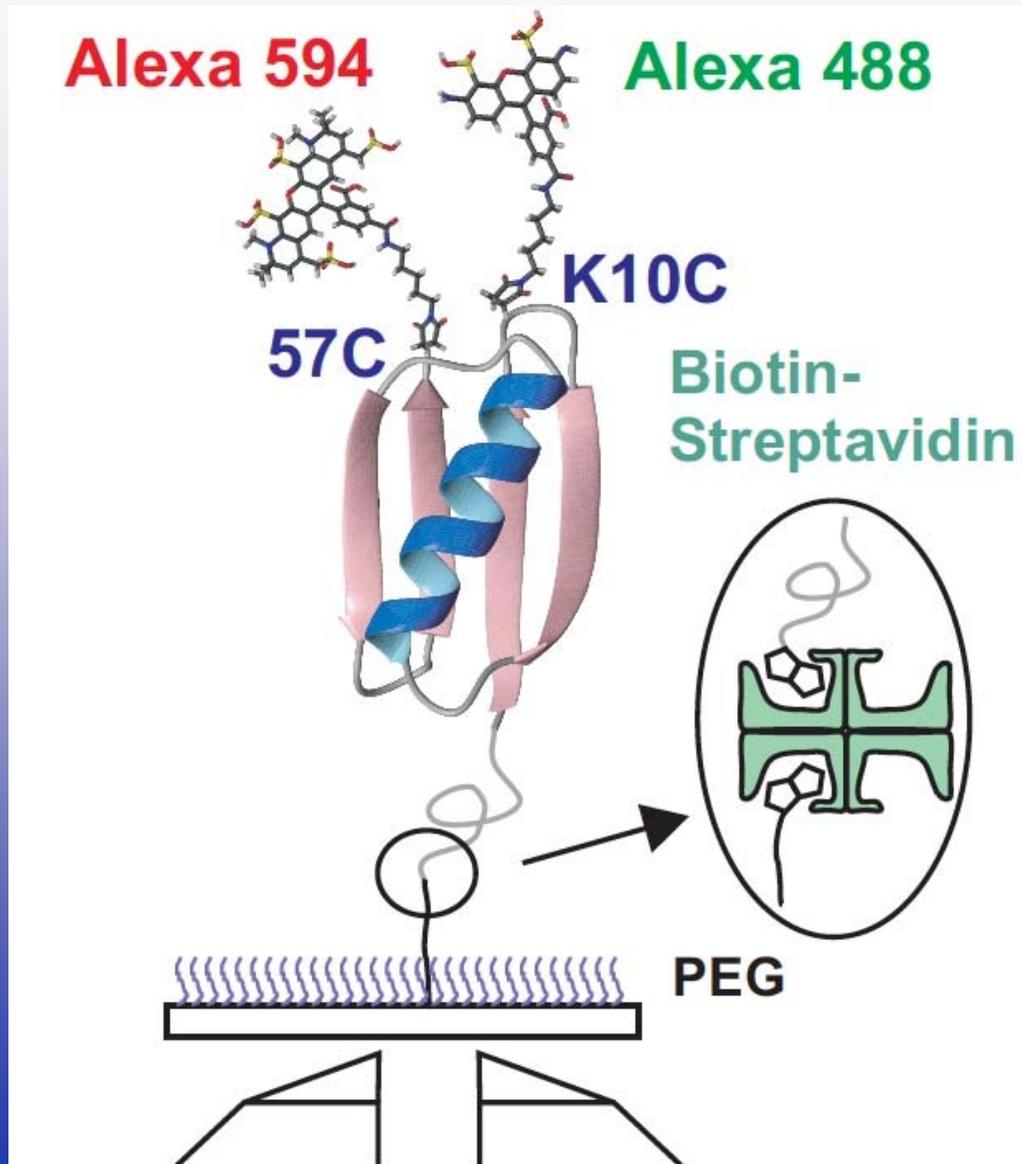
C. Seidel, Methods  
Enzymol., Vol. 475  
(2010)



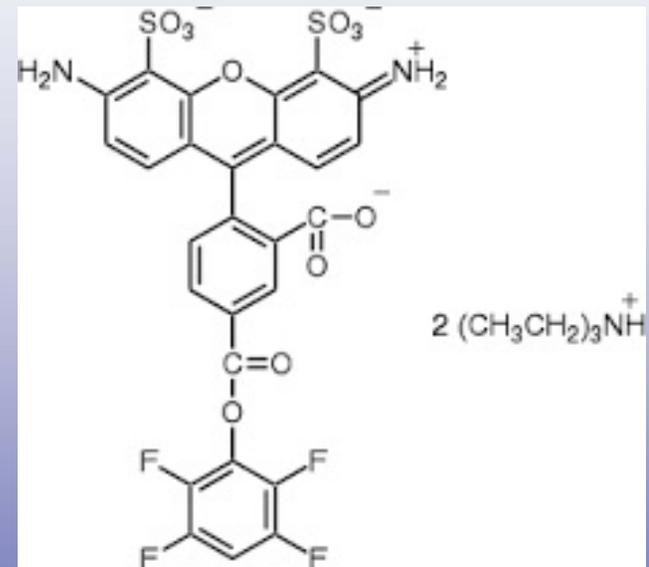
# Structure of HIV-1 reverse transcriptase



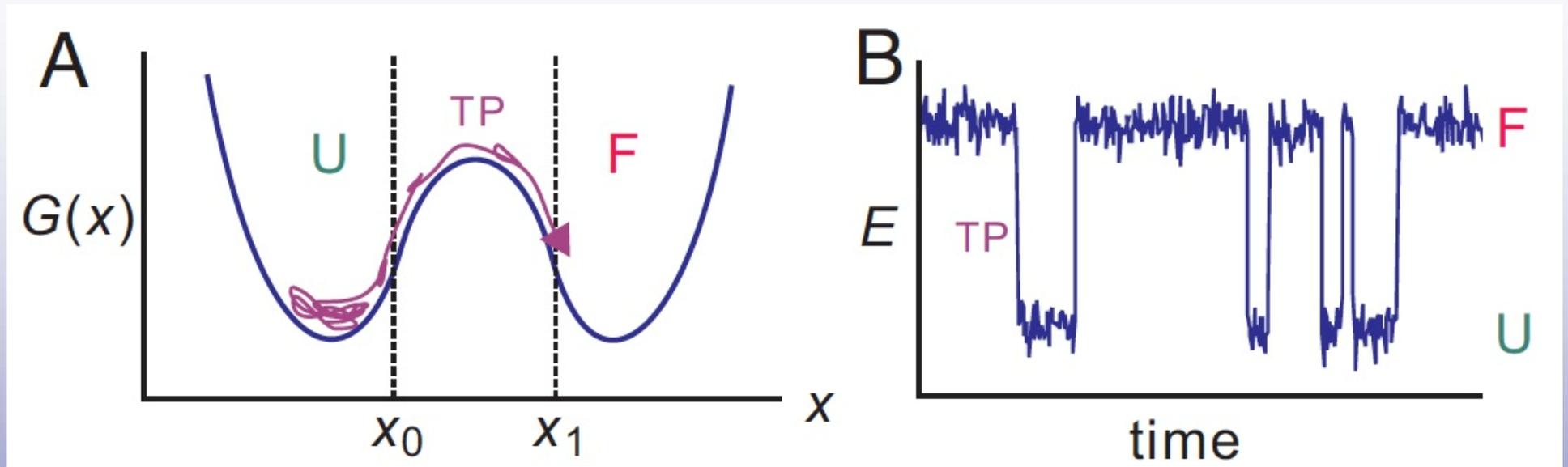
# Folding/unfolding kinetics of protein GB1, an immunoglobulin-binding protein in *Streptococcus*



## Xanthenyl dyes for FRET



W. A. Eaton, *PNAS*,  
*106*, 2009, 11837

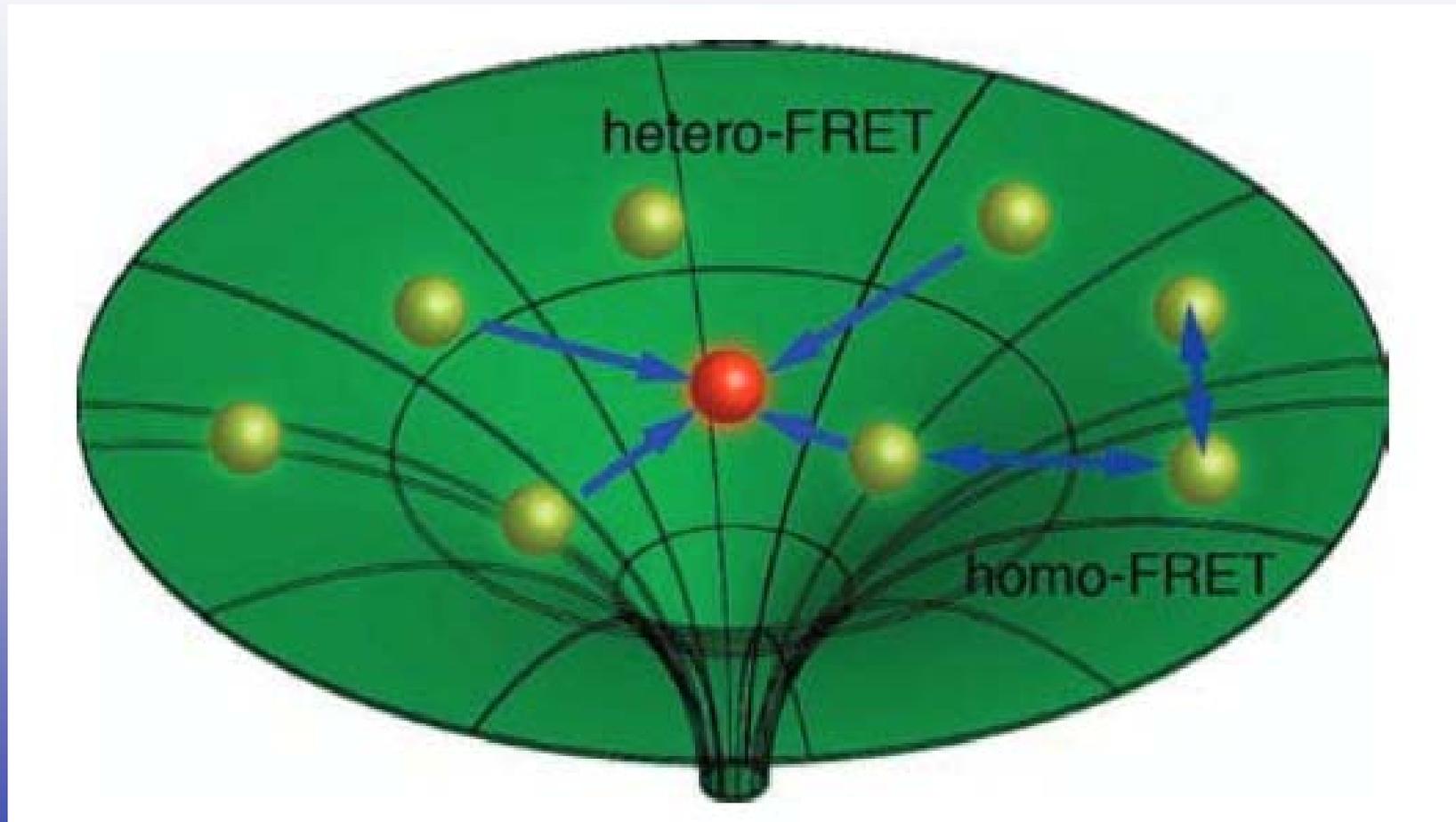


6M Urea

FRET efficiency

Transition path time  $< 200 \mu\text{s}$ ;  $> 10'000$  times shorter than the folding/unfolding rate coefficient.

# Photosynthesis antenna systems



# Conclusions

- Think! A few high-impact papers vs. publish or perish.
- FRET as a molecular ruler has a huge impact on biophysics. Be pedantic in quantity calculus.
- Scrutinizing Förster's paradoxes has revealed important chemical quenching processes.
- Proton-Transfer to C-atoms of electronically excited states can be very fast.
- The Förster-cycle provides surprising, yet reliable predictions.

