Photoremovable Protecting Groups: How fast are they really?

Hassen Boudebous, Dominik Heger, Bruno Hellrung, Yavor Kamdzhilov, Lubica Klíčová, Marek Mac, Pavel Müller, Markus Schwörer, Peter Šebej,
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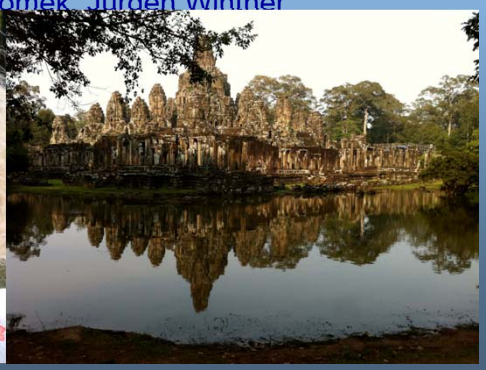
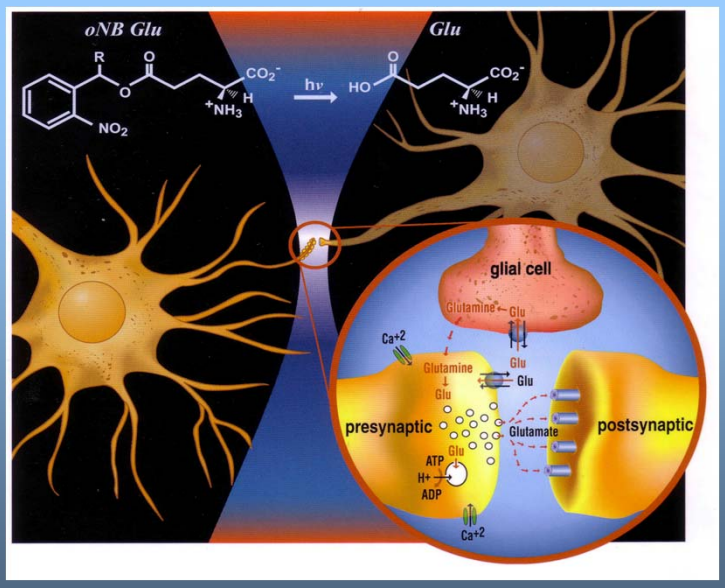
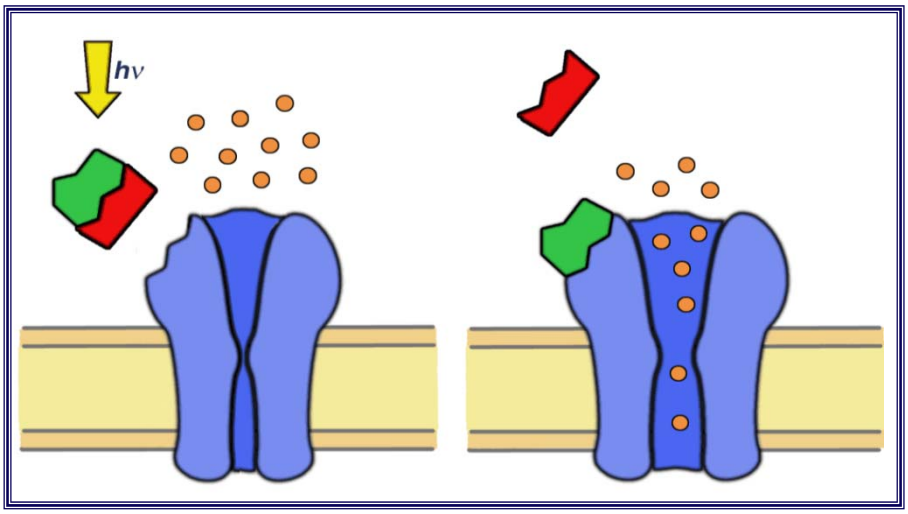


Richard Givens, Thomas Kiefhaber, Petr Klán

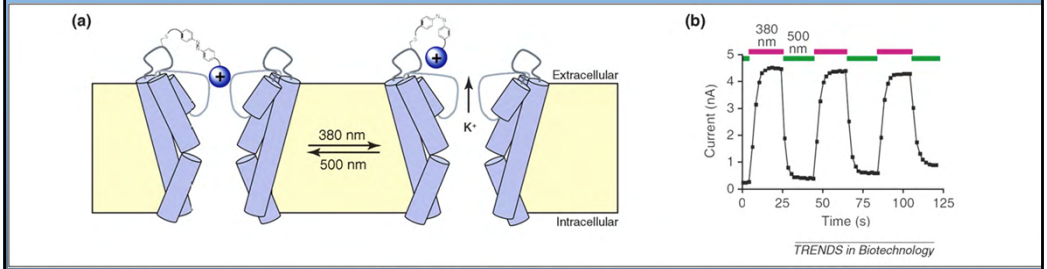


Goeldner, Givens, *Dynamic Studies in Biology,* Wiley-VCH, **2005**

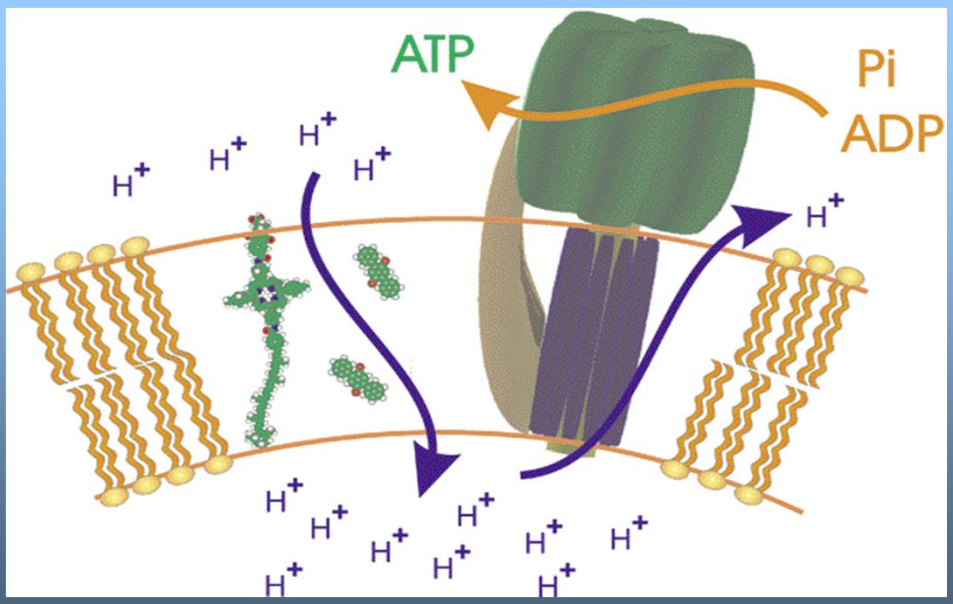
Activation of an ionotropic receptor with GABA



Remote control of ion channels



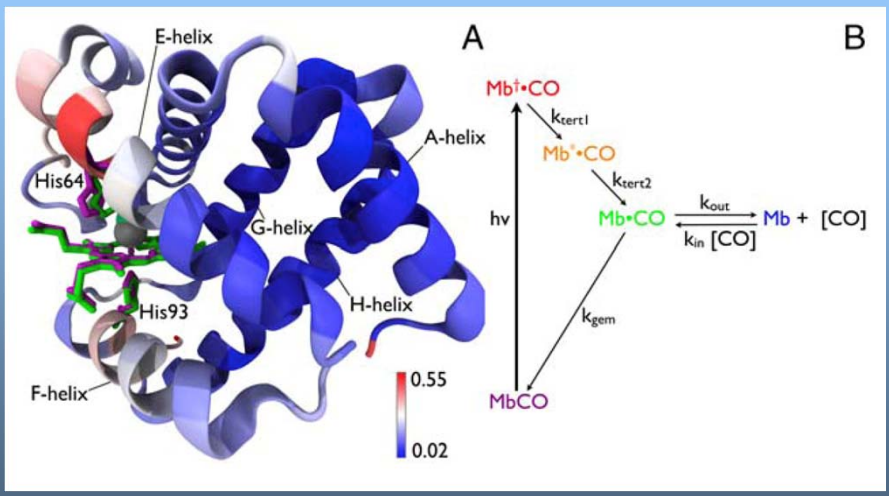
Kramer, Nat. Methods 5, **2008**, 331.



D. Gust, *Acc. Chem. Res.* **2001**, *34*, 40

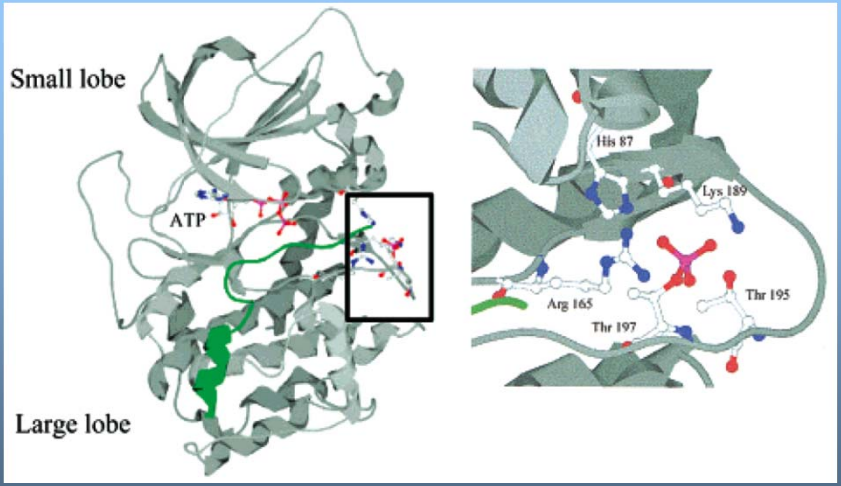
Time-resolved X-ray: Myoglobin-CO

X-ray scattering differences recorded over 8 decades of time: 100 ps to 10 ms



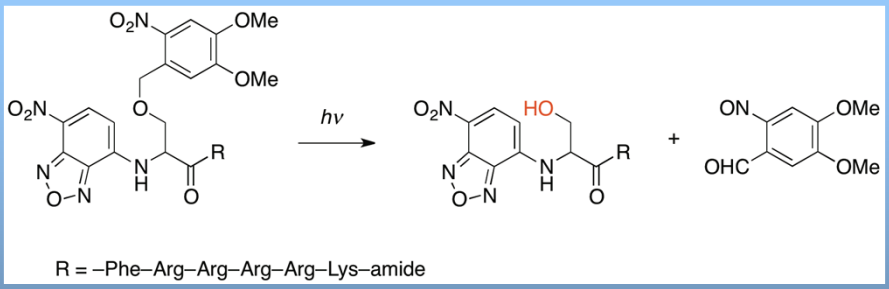
P. Anfinrud, *PNAS.* **2010**, *107*, 7281

Caged Protein Kinase A



R. S. Givens, H. Bayley, *J. Am. Chem. Soc.* **2002**, *124*, 8220

Monitoring intracellular protein kinase activity



R = –Phe–Arg–Arg–Arg–Arg–Lys–amide

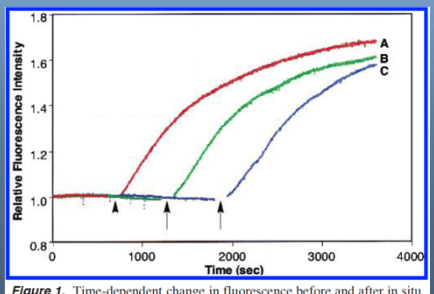
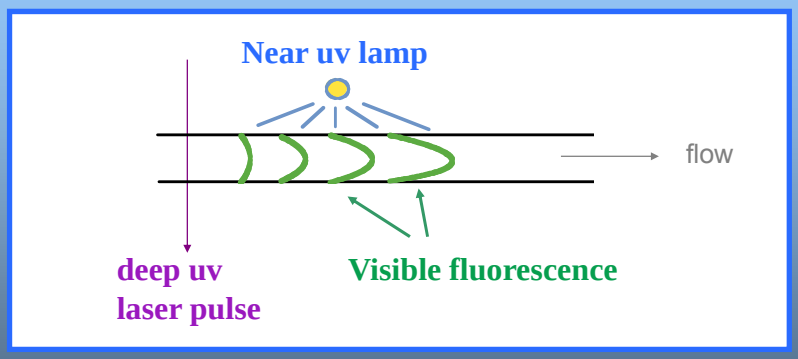


Figure 1. Time-dependent change in fluorescence before and after in situ illumination of caged peptide.

D. H. Lawrence, *JACS*, **2003**, 13358

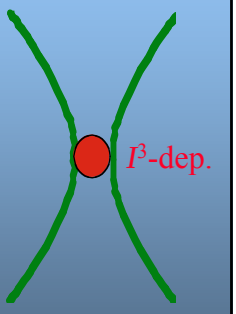
Caged fluorescers: Transport phenomena, mixing, motion



Kiskin, *Eur. Biophys. J.* **2002**, *30*, 588

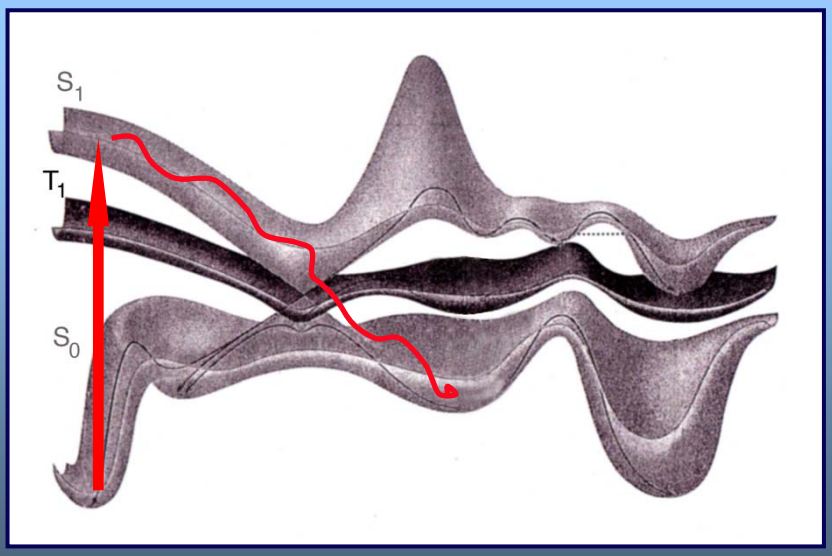
2- and 3-Photon Excitation

- Focused NIR-pulses of fs-duration
- Fluorescence excitation for imaging or release of caged reagents in addressable 0.1 fl-volumes (3-d submicron resolution)
- Little radiation damage or scattering in tissue
- Reagents diffuse out of volume within ~ ms: need fast release!

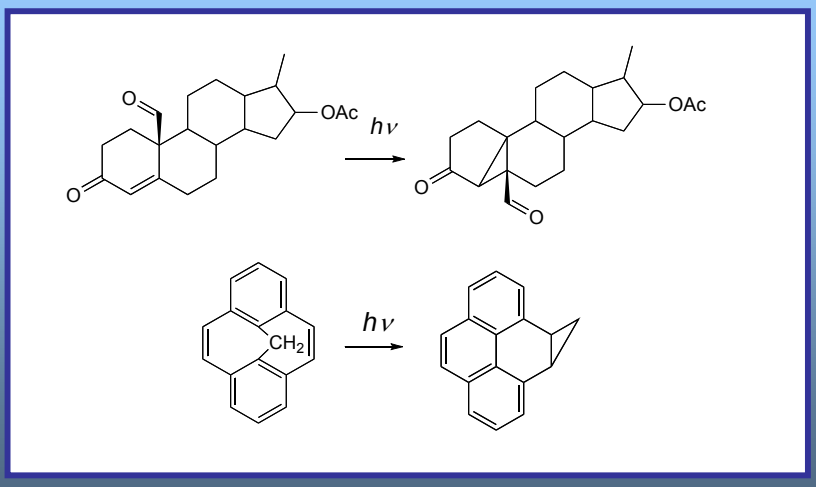


W.W. Webb, *Science Biophys. J.* **1999**, *76*, 489
M. Goeldner, *Bioorg. Med. Chem.* **2010**, *18*, 7753

Light brings a new dimension to chemistry

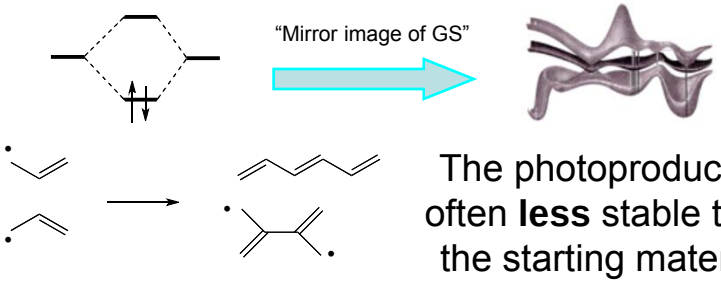


Calculations. Rules-of-thumb. Guidelines.



Rule: Opposite to ground state reactivity

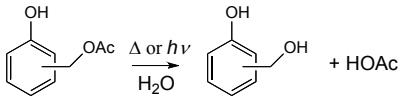
- PMO (Dewar, Dougherty)



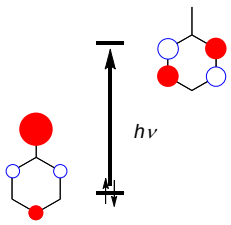
The photoproduct is often **less** stable than the starting material

Rule: Opposite to ground state reactivity

- Charge distributions (Zimmermann)

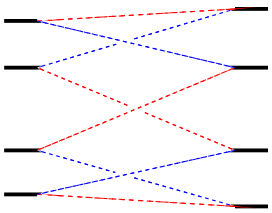


ground-state: *ortho, para* excited-state: *meta*



Rule: Opposite to ground state reactivity

- Woodward-Hoffmann rules for pericyclic reactions (retaining some symmetry elements along the reaction path)



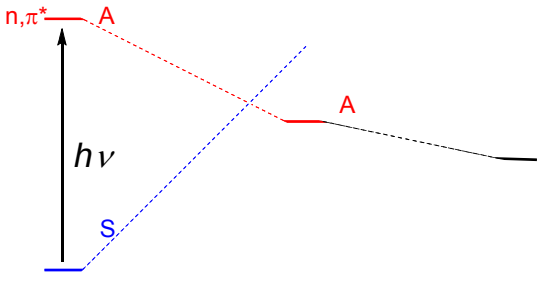
excited-state allowed
ground-state forbidden

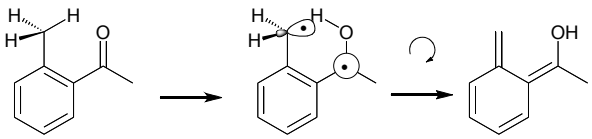
ground-state allowed
excited-state forbidden

No exceptions?

An even simpler use of symmetry:

- Electron count (Salem, Dauben, Turro)



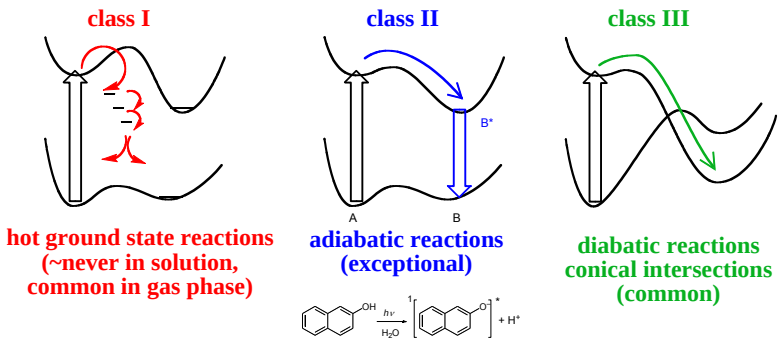


Note: The path retaining symmetry is probably **not** the best (**not** the most downhill)!

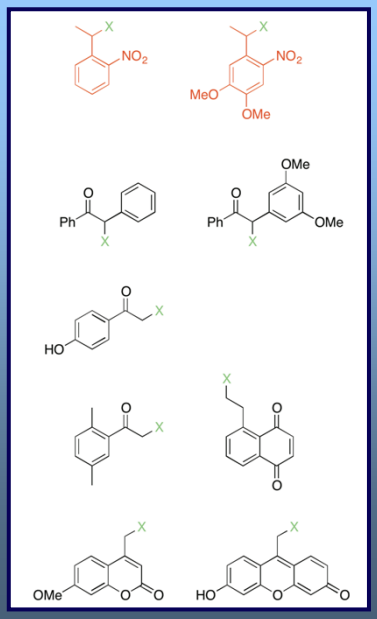
It is chosen merely because symmetry arguments are so simple to use.

At geometries with no symmetry (C_1), all wavefunctions belong to the totally symmetric representation. Do potential energy surfaces of the same symmetry cross?

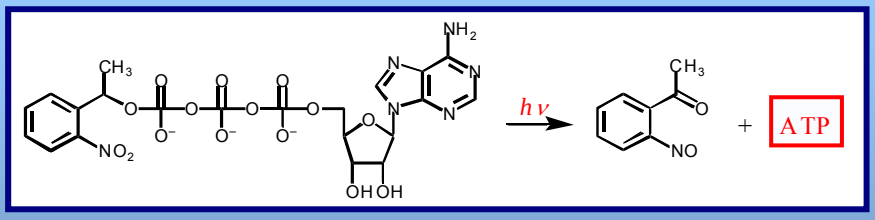
Adiabatic versus diabatic reaction paths (Förster)



Markus Schwörer
Yuri Il'itchev
Marek Mac



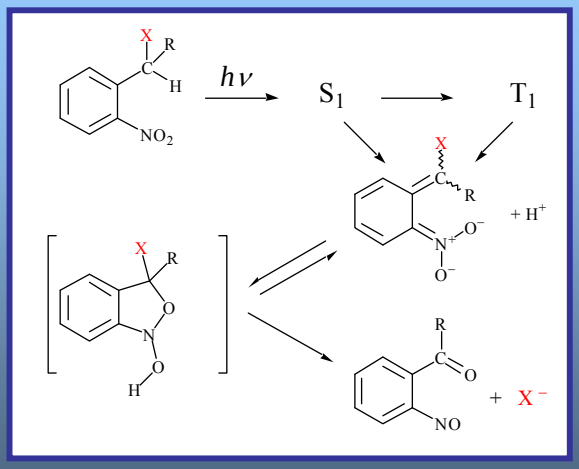
Caged ATP: The „gold standard“



- Time resolved FTIR detection using ^{13}C, ^{15}N, ^{18}O isotopomers
- Release of ATP single exponential, „synchronous“ with *aci*-nitro anion decay
- k_{app}(ATP) ≈ 52 s^{-1} at pH 7 and 10 °C

Kaplan/ Trentham/ Corrie/ Mäntele/ Gerwert
JACS 110 (**1988**) 7170; *117* (**1995**) 10311; *119*, 4149 (**1997**)

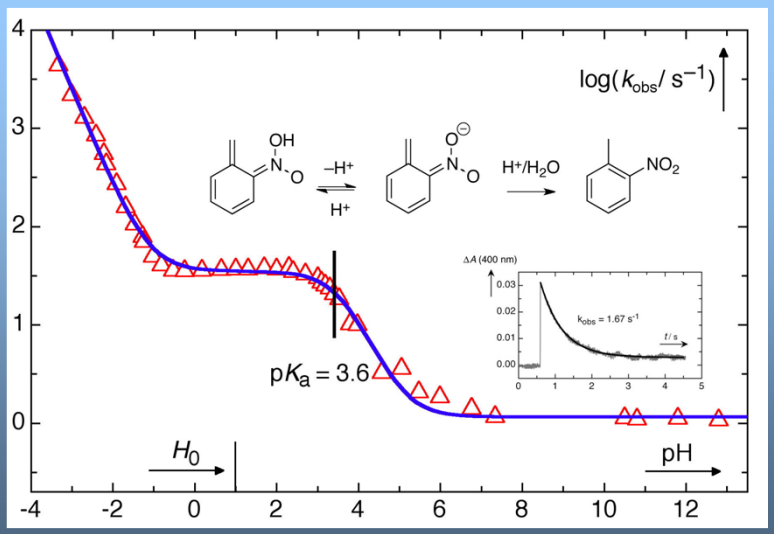
Reaction mechanism: common wisdom



aci-anion decay:
- via nitronic acid
- cycliz'n not reversible
- not necessarily rate-determining for release

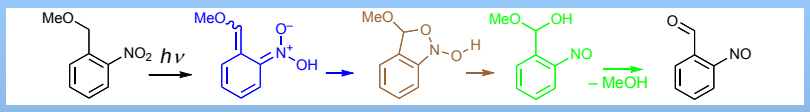
D. R. Trentham, *JACS 110*, **1988**, 7170

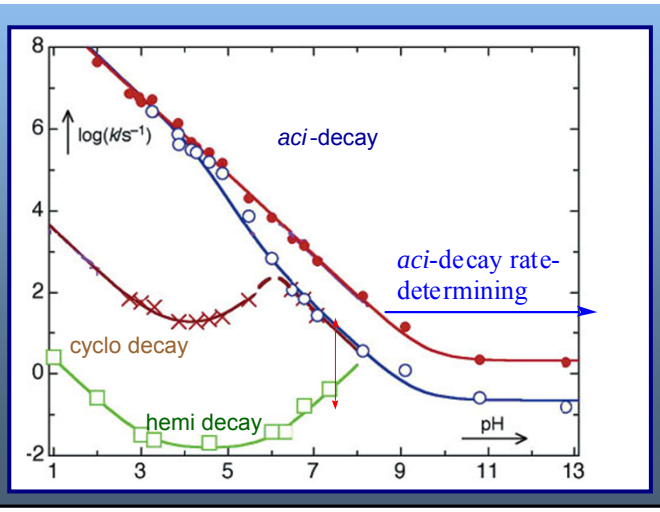
o-Nitrotoluene: pH–rate profile



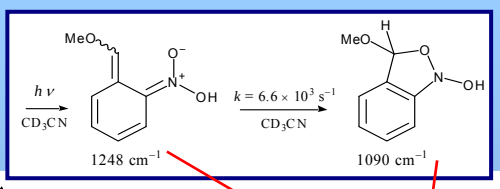
Helv. *84*, **2001**, 1441

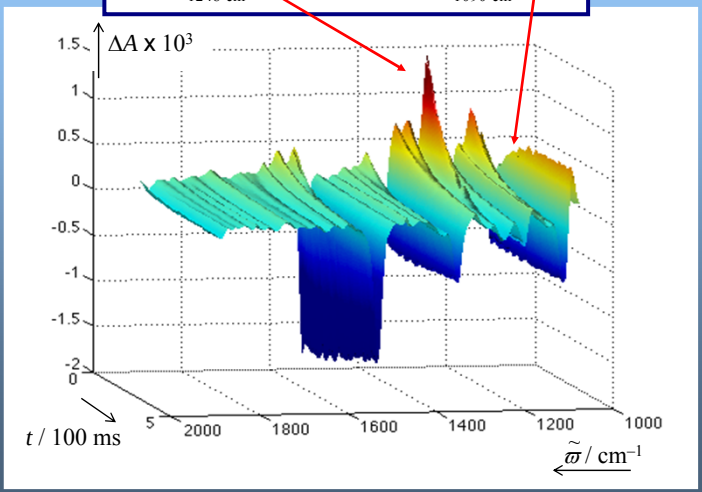
o-Nitrobenzyl methyl ether: pH–rate profile

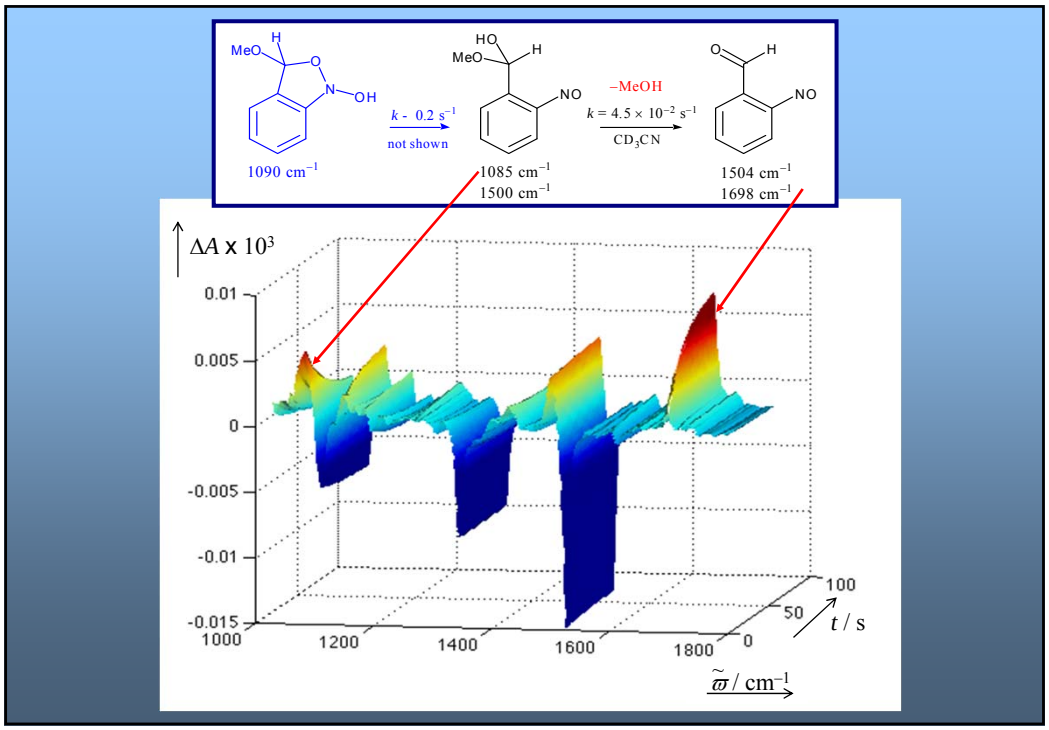


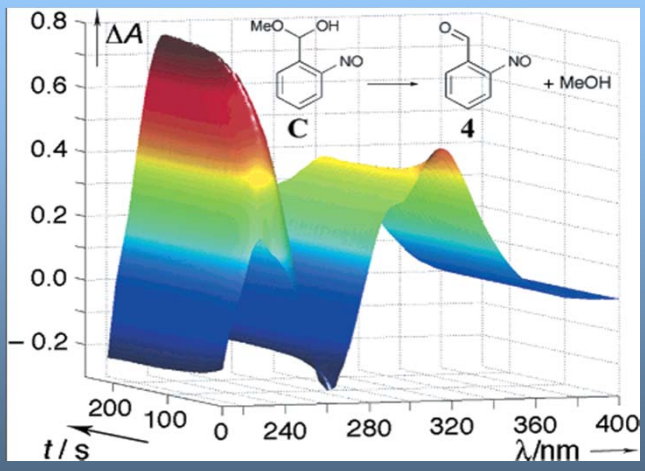


JACS **2004**, *126*, 4581



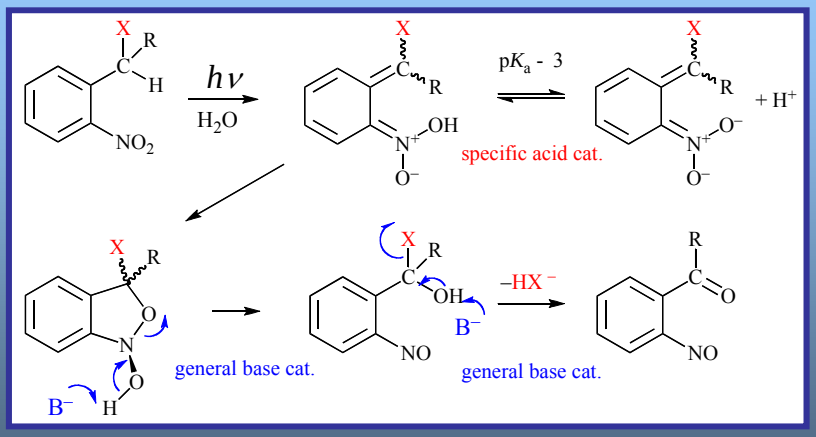






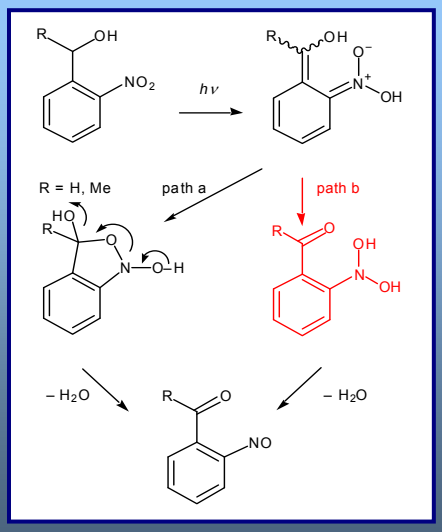
JACS **2004**, *126*, 4581

Mechanism of release

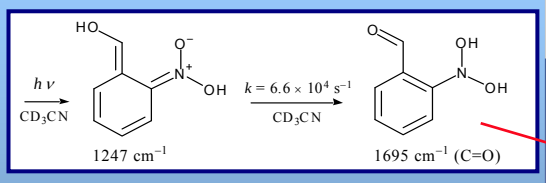


JACS **2004**, *126*, 4581

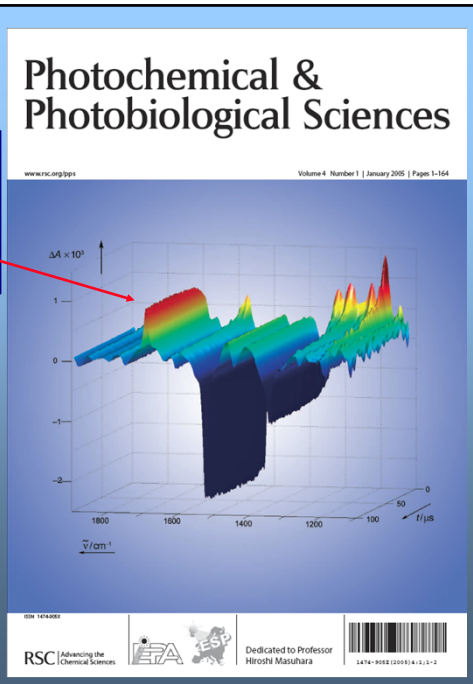
o-Nitrobenzyl alcohol



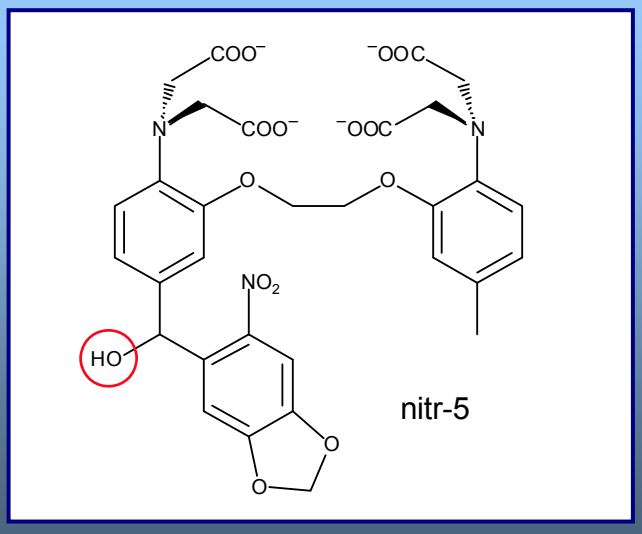
Photochem. Photobiol. Sci. **2005**, *4*, 33



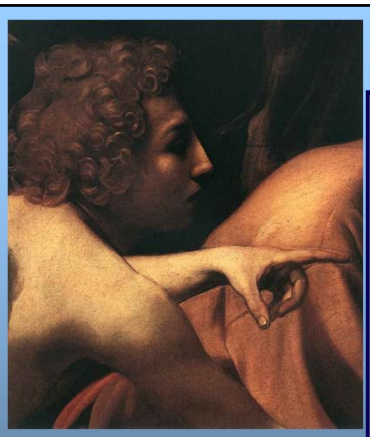
PPS, **2005**, *4*, 33



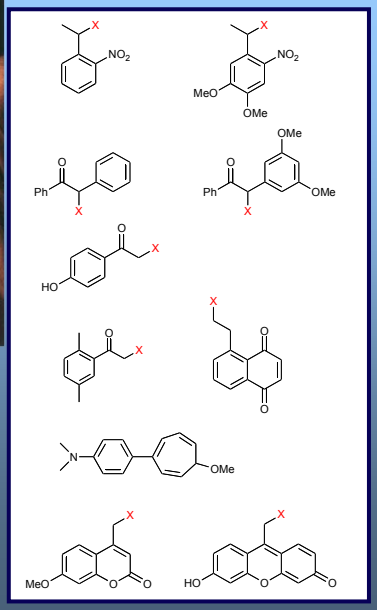
Caged Ca^{2+}



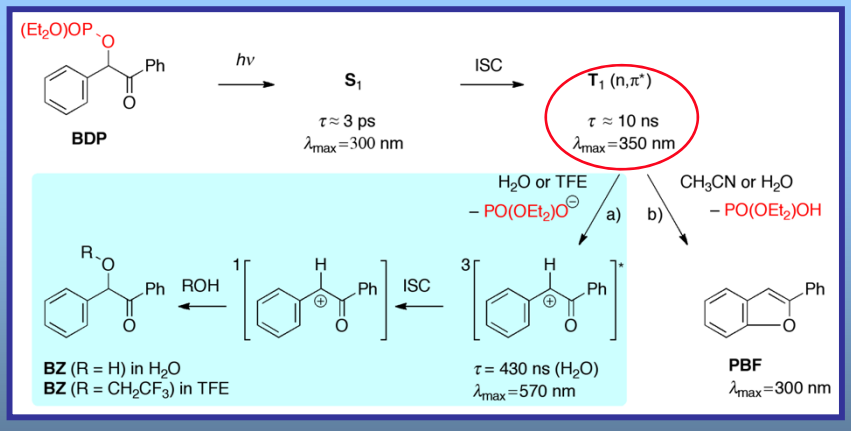
R. Y. Tsien, *JACS* **1988**, *110*, 321.

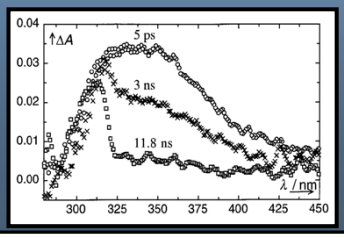


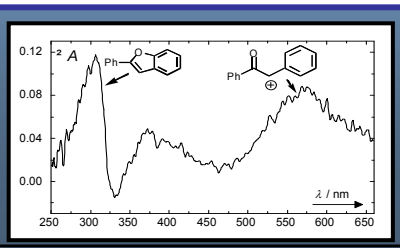
Rich Givens, Boris Sket
C. S. Rajesh
Hassen Boudebous



Benzoin phosphates



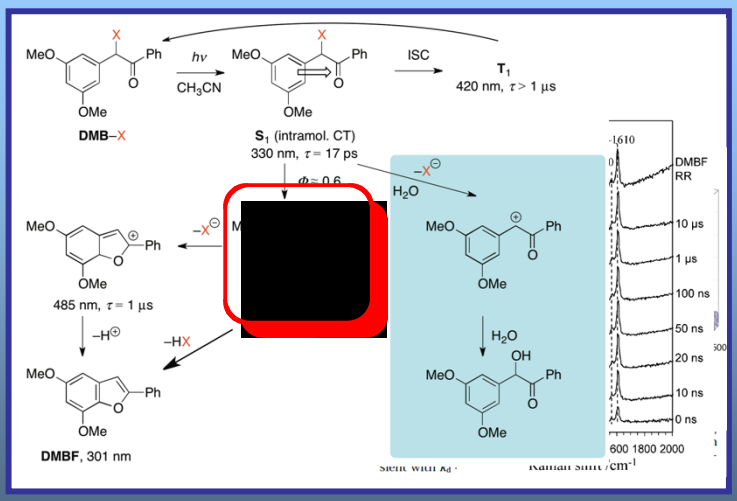




JACS **2000**, *122*, 611

D. L. Phillips, *Chem. Eur. J.* **2007**,*13*, 2290

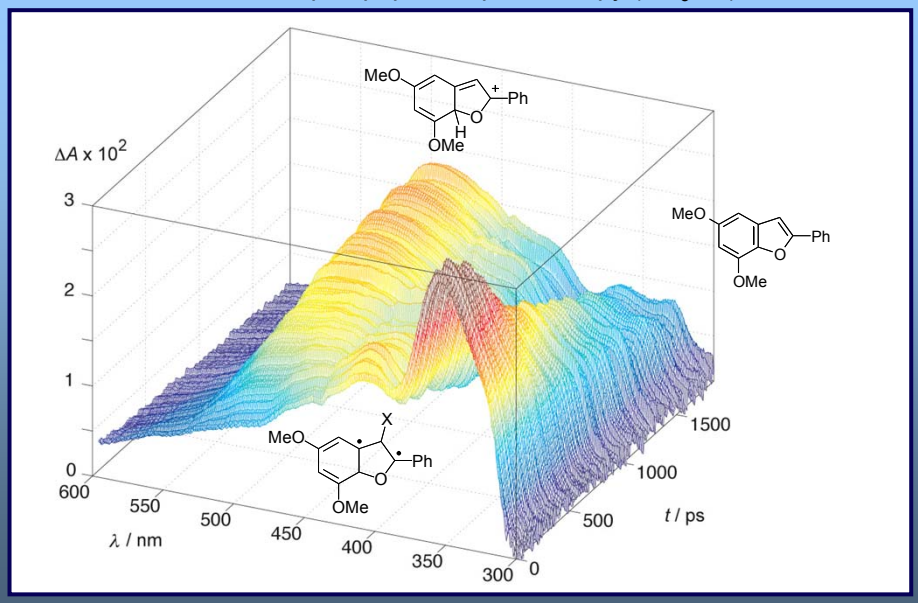
Dimethoxybenzoin cages



Sheehan, *JACS*, **1971**, *93*, 7222. Corrie, Wan, *JOC*, **1997**, *62*, 8278.

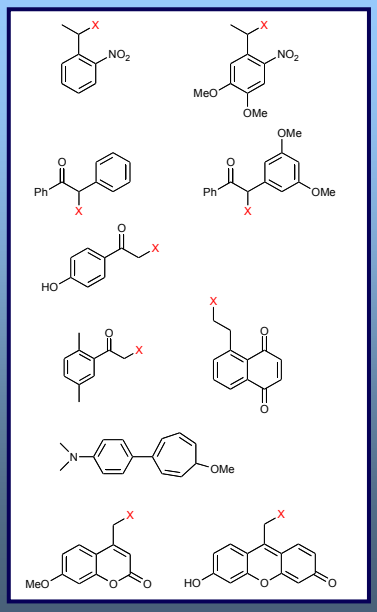
JPC *A* **2007**, *111*, 2811. Phillips, *Chem. Eur. J.* **2010**, *16*, 5102.

Picosecond pump–probe spectroscopy (CH_3CN)

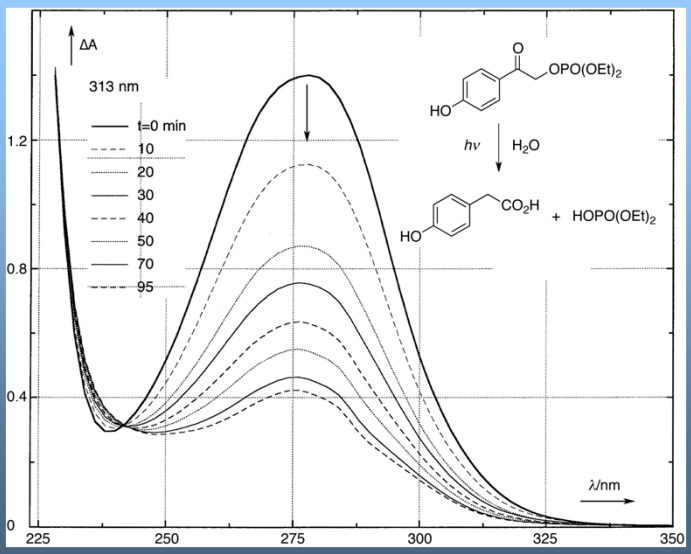


Two-wave appearance of released HX: k = 1 x 10^9 s^{-1}, 1 x 10^6 s^{-1}.

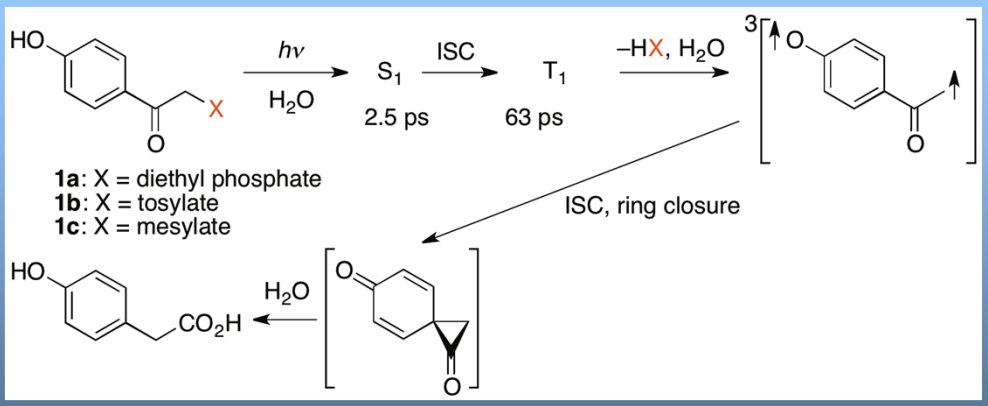
Bruno Hellrung,
Jürgen Wintner,
Dominik Heger,
Rich Givens



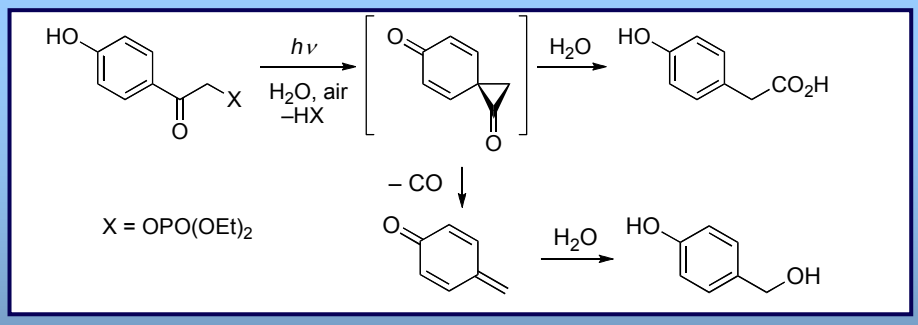
p-Hydroxyphenacyl (*p*HP) diethylphosphate

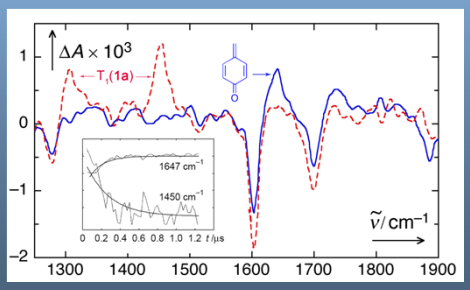


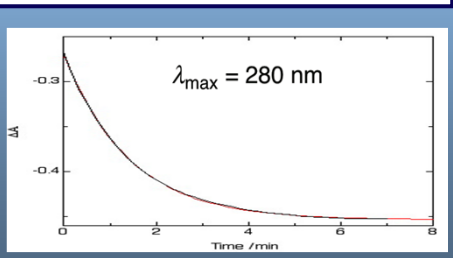
p-Hydroxyphenacyl (*p*HP) diethylphosphate



JACS, **2000**, *122*, 9346,
JACS, **2008**, *130*, 3307.

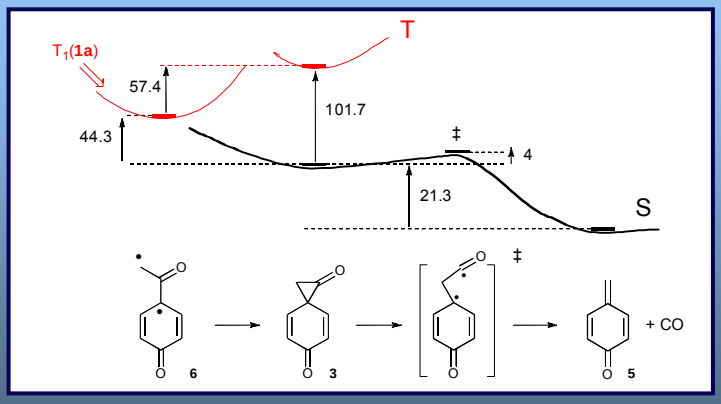






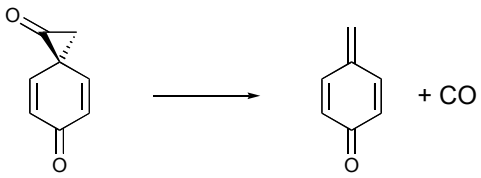
Kresge et al., *JACS*, **2002**,*124*, 6349

MP2/6-31G* (PCM acetonitrile)



$\log(k/s^{-1}) = 13 - 4.0/2.3RT = 10$

MP2 6-31G*

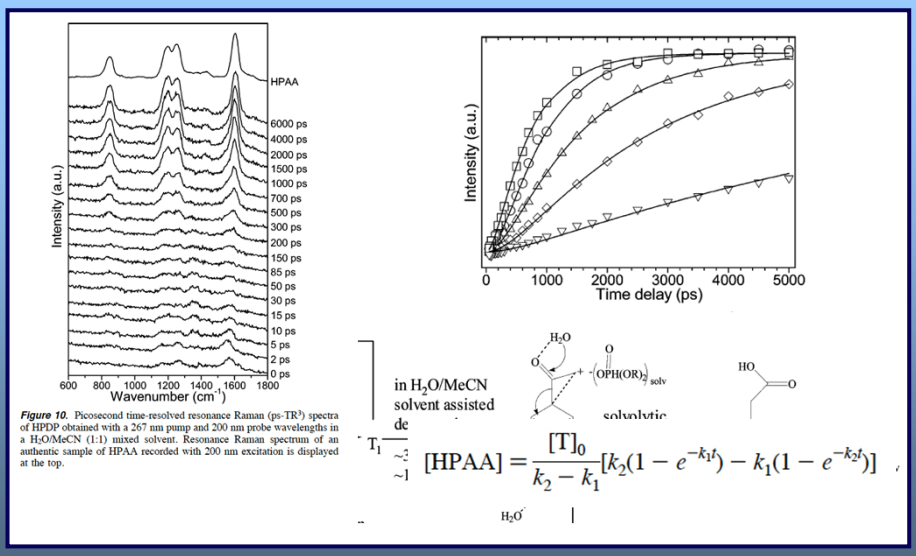


E_a (g) = 13.3 kcal/mol

E_a ("CH_3CN", PCM) = 4.0 kcal/mol

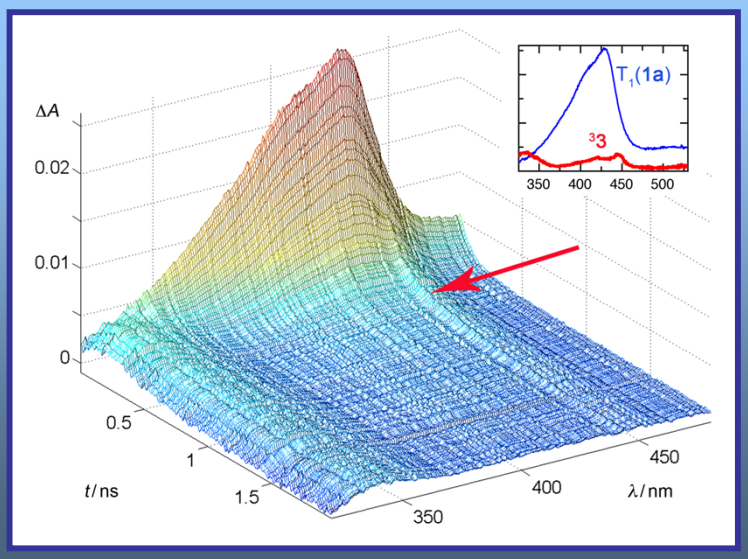
$\log(k/s^{-1}) = 13 - 4.0/2.3RT = 10$

ns-TRIR measurement: appearance of final product

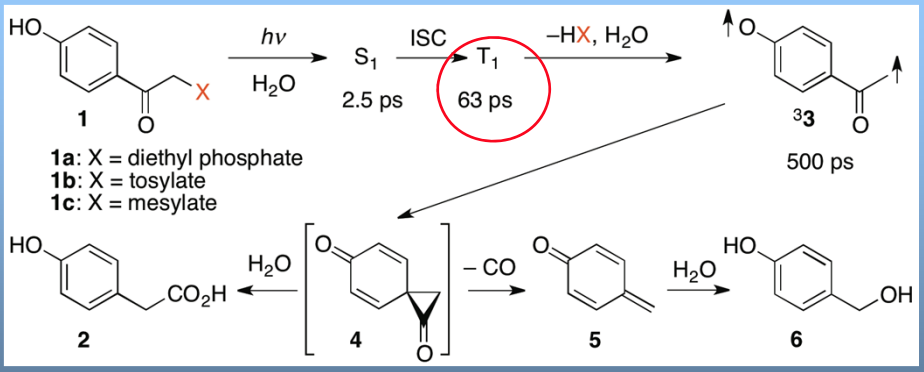


D. L. Phillips, *JACS*, **2006**, *128*, 2558

p-Hydroxyphenacyl (*p*HP) diethylphosphate

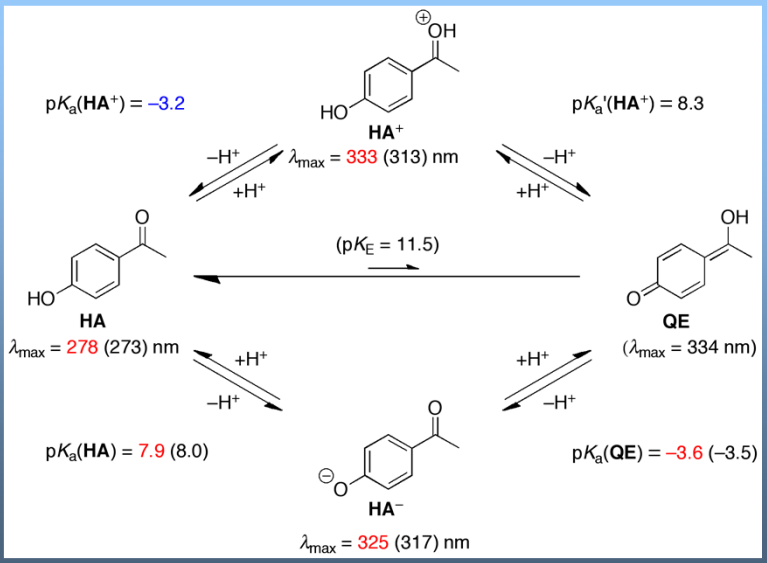


p-Hydroxyphenacyl (*p*HP) diethylphosphate

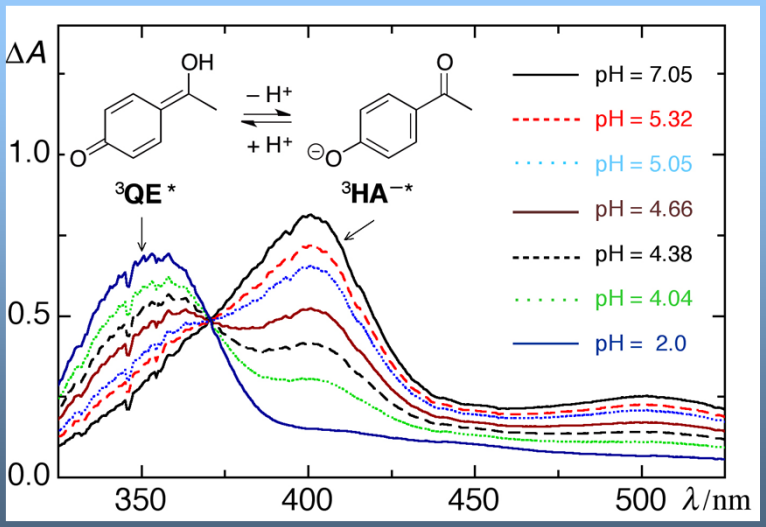


Why are quantum yields << 1?

p-Hydroxyacetophenone

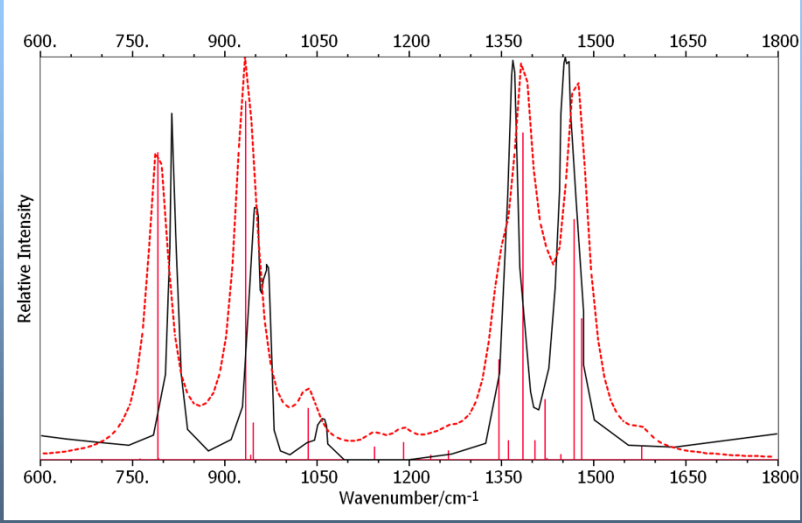


p-Hydroxyacetophenone



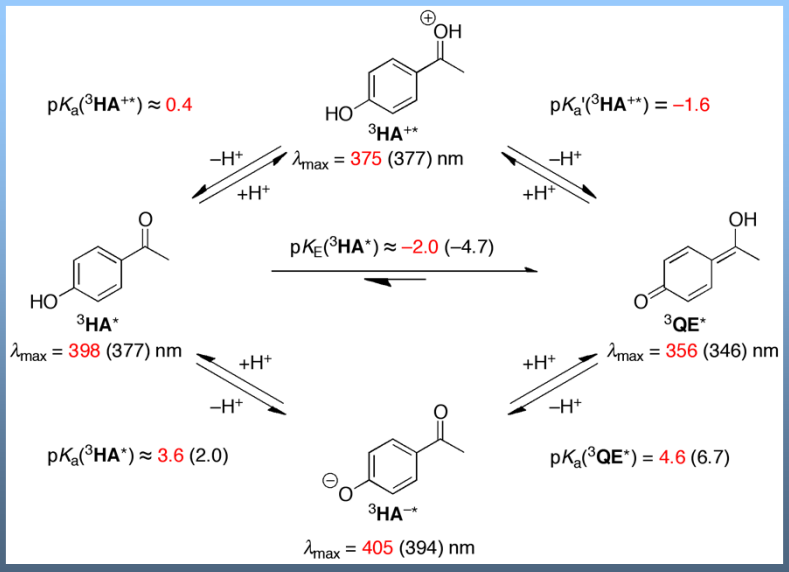
JACS **2000**, *122*, 9346

p-Hydroxyacetophenone triplet



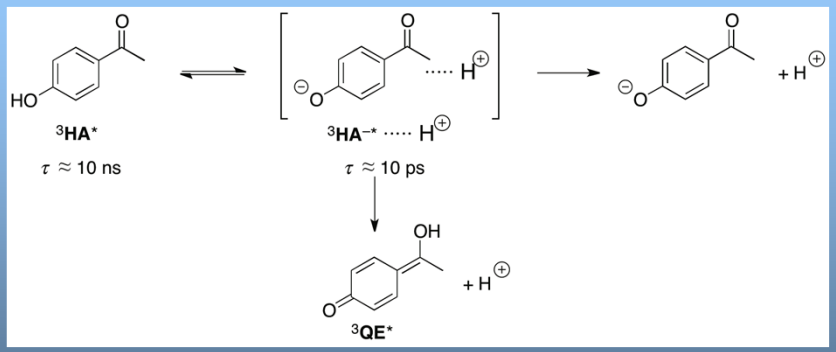
D. L. Phillips, *JOC* **2005**, *70*, 8661 Tomáš Šolomek

p-Hydroxyacetophenone triplet state in water



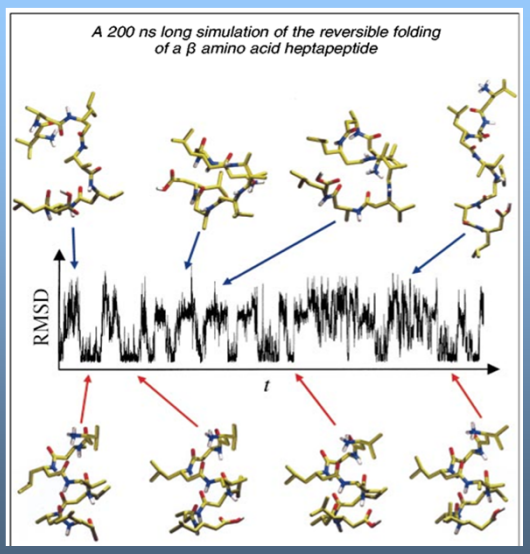
Dominik Heger, Lubica Klíčová, Peter Šebej, Tomáš Šolomek

Ionization via an ion pair intermediate



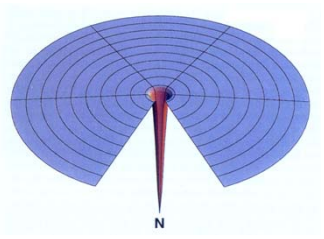
Ionization competes with release in *p*HP cages

Protein folding

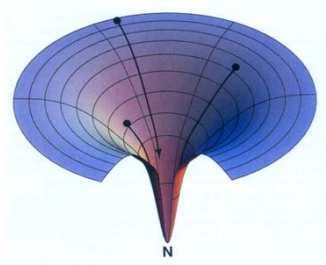


W. F. van Gunsteren, *Angew. Chemie* **2001**, *40*, 351

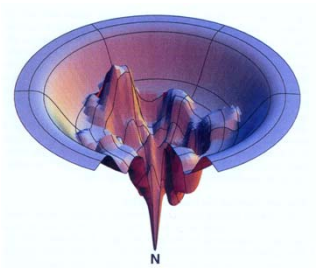
Energy Landscapes in Protein Folding



Flat Energy Surface

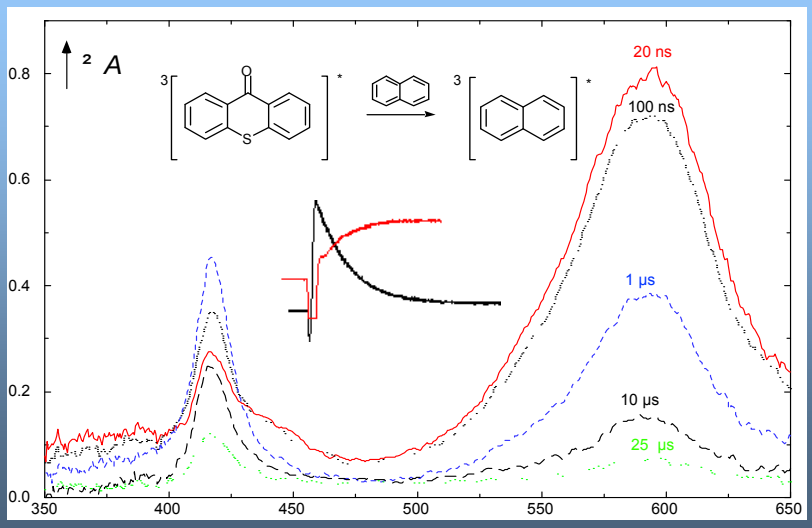


Smooth Funnel

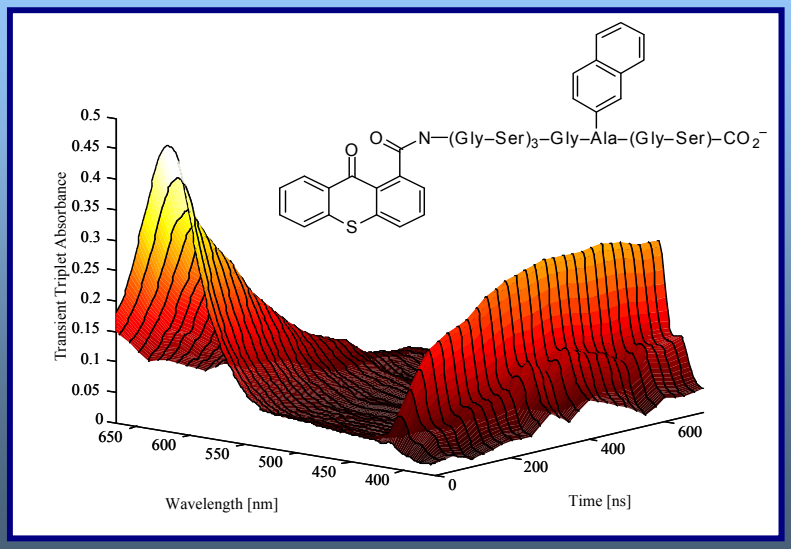


Rough Landscape

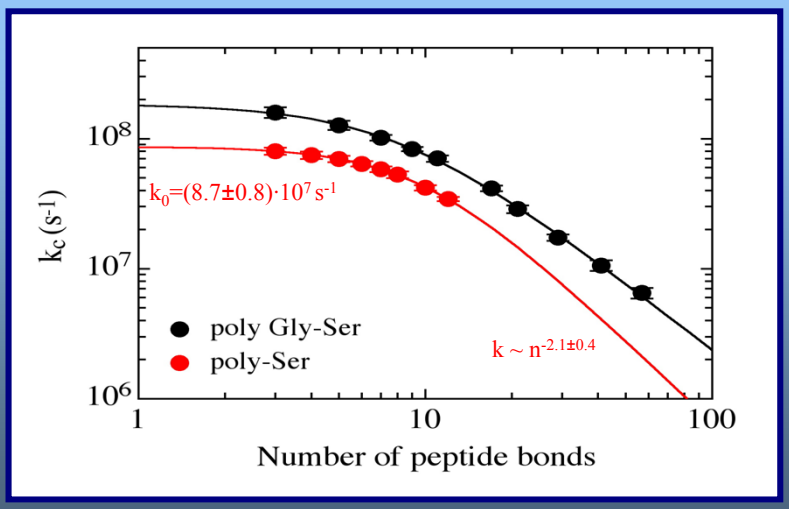
Triplet–triplet energy transfer



Triplet–triplet energy transfer

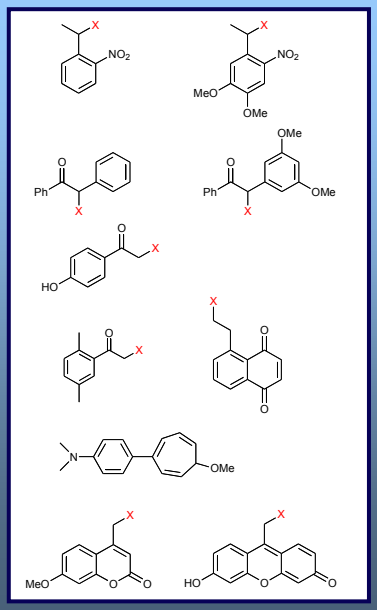


The speed limit of protein folding

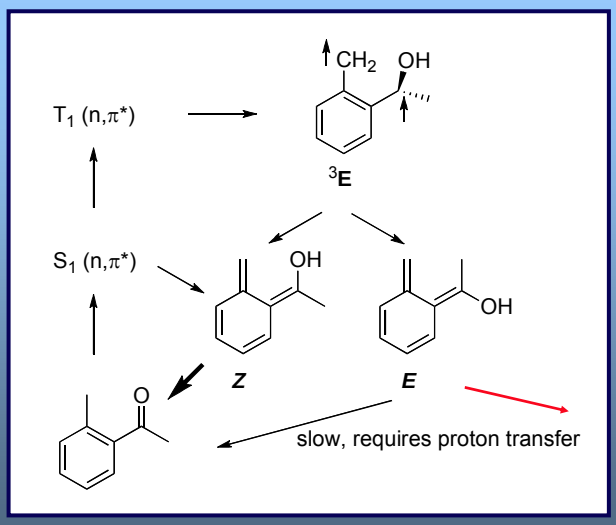


Kiefhaber, *PNAS* **1999**, *96*, 9597

Petr Klán,
Yavor Kamdzhilov

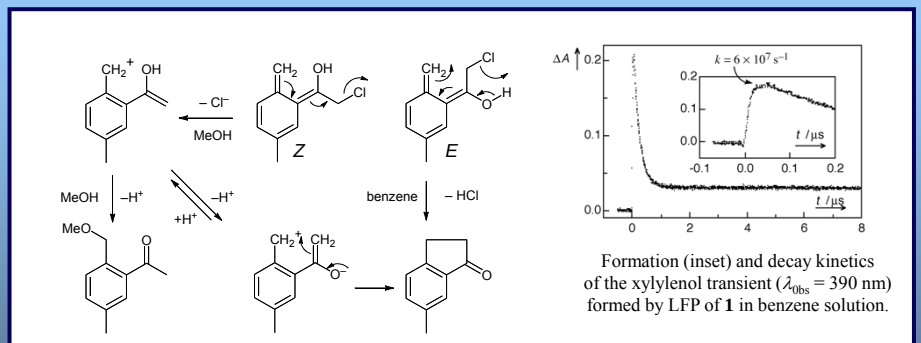


Photoenolization of 2-Methylacetophenone



Helv. Chim. Acta **1977**, *60*, 259

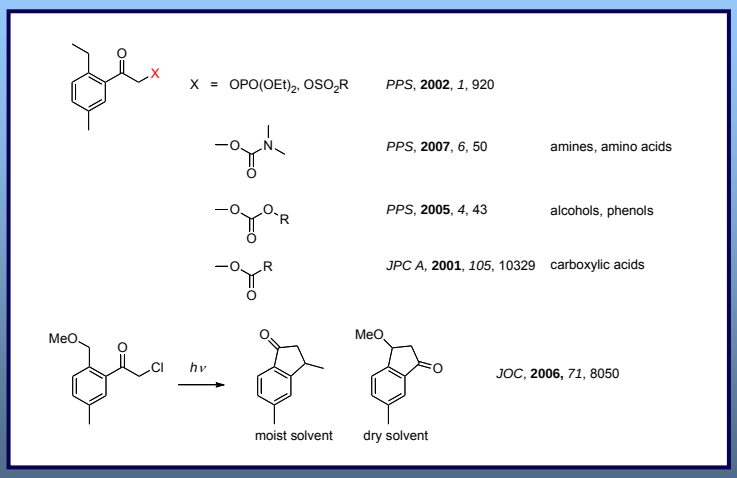
Phenacyl chloride



Formation (inset) and decay kinetics of the xylylenol transient (λ_{0bs} = 390 nm) formed by LFP of **1** in benzene solution.

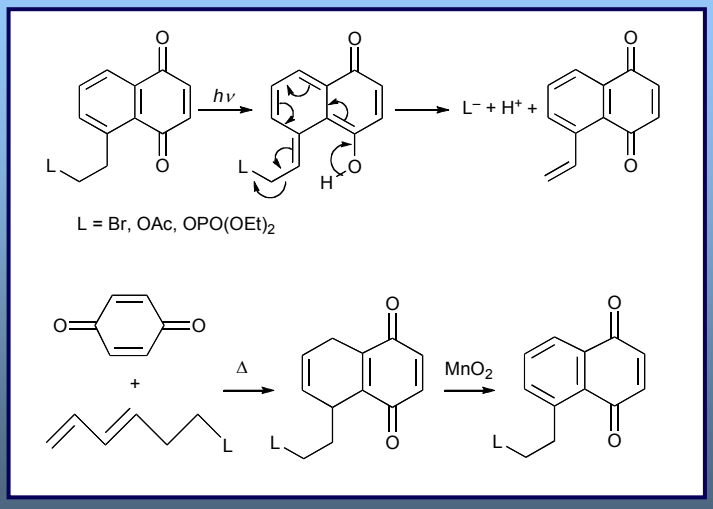
JACS **2001**, *123*, 7931

Dimethyl phenacyl cages



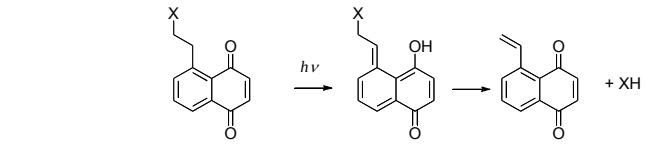
Petr Klán and coworkers, Brno

5-Ethylnaphthoquinone protecting group

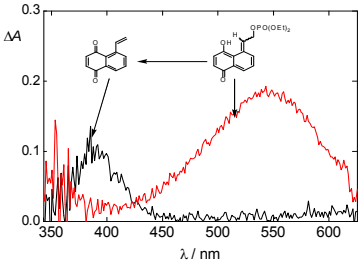


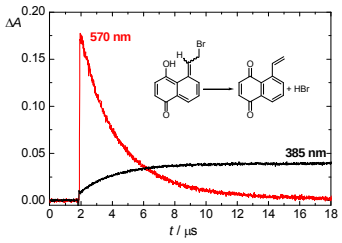
Photochem. Photobiol. Sci. **2007**, *6*, 865

5-Ethylnaphthoquinone protecting group



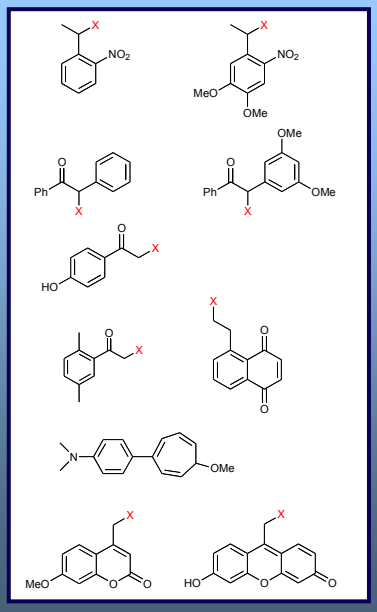
$X^- = Br^-, AcO^-, (OEt)_2OPO^-$



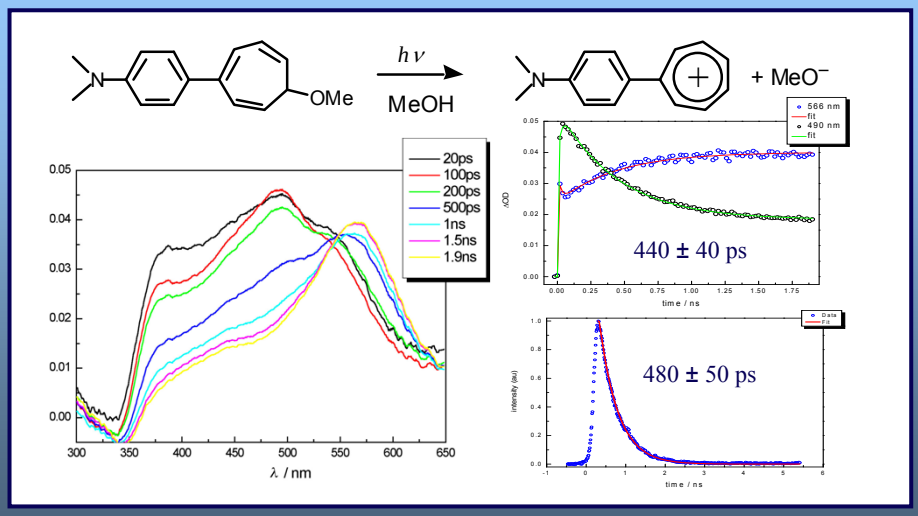


quantum yields of release in aqueous solution:
HBr (X=Br): **0.35 ± 0.06** (X=diethyl phosphate): **0.70 ± 0.08**

Bogdan Tokarczyk

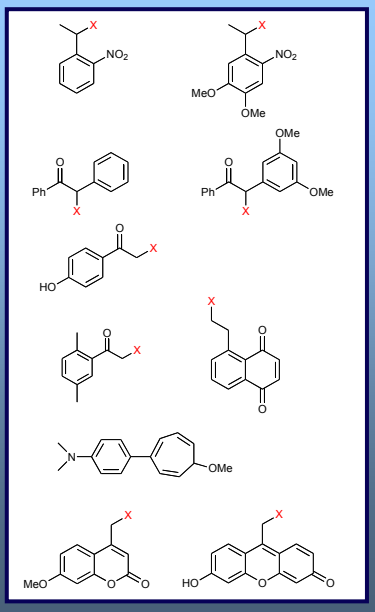


In search of fast alcohol release

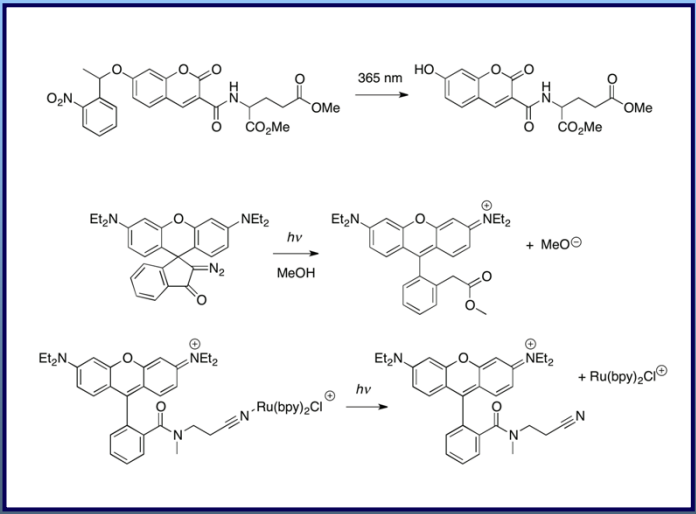


Prof. W. Abraham, Berlin

Jürgen Wintner, Pavel Müller



Caged fluorescers

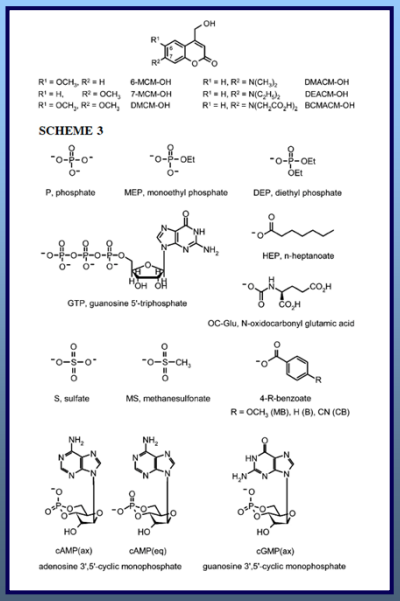


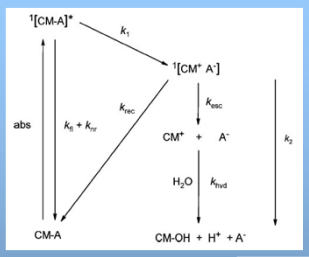
Zhao, *JACS* **2004**, *126*, 4653

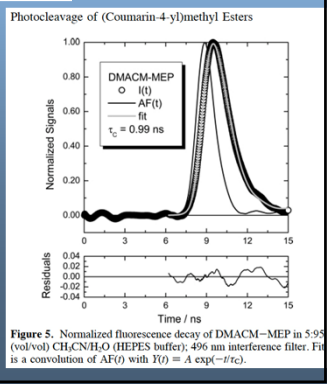
Belov, *Angew.* **2010**, *49*, 3520

del Marmol, *Anal. Chem.* **2010**, *82*, 6259

Coumarinmethyl cages

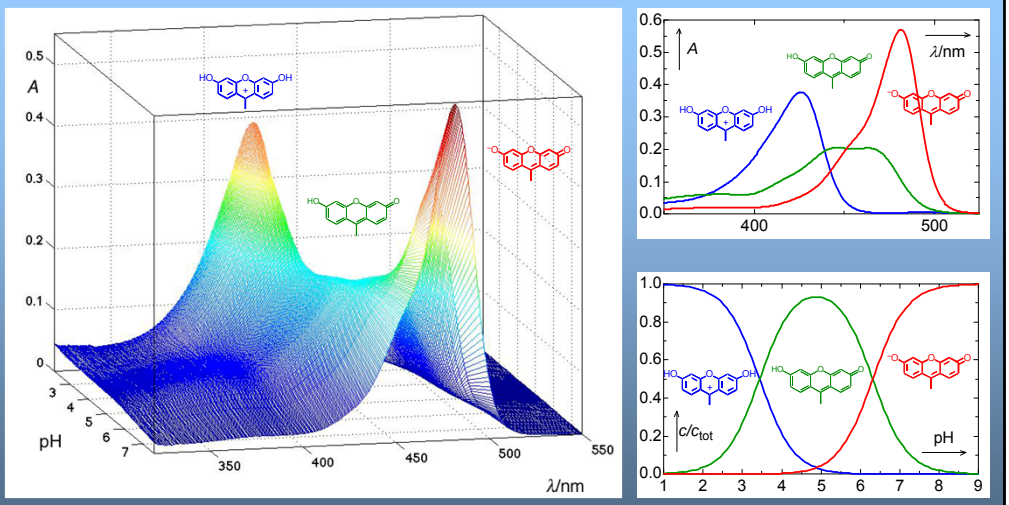




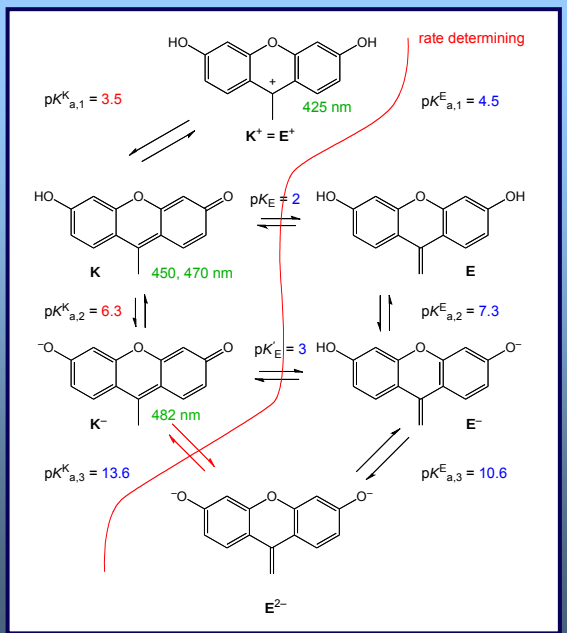


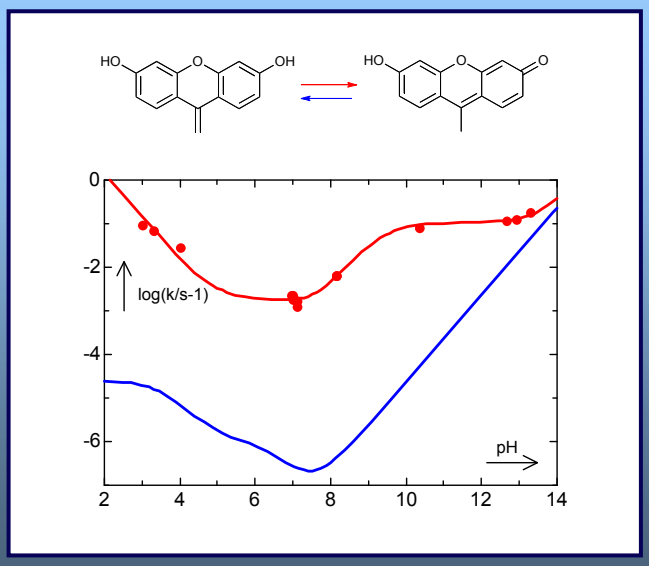
R. Schmidt, V. Hagen, *JOC* **2010**, *75*, 2790; *JPC A*, **2007**, *111*, 5768

Titration

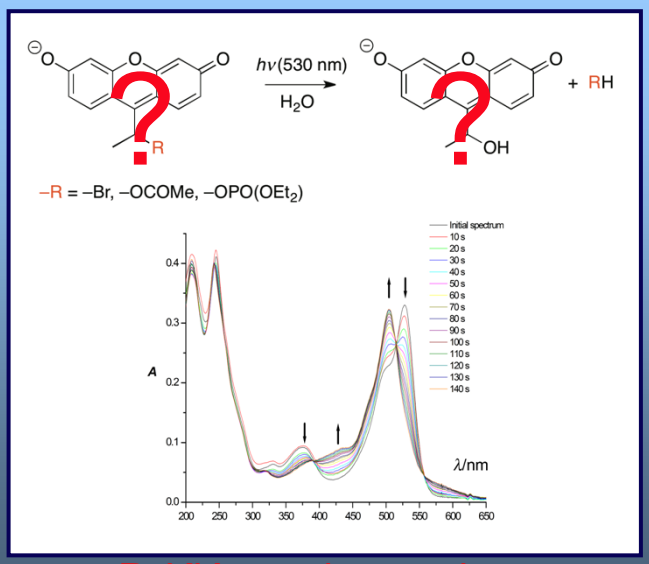


$pK_{a,1}$ = 3.44, $pK_{a,2}$ = 6.31





Preliminary work



Pavel Klán and coworkers

Desiderata

- Strong absorption >> 300 nm, large efficency of release: $\Phi_{rel}\varepsilon(\lambda_{irr})$
- Pure, stable, low activity prior to release (contrast)
- Soluble in target, affinity to target (cancer), pass barriers (cell membrane, brain)
- By-products transparent, biocompatible
- Fast release, appearance rate constant
- Simple, flexible synthesis

Conclusions

- Can YOU use phototriggers?

- Leaving group ability
- Release rate
- Solubility
- Stability in the dark

- Time-resolved X-ray of Enzymes
- Neuronal signal transmission
- Transport phenomena

Questions?