Ultrafast spectroscopy and its applications in biology and physical chemistry

Mikas Vengris, Vilnius University

24/02/2017

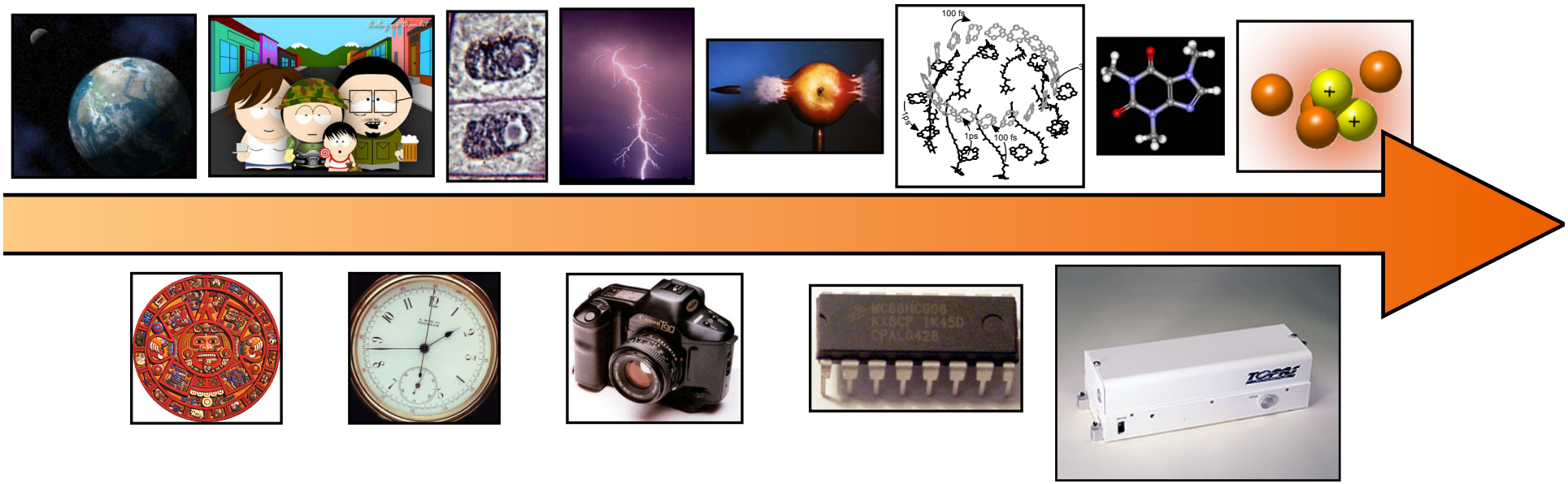
for the PowerPoint version please contact: mikas.vengris@ff.vu.lt

Outline

- What is it for?
- Elementary techniques with applications
  - Time-resolved fluorescence spectroscopy
  - Time resolved absorption
  - Data analysis techniques
  - Applications
- Adding a twist to ultrafast spectroscopy
  - Multi-pulse transient absorption
  - Femtosecond stimulated Raman scattering spectroscopy
  - Transient grating and photon echo
  - 2D electronic spectroscopy

Photosynthesis

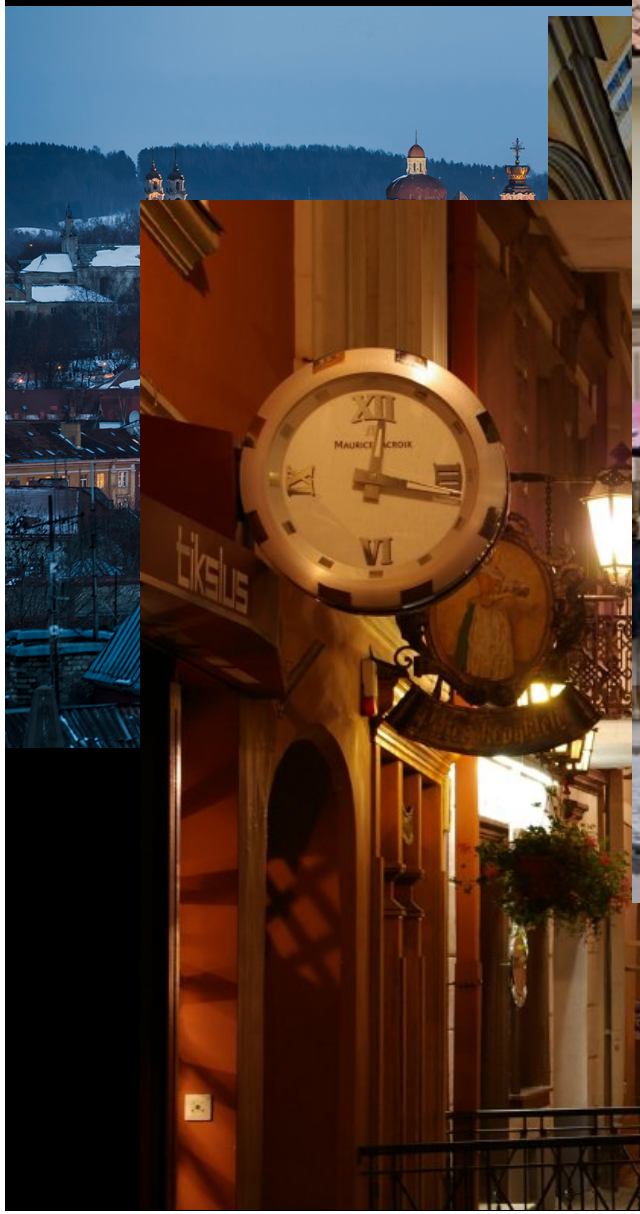
Photochemistry and photobiology

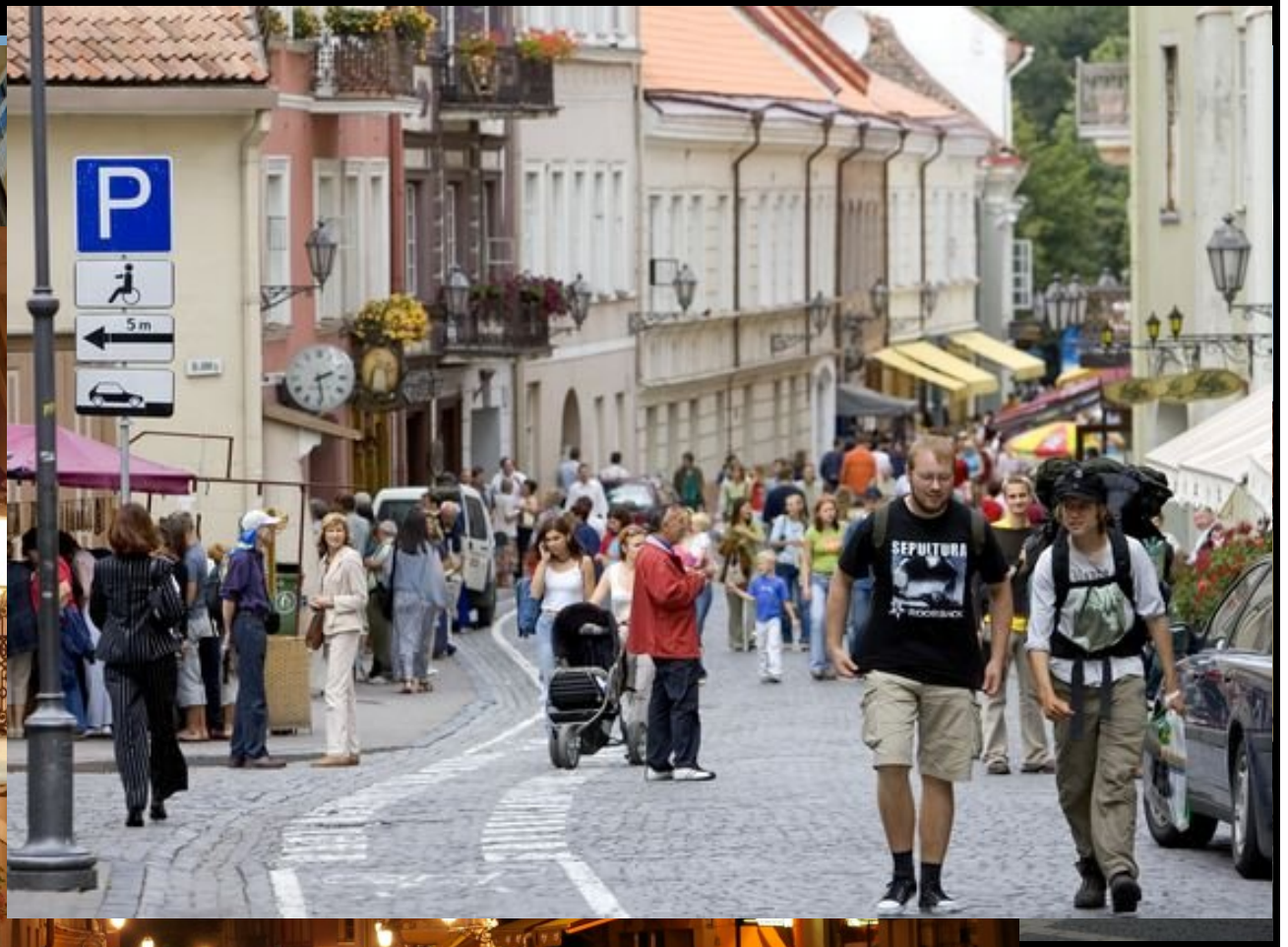


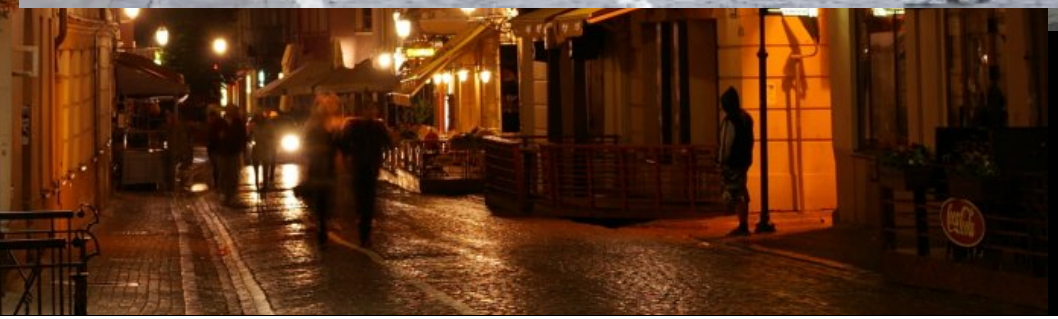
Giga-	Mega-	Kilo-	**sek.**	mili-	mikro-	nano-	piko-	femto-	ato-
10^{9}	10^{6}	10^{3}	10^{0}	10^{-3}	10^{-6}	10^{-9}	10^{-12}	10^{-15}	10^{-18}

Do all four feet of a galopping horse leave the ground at the same time?

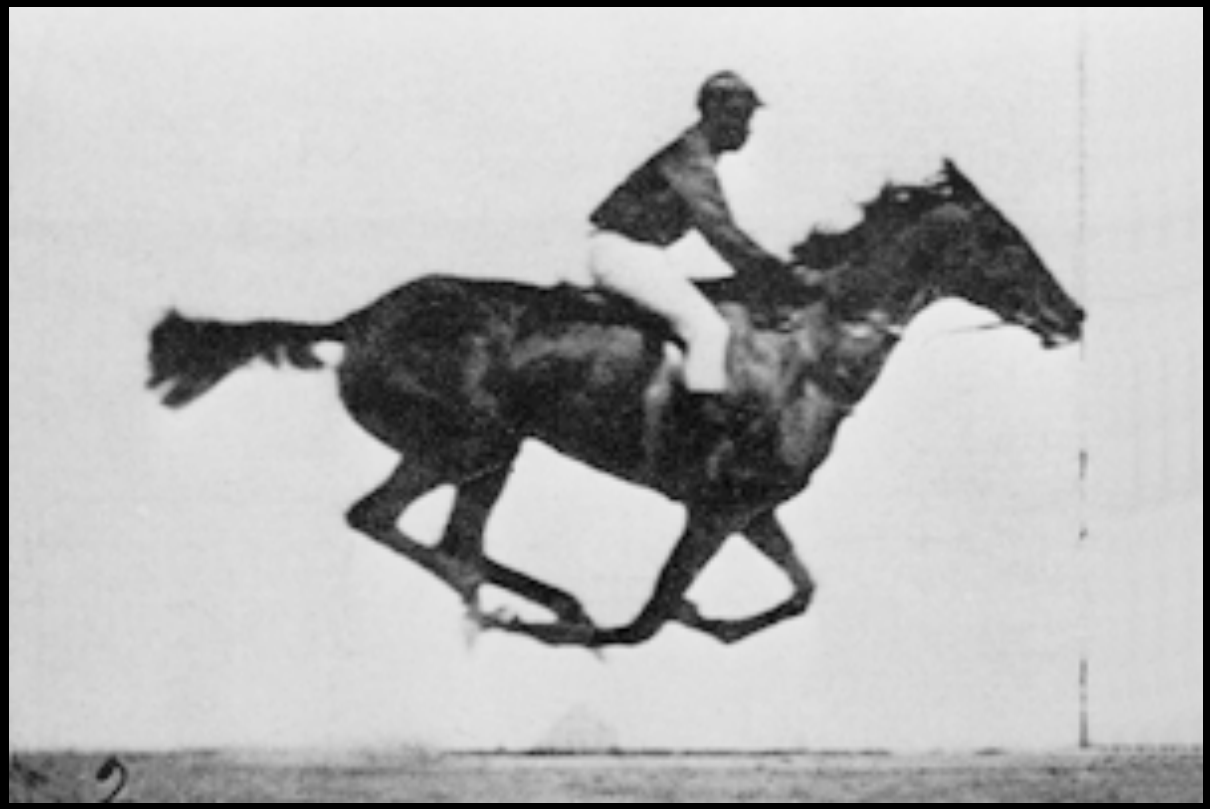
Come to visit!



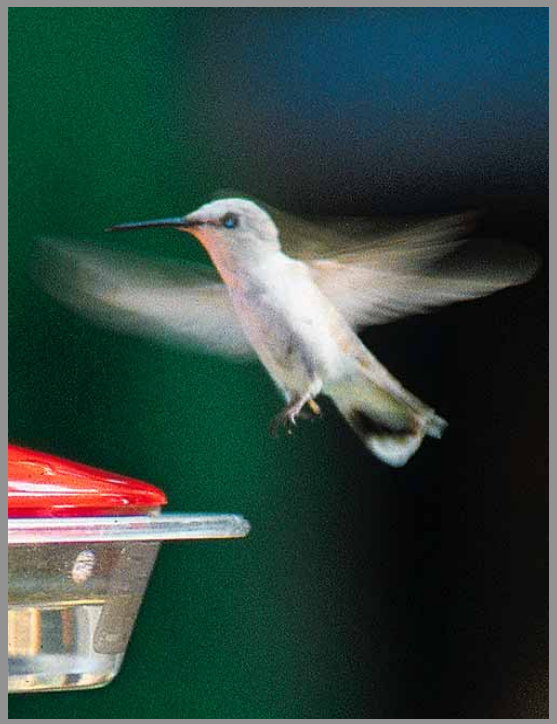




Do all four feet of a galopping horse leave the ground at the same time?



Following fast processes



Flash photolysis: the beginning

Primary photoprocesses in quinones and dyes

II. Kinetic studies

By N. K. Bridge and G. Porter*

The British Rayon Research Association, Heald Green Laboratories, Wythenshawe, Manchester

(*Communicated by R. G. W. Norrish, F.R.S.—Received* 10 *October* 1957)

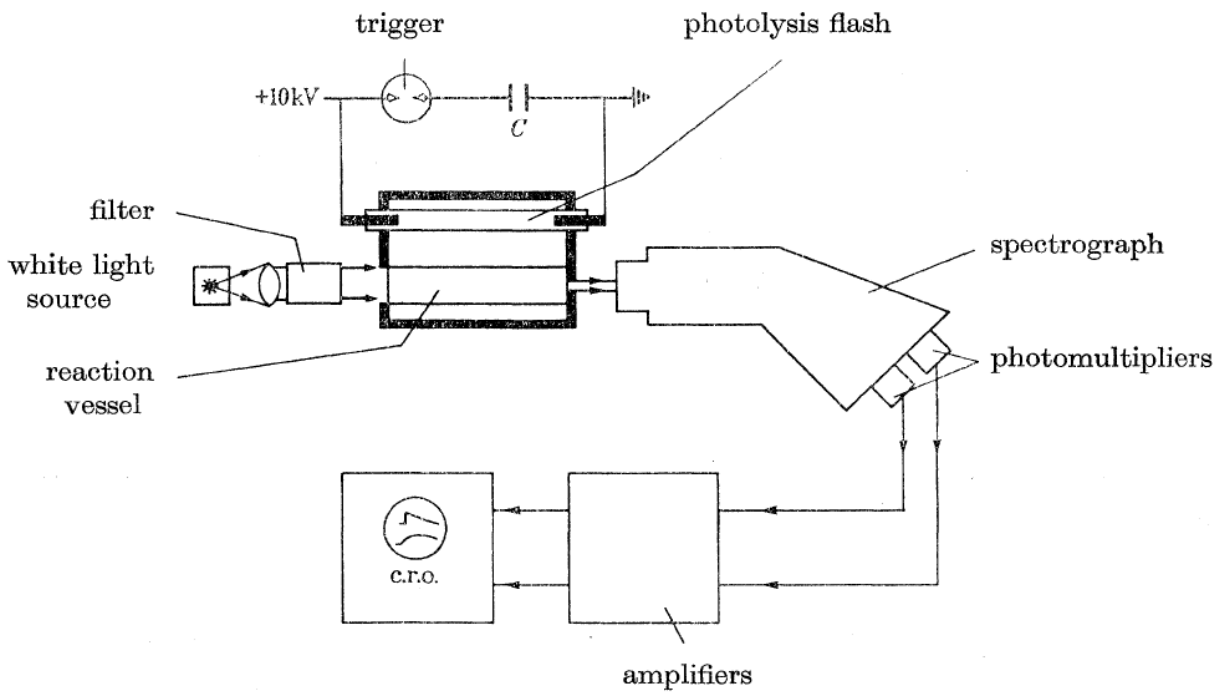
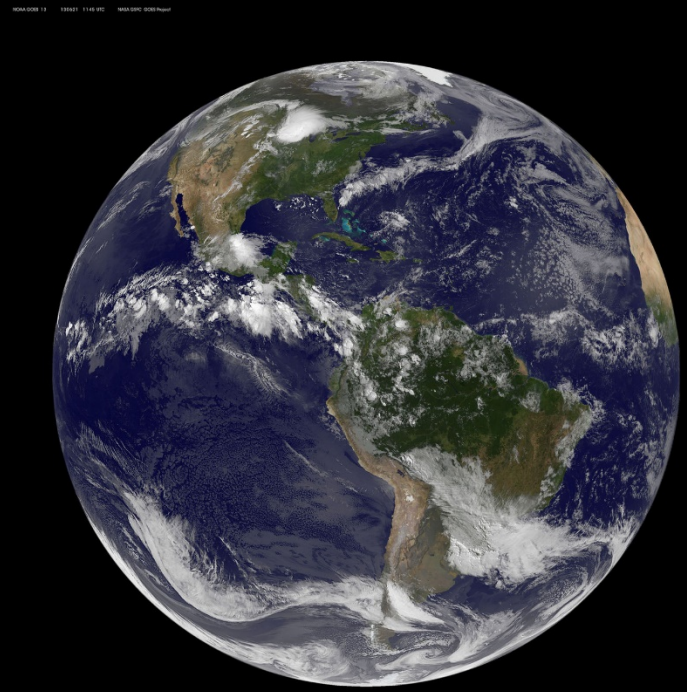


Figure 1. Flash photolysis photomultiplier apparatus.

Nobel prize 1967 :
G.Porter et al.

Apollo 17, NOAA GOES13 satellite

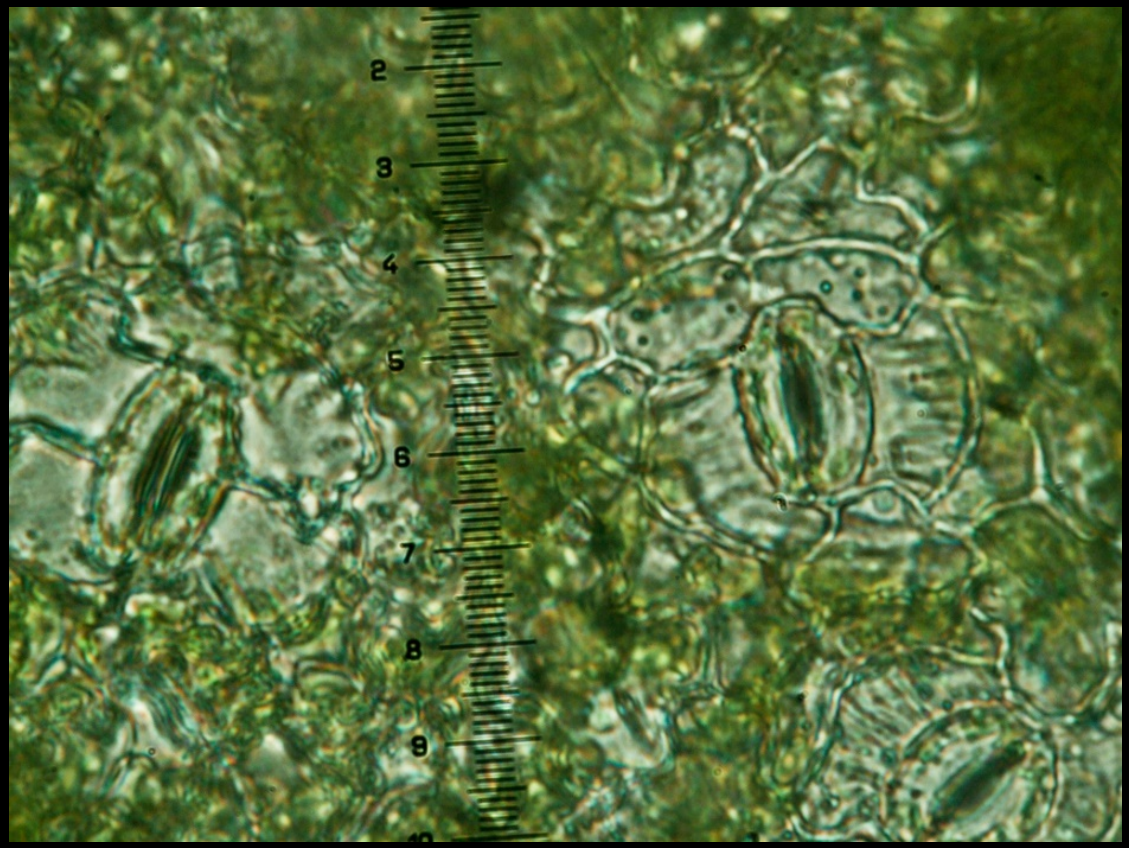


Why is it so green?

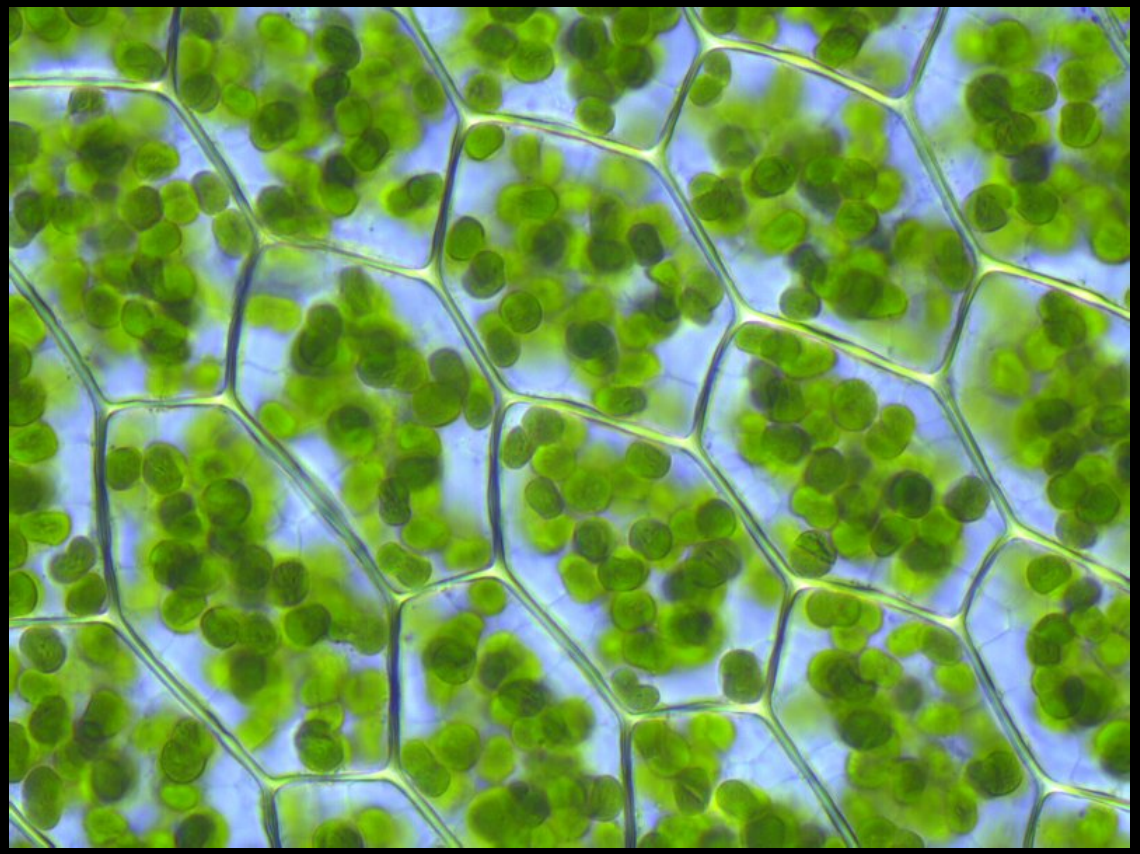
Getting closer to the green stuff

Closer yet

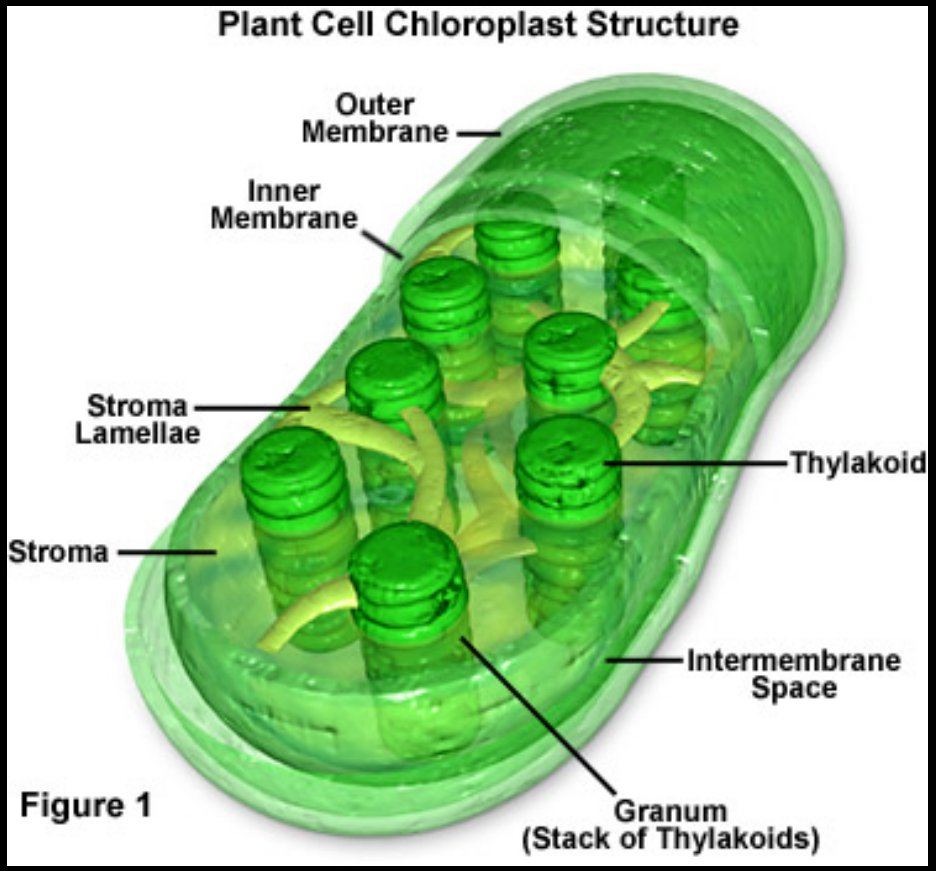
Bring out the microscope, what's the green stuff?



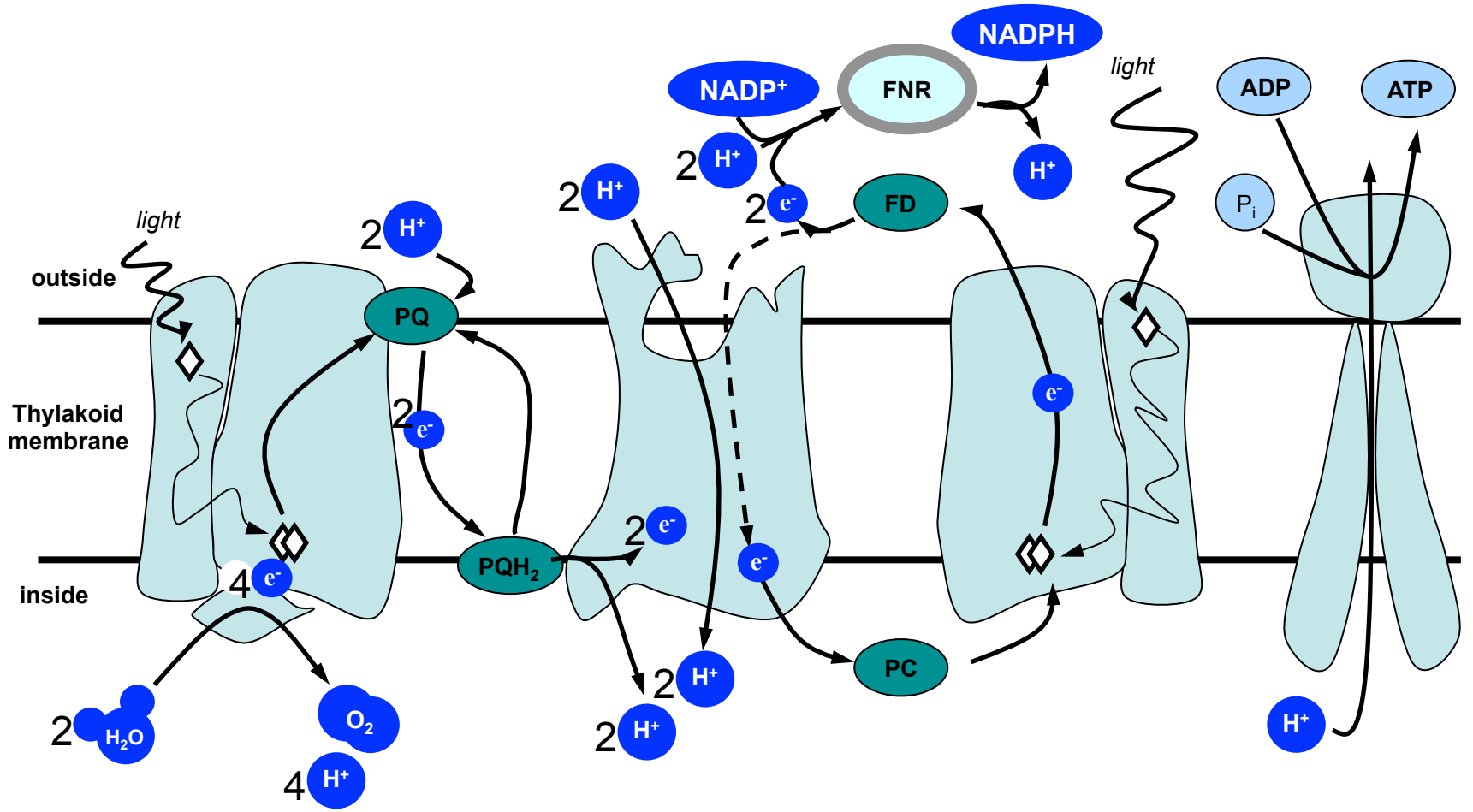
Chloroplasts



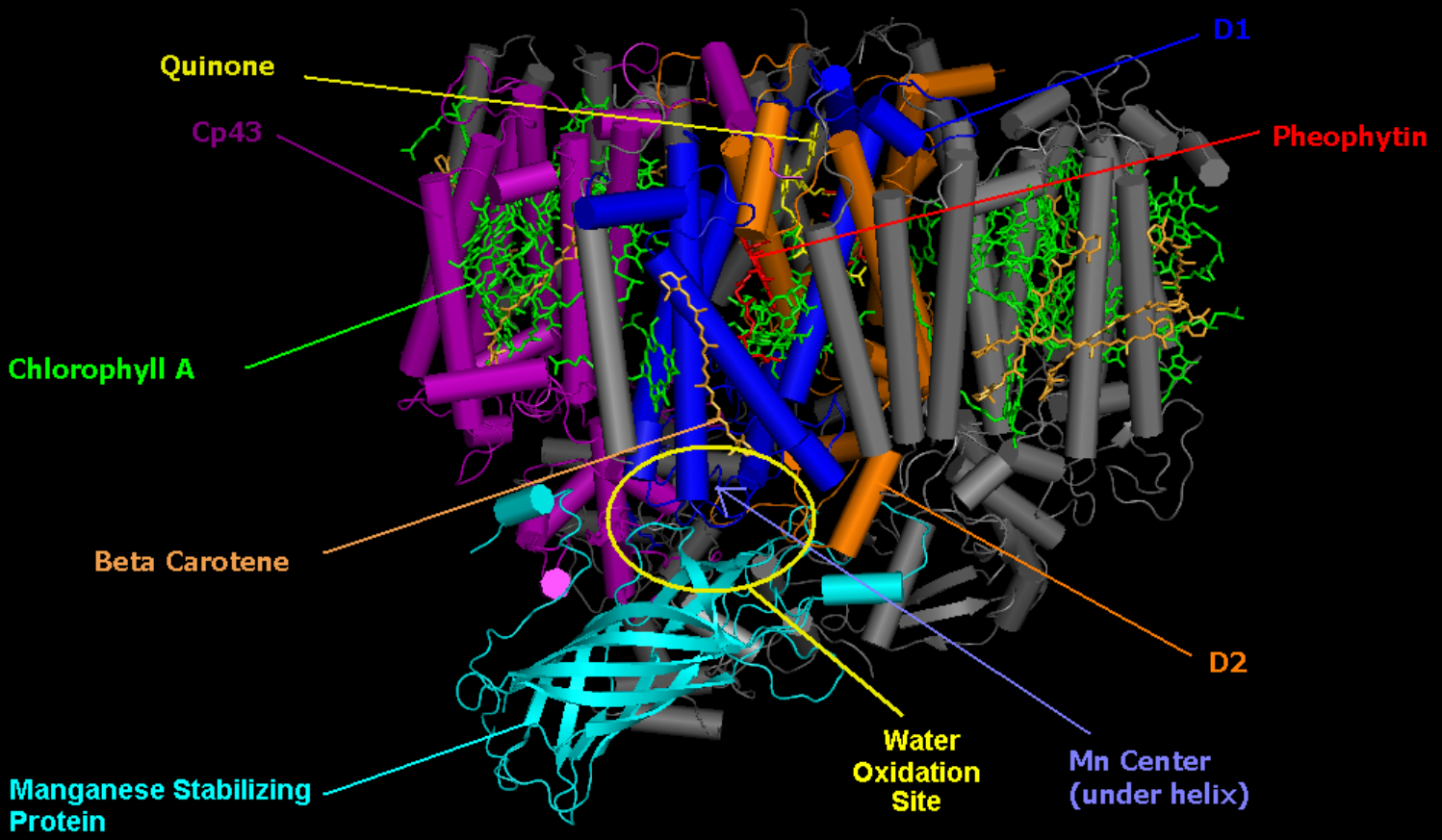
Chloroplastai



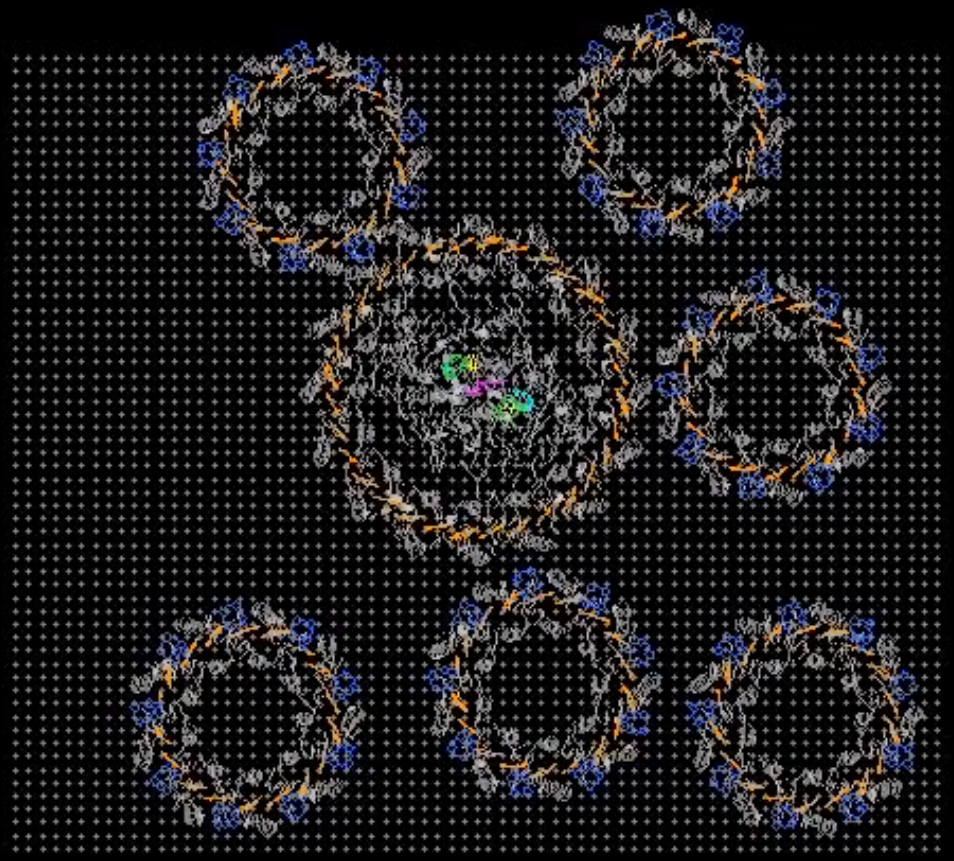
Thylakoid membrane



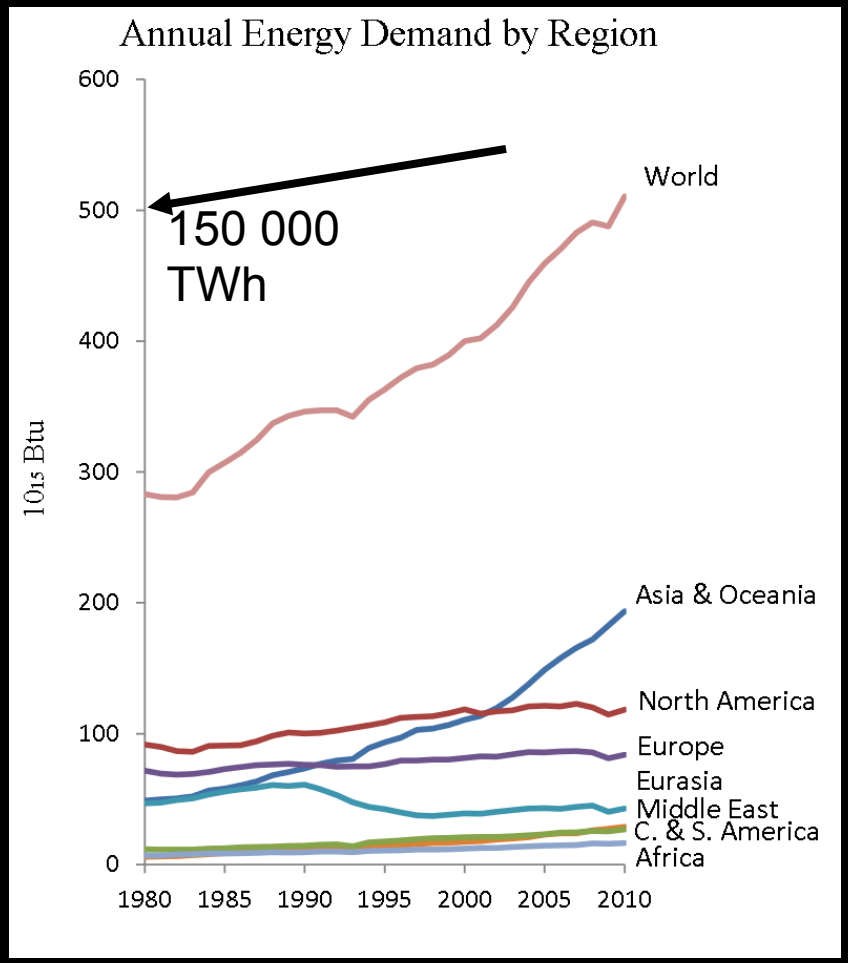
PSII


Quinone
Cp43
Chlorophyll A
Beta Carotene
Manganese Stabilizing Protein
D1
Pheophytin
D2
Water Oxidation Site
Mn Center (under helix)

Bacterial photosynthesis: structures more symmetric, but the mechanisms are the same



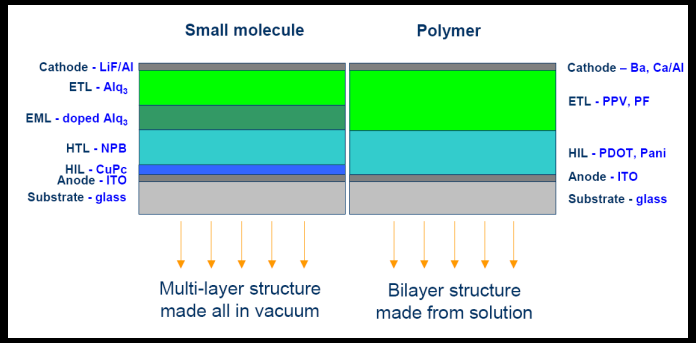
Photosynthesis = Solar energy

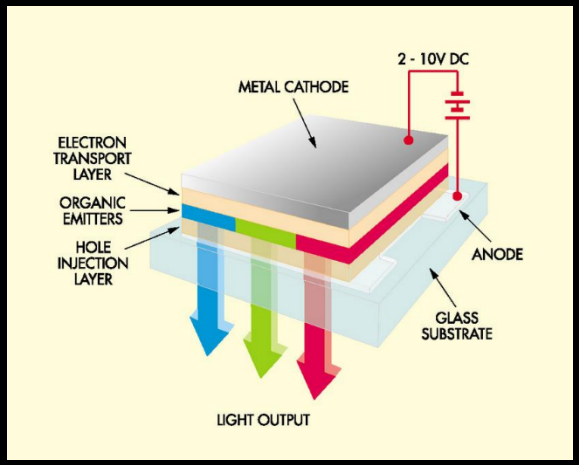


Photosynthesis: 130 TW x 365 days/year x 24 h/day =1 140 000 TWh

Natural plants, algae and bacteria capture 7x energy required by the humanity

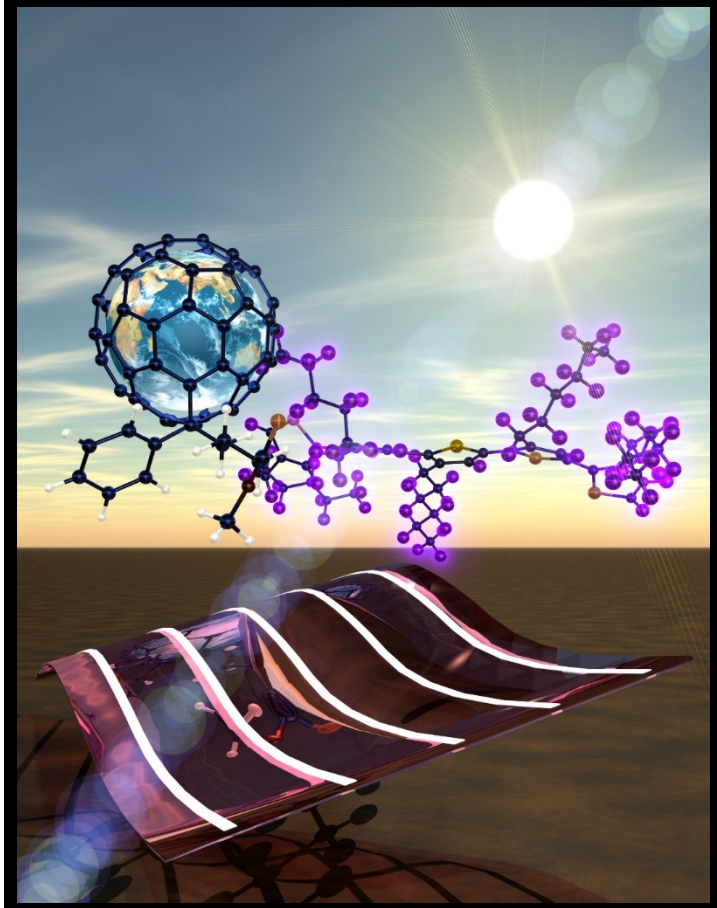
Semiconductors

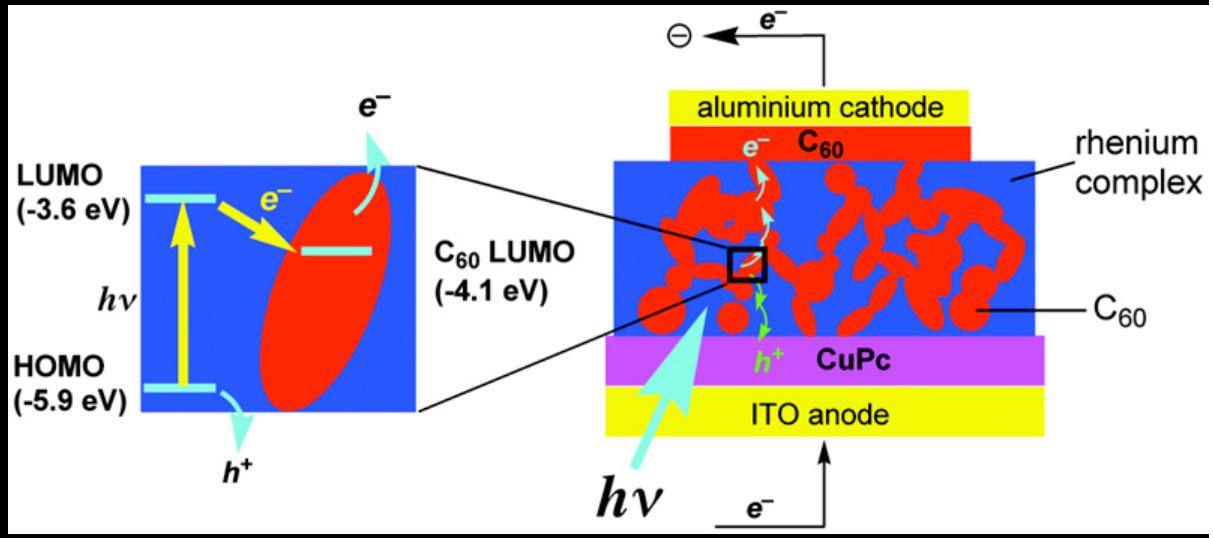




Organic LEDs (OLEDs) are the hybrid structures made of organic molecules and semiconductors for conversion from electricity to light.

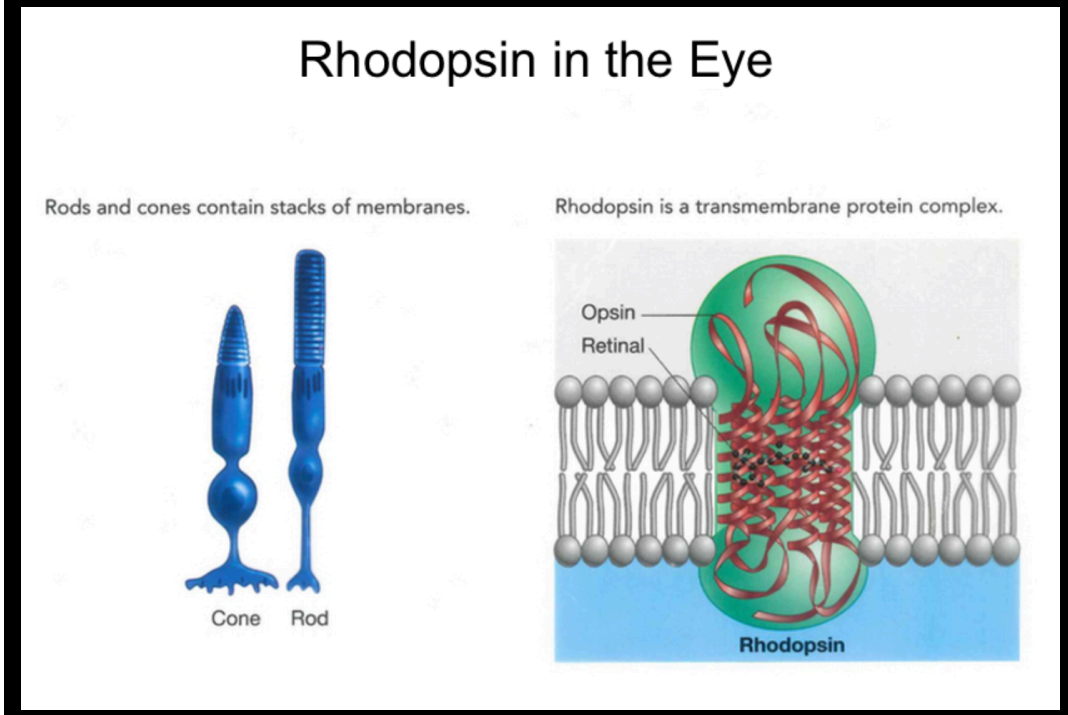
Photochemistry: organic photovoltaic cells

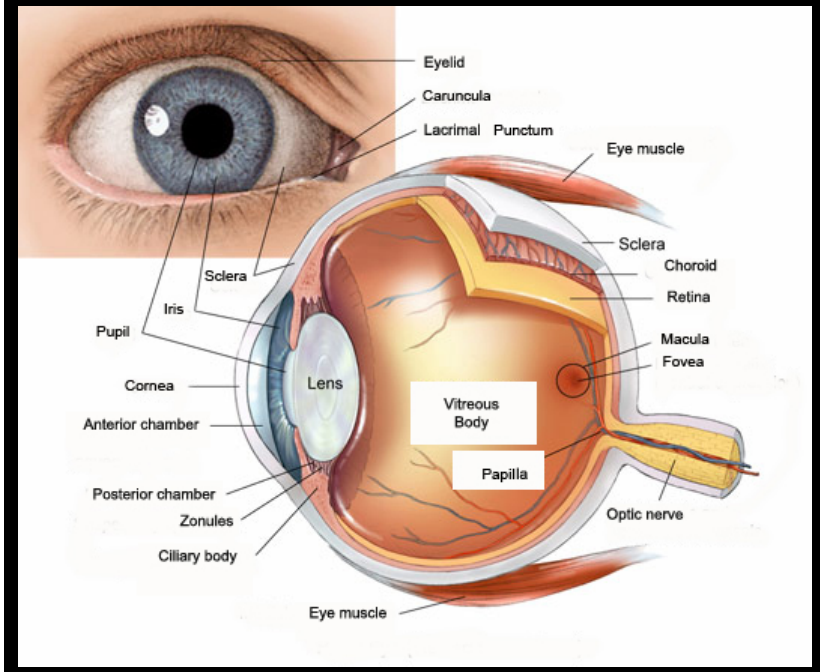




Carrier extraction must be faster than recombination of excited carriers (typically about 1 ns)

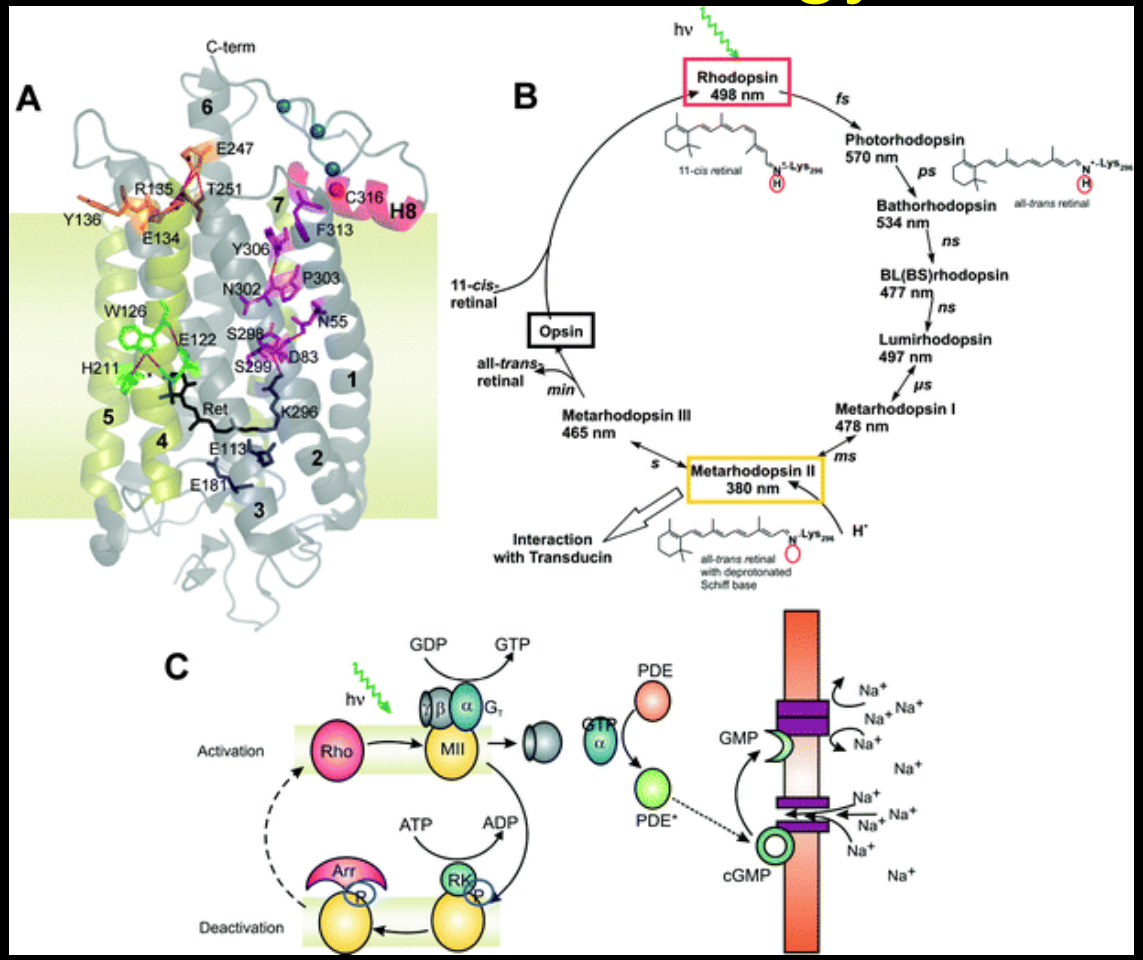
Photobiology



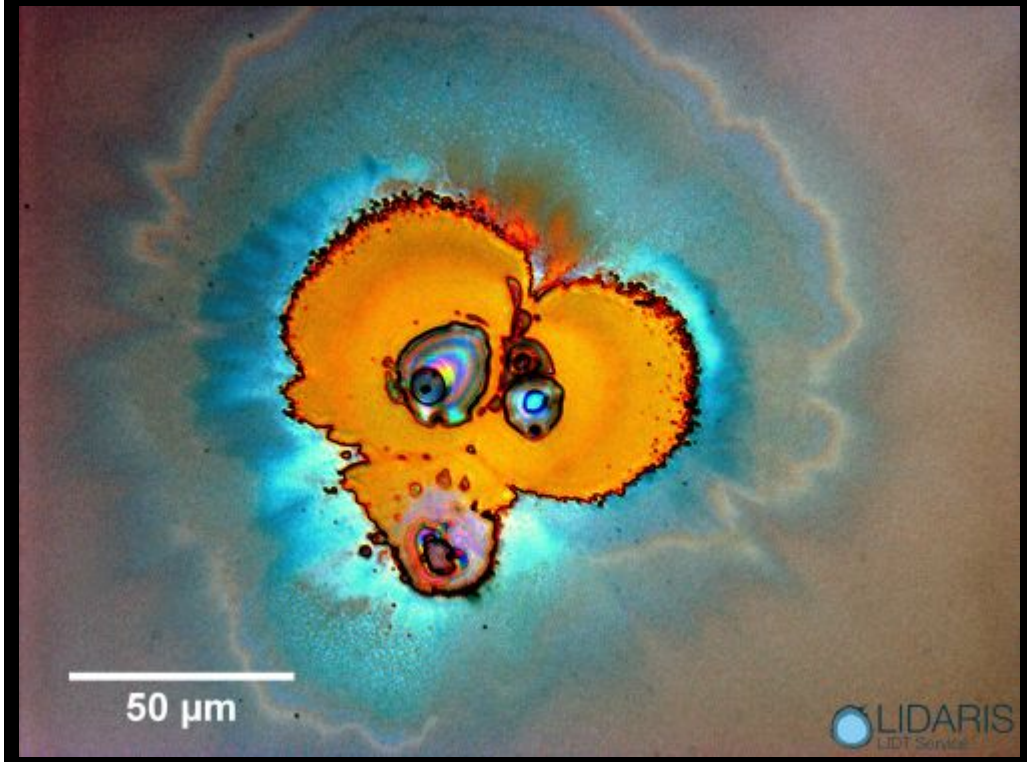


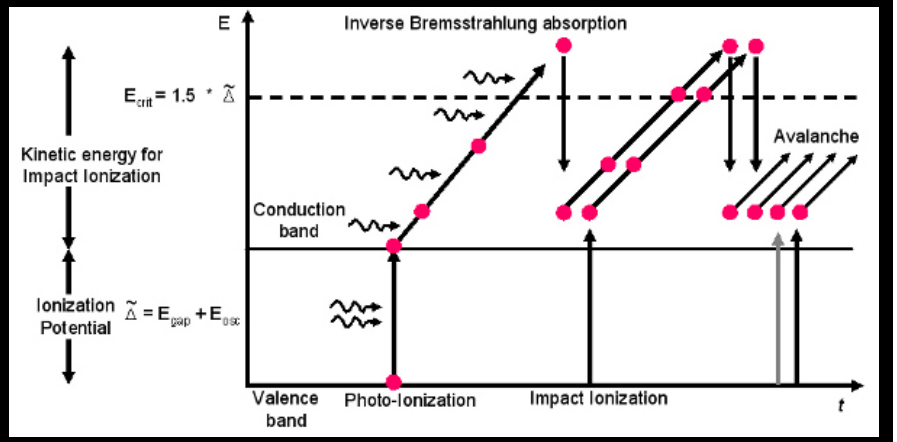
If you mutate rhodopsin-like protein into neuron, you can trigger a brain using light - optogenetics

Photobiology



Laser physics





Direct laser writing by multi-photon photopolymerization

132 OPTICS LETTERS / Vol. 22, No. 2 / January 15, 1997

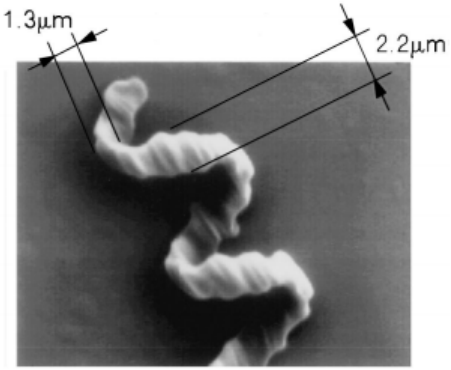
Three-dimensional microfabrication with two-photon-absorbed photopolymerization

Shoji Maruo, Osamu Nakamura, and Satoshi Kawata

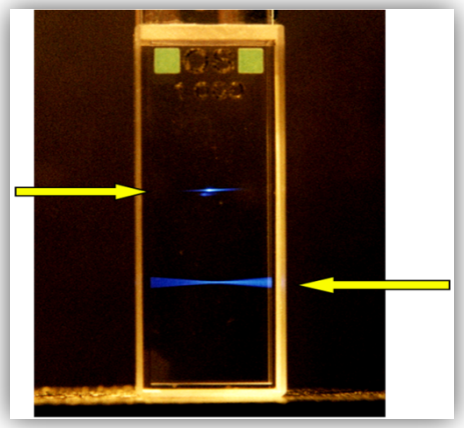
Department of Applied Physics, Osaka University, Suita, Osaka 565, Japan

Received October 1, 1996

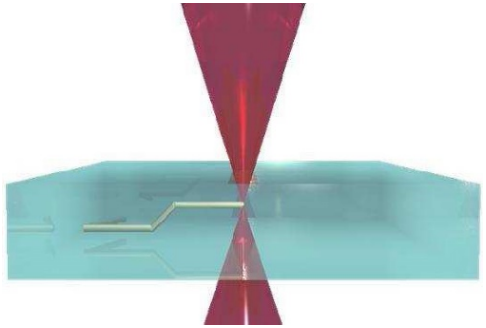
We propose a method for three-dimensional microfabrication with photopolymerization stimulated by two-photon absorption with a pulsed infrared laser. An experimental system for the microfabrication has been developed with a Ti:sapphire laser whose oscillating wavelength and pulse width are 790 nm and 200 fs, respectively. The usefulness of the proposed method has been verified by fabrication of several kinds of microstructure by use of a resin consisting of photoinitiators, urethane acrylate monomers, and urethane acrylate oligomers.



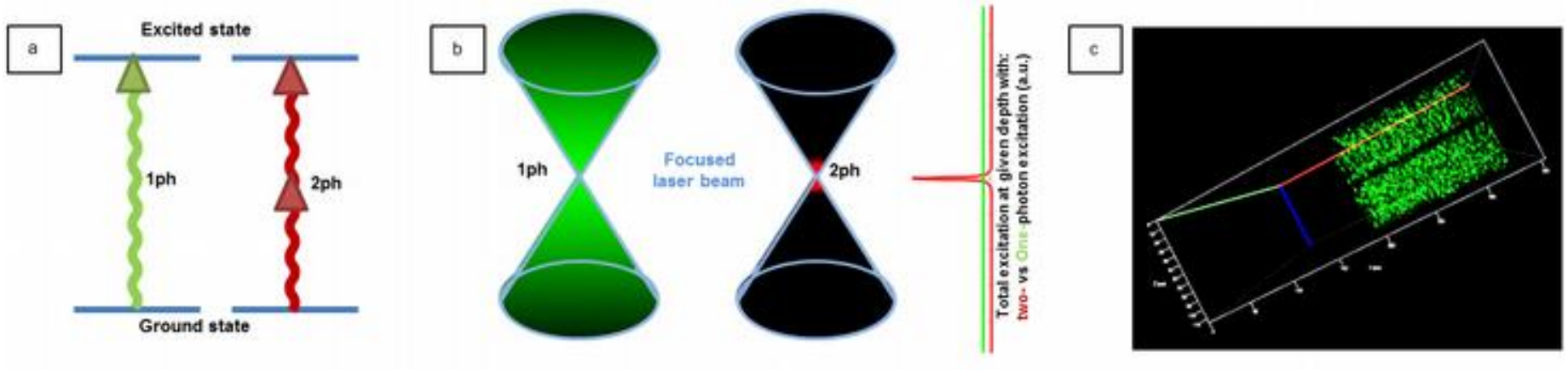
Nonlinear absorption

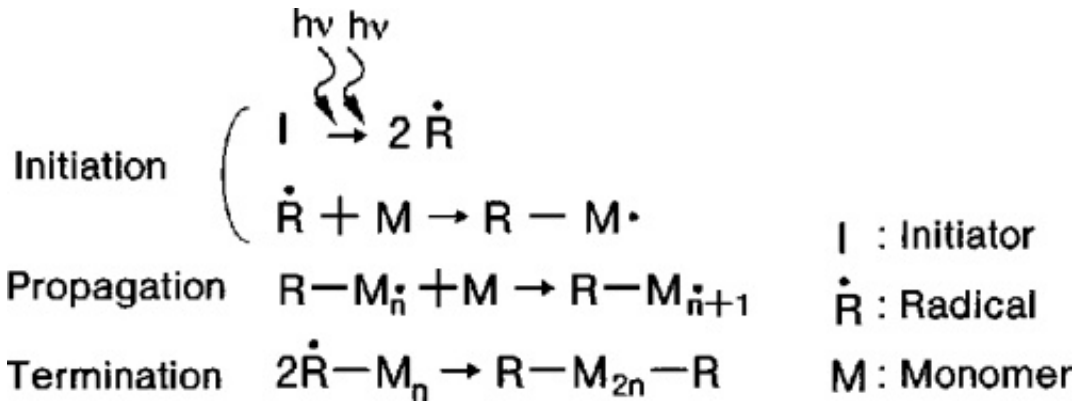


Linear absorption



What is this polymerization?





Spectroscopy←→quantum structure of material

Time-resolved spectroscopy←→quantum functioning of material

Laser spectroscopy: the childhood

Volume 84, number 3 CHEMICAL PHYSICS LETTERS 15 December 1981

PICOSECOND TIME-RESOLVED KINETIC STUDIES ON THE FORMATION OF SHORT-LIVED PSEUDOISOCYANINE IODIDE PHOTOISOMERS IN METHANOL AND ETHYLENE GLYCOL

Sabine K. RENTSCH
Department of Physics, Friedrich-Schiller-University, 6900 Jena, GDR

and

Romas V. DANIELIUS and Roaldas A. GADONAS
Department of Physics, Kapsukas University, 232054 Vilnius, USSR

Received 3 August 1981

Laser spectroscopy: the childhood

of the bleaching and transient absorption spectra of PIC in methanol and in ethylene glycol solutions. A new absorption band was studied in detail and interpreted as a photoisomer spectrum of pseudoisocyanine iodide.

2. Experimental apparatus

The excite-and-probe spectrometer described in detail in ref. [8] consisted of a Nd–YAG laser and an amplifier, a second-harmonic generator and a parametric picosecond generator with an amplifer (KDP). The parametric generator provided tunable exciting radiation in the near infrared and, after doubling, in the visible region as well. The excitation pulse duration was 20 ps and the energy 0.1–1 mJ. The probing light was generated either by a second parametric generator ($LiNbO_3$) with low radiation energy for the performance of exact kinetic measurements, or by a picosecond continuum in D_2O for measurements of the transient spectra at a fixed delay time. The transmission of the sample cell was measured with excitation and without it by means of a multiplier relative to a reference signal of the probe light on a second detector. The measuring data were processed by a minicomputer which, in addition, controlled the parameters of the experiment.

The differential optical density

$$\Delta D = D_{\text{with}} - D_{\text{without}} ,$$

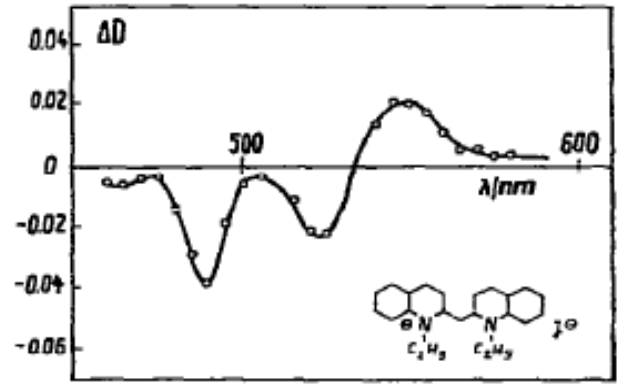
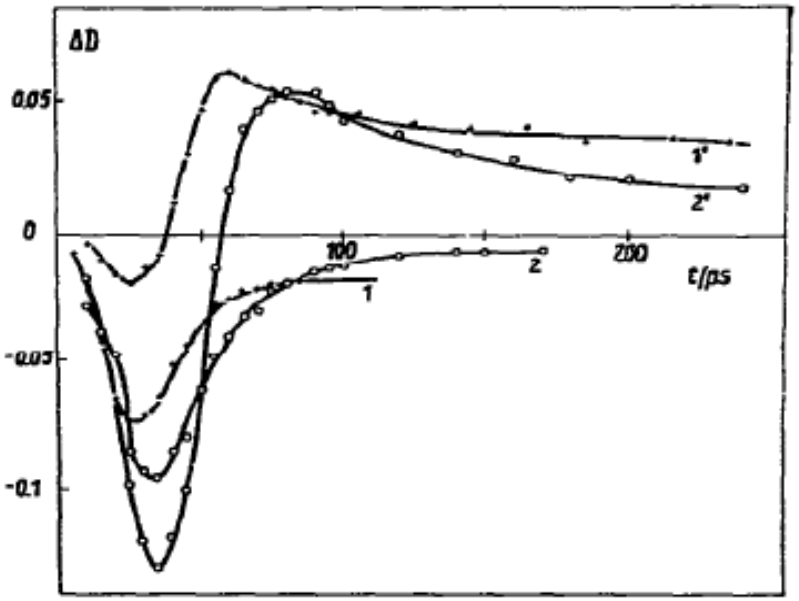
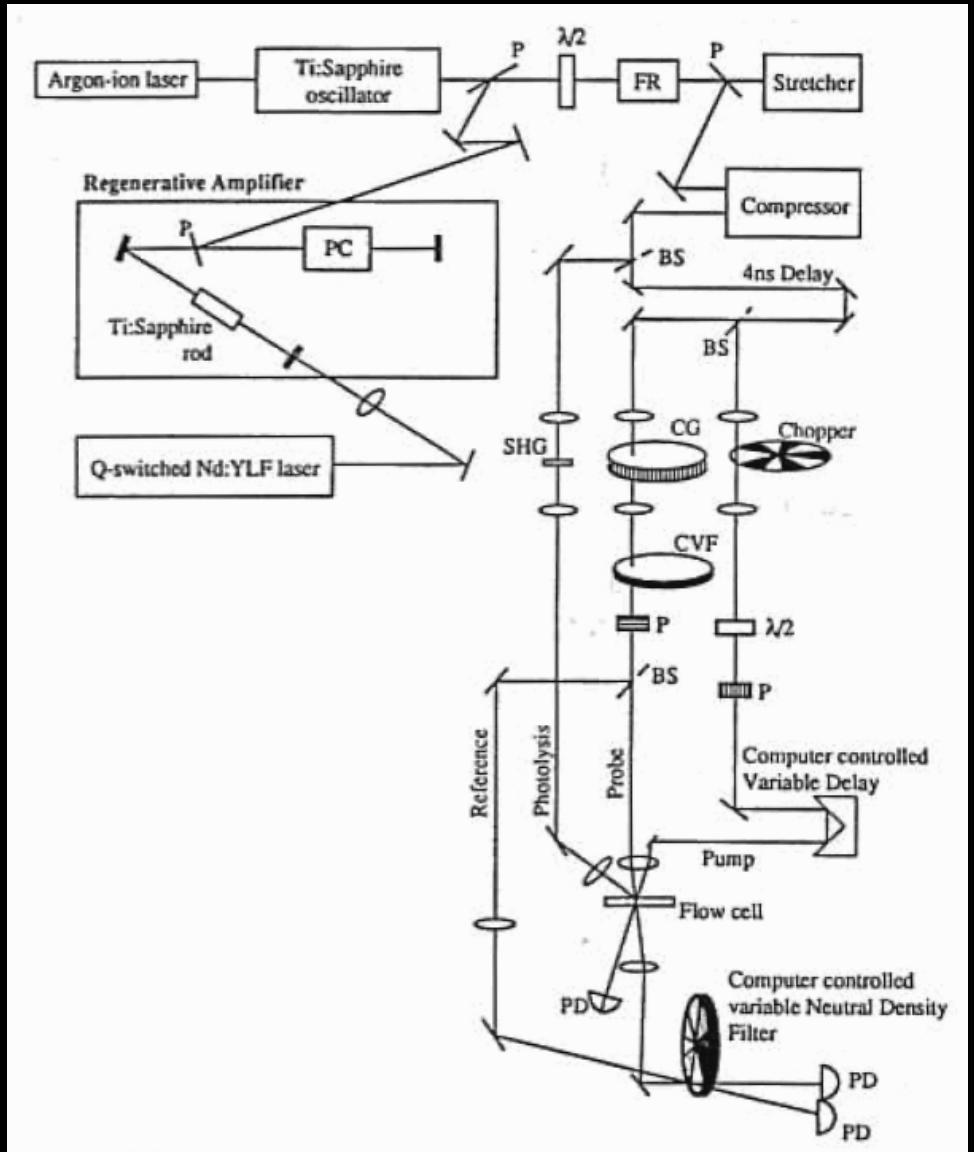


Fig 1. Bleaching and transient absorption of pseudoisocyanine iodide in methanol (λ_{ex} = 530 nm, $c = 4 \times 10^{-5}$ mol/ℓ).



Laser spectroscopy: the puberty



Chapter 4

Ultrafast Spectroscopy of Photosynthetic Systems

Ralph Jimenez and Graham R. Fleming*
Department of Chemistry and the James Franck Institute, The University of Chicago, 5735 S. Ellis Avenue, Chicago, IL 60637, USA

J. Amesz and A. J. Hoff (eds.), Biophysical Techniques in Photosynthesis, pp. 63–73.

Laser spectroscopy: maturity

LIGHT CONVERSION

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Ultrafast Spectrometers

HARPIA

Off-the-Shelf Pump-Probe Spectrometer

- Straightforward operation
- Modular, customizable design
- Ample sample space to fit a cryostat or flow system
- LabView based measurement automation software
- Full control over polarization, intensity, delay and wavelength

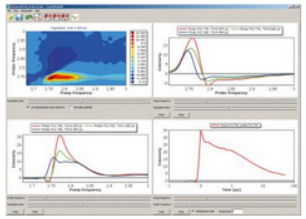
CHIMERA

Fluorescence Upconversion & Time-Correlated Single Photon Counting Spectrometer

- Straightforward operation
- Modular, customizable design
- Ample sample space to fit a cryostat or flow system
- Automated spectral scanning and upconversion crystal tuning
- Measure fluorescence dynamics from hundreds of fs to 2 µs in a single instrument

CARPETview

Spectroscopy Data Analysis Software

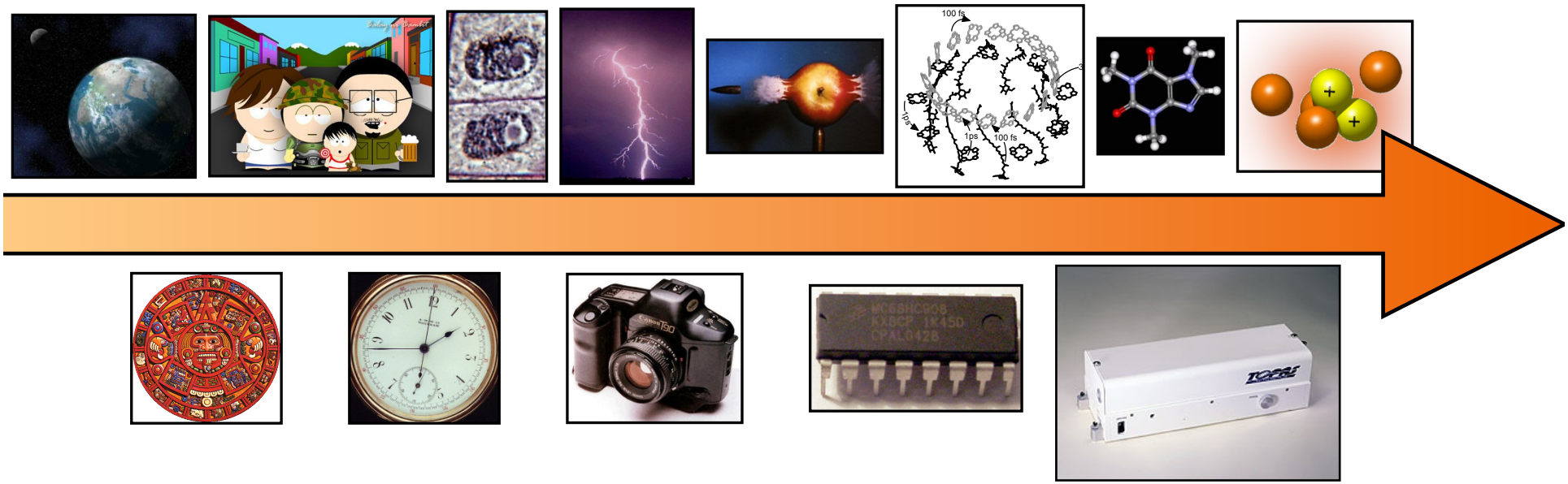


- Dedicated package for ultrafast spectroscopy data analysis
- Visualization functions: spectral slices, contour plot, comparison of several graphs and more
- Wide set of trivial data manipulations functions
- Global and target analysis of ultrafast data
- Optional 3D version

LIGHT CONVERSION

Photosynthesis

Photochemistry and photobiology



Giga-	Mega-	Kilo-	**sec.**	mili-	mikro-	nano-	piko-	femto-	ato-
10^{9}	10^{6}	10^{3}	10^{0}	10^{-3}	10^{-6}	10^{-9}	10^{-12}	10^{-15}	10^{-18}

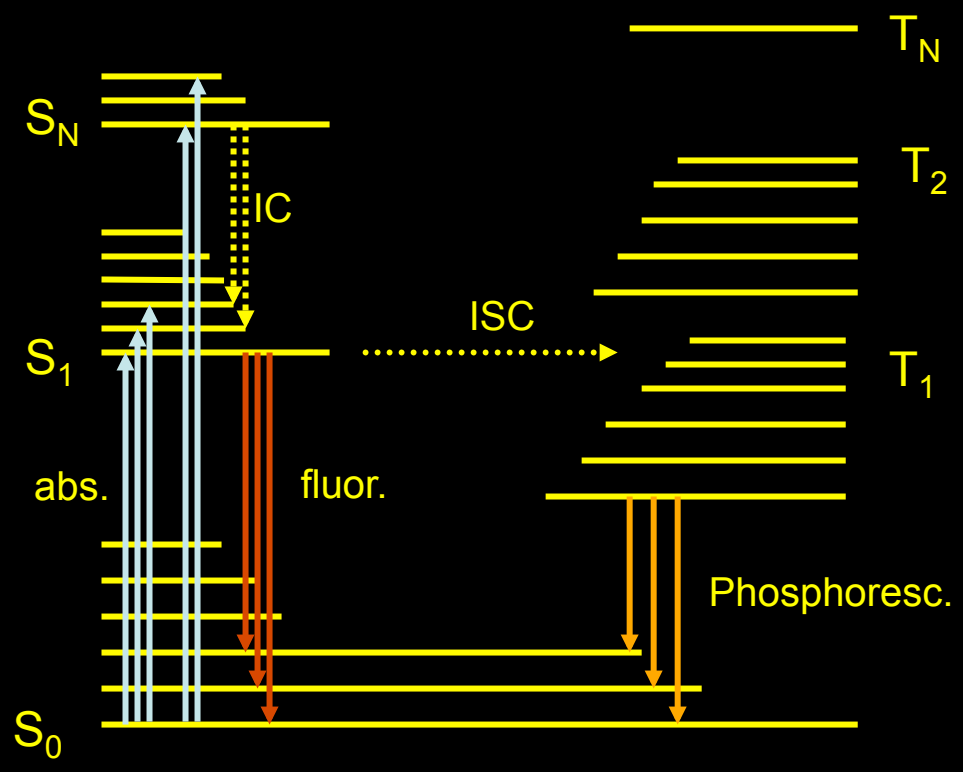
Processes explored by ultrafast spectroscopy

- Charge transfer (electron, proton)
- Solvation
- Vibrational relaxation
- Excitation energy transfer
- Photoreaction dynamics
- Carrier dynamics in semiconductors

Time-resolved fluorescence spectroscopy

- Explores the time dependence of emission spectra in:
  - Molecules
  - Solids
  - Nanostructures

Molecular energy levels: Jablonski diagram



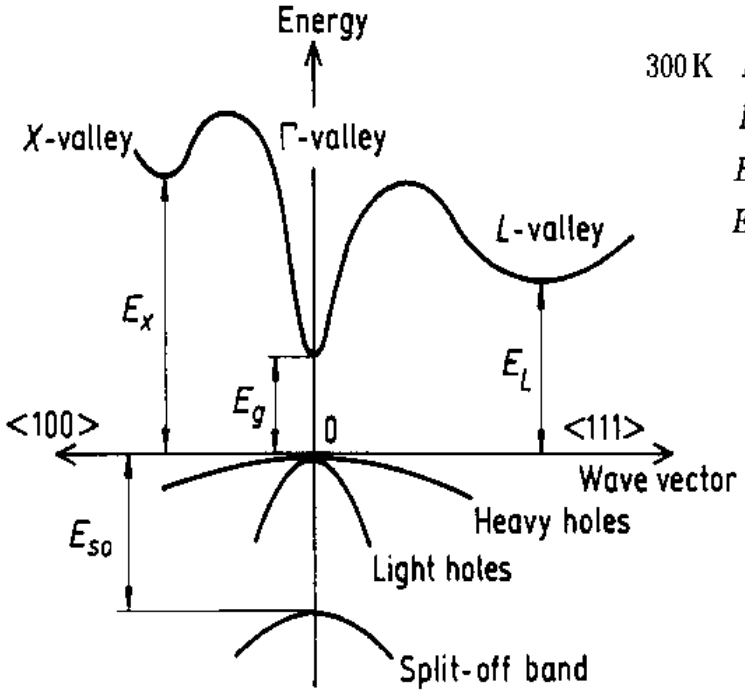
Molecule was excited. What can happen?

- Radiative relaxation
- Internal conversion
- Intersystem crossing
- Excitation energy transfer
- Solvation
- Photoinduced reaction (e.g., isomerization)

When the state of the molecule changes, emission spectrum will change. Therefore, emission is a way of observing all mentioned processes.

Only useful while excited state is preserved!

Band structures in solid state



$300\,\mathrm{K}\quad E_g = 1.42\ \mathrm{eV}$

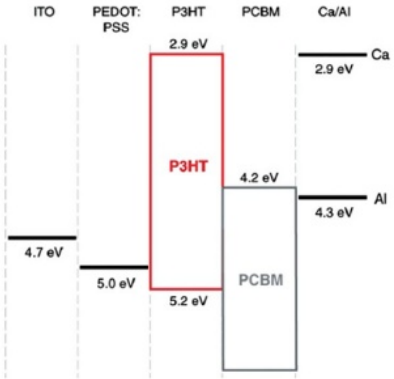
$E_L = 1.71\ \mathrm{eV}$

$E_X = 1.90\ \mathrm{eV}$

$E_{so} = 0.34\ \mathrm{eV}$

Gallium Arsenide

Energy-level band diagram



Energy-level band diagram of a typical P3HT:PCBM Organic Solar Cell

D. Ginley, Fundamentals of materials for Energy and Environmental Sustainability, page 233

Organic solar cell (dispersed heterojunction)

A solid-state sample (semiconductor, dielectric, metal, amorphous/organic or crystalline) was excited. What can happen?

- Band-to-band radiative recombination (light output)
- Shokley-Read-Hall (trap-assisted) recombination
- Auger recombination
- Electron cooling and re-quilibration
- Trap luminescence (light output)
- Non-radiative recombination (light disappearance)

If at least a fraction of carriers recombine in a radiative manner, the carrier dynamics can be observed in time-resolved fluorescence experiments.

Again, when the light output stops, we stop seeing it.

A quantum well/wire/dot was excited. What can happen?

- A hybrid behavior between solid state (bands, state continuum) and molecules (discreet states).

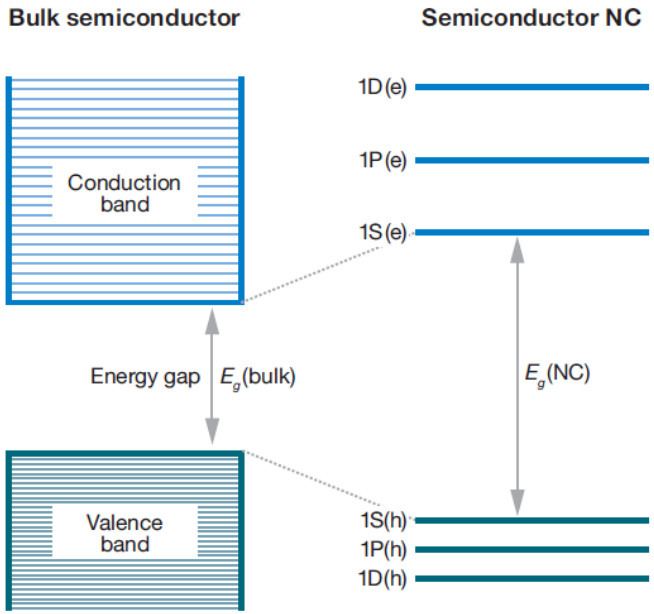
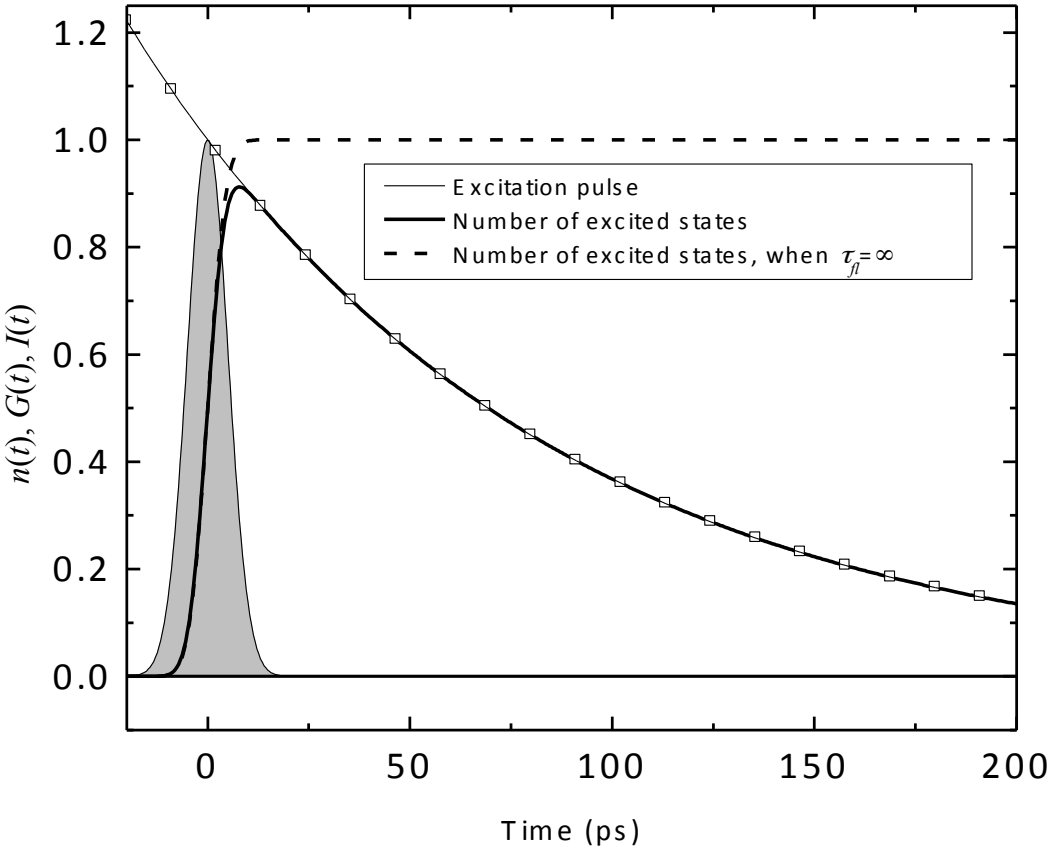


Figure 1

A bulk semiconductor has continuous conduction and valence energy bands separated by a fixed energy gap, E_g, whereas a semiconductor nanocrystal (NC) is characterized by discrete atomic-like states and an NC size-dependent energy gap. In a simple model of a spherical quantum well with an infinite barrier, the NC energy gap, E_g(NC), relates to the bulk semiconductor energy gap, E_g(bulk), by the following expression: $E_g(\text{NC}) = E_g(\text{bulk}) + \frac{\pi^2\hbar^2}{2m_r R^2}$, where R is the NC radius, $m_r = (m_e^{-1} + m_h^{-1})^{-1}$, and m_e and m_h are the electron and hole effective masses, respectively. The NC energy structures are shown for the model case of a two-band semiconductor, which has a single parabolic conduction band and a single parabolic valence band.

V. Klimov Annu. Rev. Phys. Chem. 2007. 58:635–73

Simple kinetics



$$n(t) = G(t) \cdot e^{-\frac{t}{\tau_{fl}}}$$

$$G(t) \sim 1 + erf\left(\frac{t}{\tau}\right)$$

$$erf(t) \equiv \frac{2}{\sqrt{\pi}} \int_0^t e^{-t^2} dt$$

Time resolved fluorescence techniqes:

Time-correlated single photon counting

Fluorescence consists of single photons

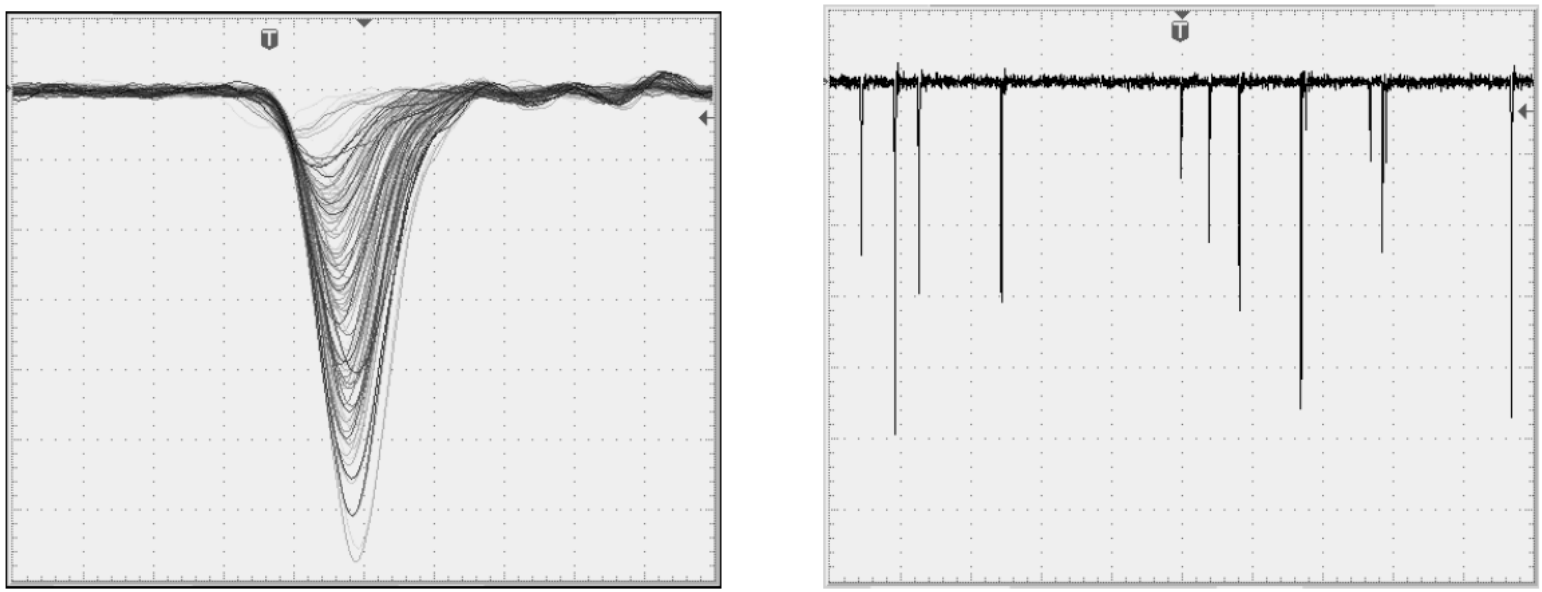


Fig. 118: Single-photon pulses delivered by a R5900 PMT (left, 1 ns / div) and output signal of the PMT at a photon detection rate of 10^7 s^{-1} (right, 100 ns / div). Operating voltage -900V, signal line terminated with 50 Ω.

Excite the sample with high rep.rate laser

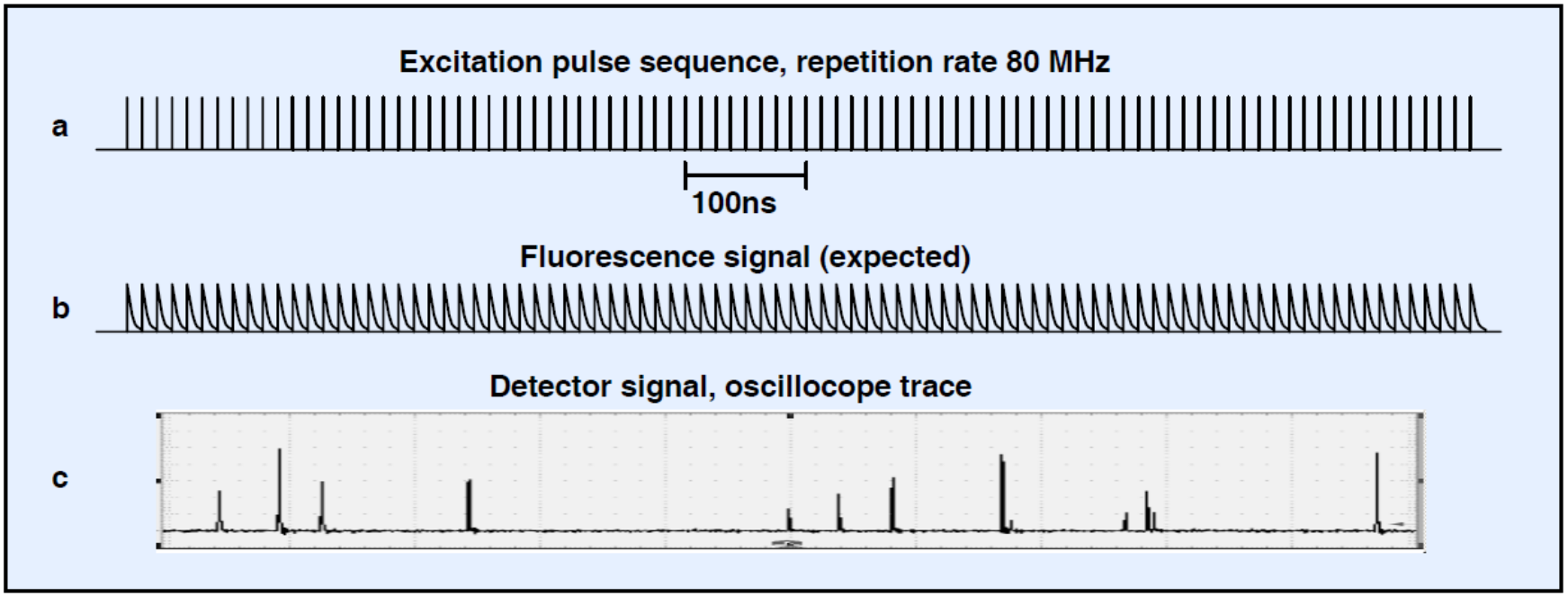
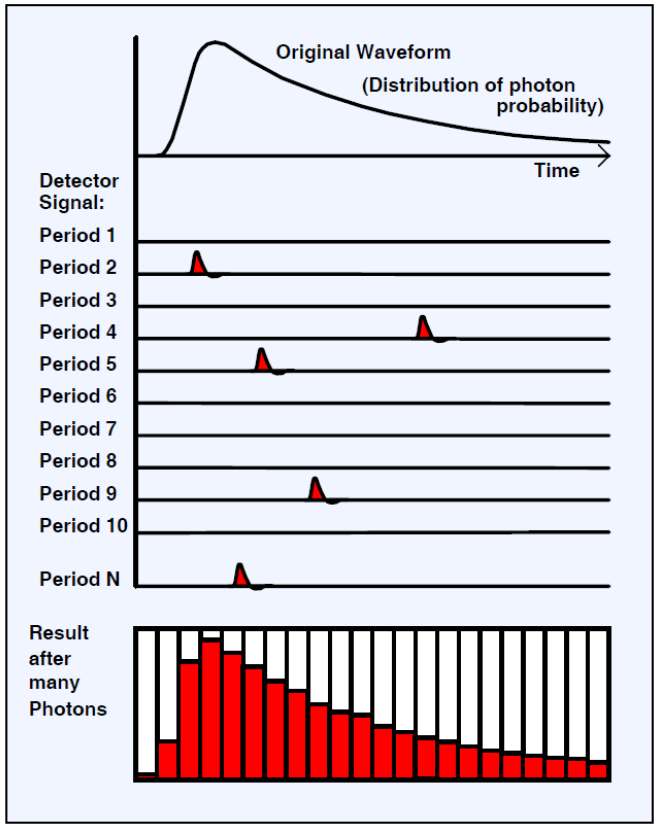


Fig. 119: Detector signal for fluorescence detection at a pulse repetition rate of 80 MHz

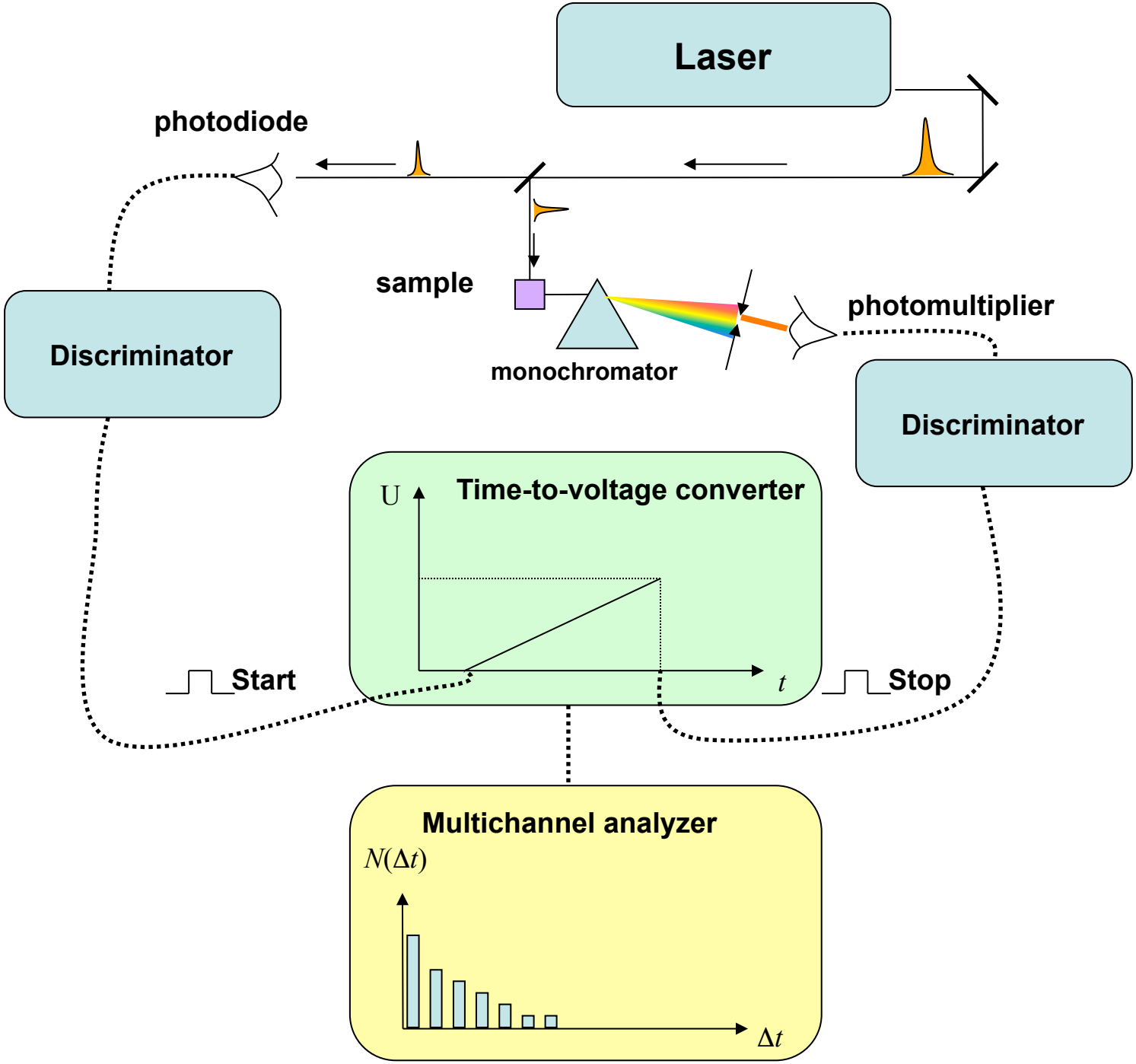
Excite the sample with high rep.rate laser



The histogram of photon arrival times (with respect to the corresponding laser pulses) is the fluorescence decay curve.

The method relies on statistics

Only the timing (not signal amplitude) noise is important


Laser
photodiode
sample
monochromator
photomultiplier
Discriminator
Discriminator
Time-to-voltage converter
U
t
Start
Stop
Multichannel analyzer
N(Δt)
Δt

Constant fraction discriminator: a way to avoid timing noise in variable amplitude signal

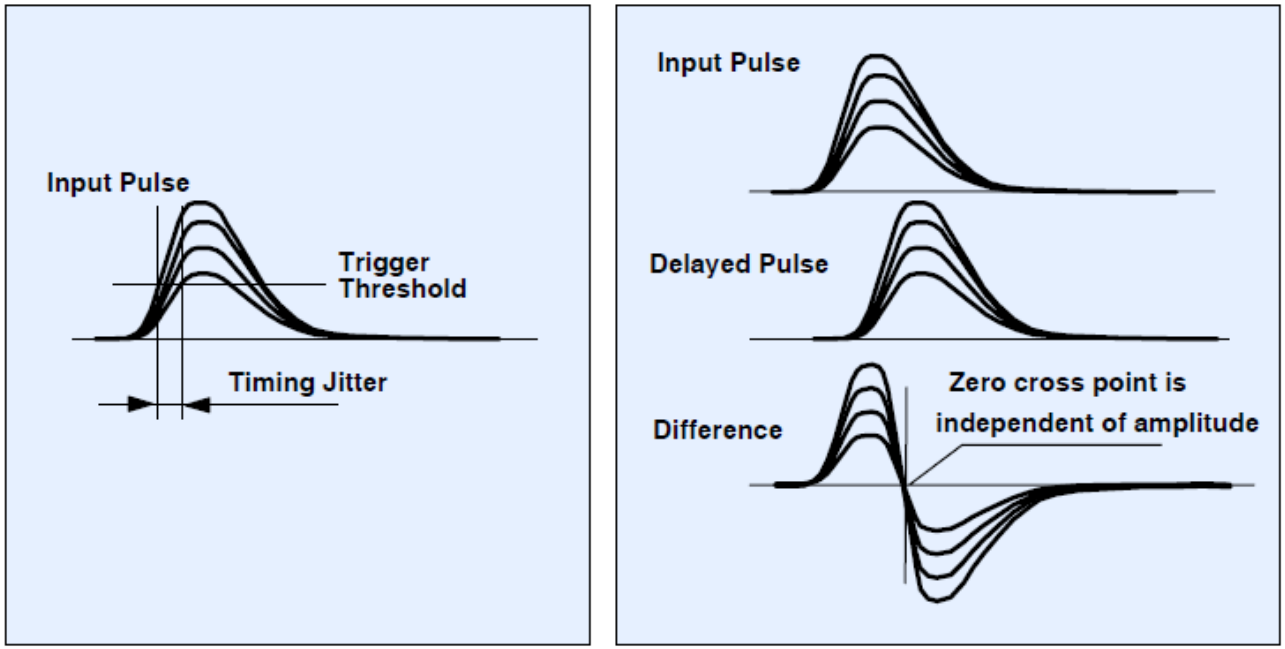


Fig. 158: Leading-edge triggering (left) and constant-fraction triggering (right)

Constant fraction of the total amplitude of a particular pulse. The circuit also discriminates on the total amplitude (threshold) to reject very small spurious pulses.

TCSPC: features

- Time resolution 50-150 ps (limited by electronic jitter of the detector)
- ‘Cheap’
- Good signal-to-noise (as good as you are willing to wait)
- No intense lasers necessary (semiconductor lasers are enough)
- Single-color
- Suitable for imaging (FLIM)

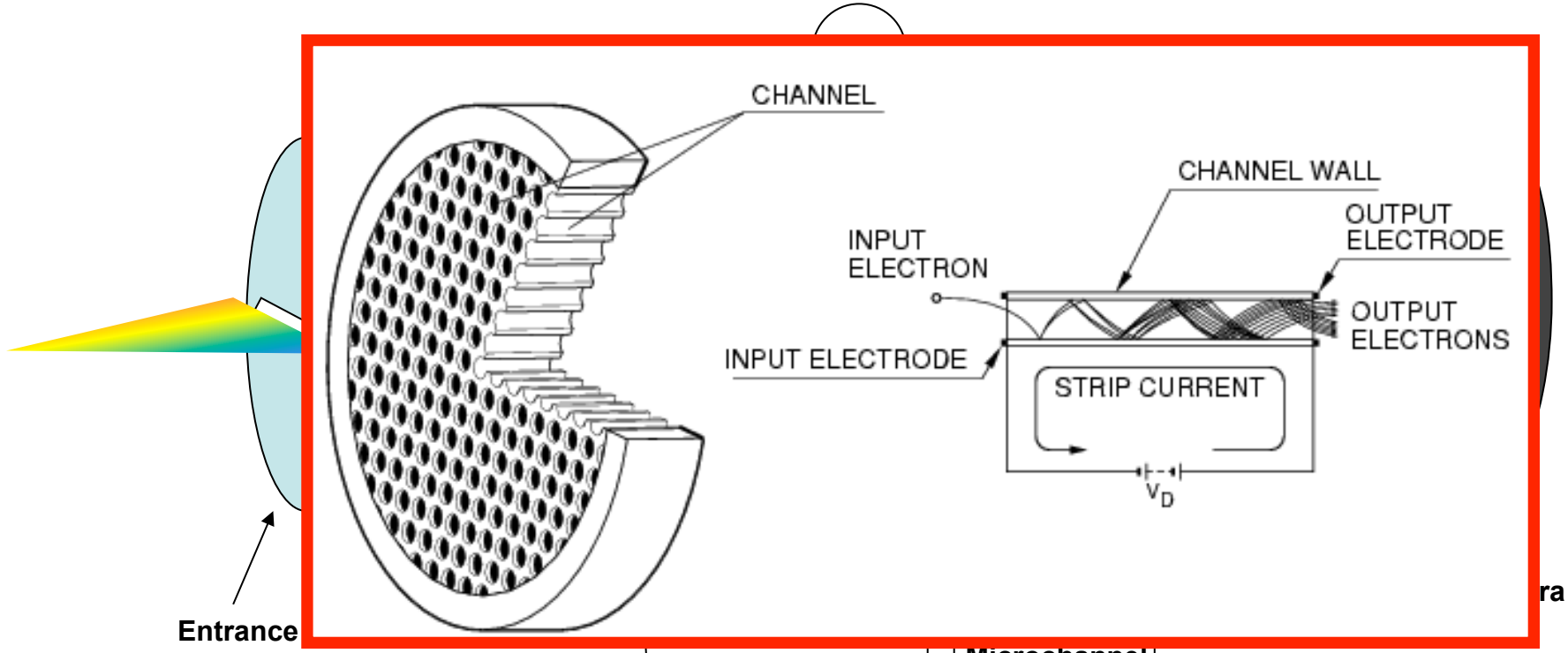
Time resolved fluorescence techniqes:

Streak camera

Light

Electrons

Light



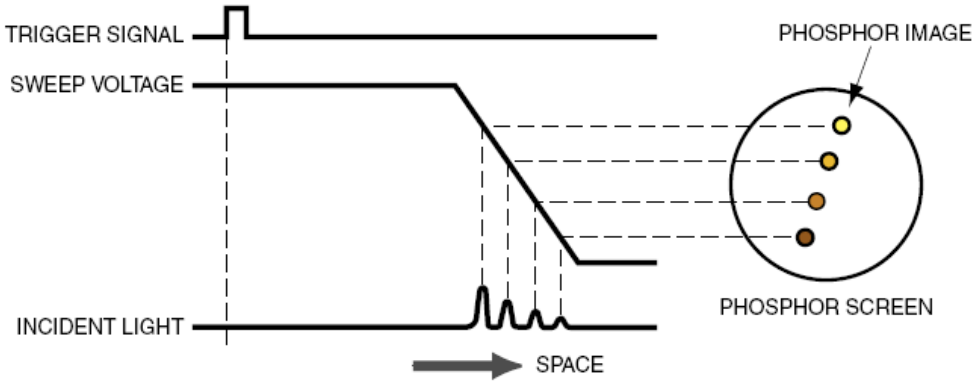
Entrance

Acceleration grid

Microchannel plate

Deflection electrodes

Phosphor screen

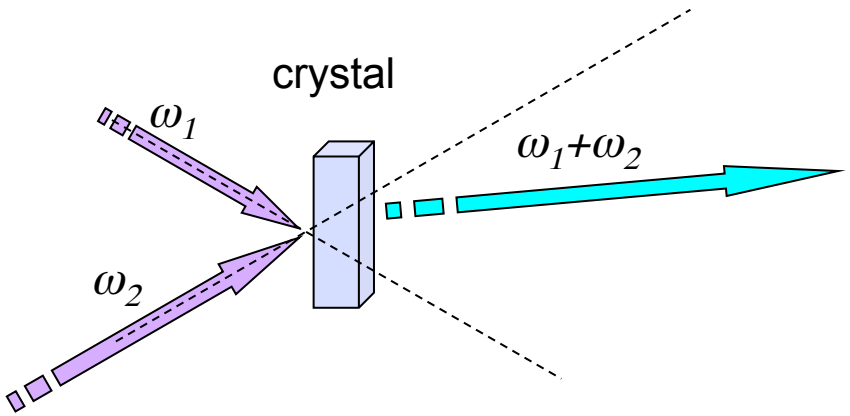


Streak camera

- Simultaneous measurement of the spectrum and the kinetics.
- Very sensitive
- Time resolution of synchroscan cameras down to 1-2 ps.
- Expensive (~500 k€) ;

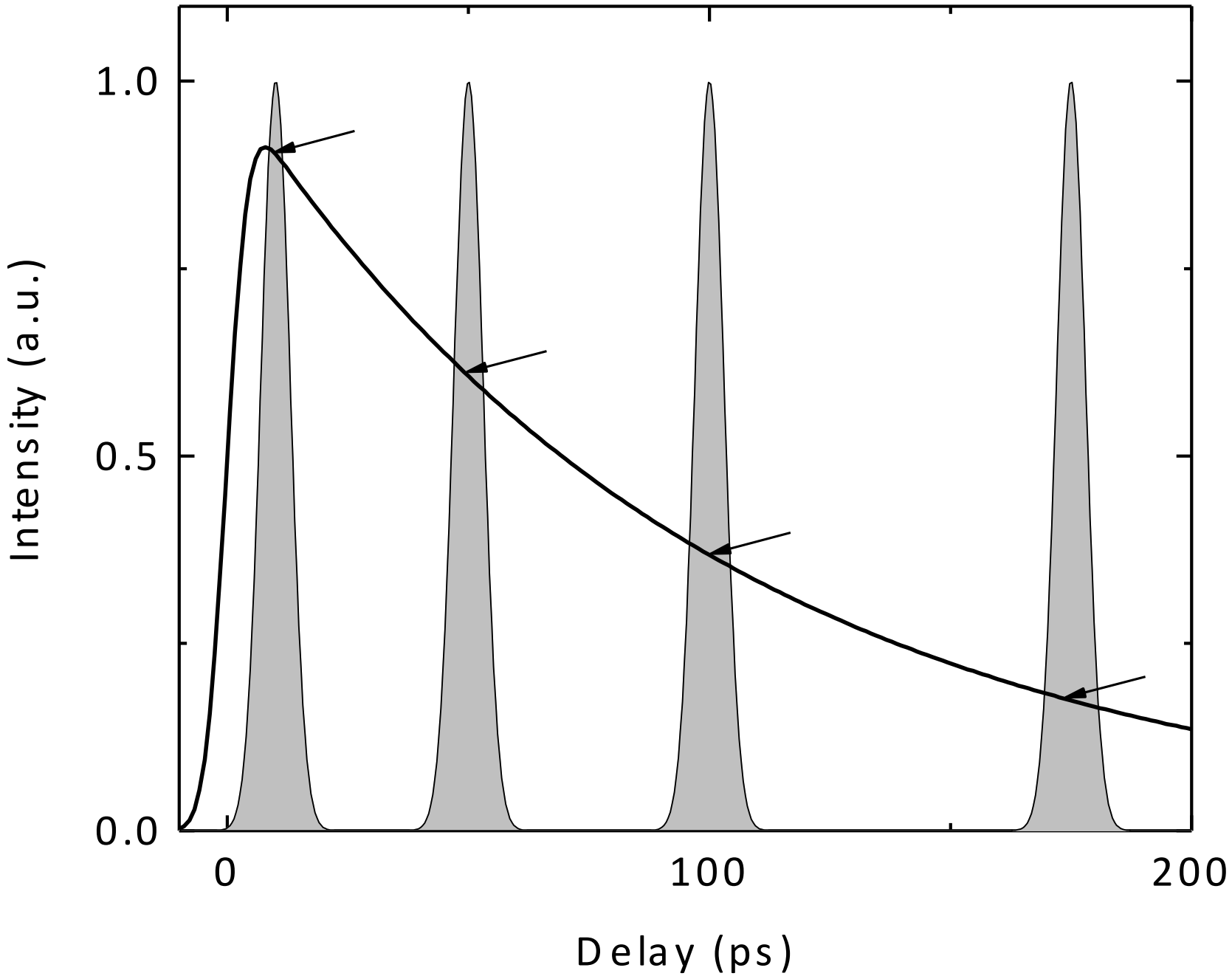
Time resolved fluorescence techniqes:

upconversion

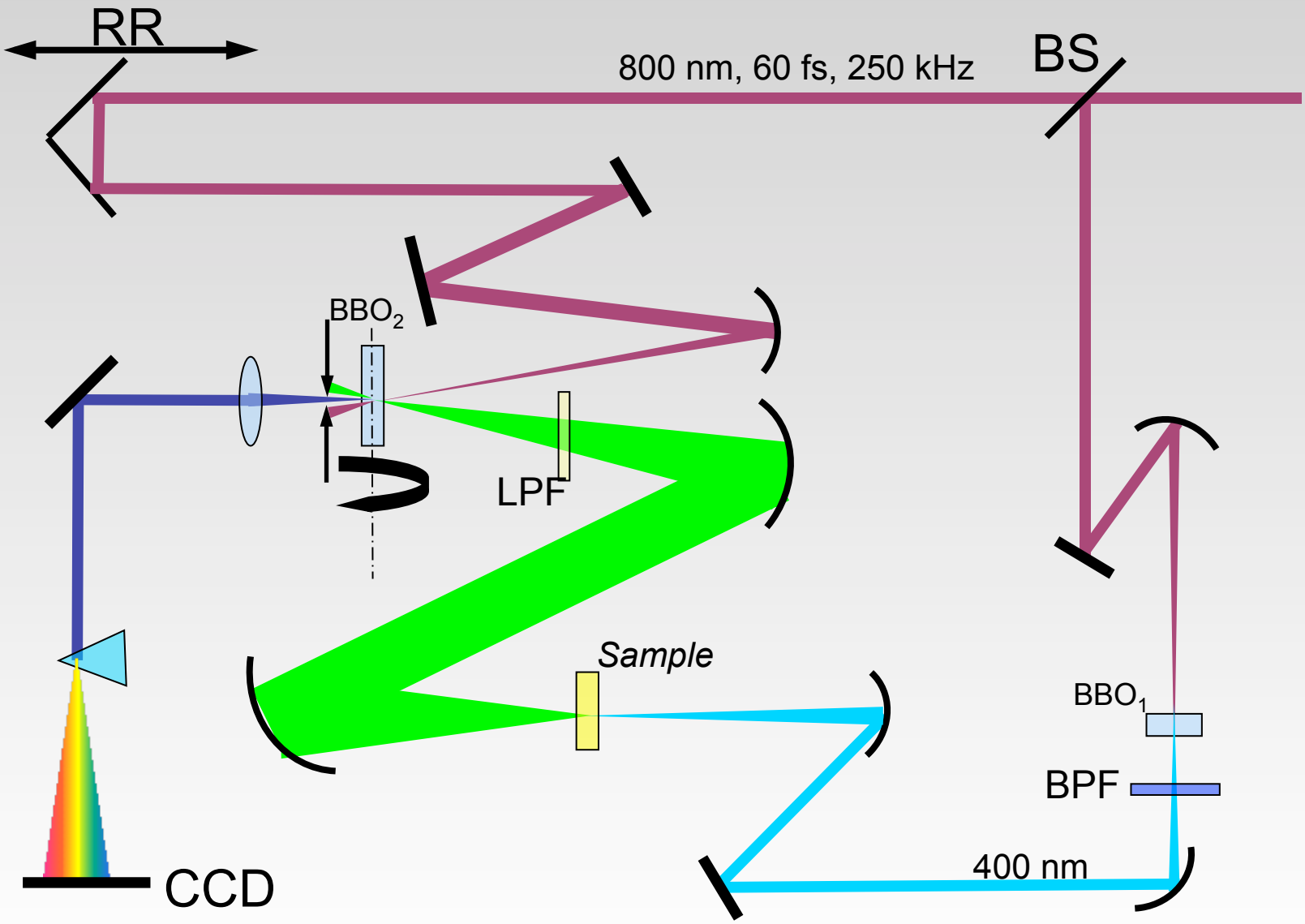


$$I_{sum}(t) = I_{gate}(t) \times I_{fluoresc.}(t)$$

$$\frac{1}{\lambda_{sum}} = \frac{1}{\lambda_{gate}} + \frac{1}{\lambda_{fluoresc.}}$$


1.0
0.5
0.0
Intensity (a.u.)
0
100
200
Delay (ps)

Dispersed Fluorescence Upconversion Setup



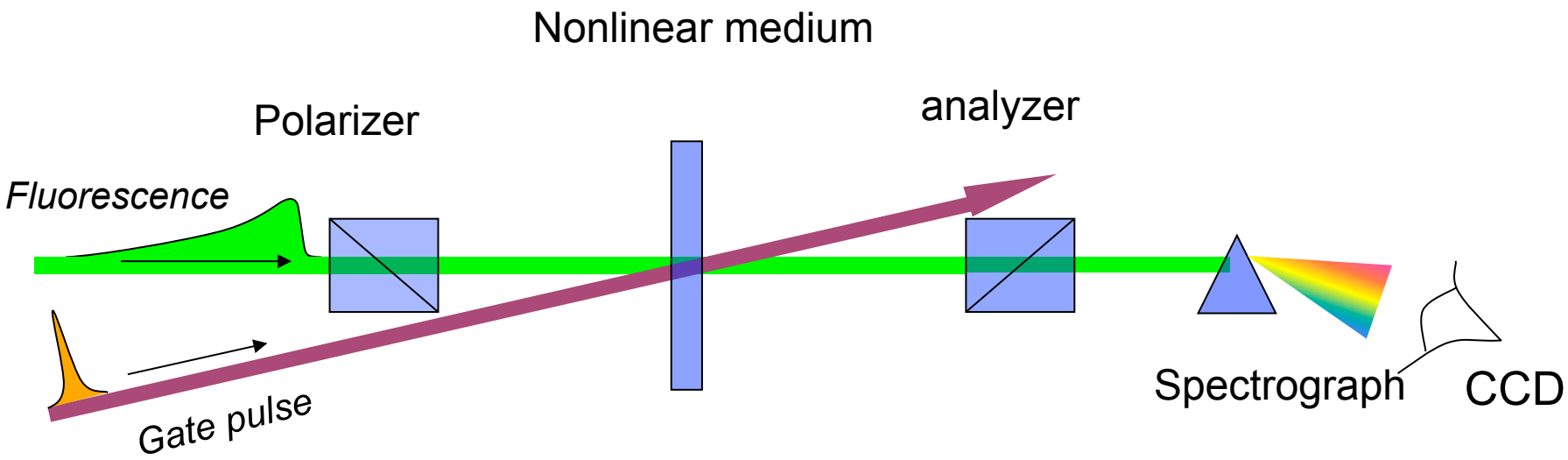
Fluorescence upconversion

- Problem: calibration of spectral sensitivities at different wavelengths
- Time resolution down to 50fs!
- A lot of excitation light required (bad for the samples)
- Experiments take time (one wavelength is phasematched at a time)
- Wavelength resolution limited by the spectral width of the gate pulse.

Time resolved fluorescence techniqes:

Optical Kerr shutter

Kerr shutter



$$n = n_0 + n_2 I$$

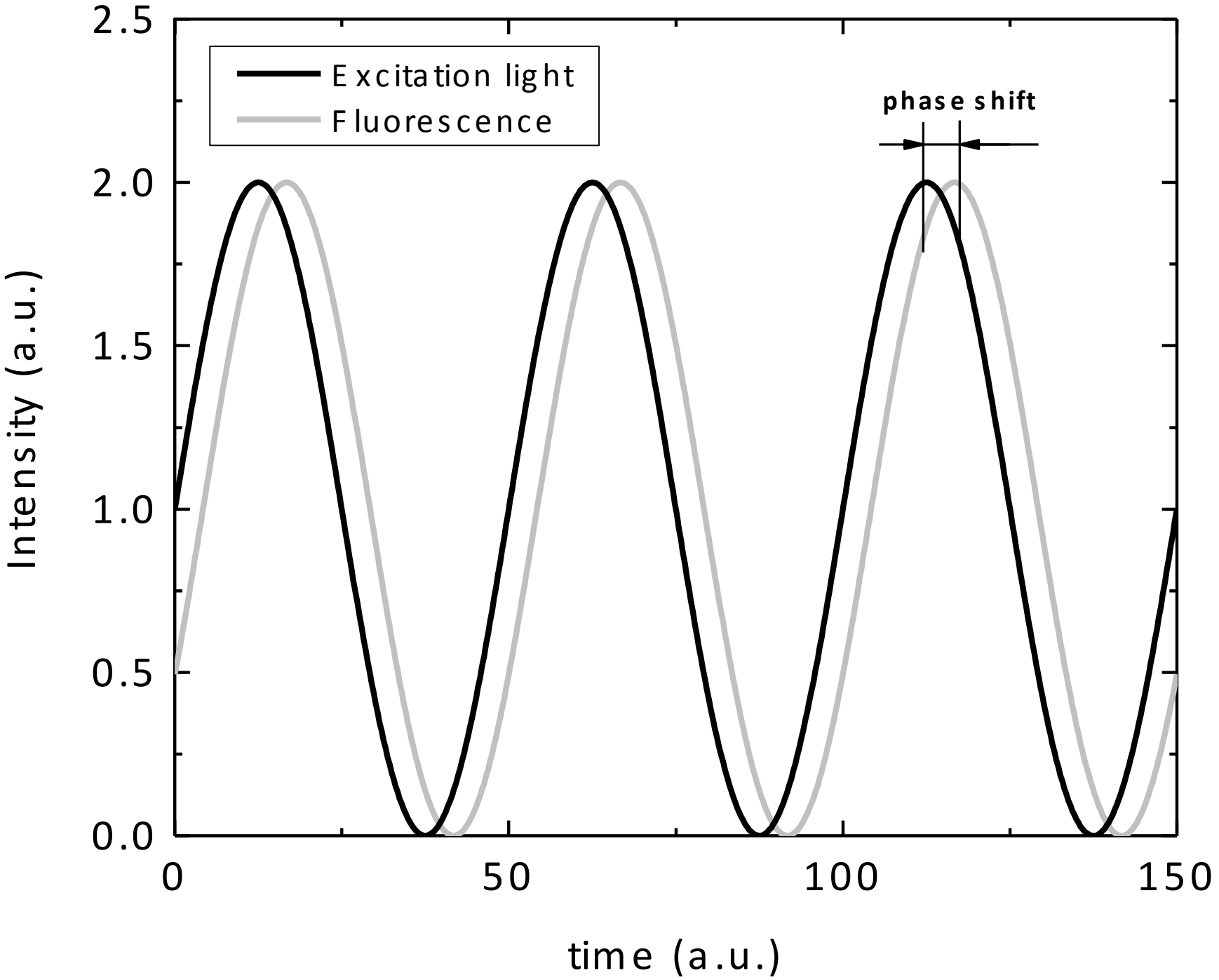
Optical Kerr effect – birefringence induced by the (polarized) electric field.
Nonlinear medium operates as a shutter that is open only during the gate pulse.

Optical Kerr shutter

- Entire fluorescence spectrum measured at a time
- Time resolution down to 50 fs
- Extremely high laser intensities required
- Materials with large Kerr effect have intertial response (CS_2, water)
- Troublesome experimental implementation

Time resolved fluorescence techniques:

Phase fluorimetry (a.k.a. frequency domain fluorescence lifetime measurement)


Excitation light
Fluorescence
phase shift
Intensity (a.u.)
time (a.u.)
0.0
0.5
1.0
1.5
2.0
2.5
0
50
100
150

More frequencies – more complex decays can be disentangled

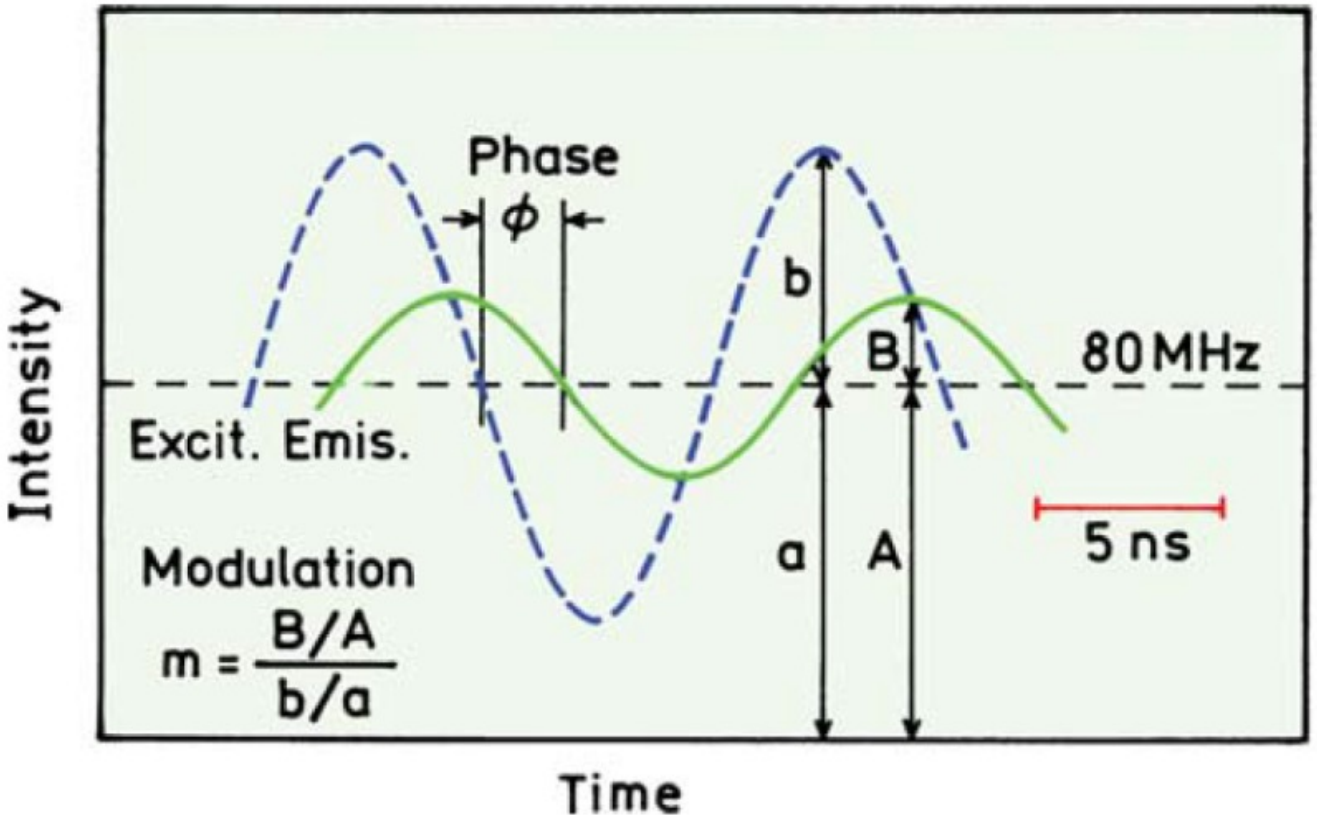
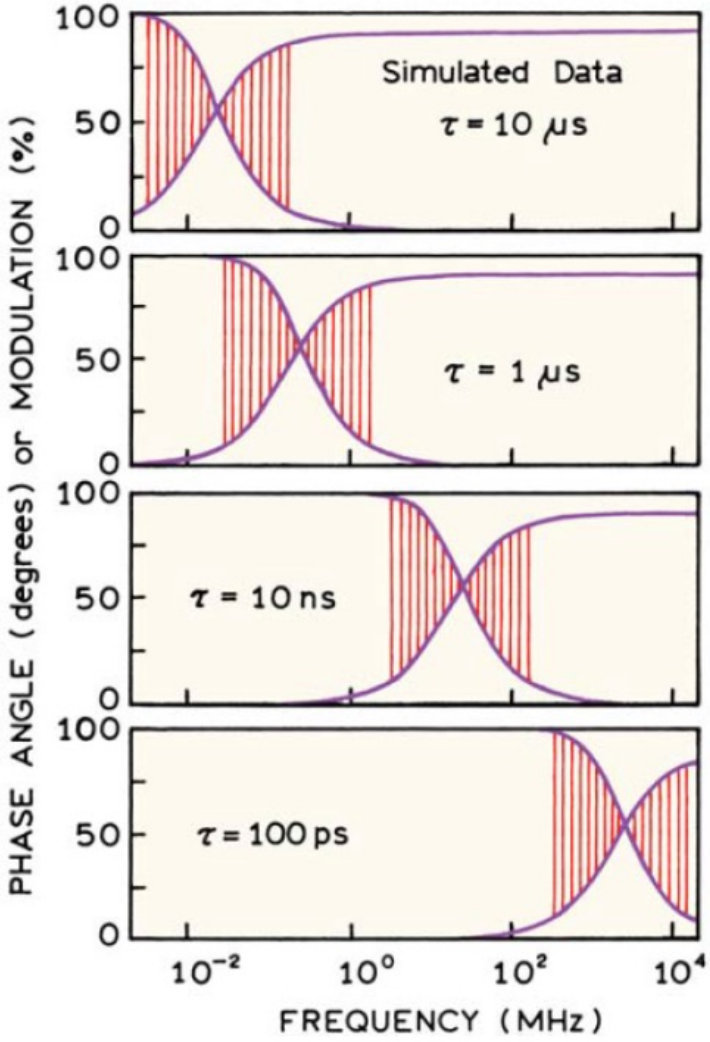


Figure 5.2. Definitions of the phase angle and modulation of emission. The assumed decay time is 5 ns and the light modulation frequency is 80 MHz.

Molecules are acting as an integrating filter in electronics



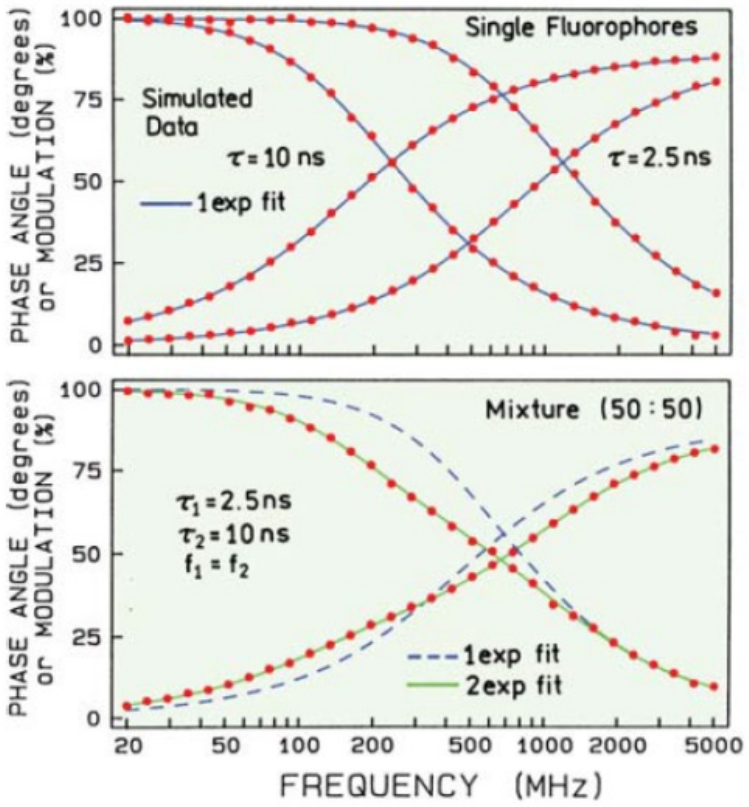


Figure 5.5. Simulated frequency-domain data for single- (top) and double- (bottom) exponential decays. The phase angle increases and the modulation decreases with increasing modulation frequency. The datapoints indicate the simulated data. **Top**: The solid lines show the best fits to a single decay time. **Bottom**: The dashed and solid lines show the best single- and double-exponential fits, respectively.

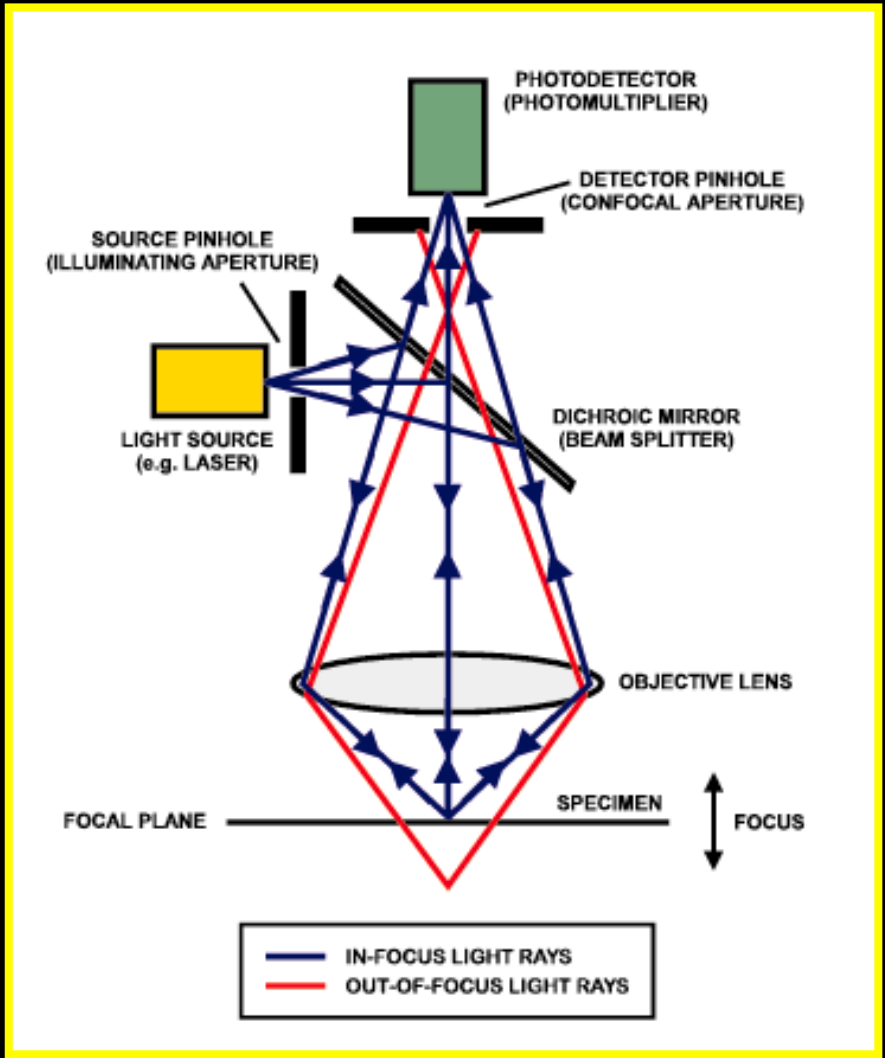
Phase fluorimetry (time domain lifetime measurements)

- Current modulation techniques allow wideband sweeping of the modulation frequency to de-construct response curves.
- Non-intuitive artifacts.
- Decay recovery is based on the assumptions on the (exponential?) decay of emission.
- No expensive equipment required (?)
- Good choice for ‘quick and dirty’ analysis of multiple samples.

Time resolved fluorescence: applications

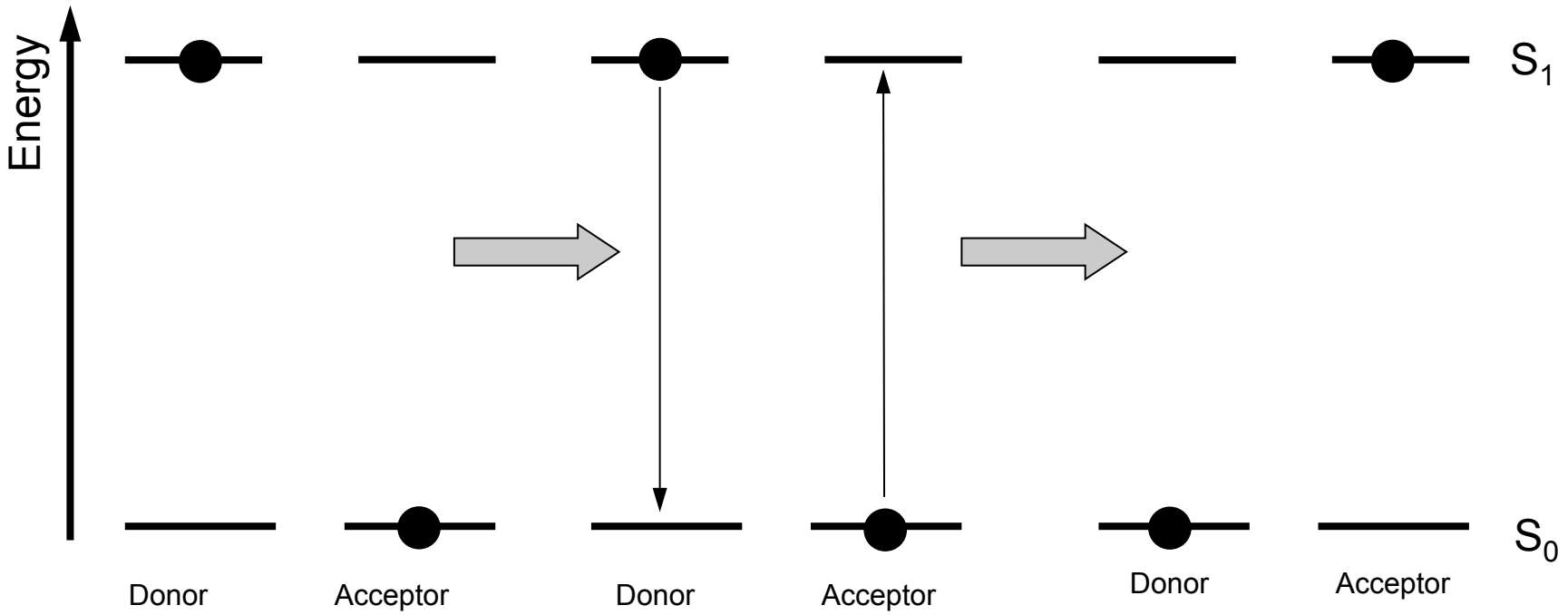
FLIM for FRET

Technical implementation of FLIM:



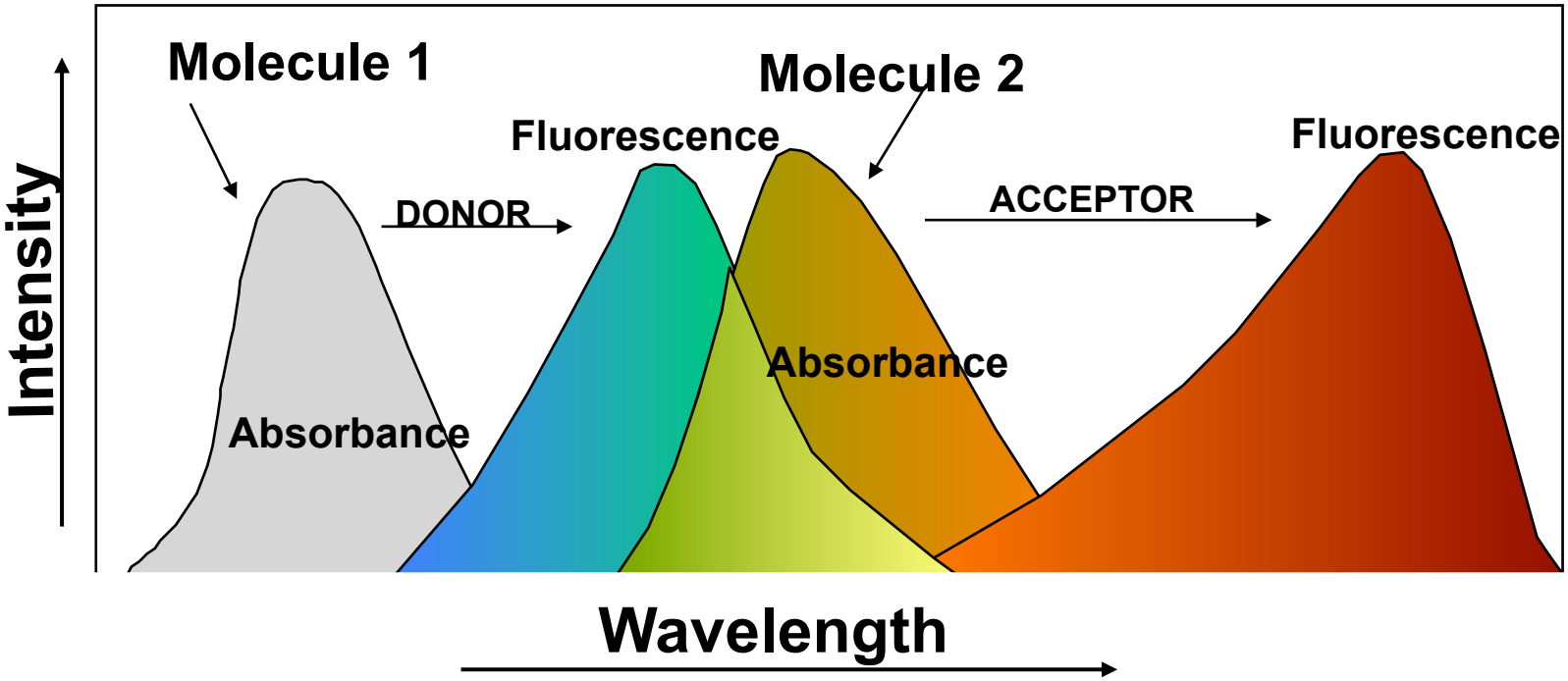
In your confocal microscope, replace excitation laser by a picosecond diode laser, and use a photon counting detector connected to TCSPC electronics

Förster energy transfer



Förster Resonance Energy Transfer

$$k_{DA} = \frac{9\ln(10)\kappa^2 c^4 \phi_D}{80\pi n^4 N_{av} \tau_D R^6} \int \frac{F_D(\omega) A_A(\omega)}{\omega^4} d\omega$$



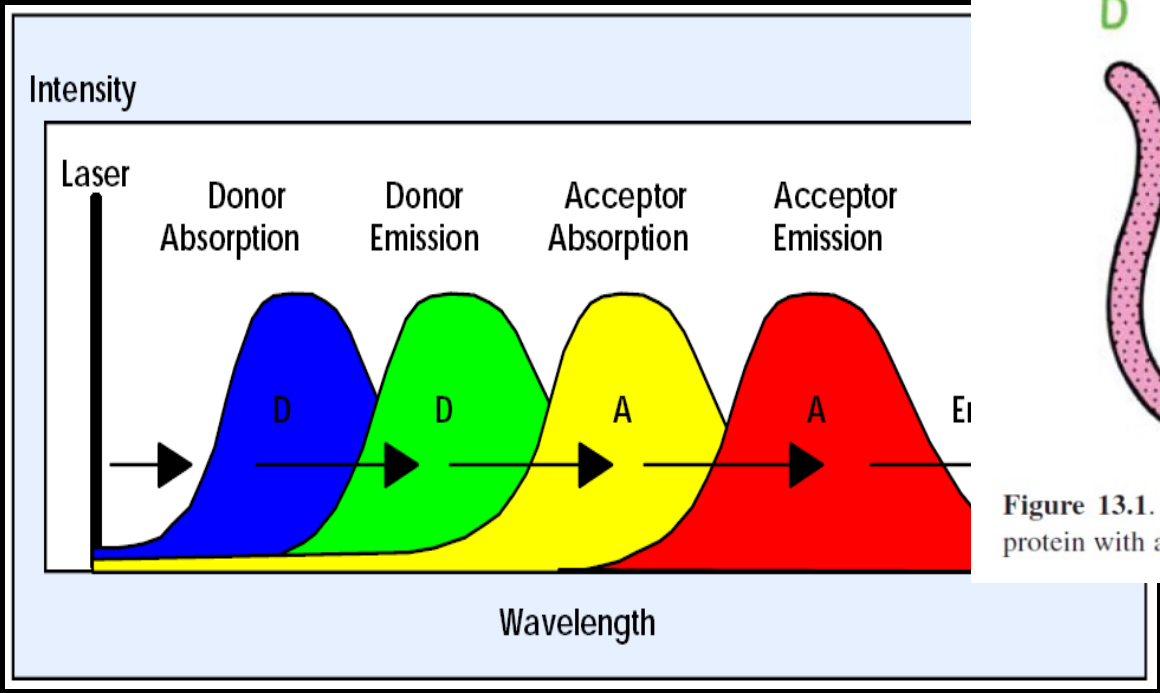
Assumptions

- When transfer is over, the correlation between donor and acceptor state is lost
- No orbital overlap between donor and acceptor (large distances)
- Dipole-dipole coupling
- Donor has relaxed to the bottom of its emissive state

After all these assumptions it is almost a miracle that the model works, but it does, and does it amazingly well.

FLIM and FRET

- Optical ruler for ~10 nm distances.



$$k_T(r) = \frac{1}{\tau_D}\left(\frac{R_0}{r}\right)^6 = \text{TRANSFER RATE}$$

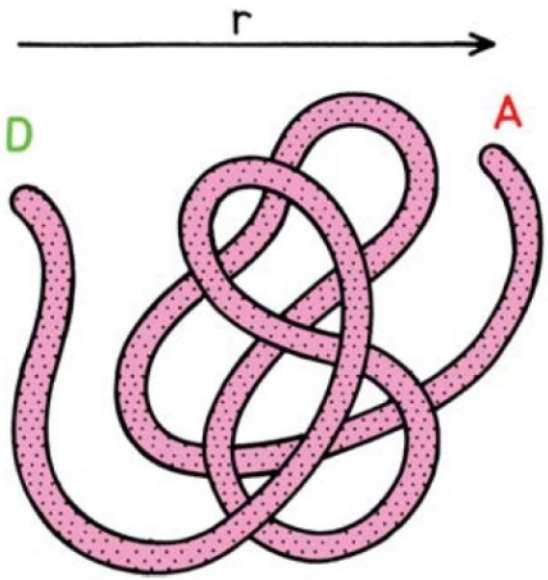
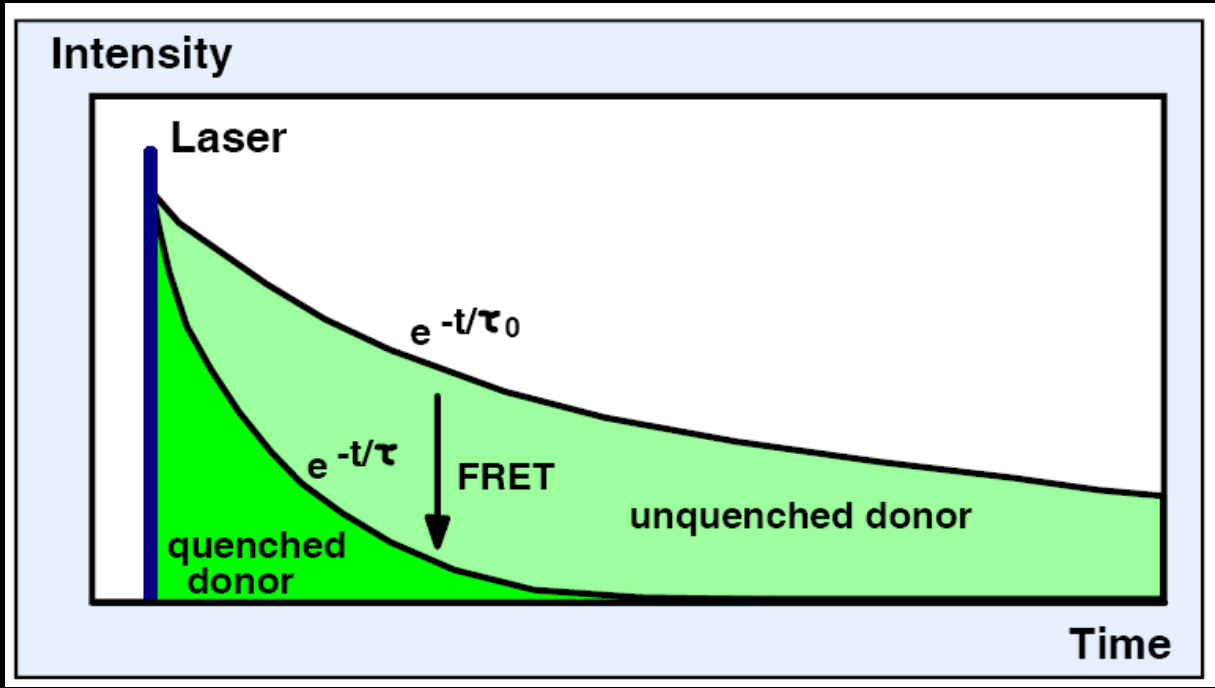


Figure 13.1. Fluorescence resonance energy transfer (FRET) for a protein with a single donor (D) and acceptor (A).

Energy transfer and donor lifetime



Donor fluorescence lifetime is reduced, because the acceptor is “sucking away” excited states

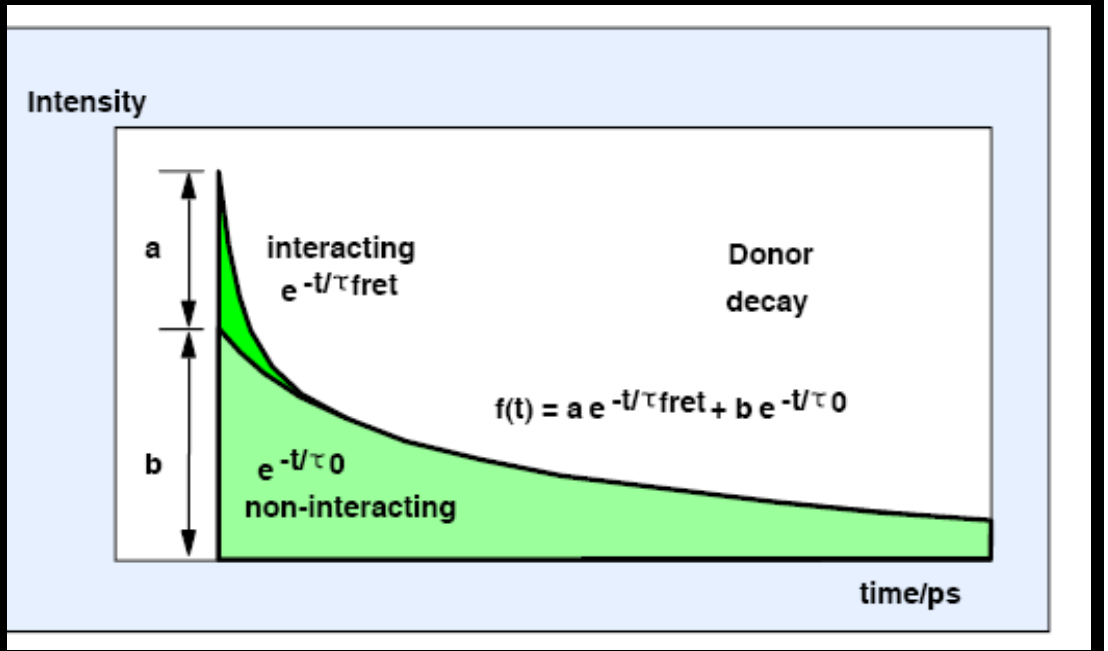
You could just look at the acceptor intensity, but

- Donor and acceptor concentrations in the cells are not known precisely
- Absorption spectra may overlap
- Calibration and control is tricky

Time-resolved FRET

- Direct measurement of the reduction in donor lifetime

1. No FRET – slow donor decay
2. Yes FRET – part of the donors disappear quickly.



Time-resolved FRET – detailed spatial information on molecular interactions

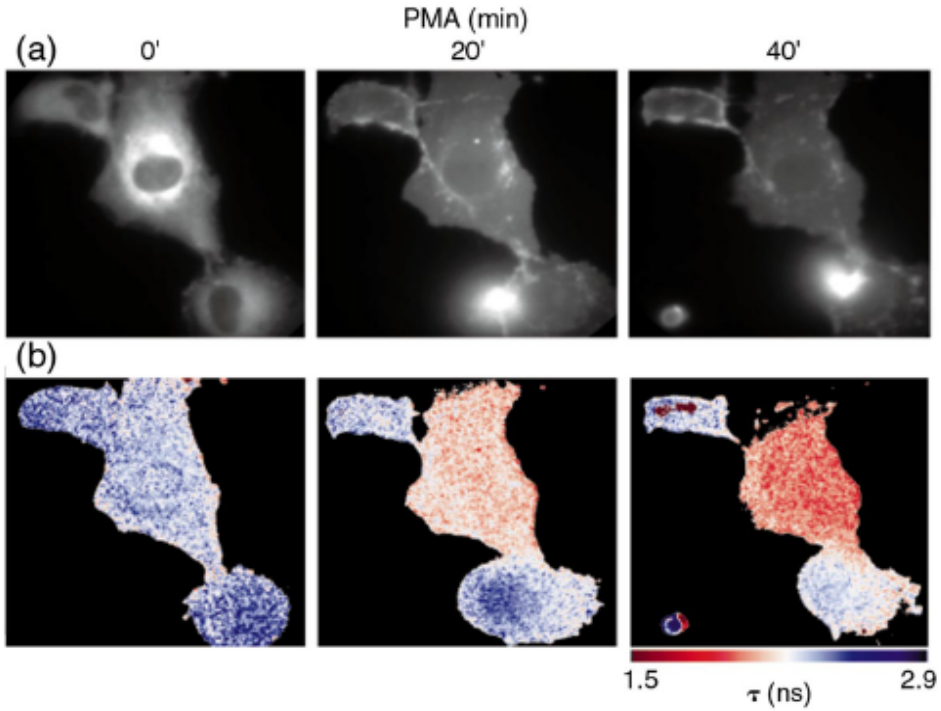


FIGURE 3

Activation of green fluorescent protein (GFP)-tagged protein kinase C alpha (PKCα) in live Cos7 cells measured by autophosphorylation using fluorescence lifetime imaging microscopy (FLIM). Only the middle cell was microinjected with site-specific IgG–Cy3.5. The cells were stimulated with 100 nM phorbol myristate acetate (PMA) and fluorescence lifetime images acquired at the times indicated. (a) Fluorescence images of GFP–PKCα; (b) fluorescence lifetime images of GFP–PKCα. Note that the lifetimes only decrease in the middle microinjected cell owing to fluorescence resonance energy transfer (FRET) between GFP–PKCα and IgG–Cy3.5.

P.I.H. Bastiaens and A. Squire, *Trends in Cell Biology*, 1999. **9**(2): p. 48-52.

Isomerization of retinal in bacteriorhodopsin

Bacteriorhodopsin, summary of:

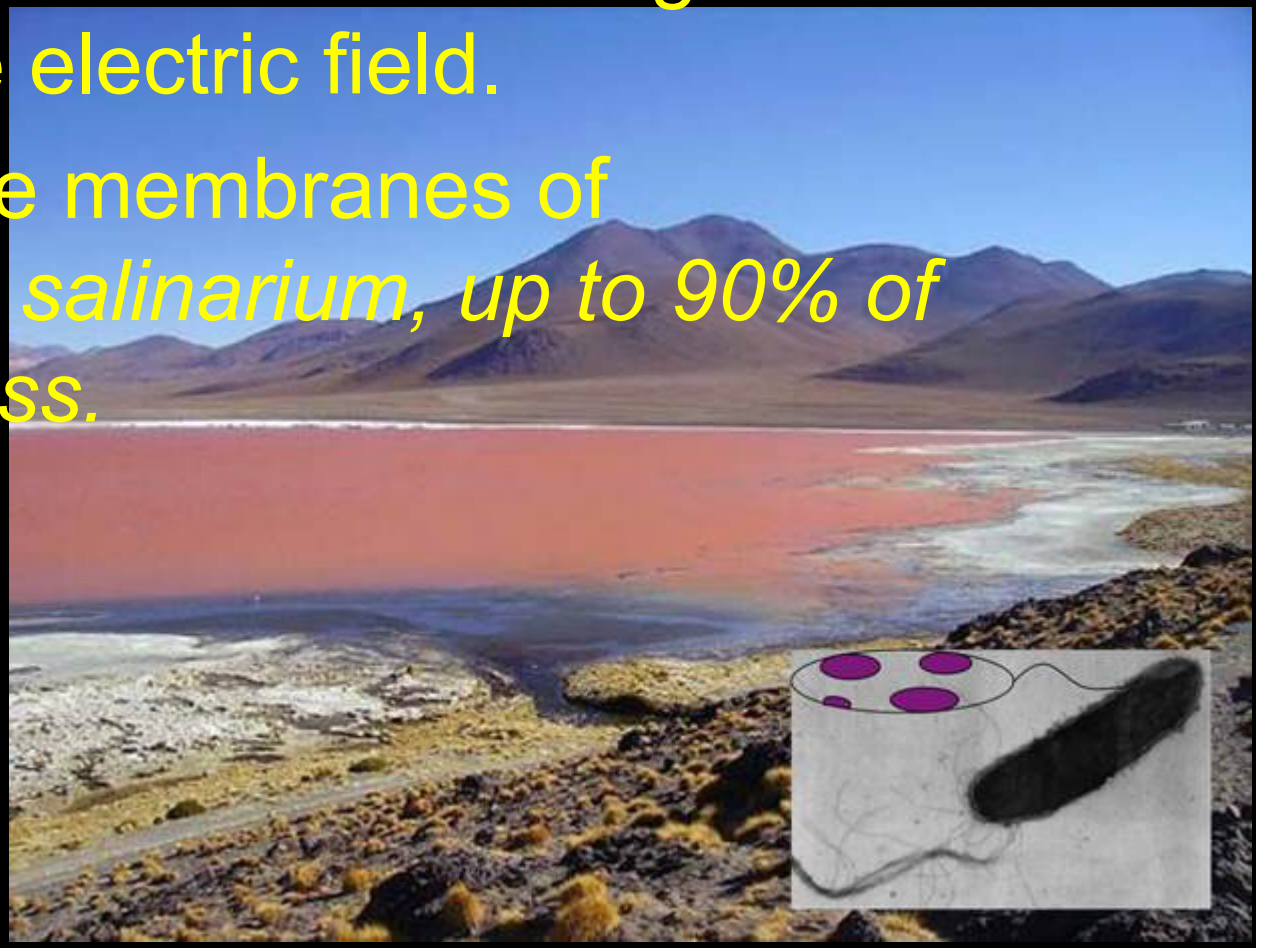
- Widely investigated:

  – Nature – 34 papers (1990 – 2012)
  – Science – 43 papers(1990 – 2012)
  – PNAS -173 papers (1990 – 2012)

  (Source: Web of science)

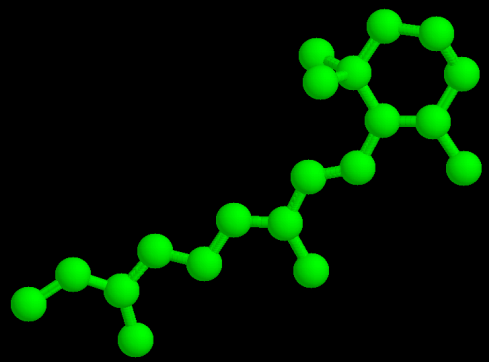
Function:

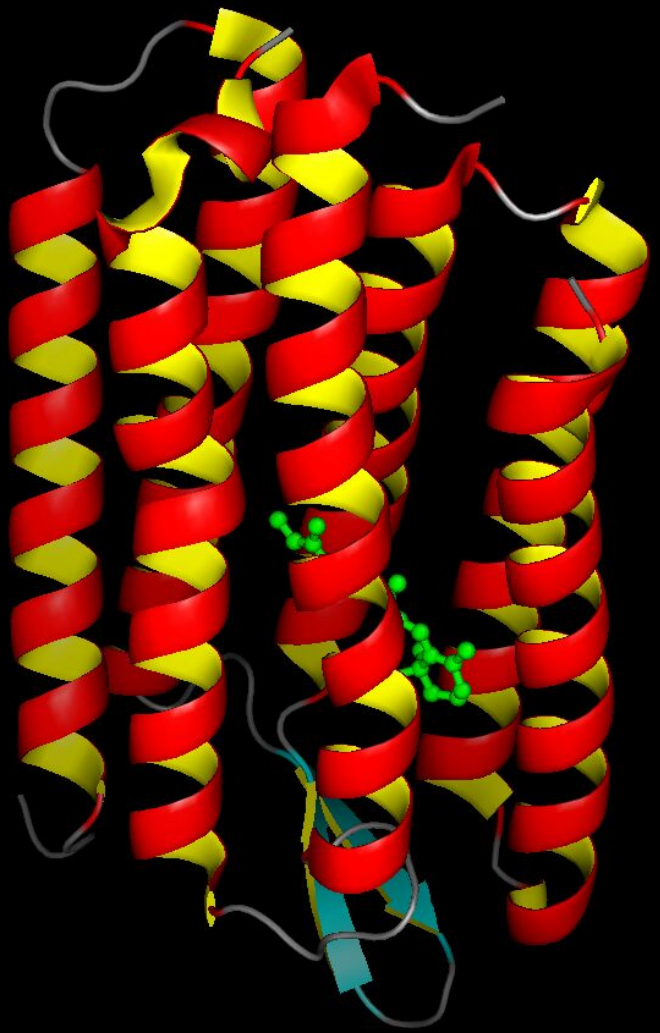
- Light drive proton pump that pushes protons across the membrane against the direction of the electric field.
- Found in purple membranes of *Halobacterium salinarium, up to 90% of membrane mass.*



Structure: membrane protein

- Structure resolved to 1.65 Å.
- 7 alpha helices containing a retinal chromophore

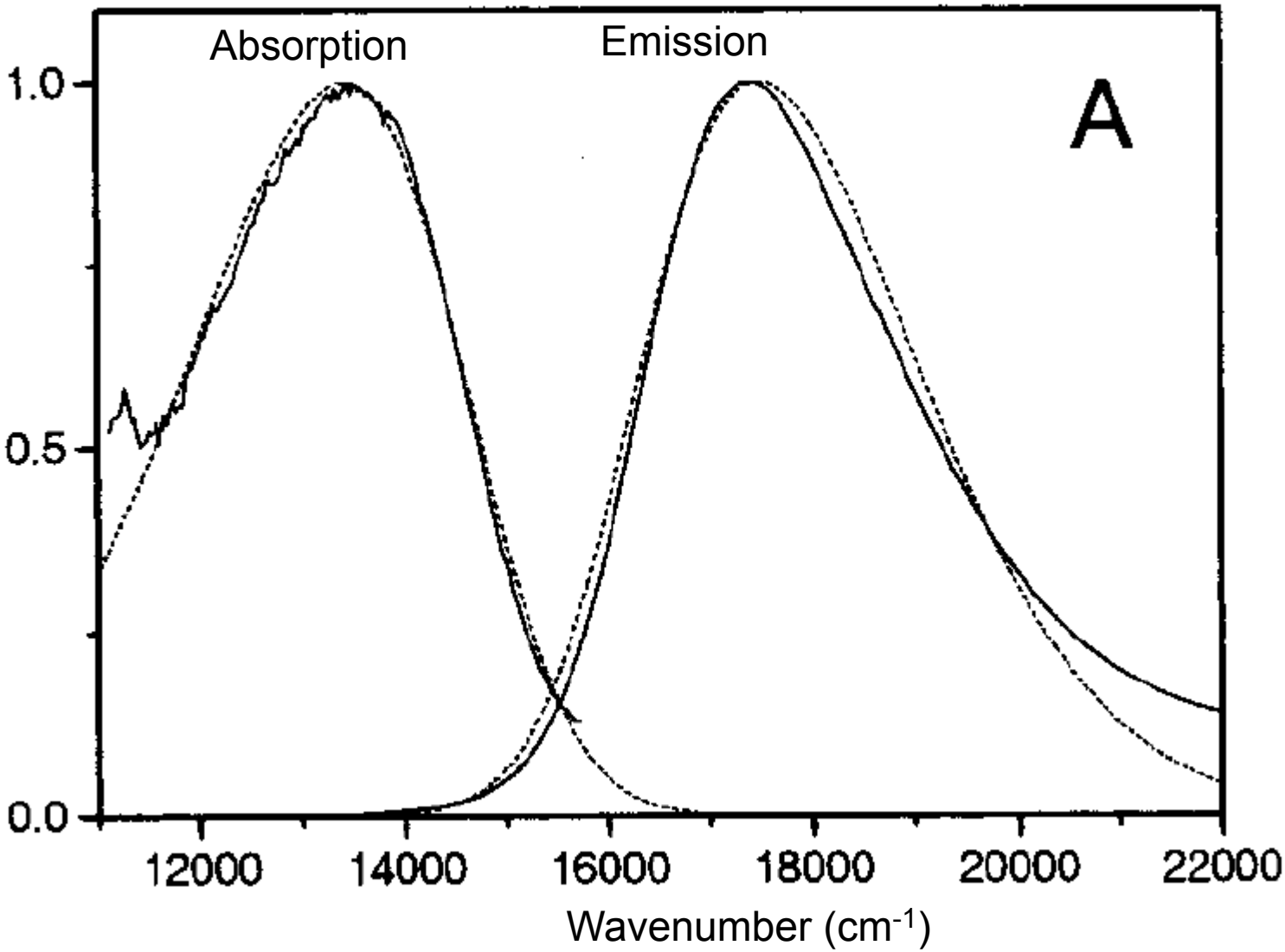


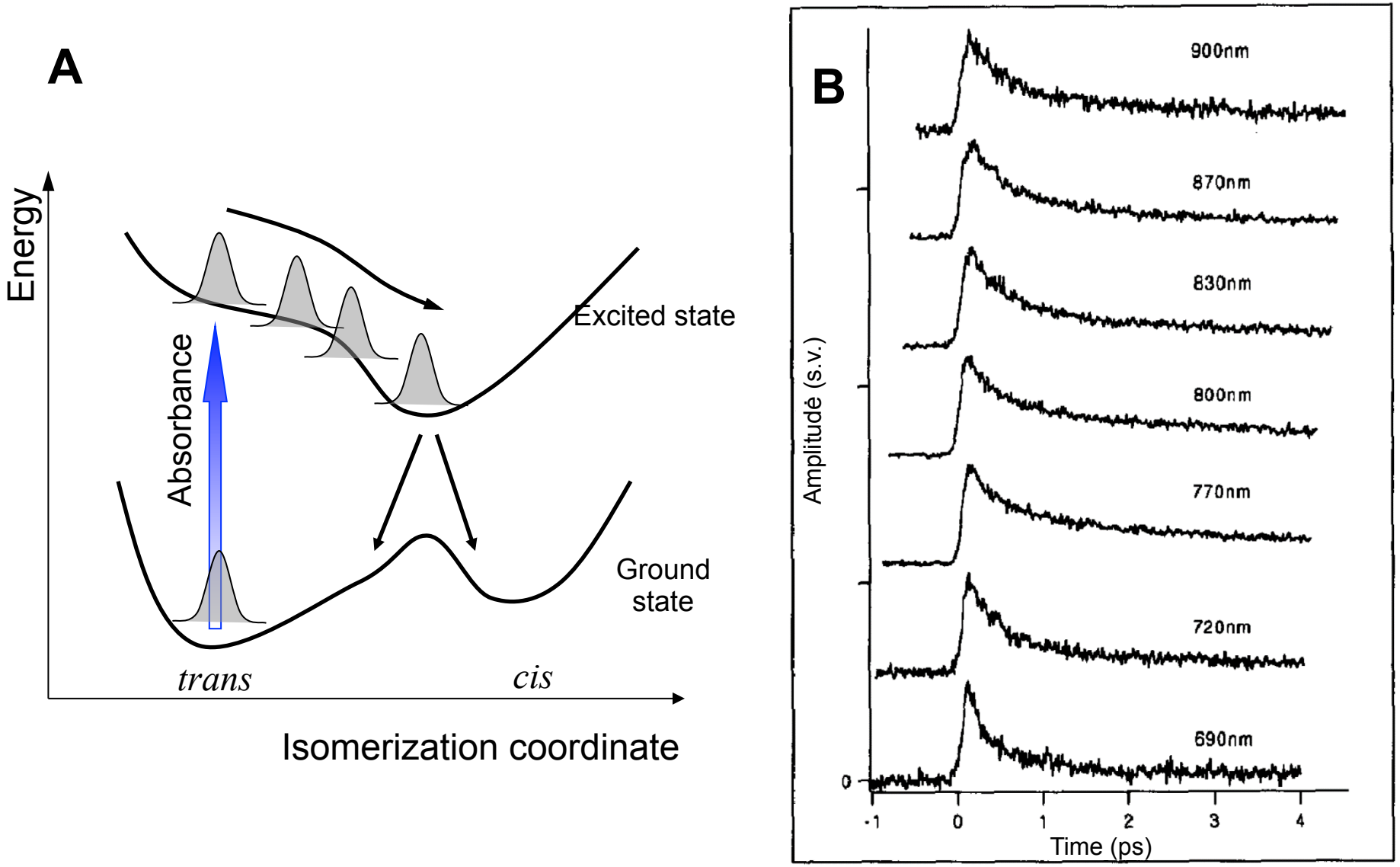


Advantages:

- Chemically stable and photostable
- Well known structure, easy to crystalize
- Bacteria grow a lot of it
- Fast, photoactive and therefore interesting
- Can be used as biomolecular tool or a model system for photoreactions

Absorption and emission

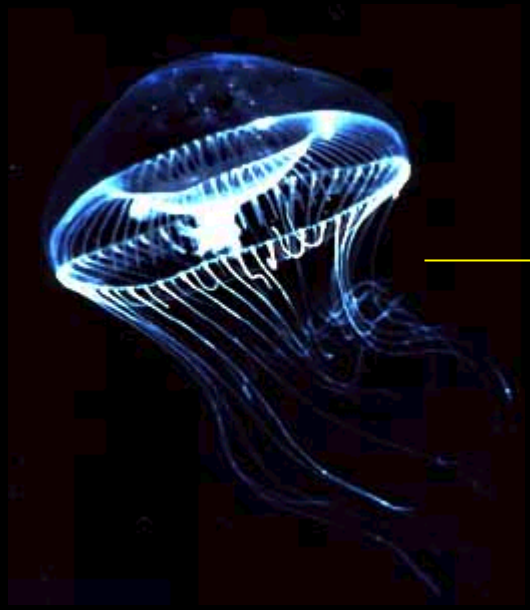




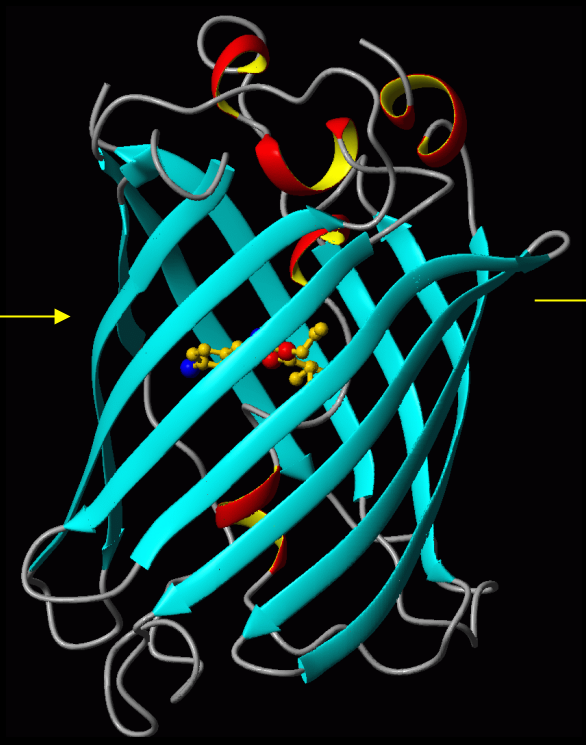
M. Du and G.R. Fleming, *Biophysical Chemistry*, 1993. **48**(2): p. 101-111.

Green fluorescent protein

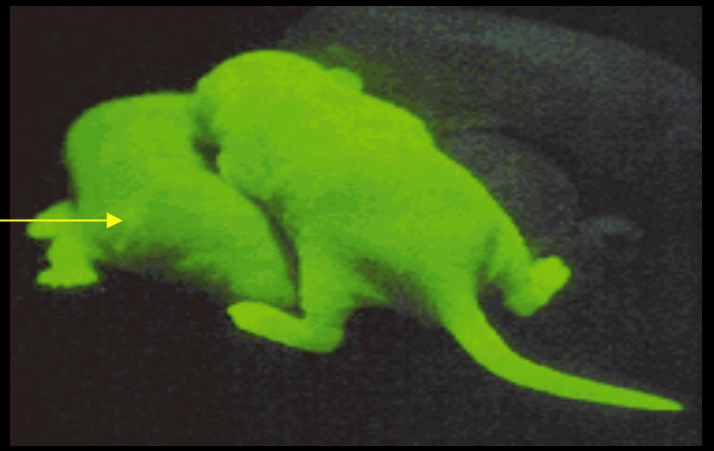
Aequorea victoria: jellyfish



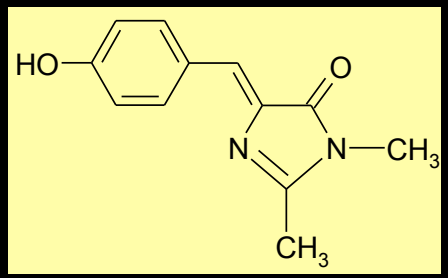
GFP protein

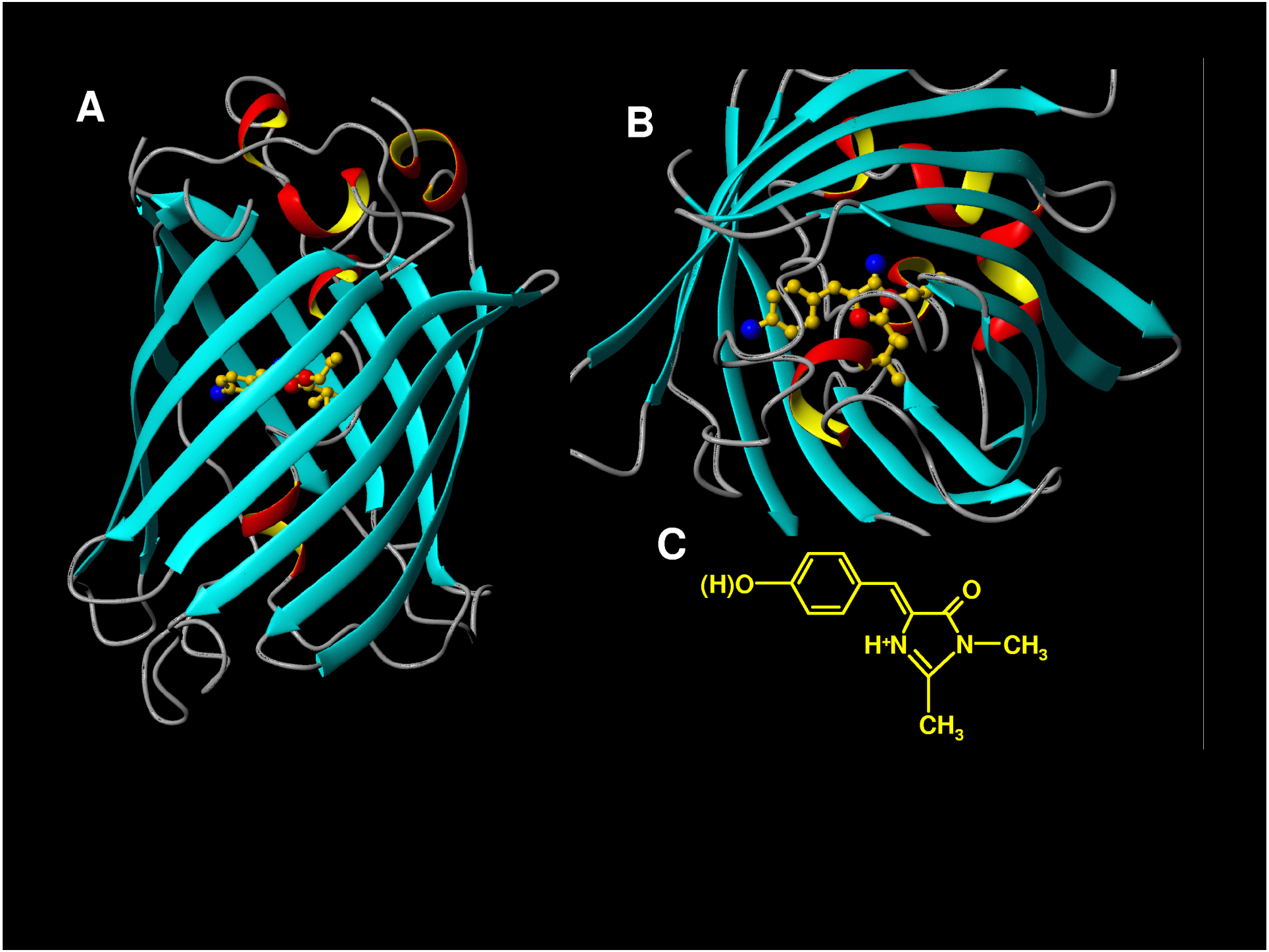


Marker for molecular biology

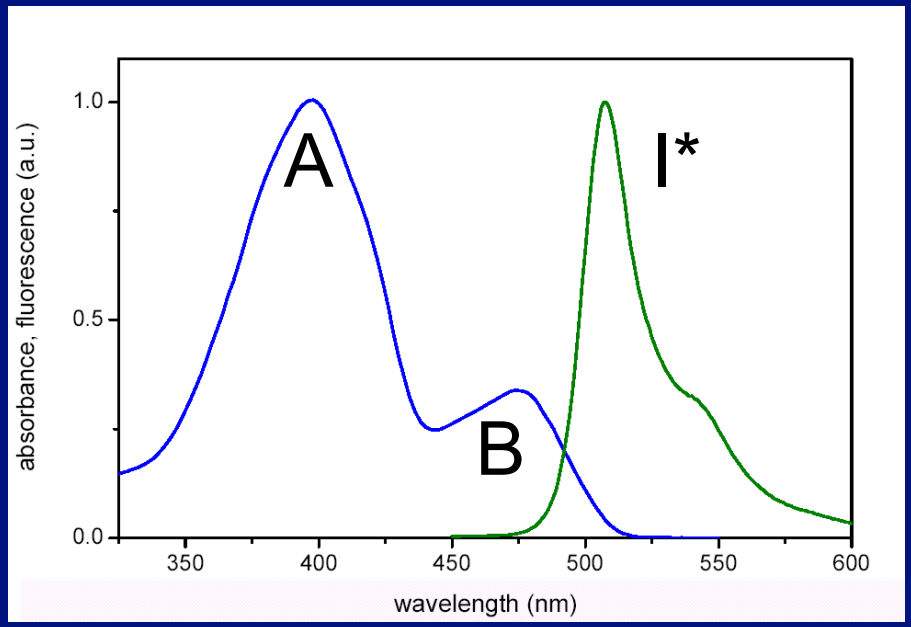


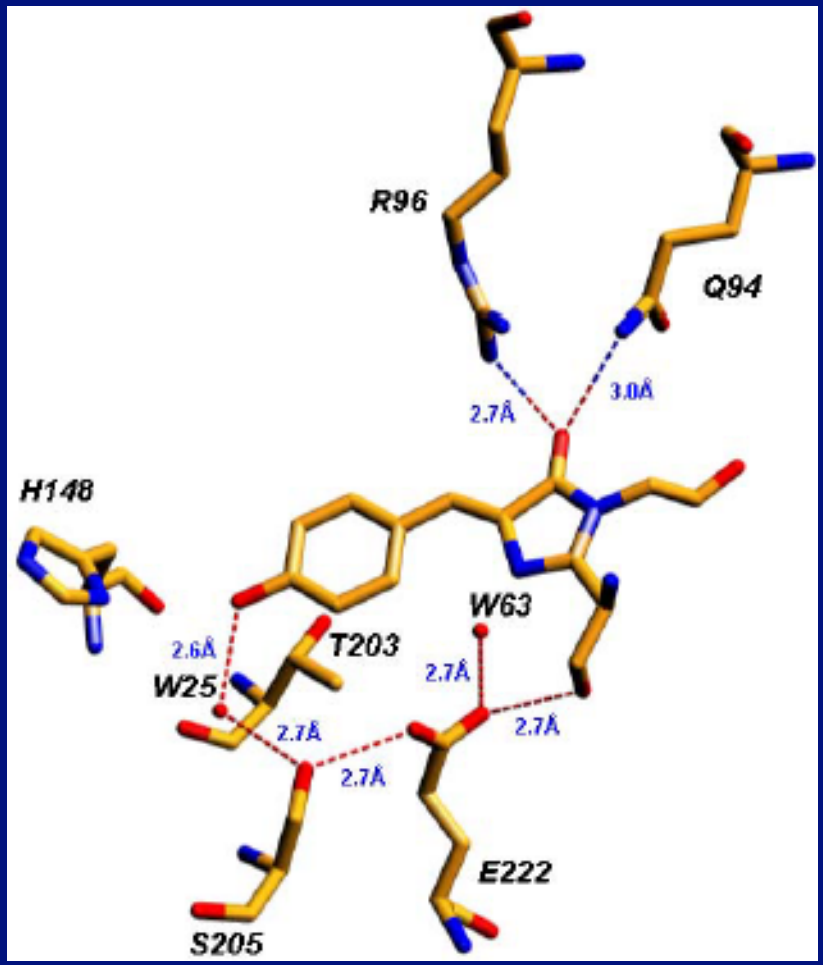
GFP chromophore

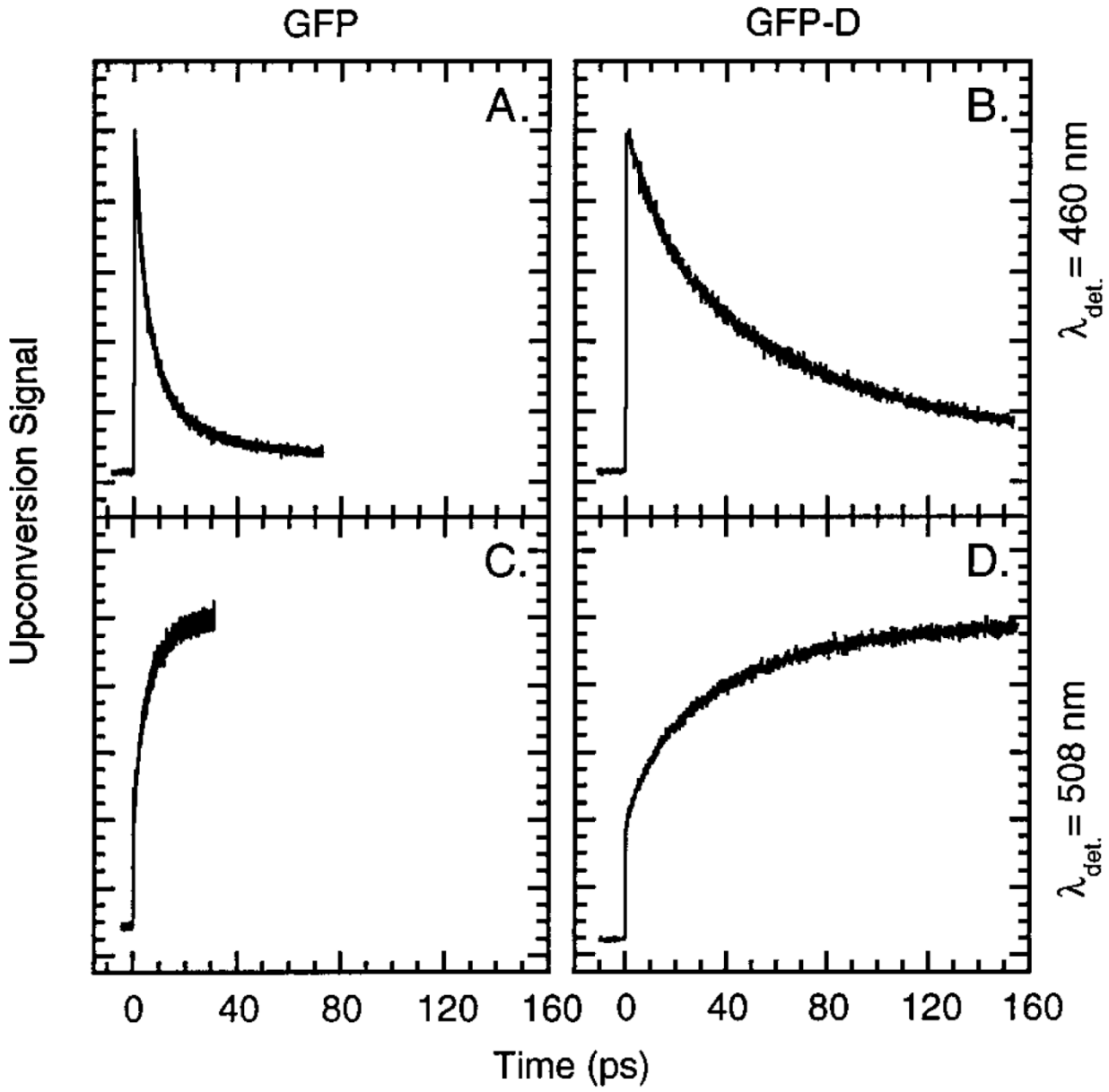



A
B
C
(H)O
O
H+N
N—CH3
CH3

Fluorescence from GFP originates from 'I': Excited-state proton transfer*



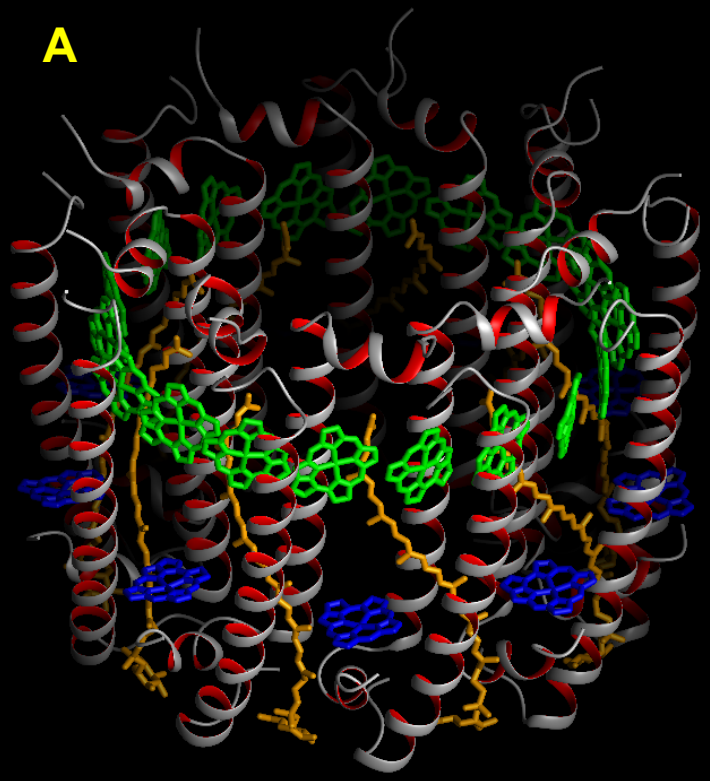




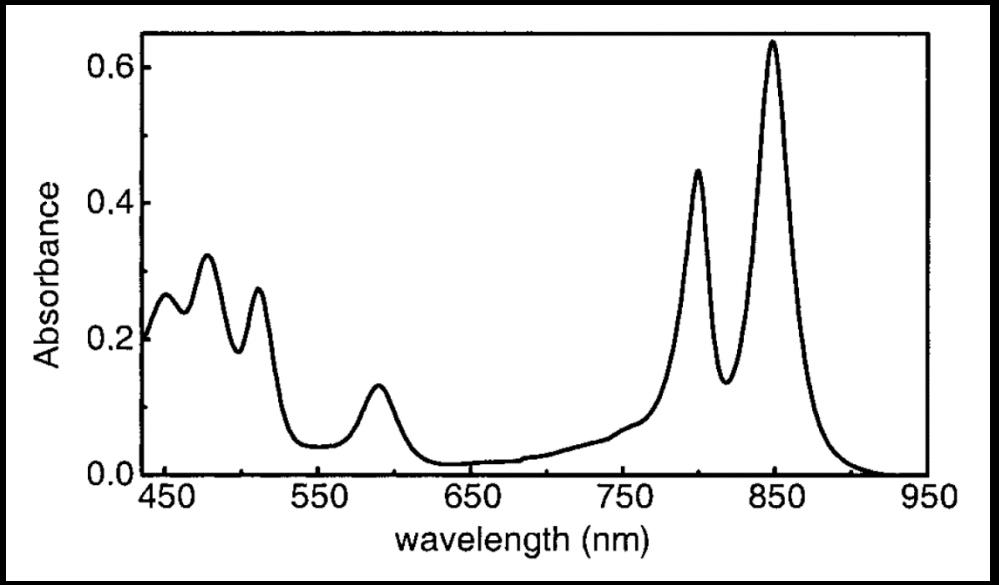
M. Chattoraj, B.A. King, G.U. Bublitz, and S.G. Boxer, *Proceedings of the National Academy of Sciences (USA)*, 1996. **93**: p. 8362-8367.

Energy transfer in LH2 of purple bacteria

A

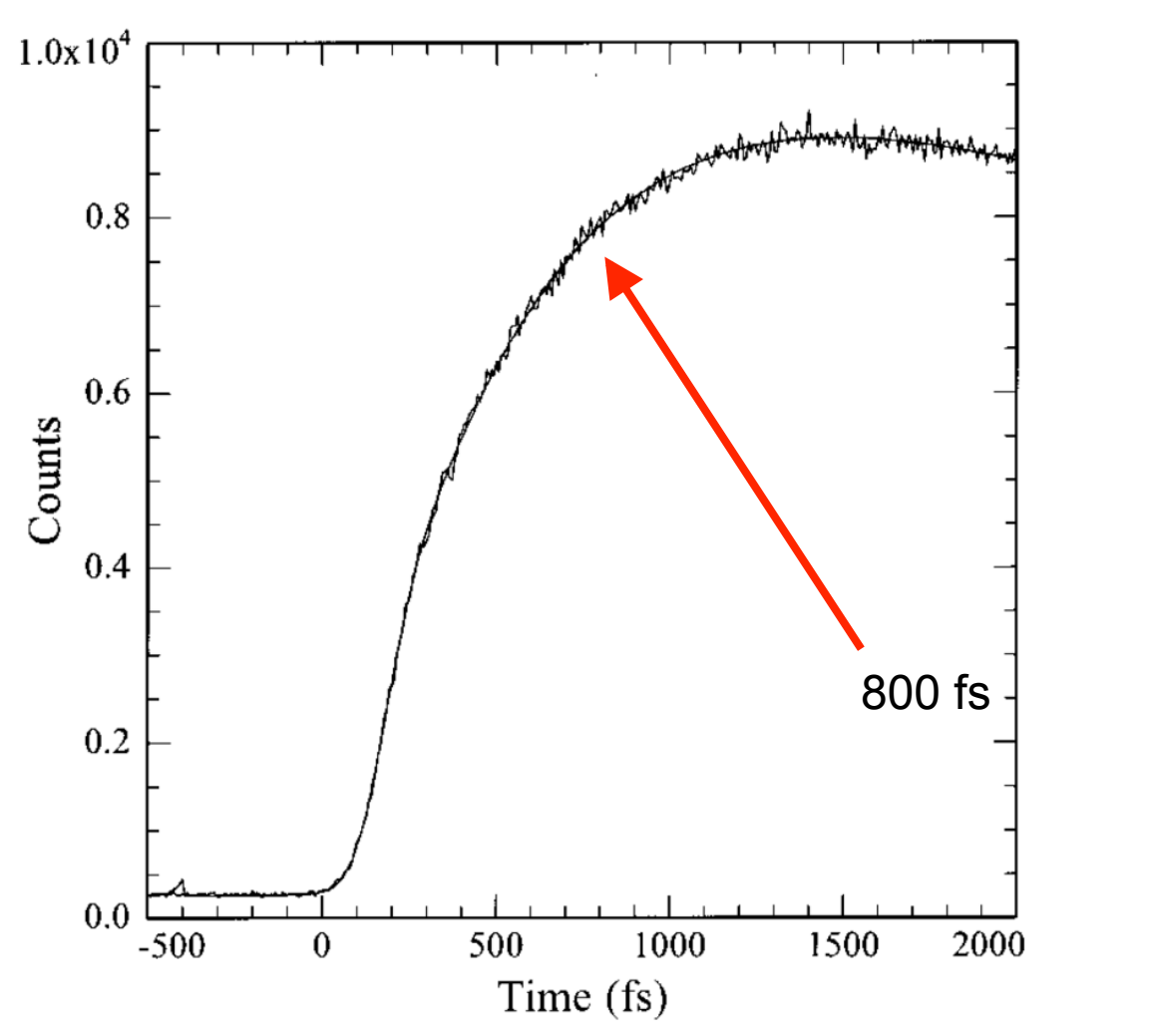


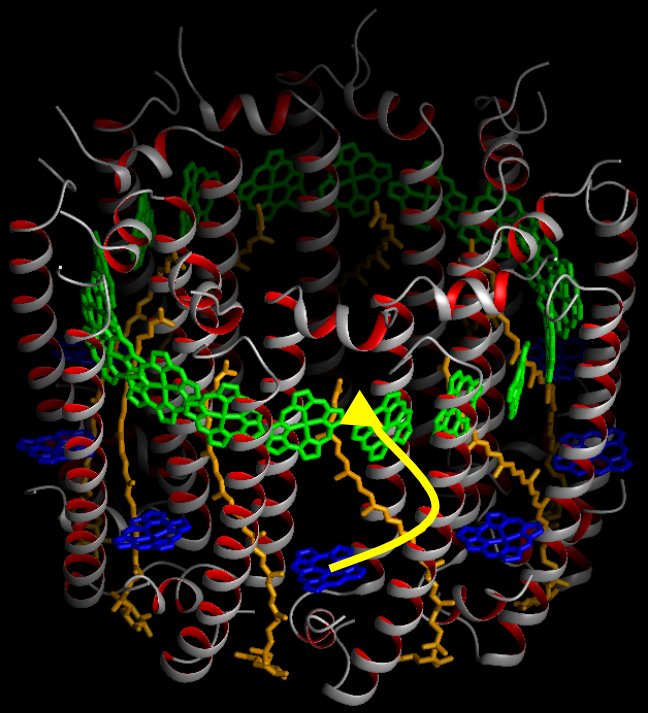
B



R. Jimenez, S.N. Dikshit, S.E. Bradforth, and G.R. Fleming, *Journal of Physical Chemistry*, 1996. **100**(16): p. 6825-6834.

Fluorescence of the acceptor pigment develops with the delay after the excitation. The delay is equal to energy transfer time.





Energy transfer between isoenergetic pigments

- If the excitation energies of different pigments are equal, emission wavelength does not change due to energy transfer;
- Therefore, the fluorescence appears at that wavelength without any delay.
- However, the polarization of the emission will change!

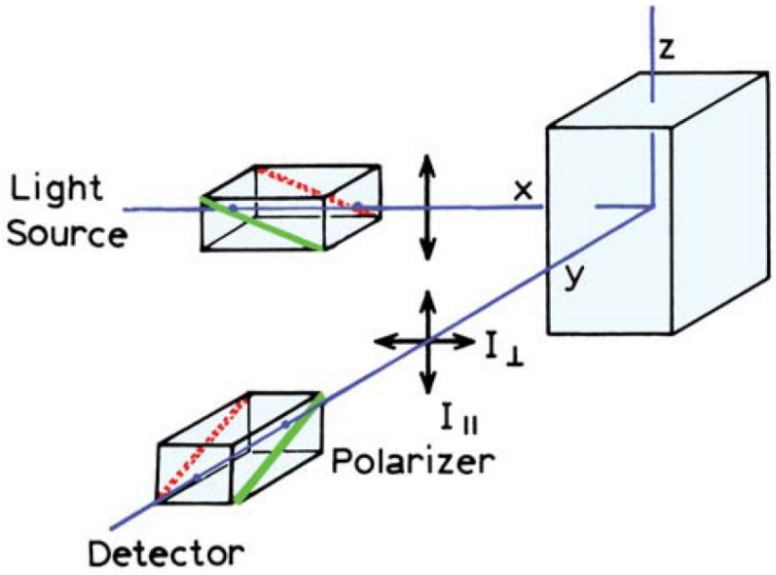
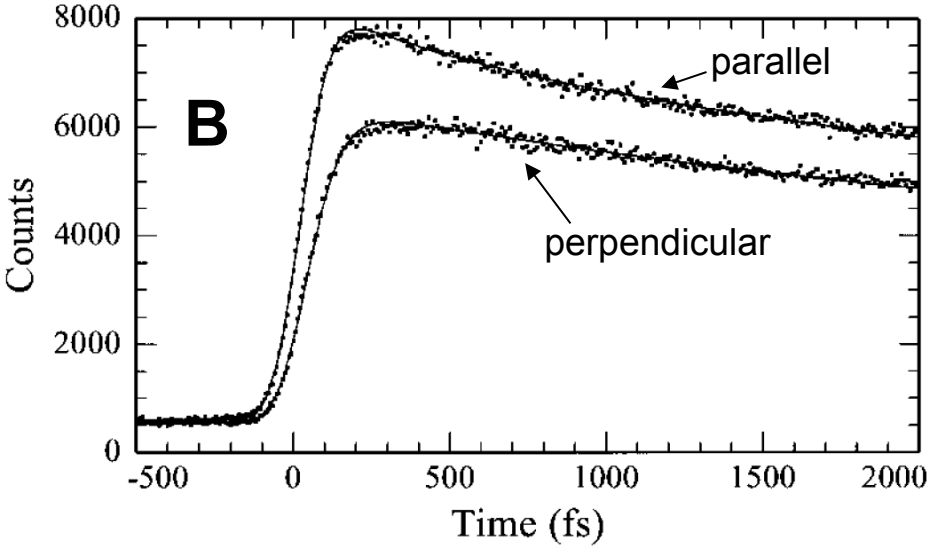
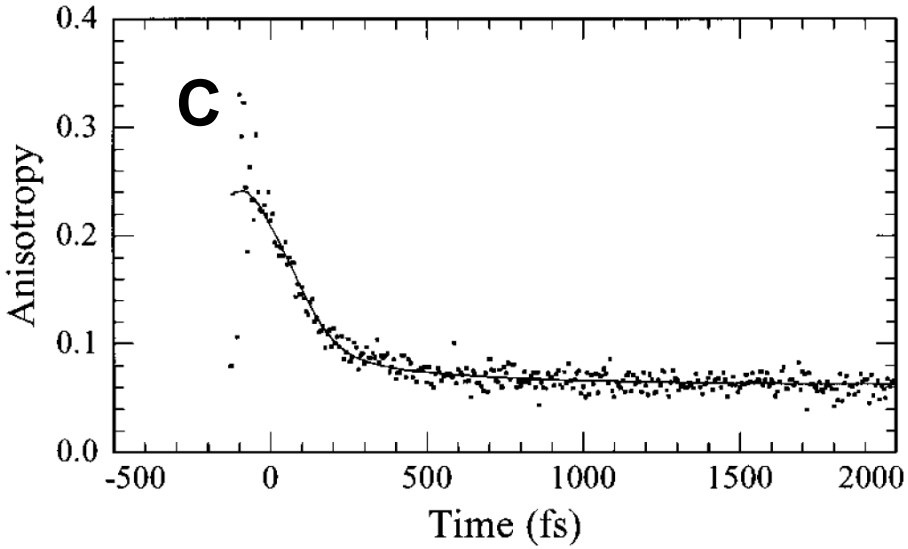


Figure 10.1. Schematic diagram for measurement of fluorescence anisotropies.

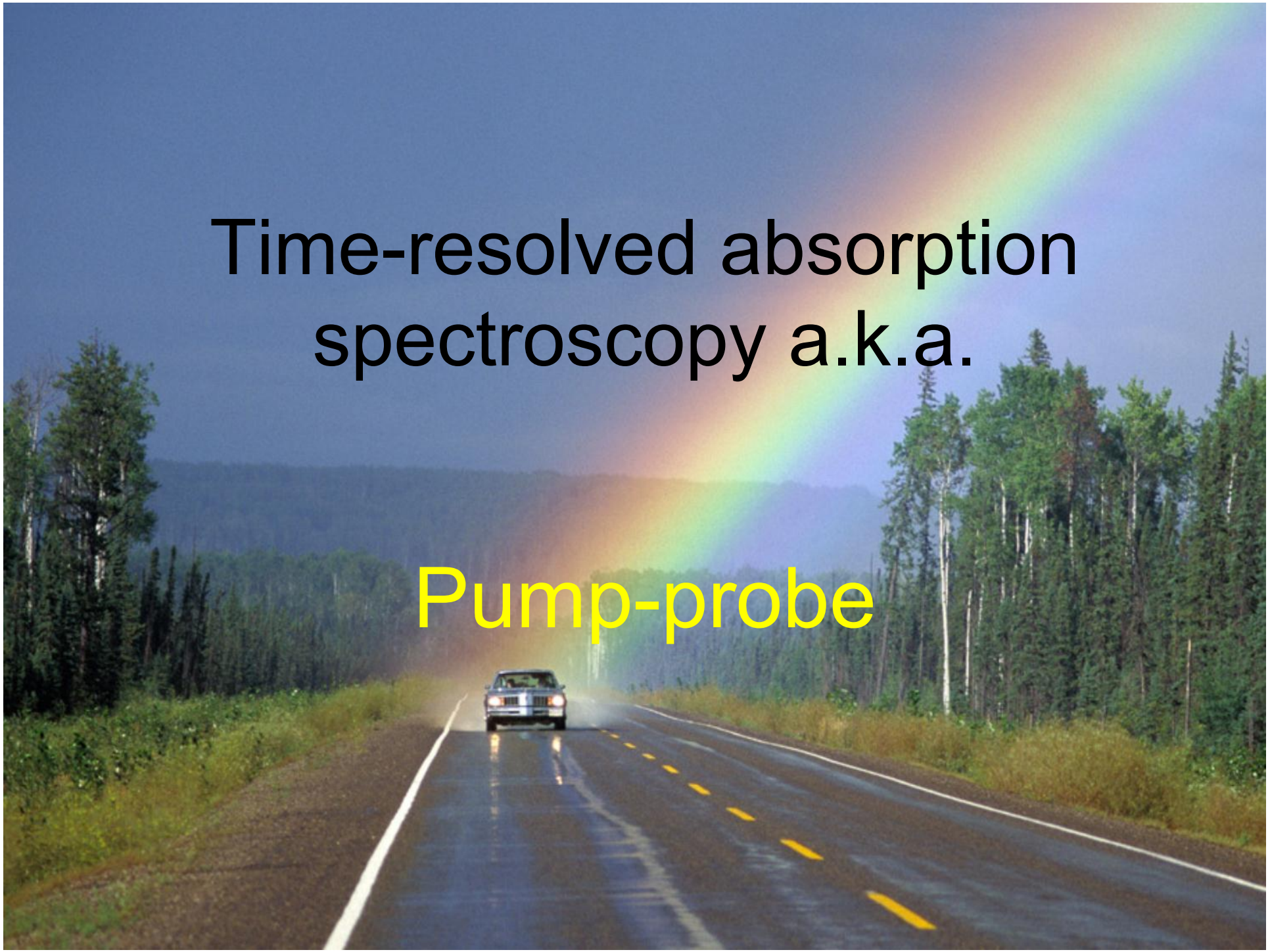




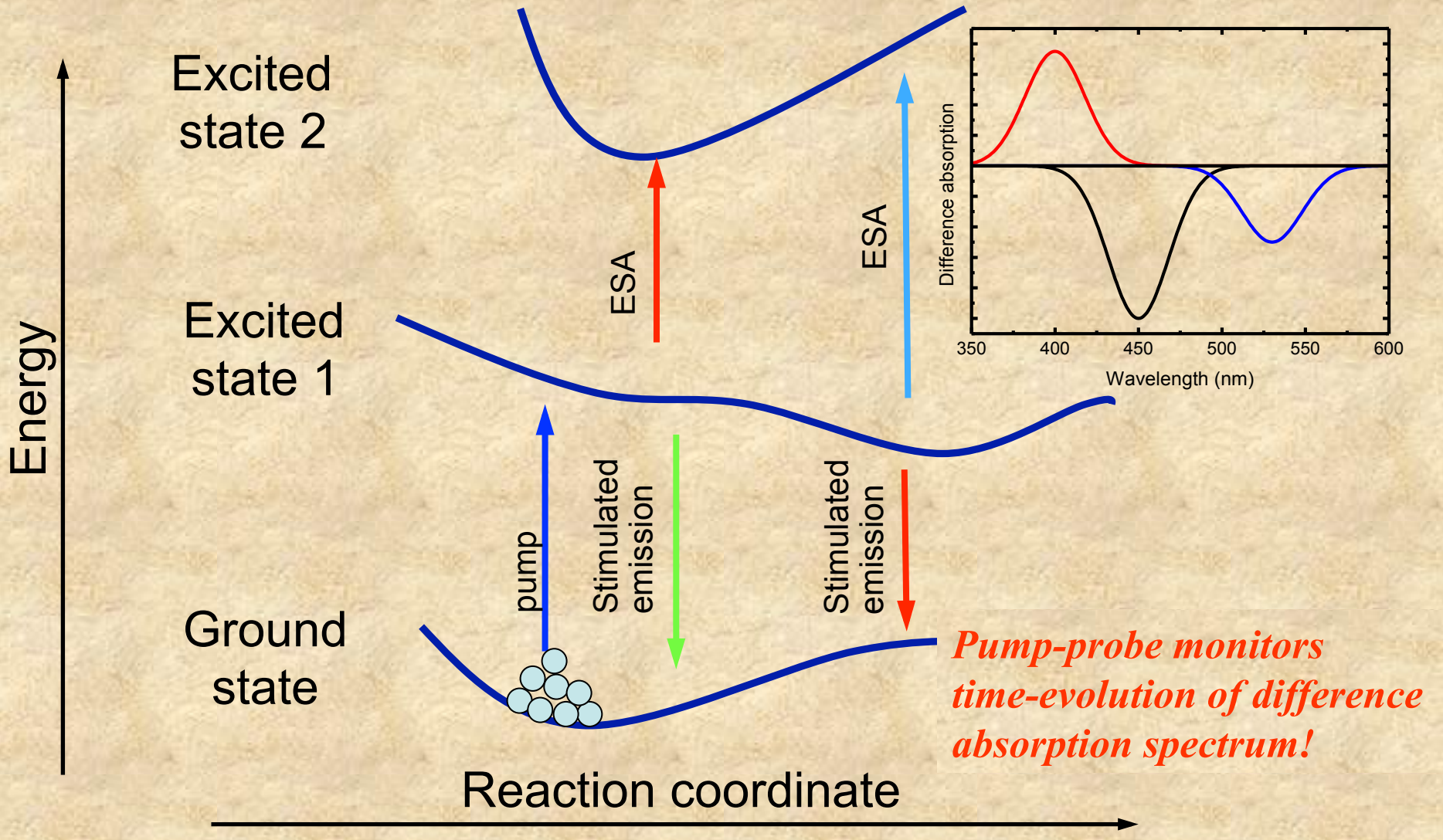
Fluorescence is good, but...

- Contains only the information about the excited states, whereas interesting things happen in ground state as well...

Therefore we switch to...

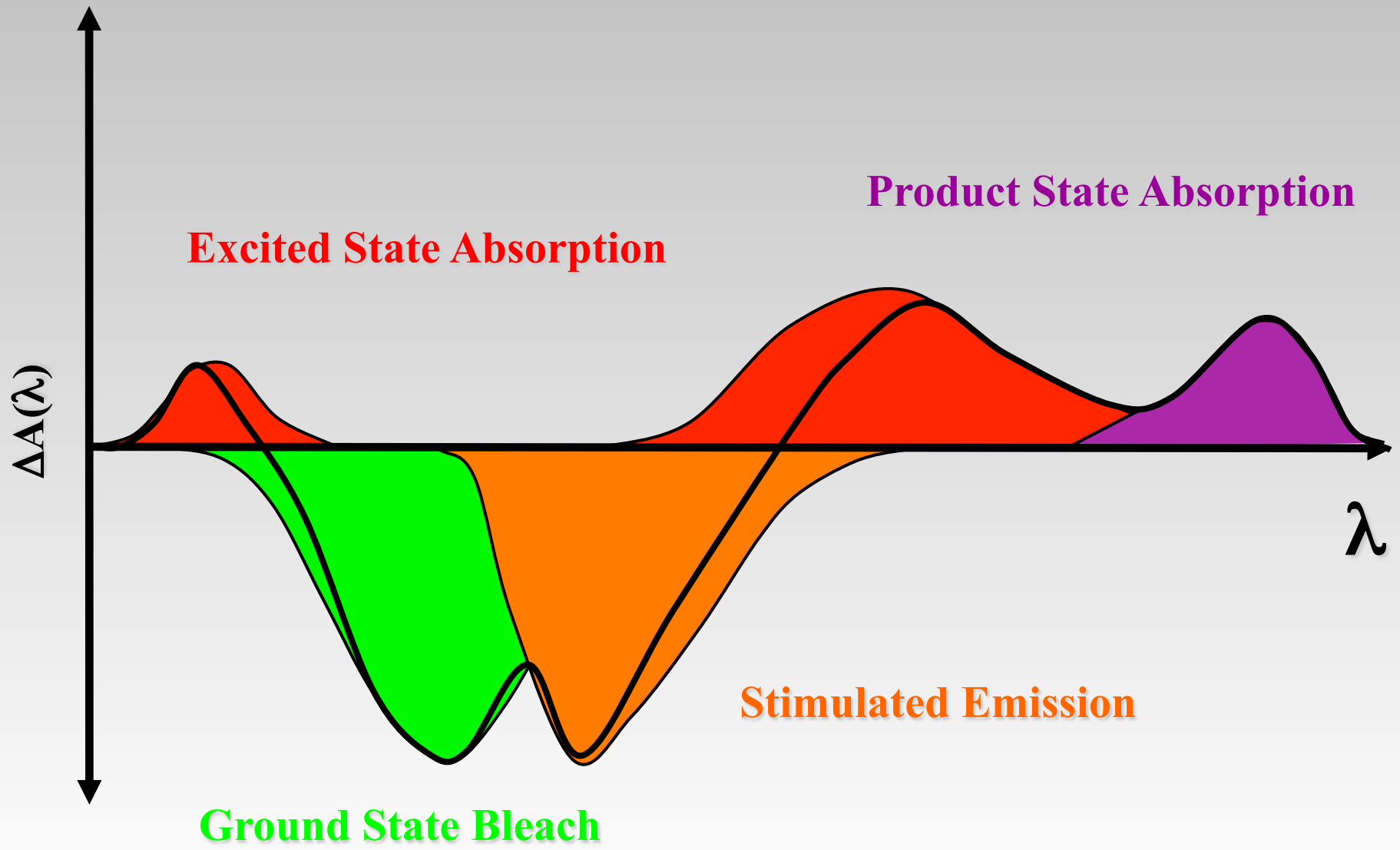

Time-resolved absorption
spectroscopy a.k.a.
Pump-probe

Pump-probe: a traditional technique of monitoring photodynamics



Pump-probe monitors time-evolution of difference absorption spectrum!

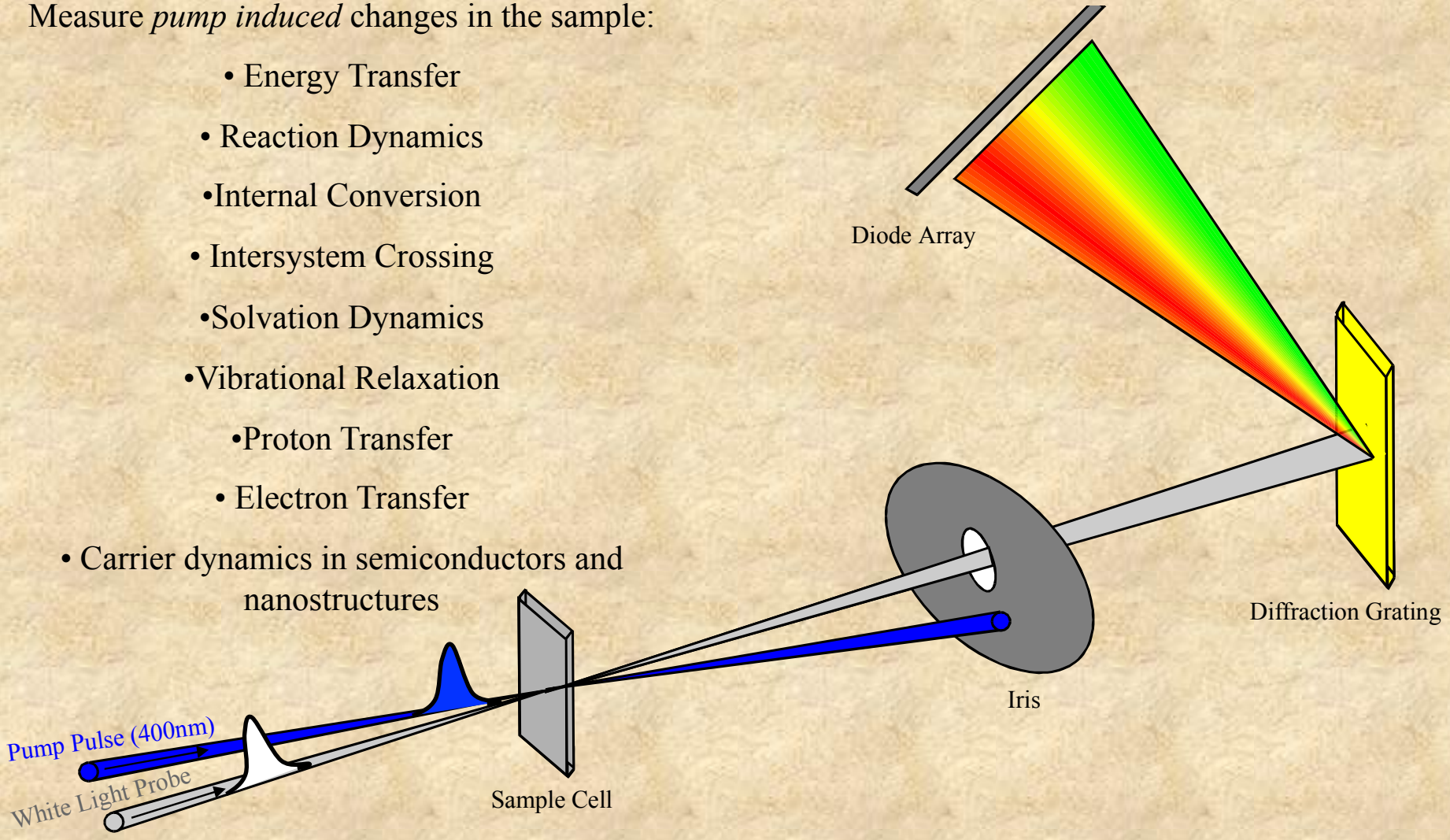
Decomposing Transient Absorption Spectra

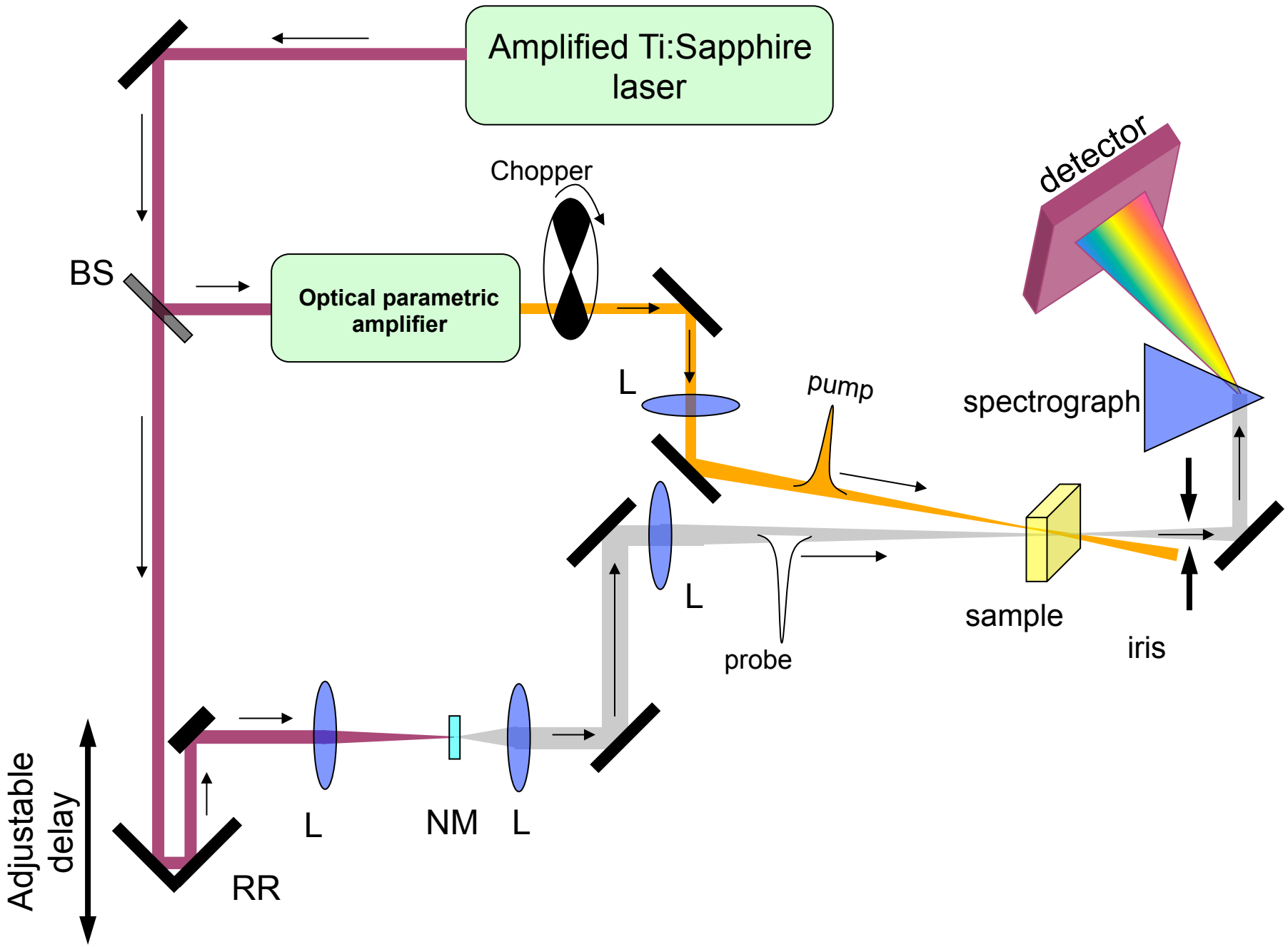


Dispersed Pump-Probe Experimental Setup

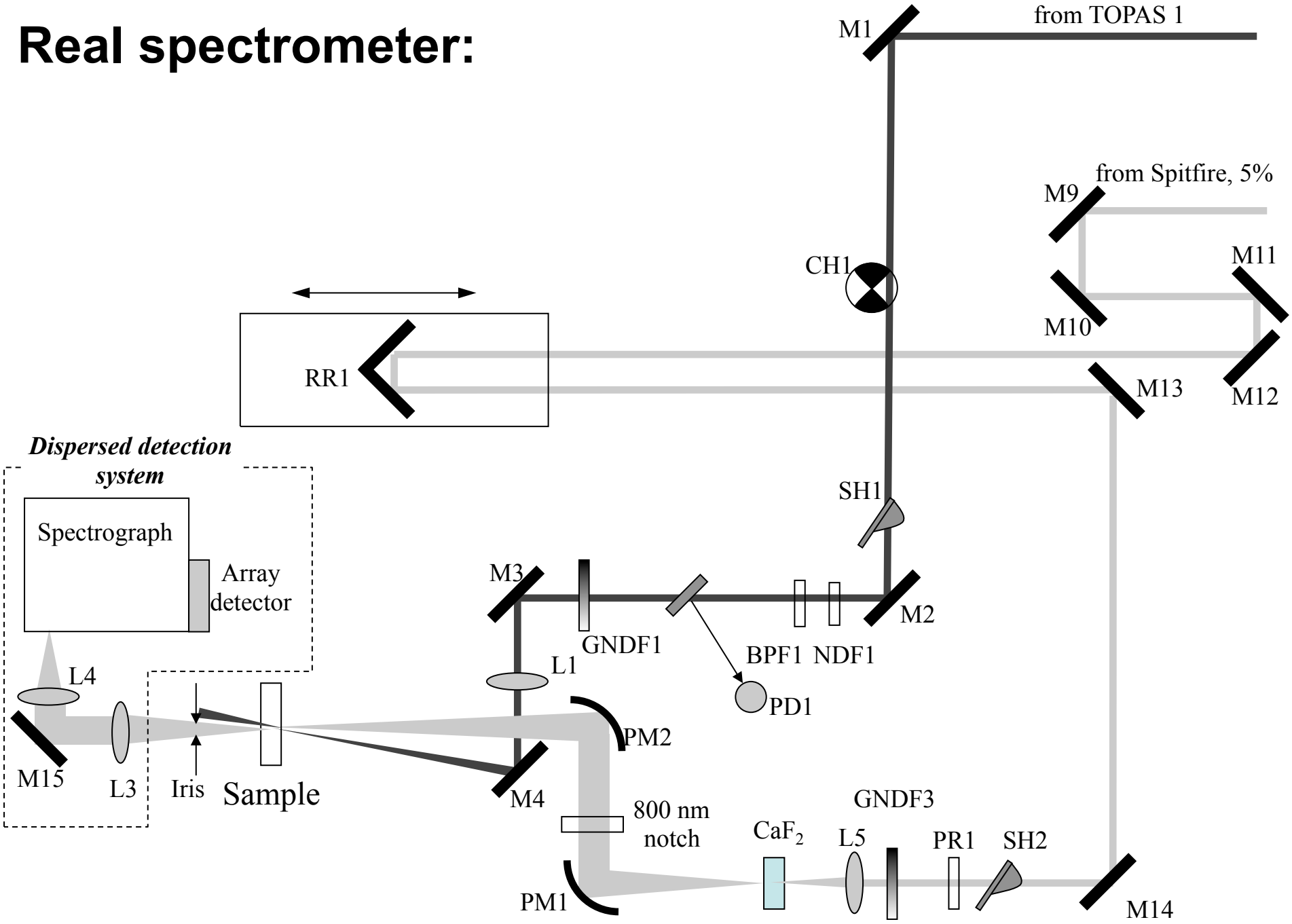
Measure *pump induced* changes in the sample:

- Energy Transfer
- Reaction Dynamics
- Internal Conversion
- Intersystem Crossing
- Solvation Dynamics
- Vibrational Relaxation
- Proton Transfer
- Electron Transfer
- Carrier dynamics in semiconductors and nanostructures



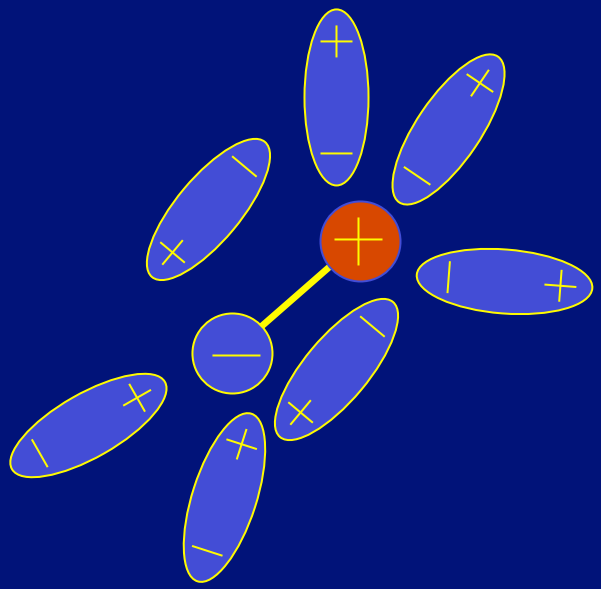

Amplified Ti:Sapphire laser
Chopper
detector
BS
Optical parametric amplifier
L
pump
spectrograph
L
sample
probe
iris
L
NM
L
Adjustable delay
RR

Real spectrometer:

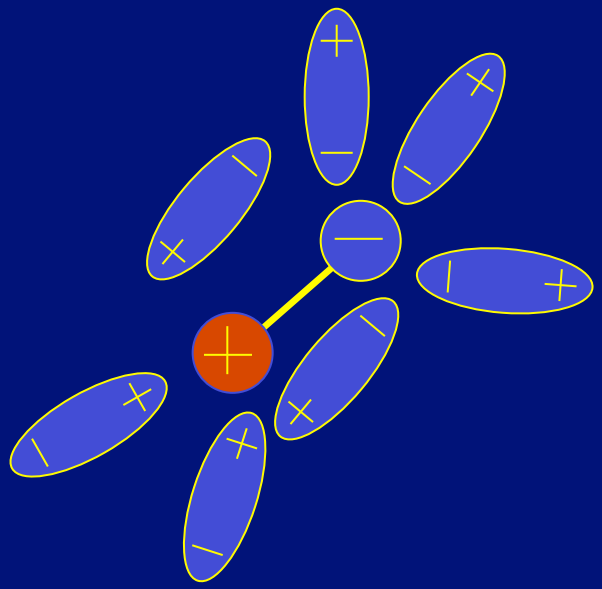


Conceptual example: solvation

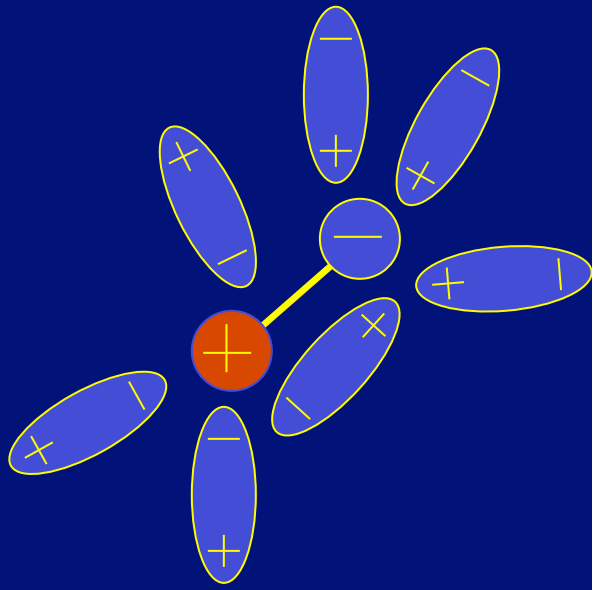
Ground state



Excited state at t=0

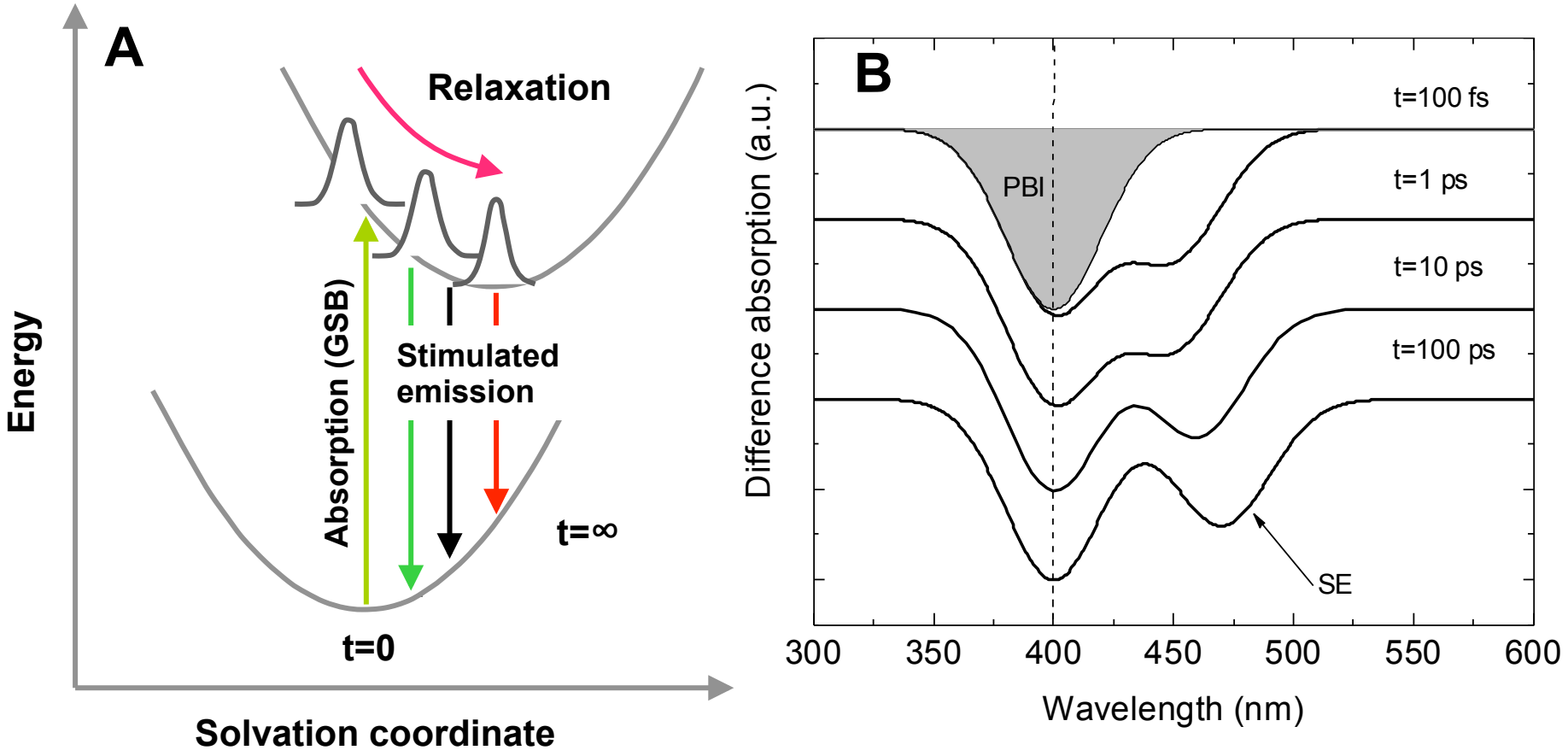


Excited state after some time

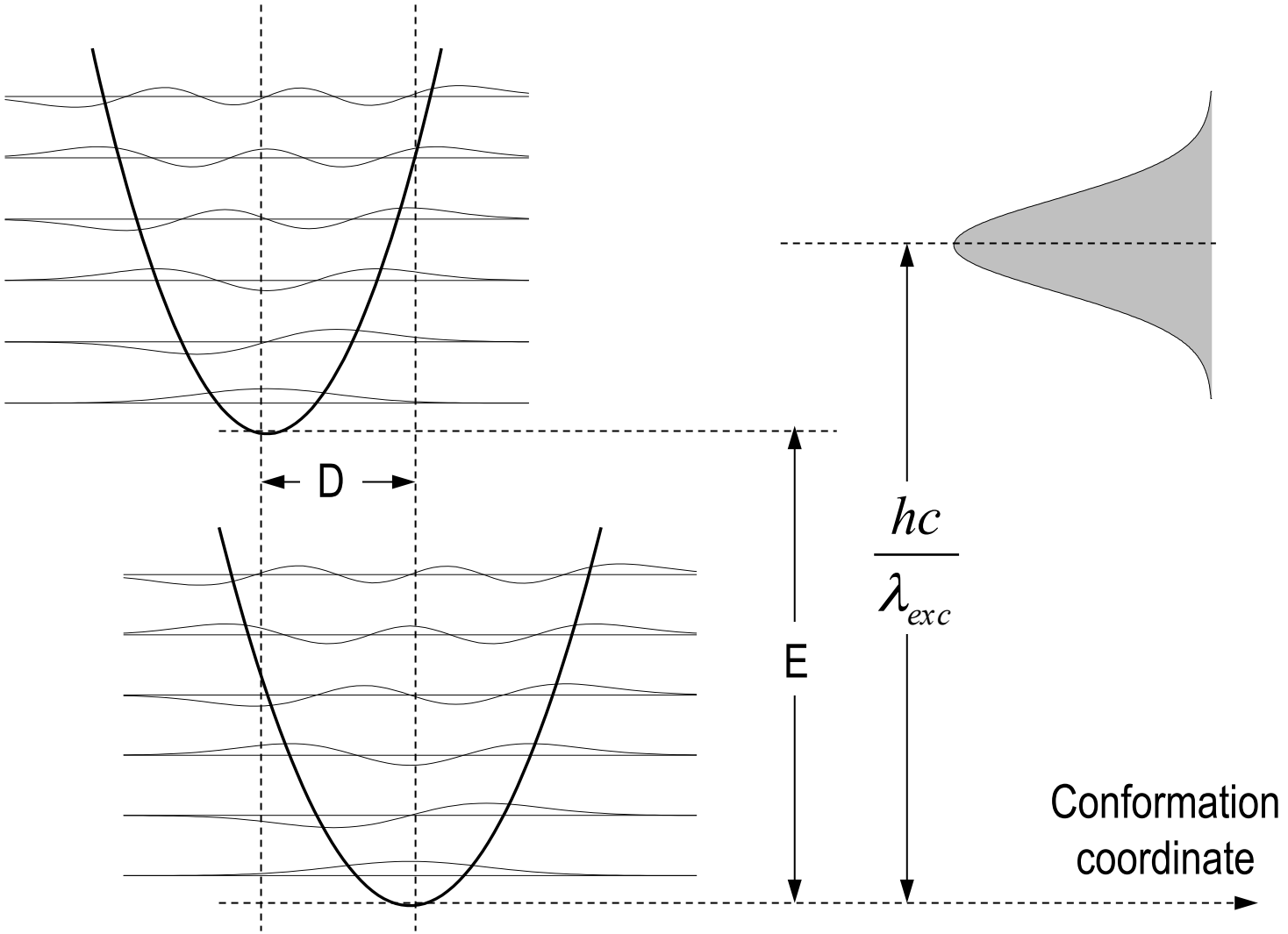


Adjustment of solvent molecules around the solute to minimize the overall system energy.

Conceptual example: solvation



Conceptual example 2: two electronic levels coupled to 1 vibrational mode



Some basic quantum mechanics...

• The pulse excites several vibrational sub-states at once (forms so-caled excited-state wavepacket):

$$\Psi_{exc}(x,t) = \sum_j a_j(t)\psi_j(x)$$

Where $\psi_j(x)$ - oscillator eigenfunctions

$a_j(t)$ - time-dependent amplitudes

Some basic quantum mechanics...

- After plugging this into time dependent Schrödinger equation

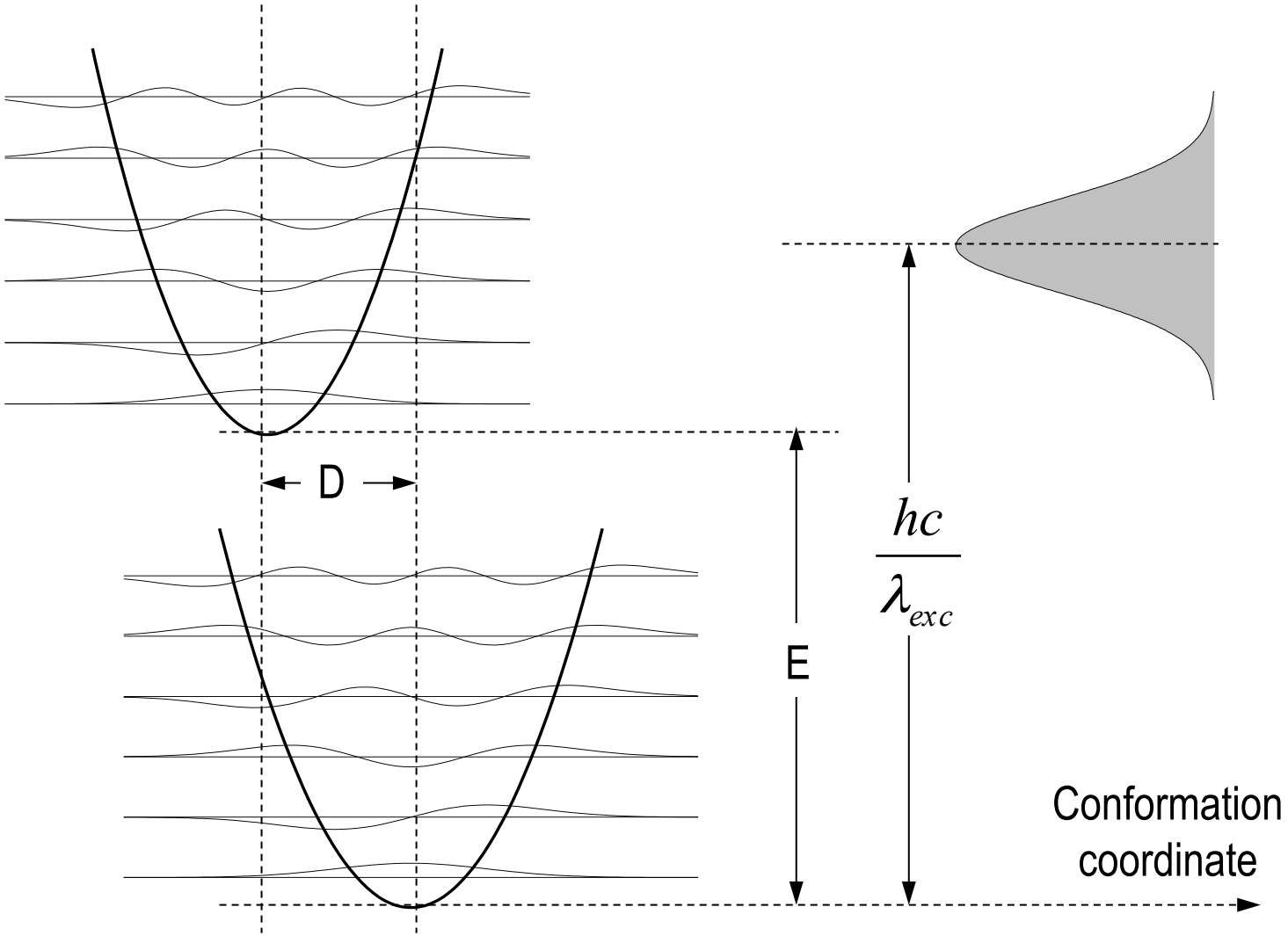
$$i\hbar \frac{\partial}{\partial t}\Psi = \hat{H}\Psi$$

$$\cdot \psi_k^*(x), \int \ldots dx \quad \Big| \quad i\hbar \sum_j \psi_j(x) \frac{\partial}{\partial t} a_j(t) = \hat{H} \sum_j a_j(t) \psi_j(x)$$

$$\frac{\partial}{\partial t} a_k(t) = i\omega_v \left(\frac{1}{2} + k \right) a_k(t)$$

$$a_k(t) = a_k(0) e^{-i\omega_v \left(k + \frac{1}{2} \right) t}$$

Oversimplified model system: two electronic levels coupled to 1 vibrational mode



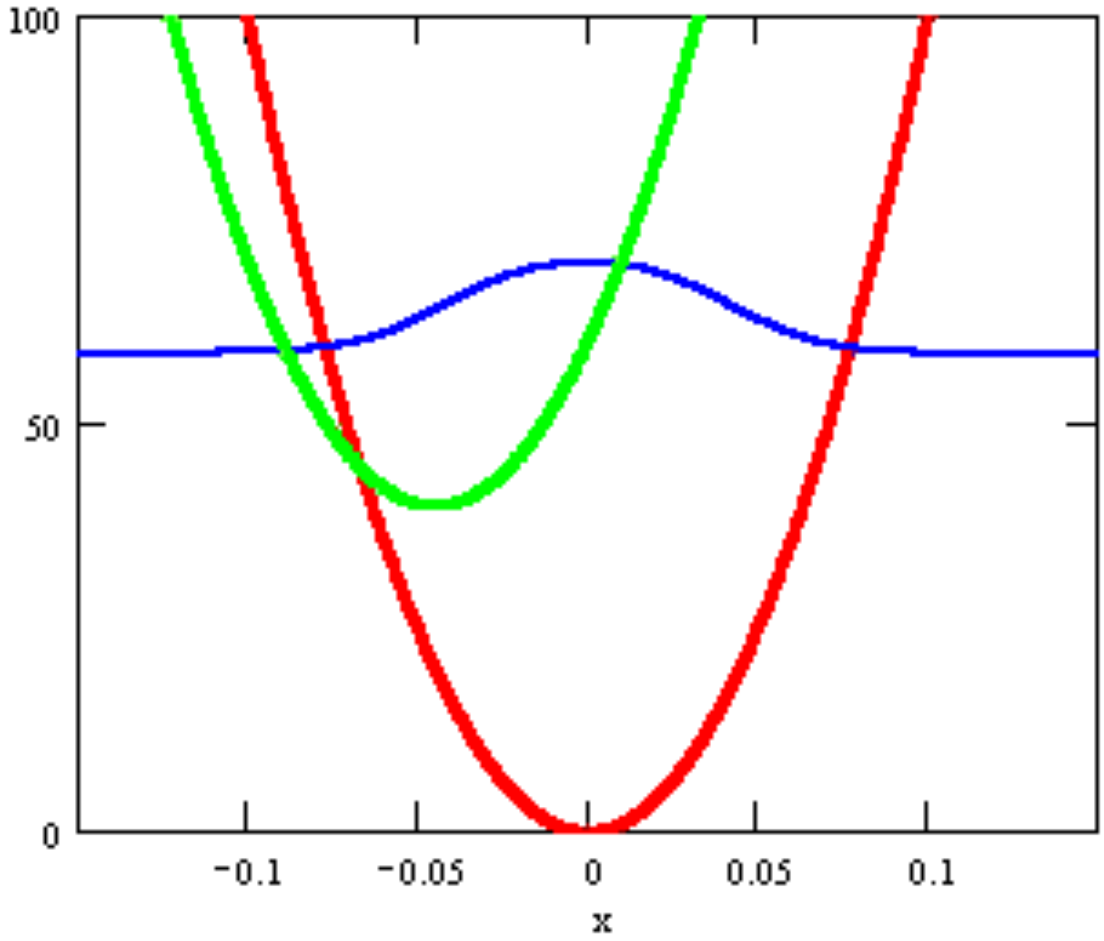
Initial population of the vibrational substates in the excited electronic state

$a_k(0)$ depend on:

- Population of ground state vibronic sublevels (Bolzman factor);
- Overlaps between ground- and excited-state vibrational wavefunctions (Frank-Condon factors);
- Width and central frequency of the excitation pulse;

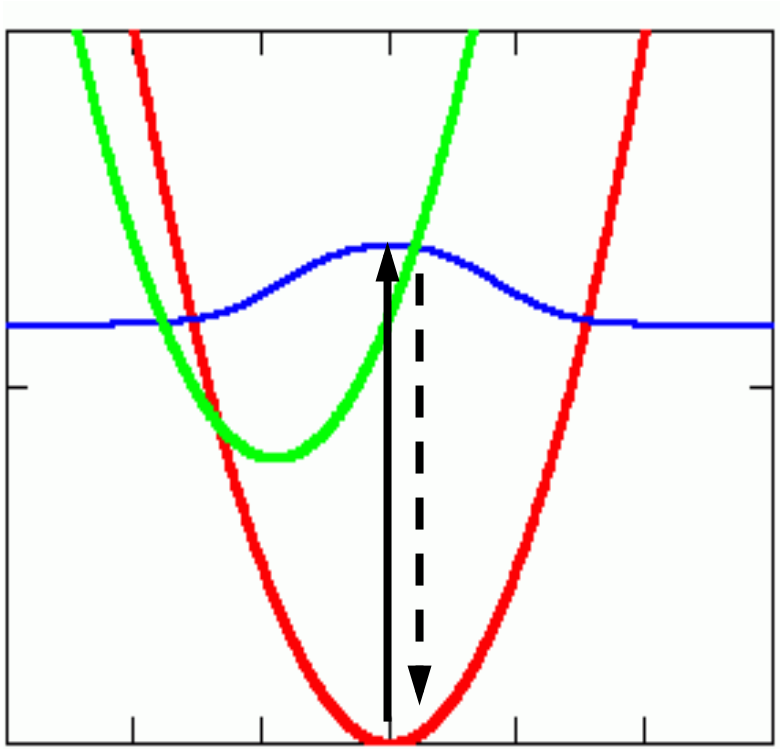
- **All of this can be calculated for our model!**

Example: red-edge excitation, 100 fs pulse, 77K

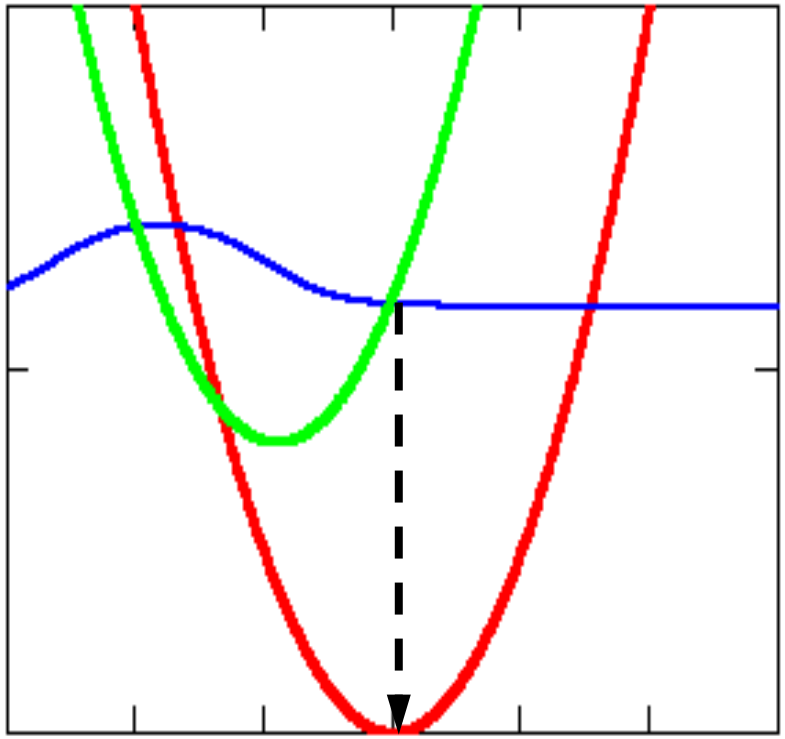


The reason for oscillations in pump-probe

Probed at time zero:



Probed after half period:



Generalization

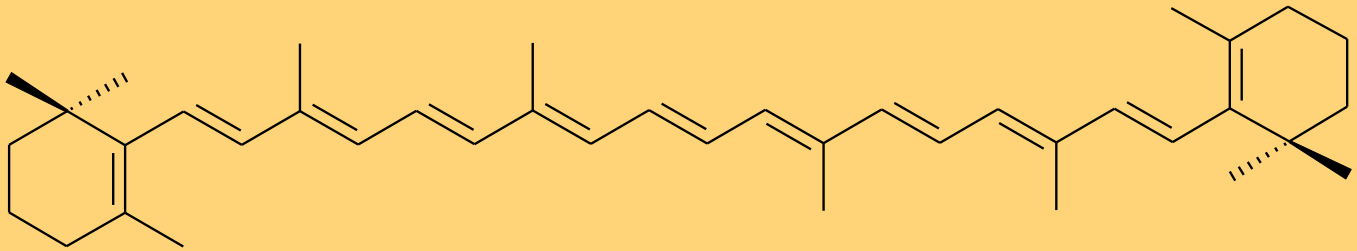
When electron-vibronic system is excited with an ultrashort pulse (its spectrum has to cover several vibrational levels), oscillations will be observed in pump-probe time dependence, corresponding to the wavepacket motion.

The lifetime of these vibrations is typically hundreds of femtoseconds, even several picoseconds (corresponds to the width of vibrational lines in Raman spectrum).

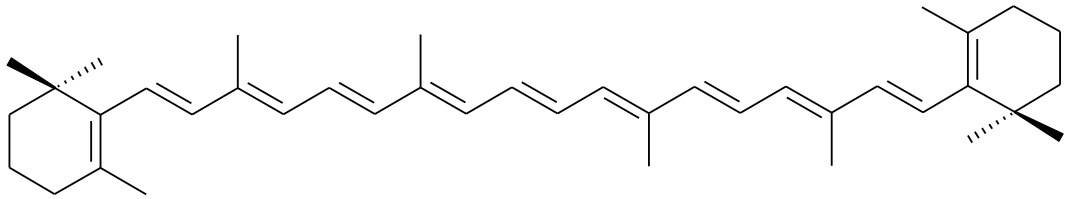
Let’s keep it in the back of our heads for later…


Healthy Origins®
LYC-O-MATO®
Natural Tomato Lycopene
15 MG "Clinical Strength"
60 SoftGel Capsules
A Dietary Supplement

β-carotene: the "model" carotenoid

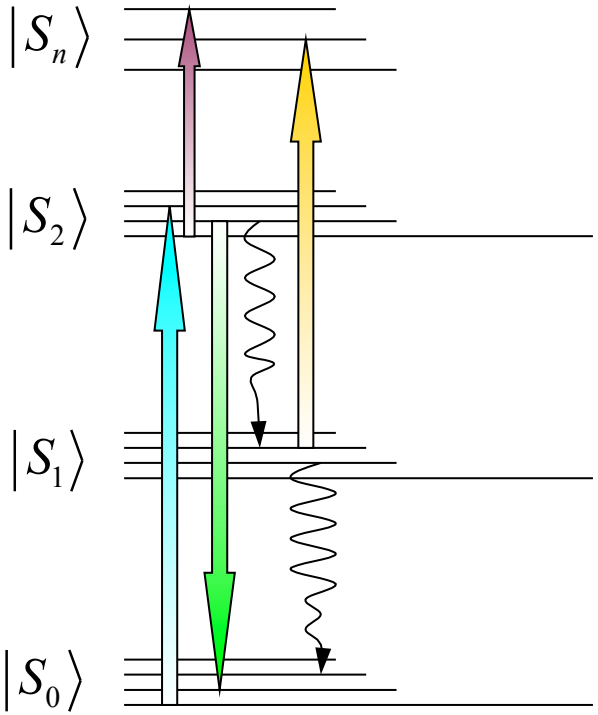


A

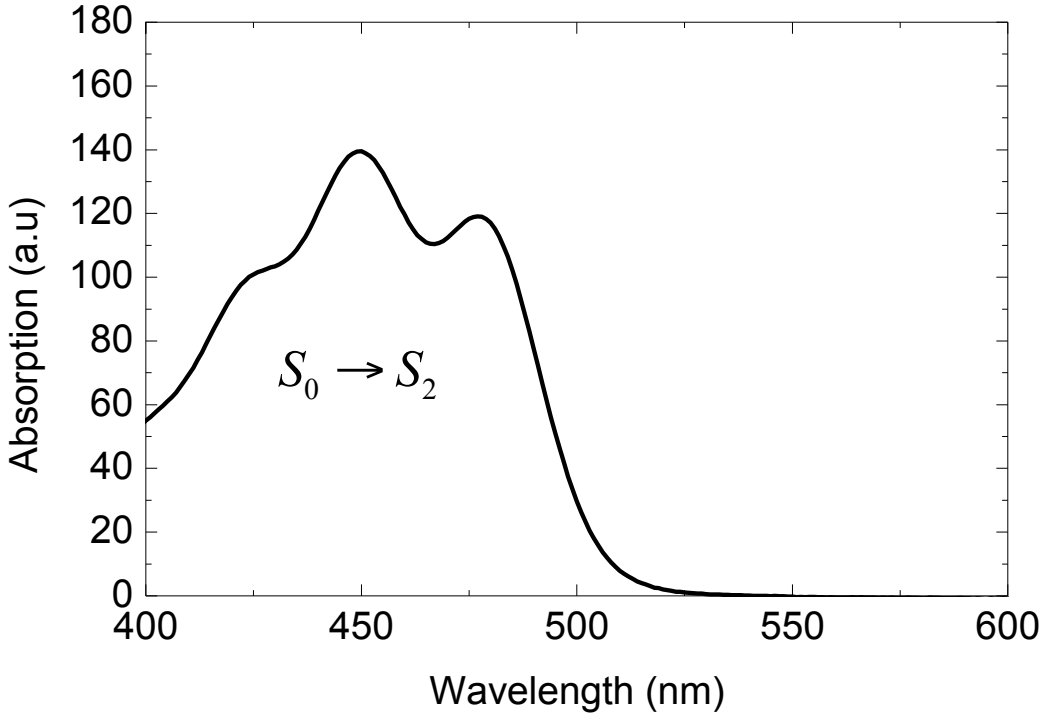


β − carotene

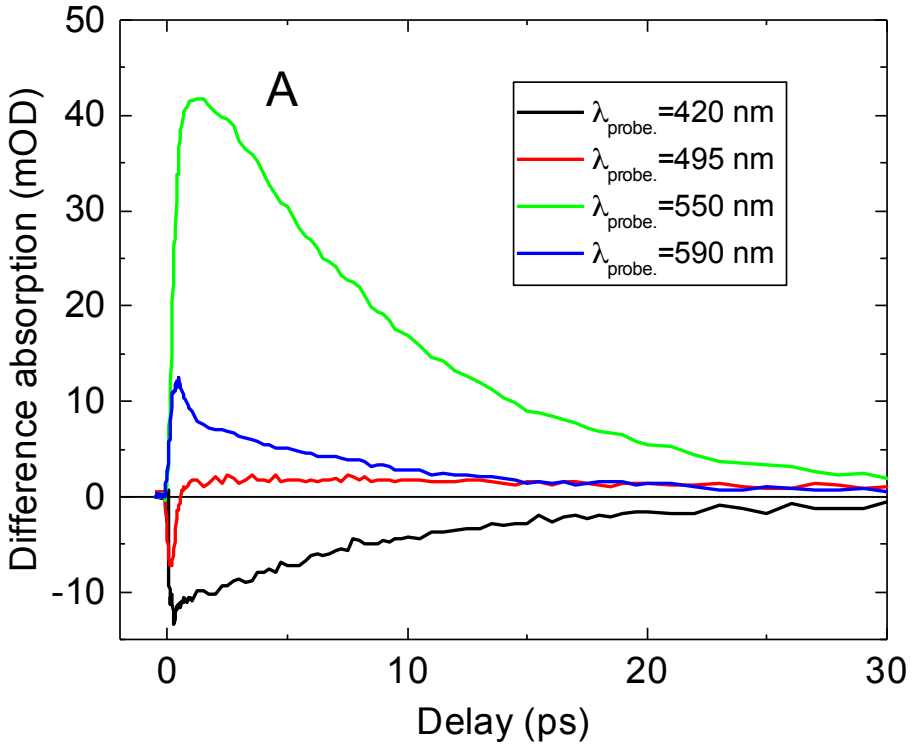
B



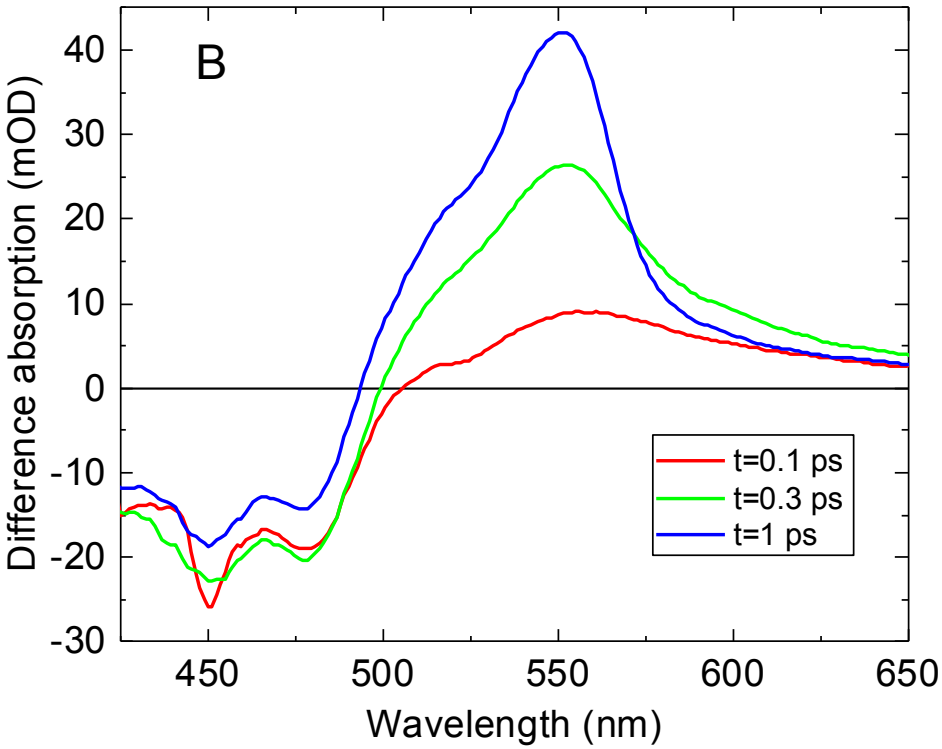
C



Time-resolved kinetics at selected wavelengths



Time-resolved spectra at selected times



Dynamics of SE and ESA bands different from GSB, which implies that it is due to energy relaxation in higher excited states.

Application:
Role of carotenoids in excitation quenching

Leaf response to prolonged illumination – reduction of fluorescence

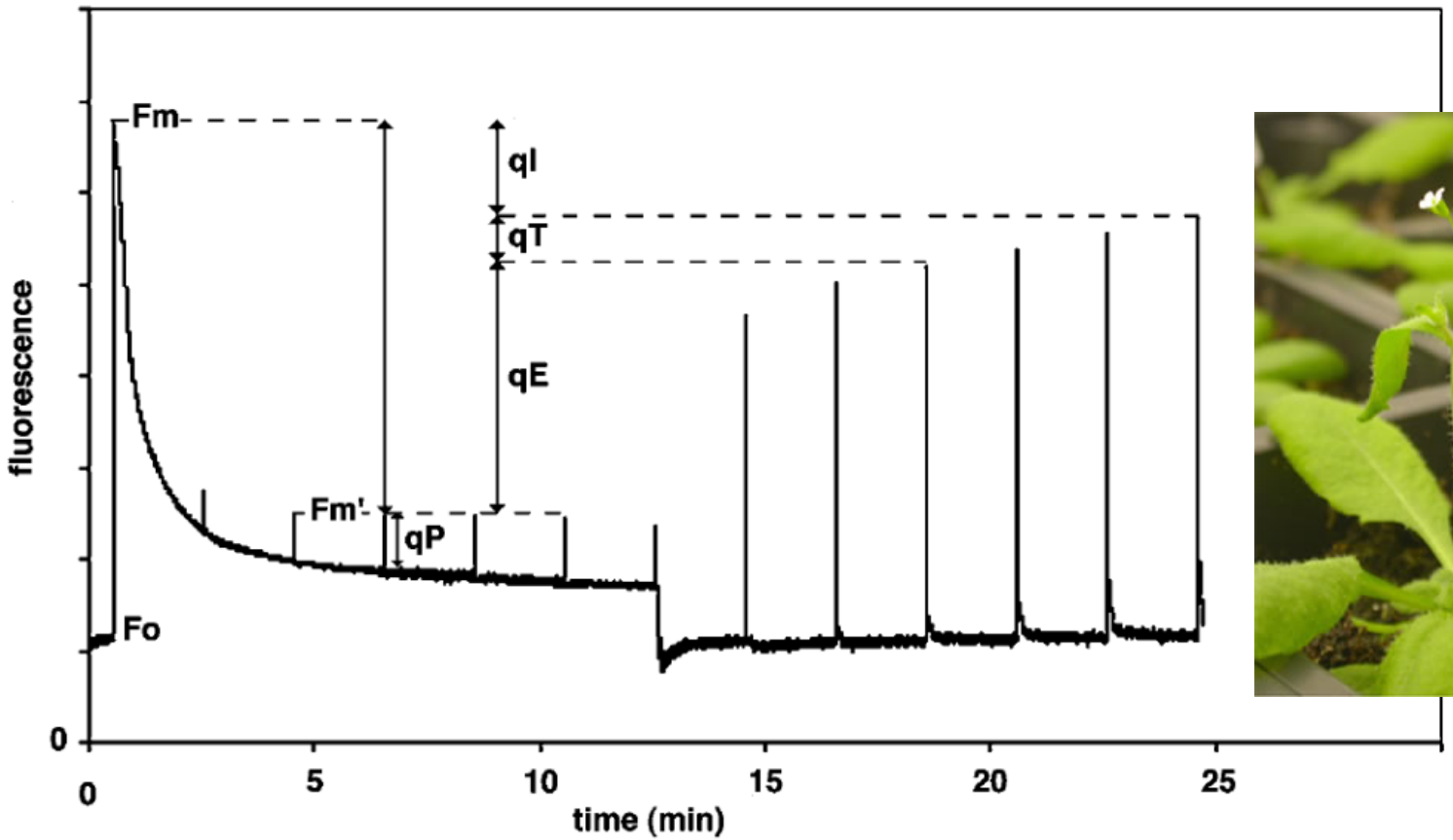
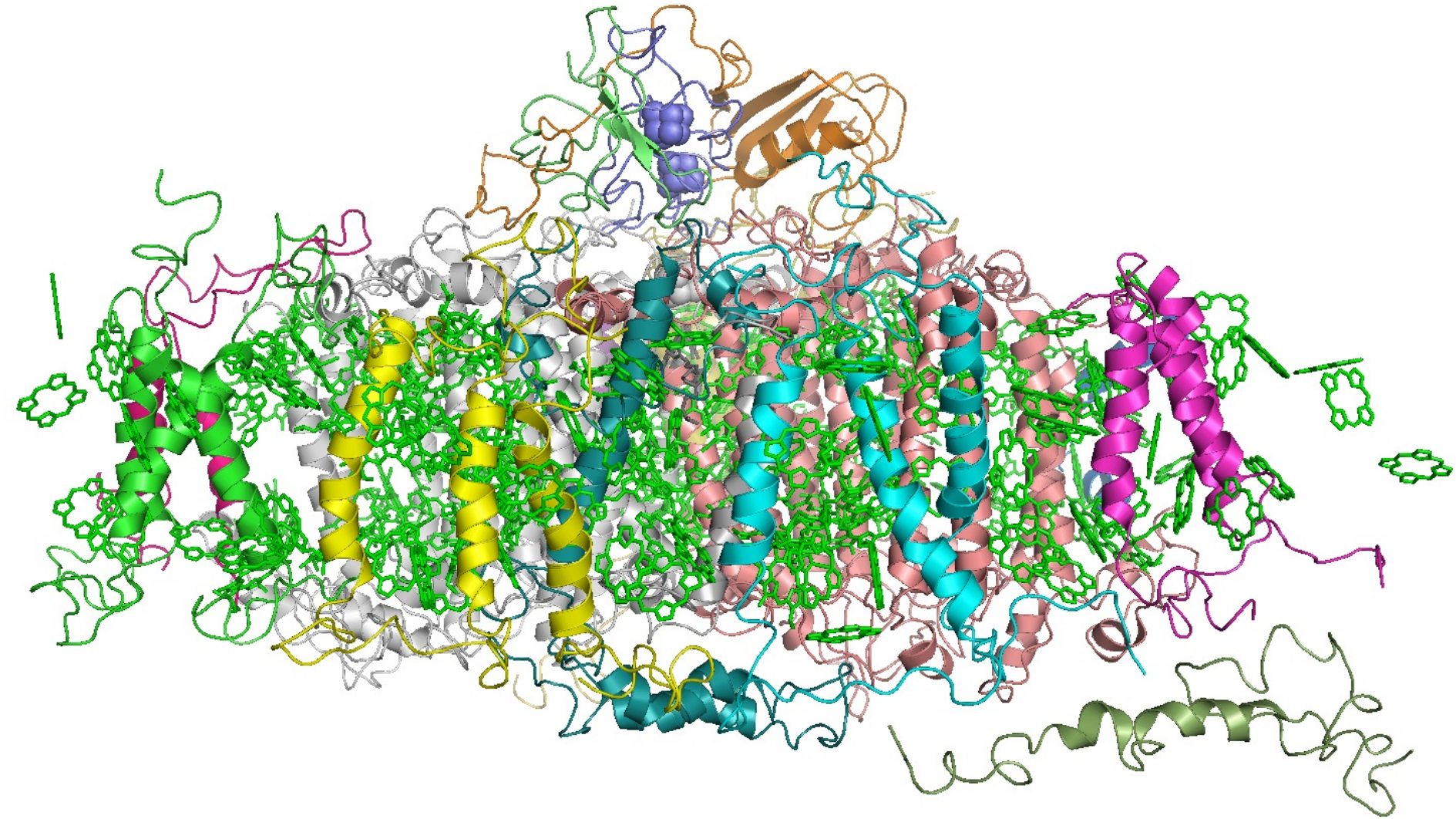
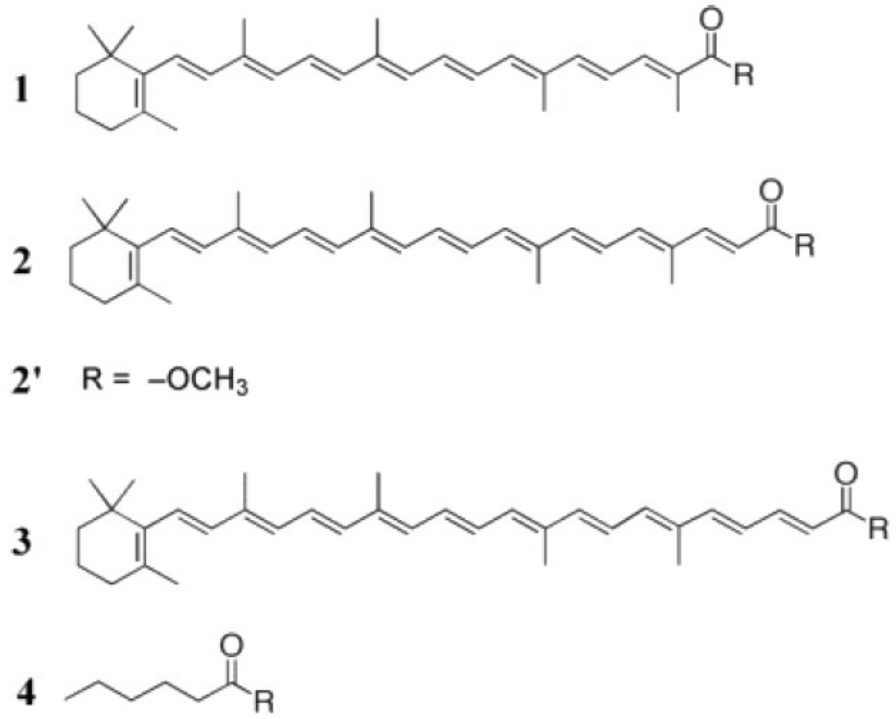
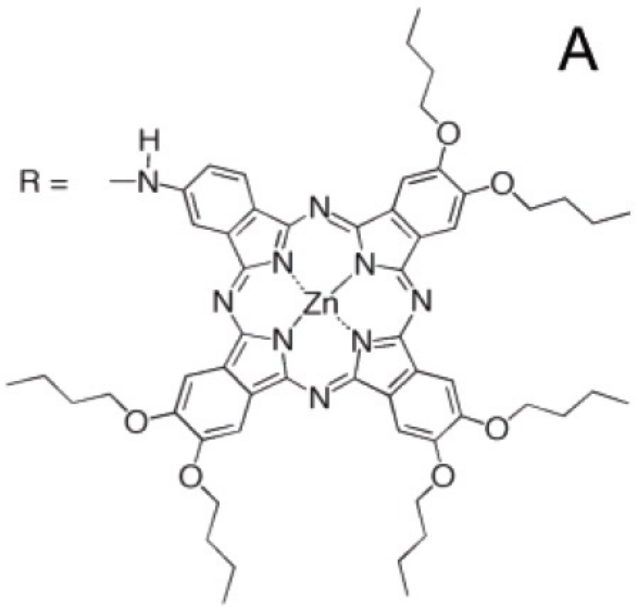
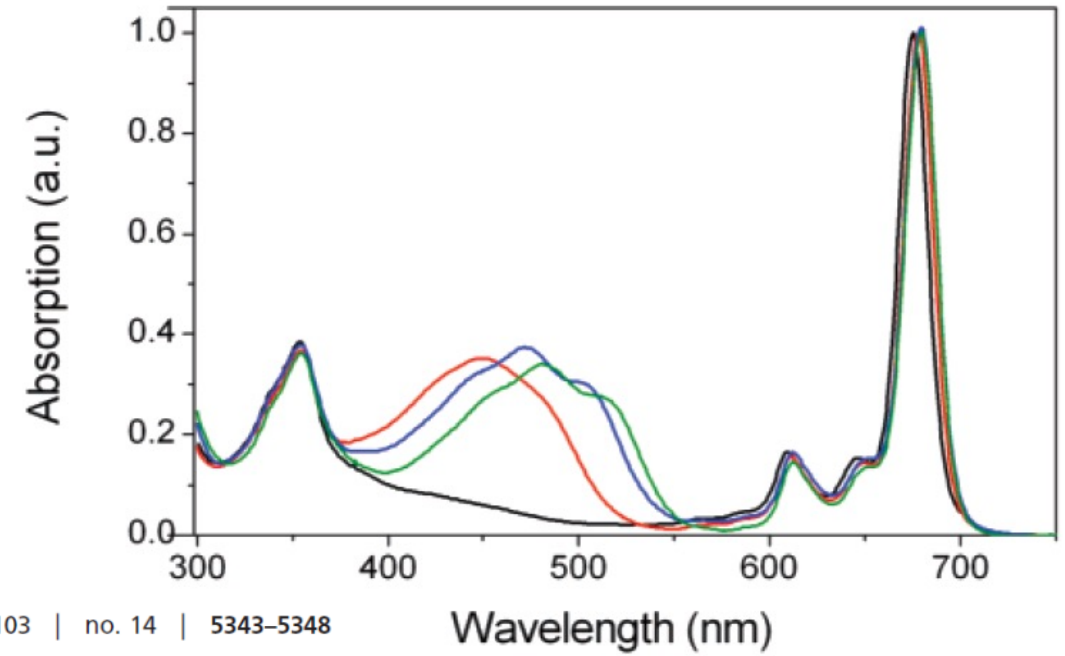


Figure 2. Chl fluorescence measurement from an Arabidopsis leaf. In the presence of only weak measuring light the minimal fluorescence (F_o) is seen. When a saturating light pulse is given, the photosynthetic light reactions are saturated and fluorescence reaches a maximum level (F_m'). Upon continuous illumination with moderately excess light (750 μmol photons m^{-2} sec^1; growth light was 130 μmol photons m^{-2} sec^{-1}), a combination of qP and NPQ lowers the fluorescence yield. NPQ (qE + qT + qI) can be seen as the difference between F_m and the measured maximal fluorescence after a saturating light pulse during illumination (F_m'). After switching off the light, recovery of F_m' within a few minutes reflects relaxation of the qE component of NPQ.



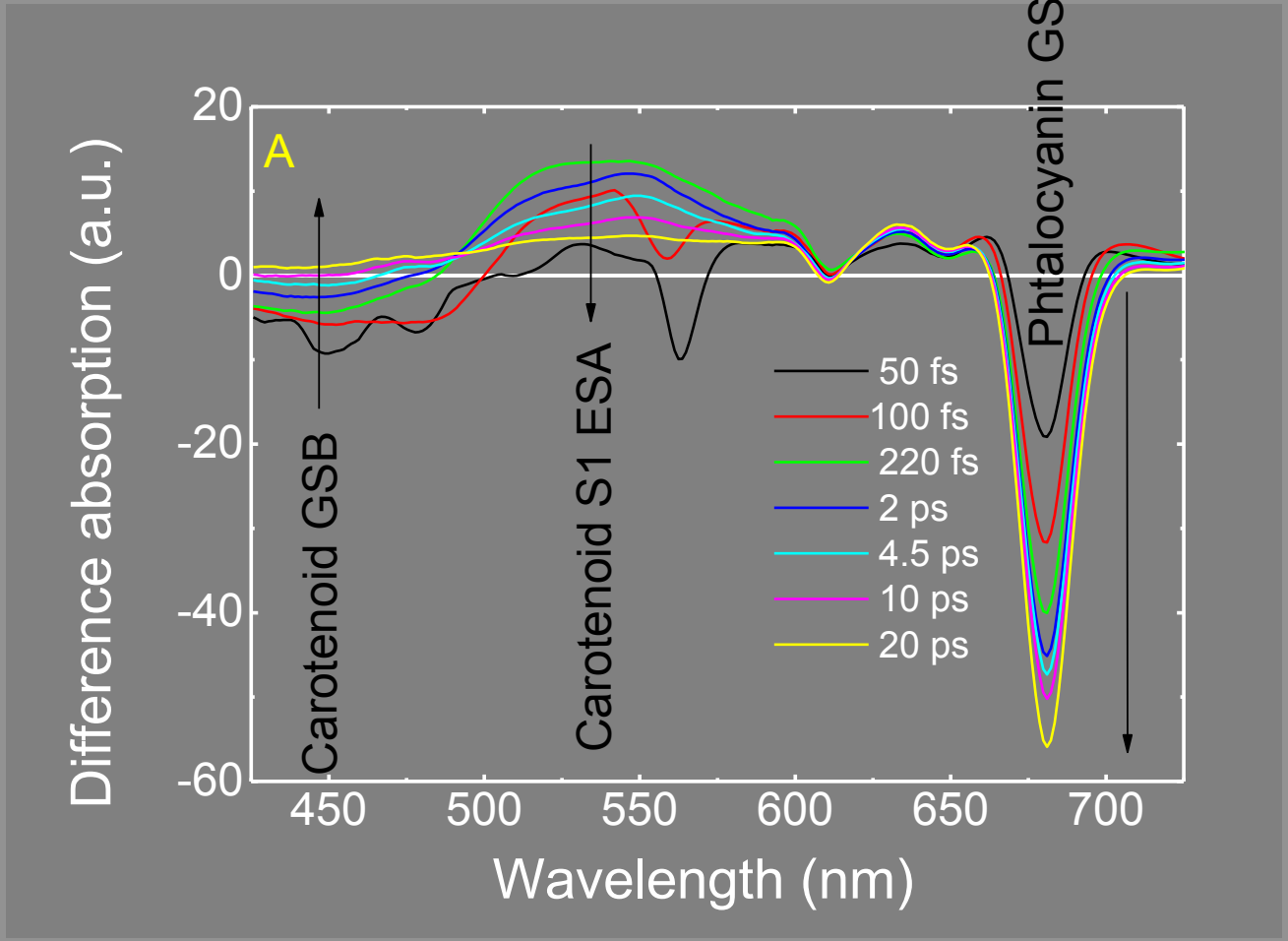

1
2
2' R = –OCH3
3
4


A
R =

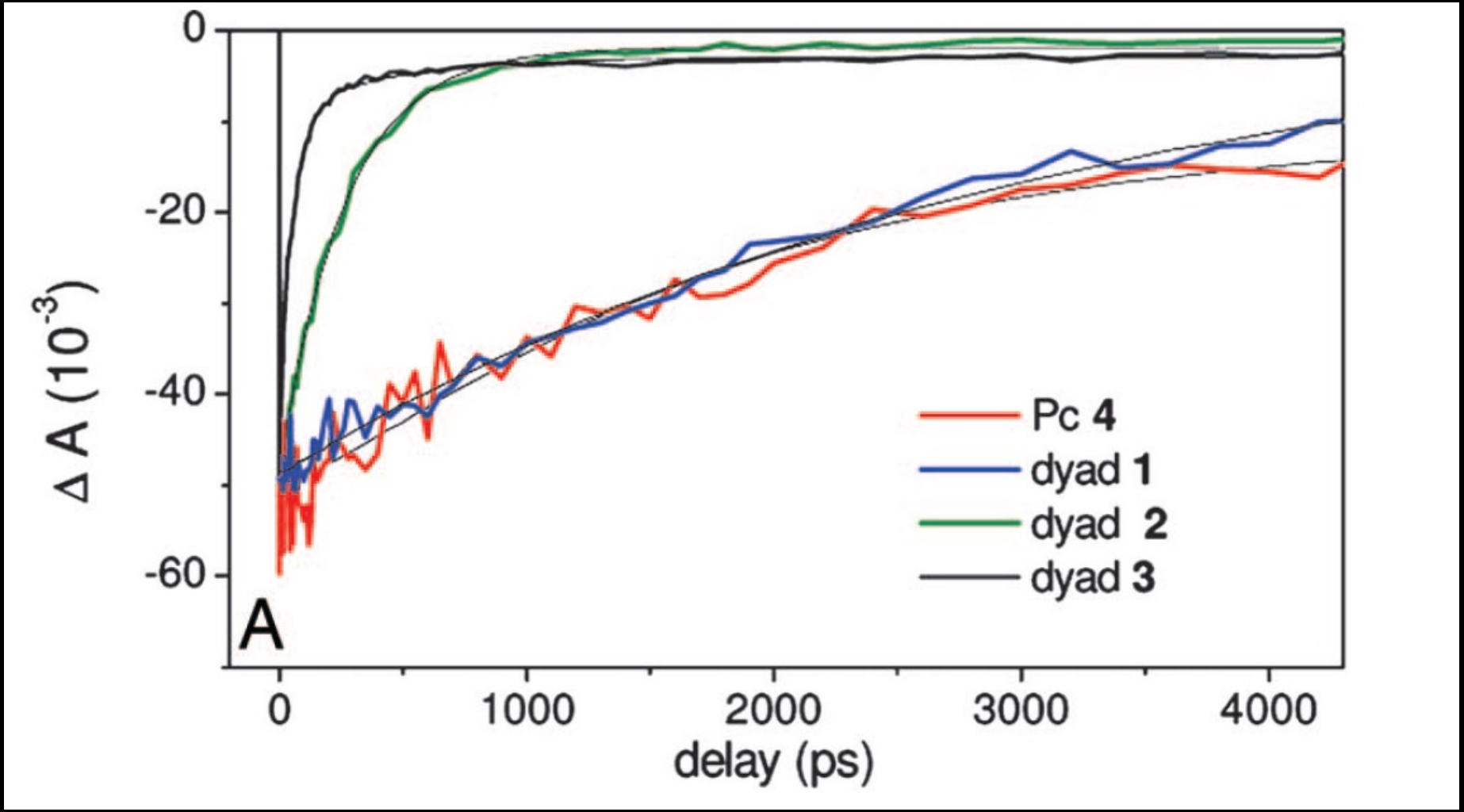

B
Absorption (a.u.)
1.0
0.8
0.6
0.4
0.2
0.0
300
400
500
600
700
Wavelength (nm)

Berera et al.

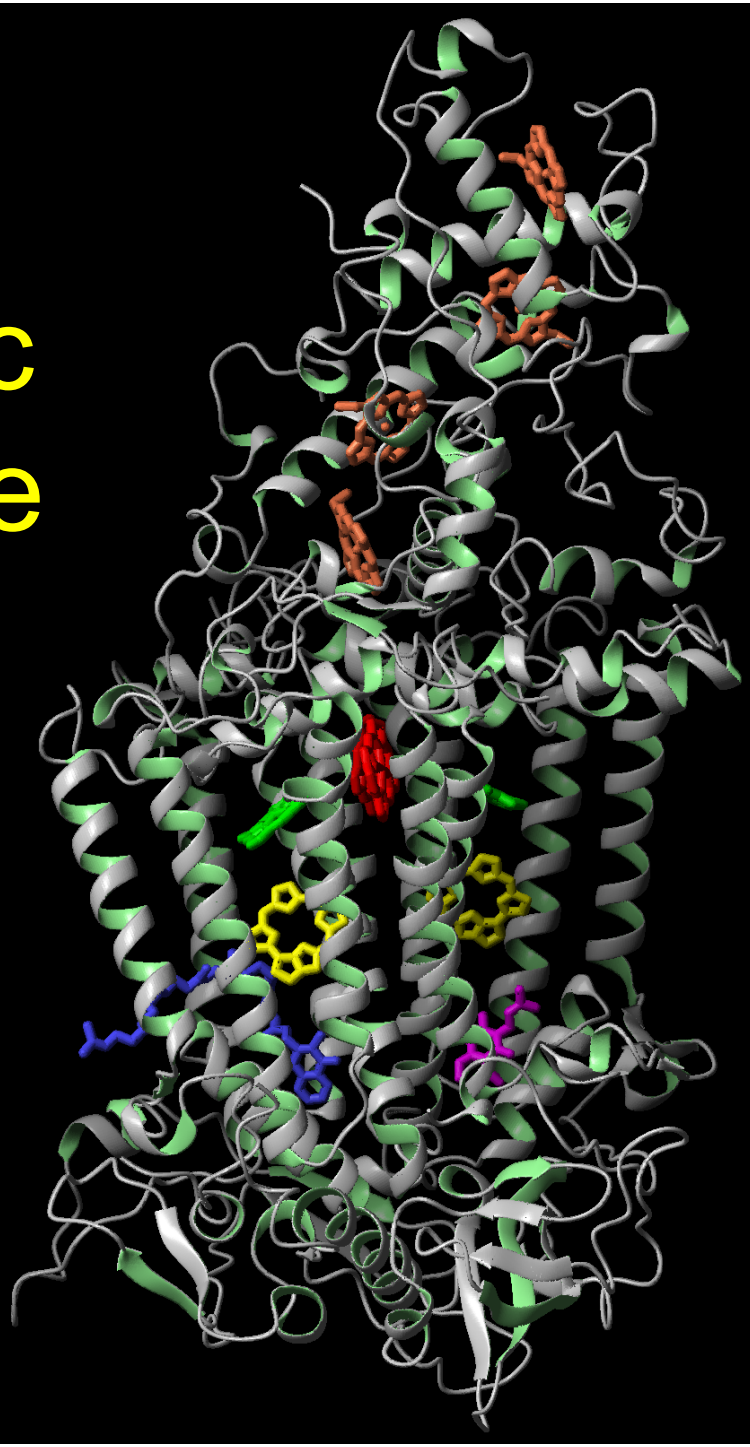
EET dynamics monitored by pump-probe spectroscopy

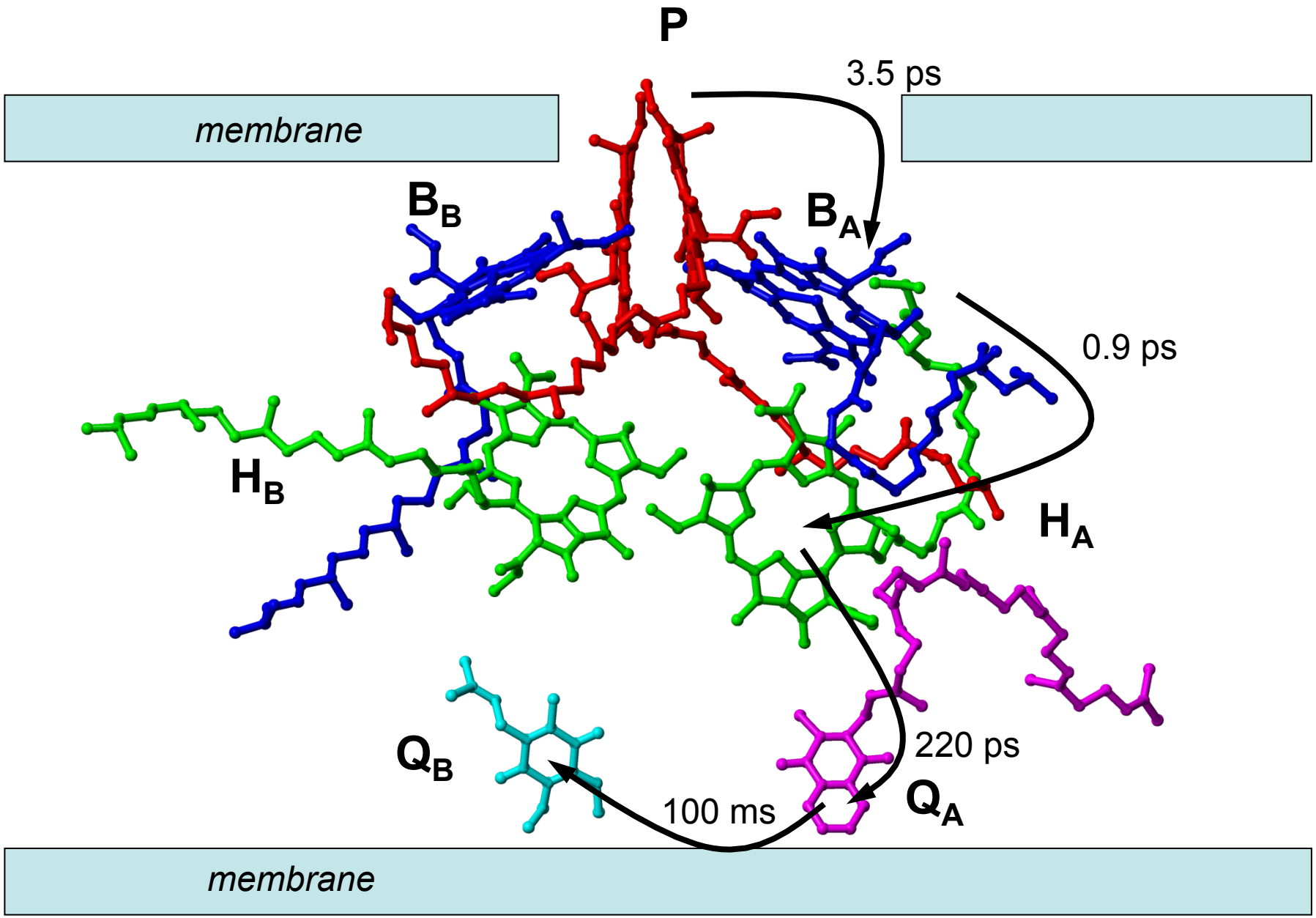


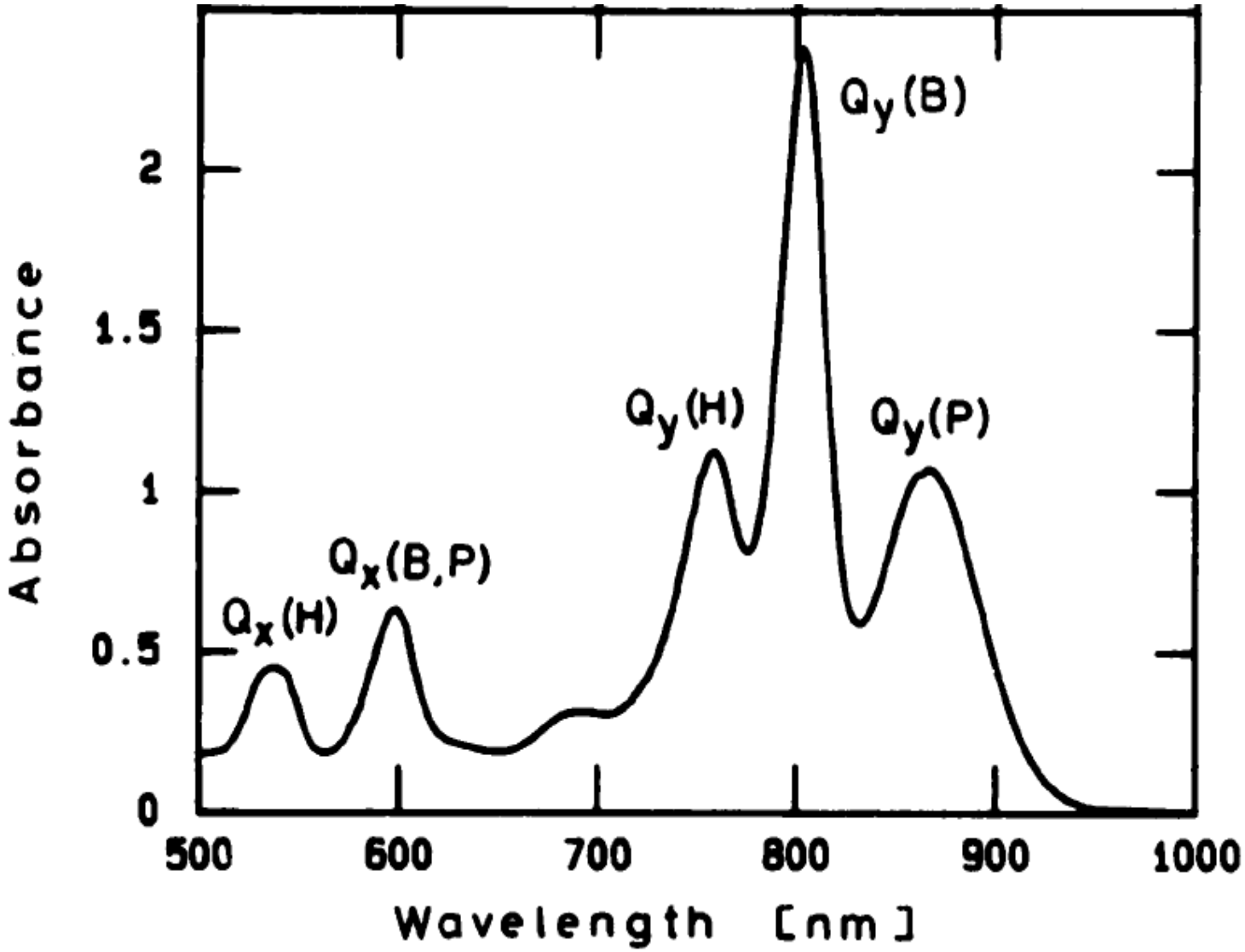
Carotenoid can be both energy donor and acceptor

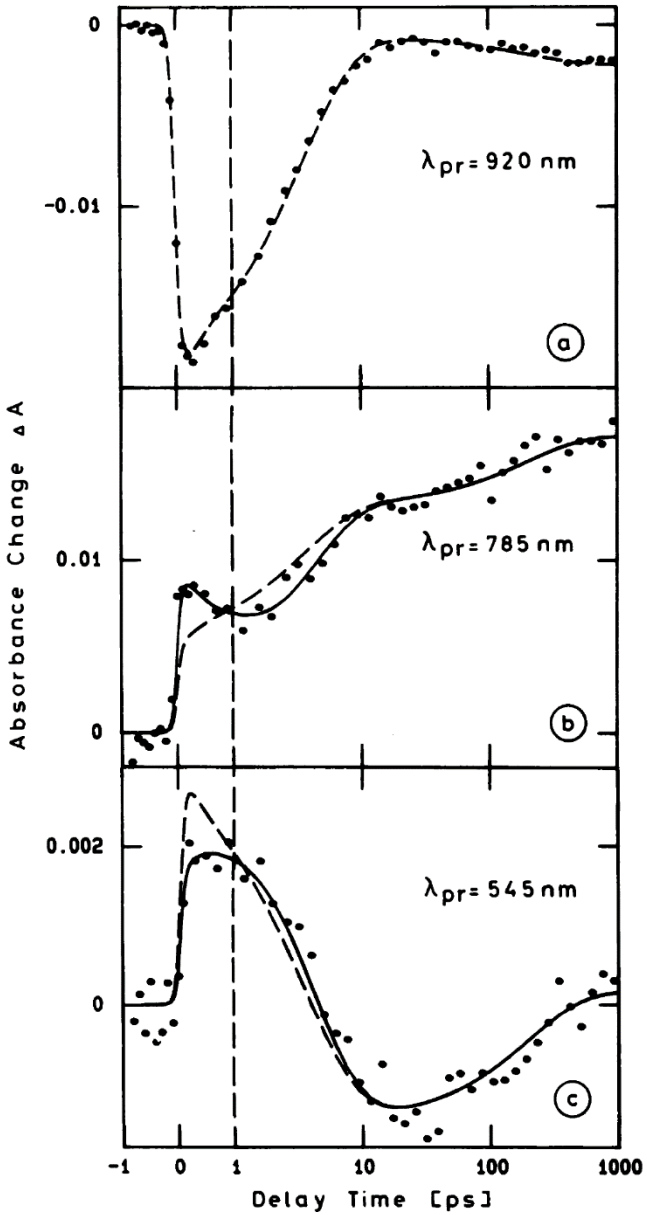


Application: photosynthetic reaction centre




P
3.5 ps
membrane
B_B
B_A
0.9 ps
H_B
H_A
Q_B
220 ps
100 ms
Q_A
membrane


Absorbance
0
0.5
1
1.5
2
500
600
700
800
900
1000
Wavelength [nm]
Qx(H)
Qx(B,P)
Qy(H)
Qy(B)
Qy(P)



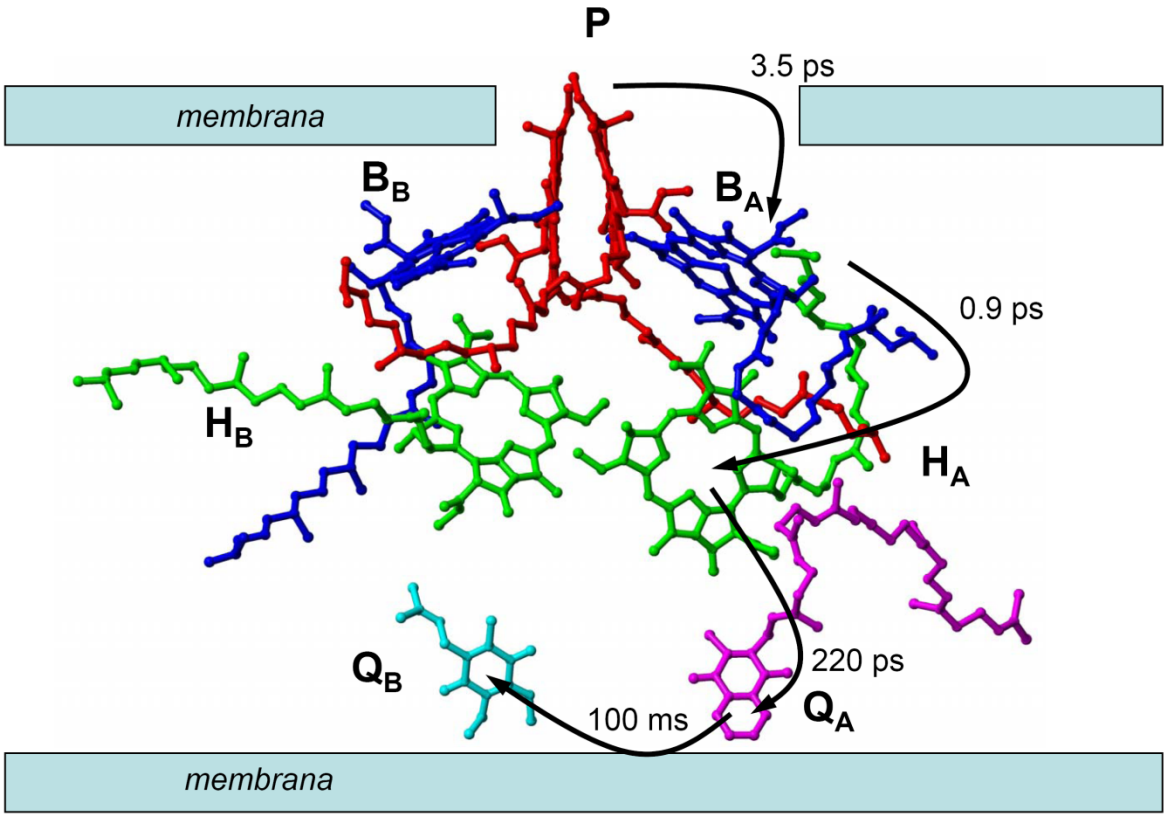
Stimulated emission of the special pair

Induced absorption of special pair and accessory bacteriochlorophylls

Bleach of bacteriopheophytins

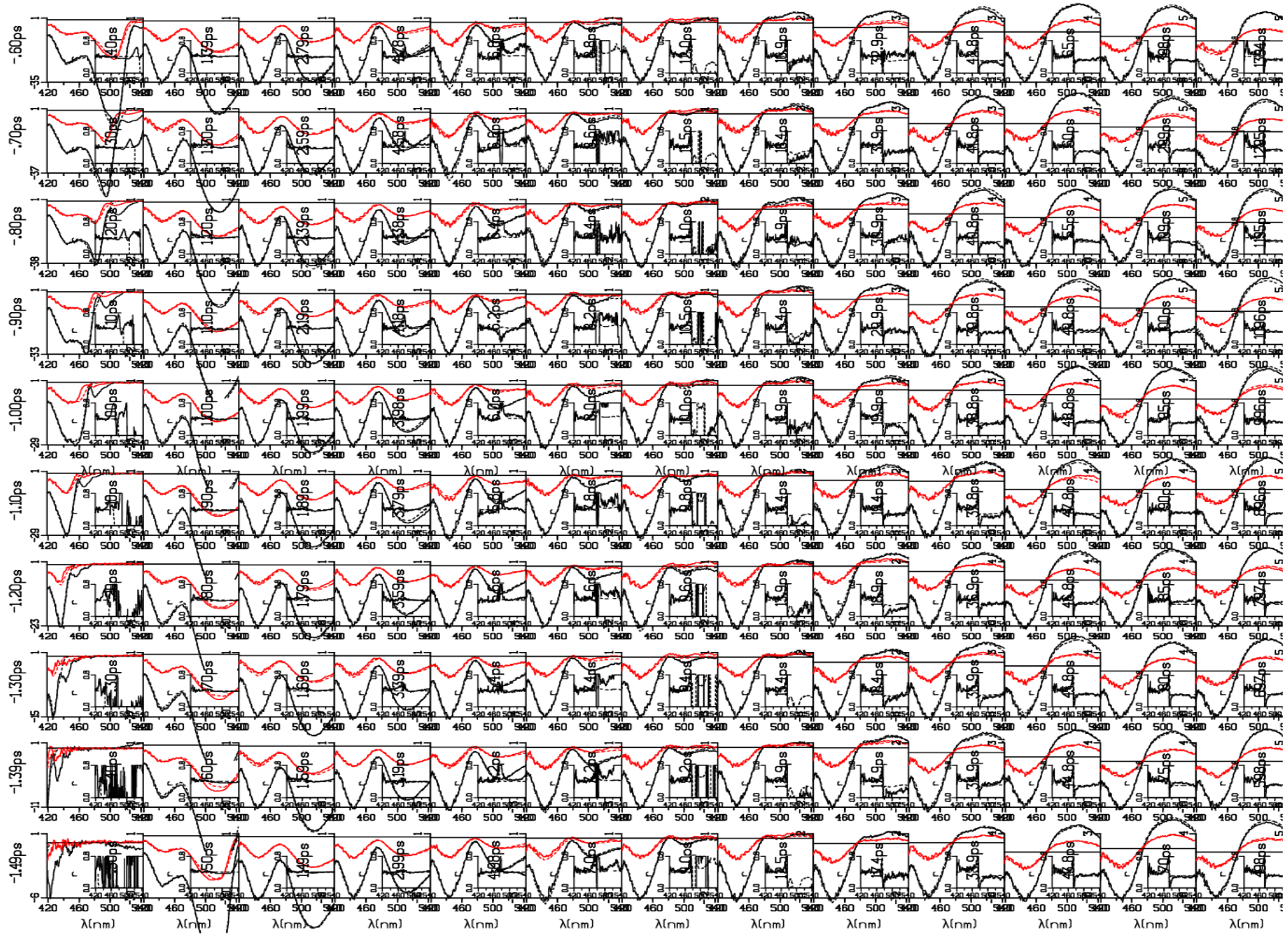
W. Holzapfel, U. Finkele, W. Kaiser, D. Oesterhelt, H. Scheer, H.U. Stilz, and W. Zinth, *Proceedings of the National Academy of Sciences of the United States of America*, 1990. **87**(13): p. 5168-5172.

W. Zinth et al. conclusion

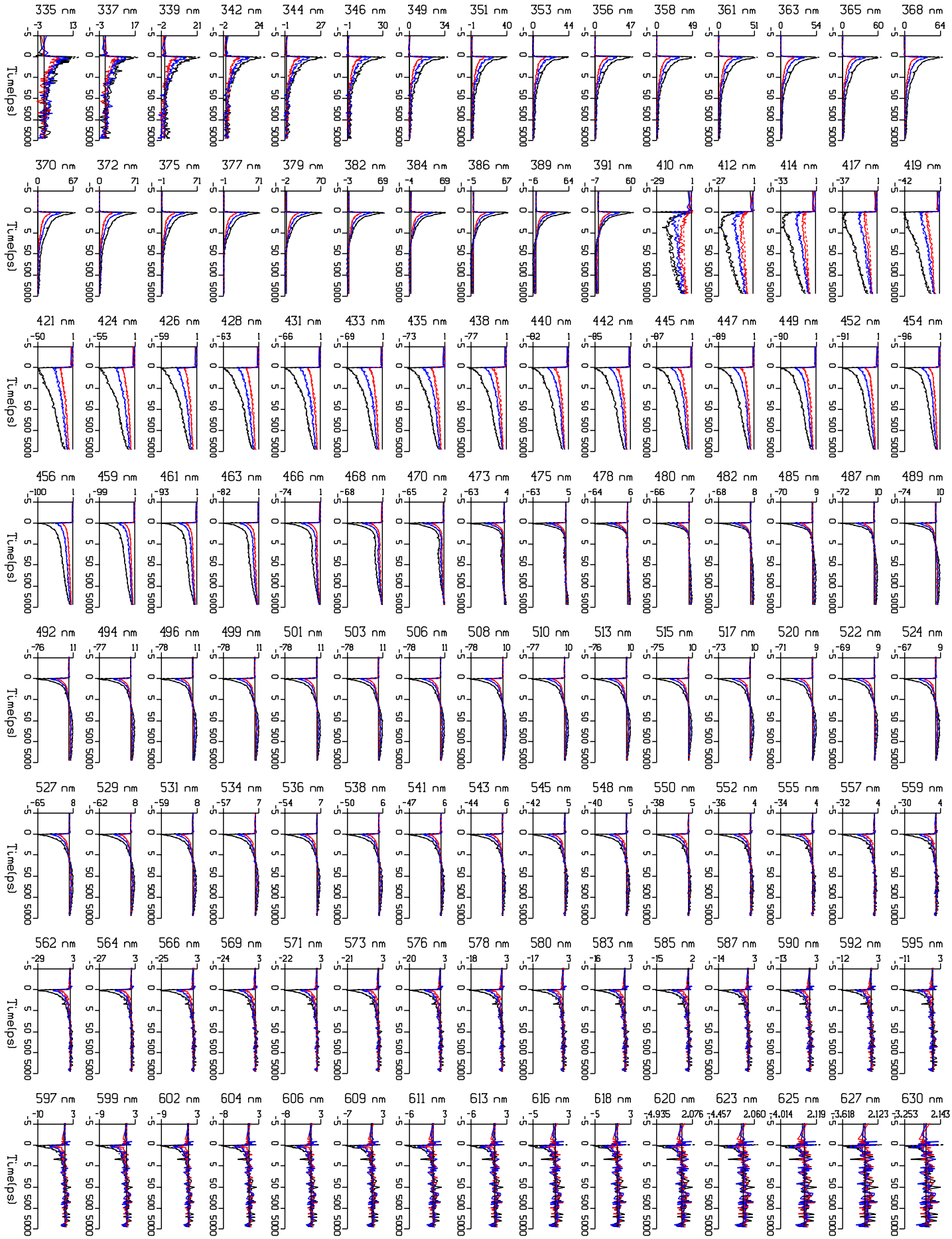


$$P* \xrightarrow{3.5\,ps} P^+B^- \xrightarrow{0.9\,ps} P^+H^- \xrightarrow{220\,ps} P^+Q_A^-$$

A word on data analysis

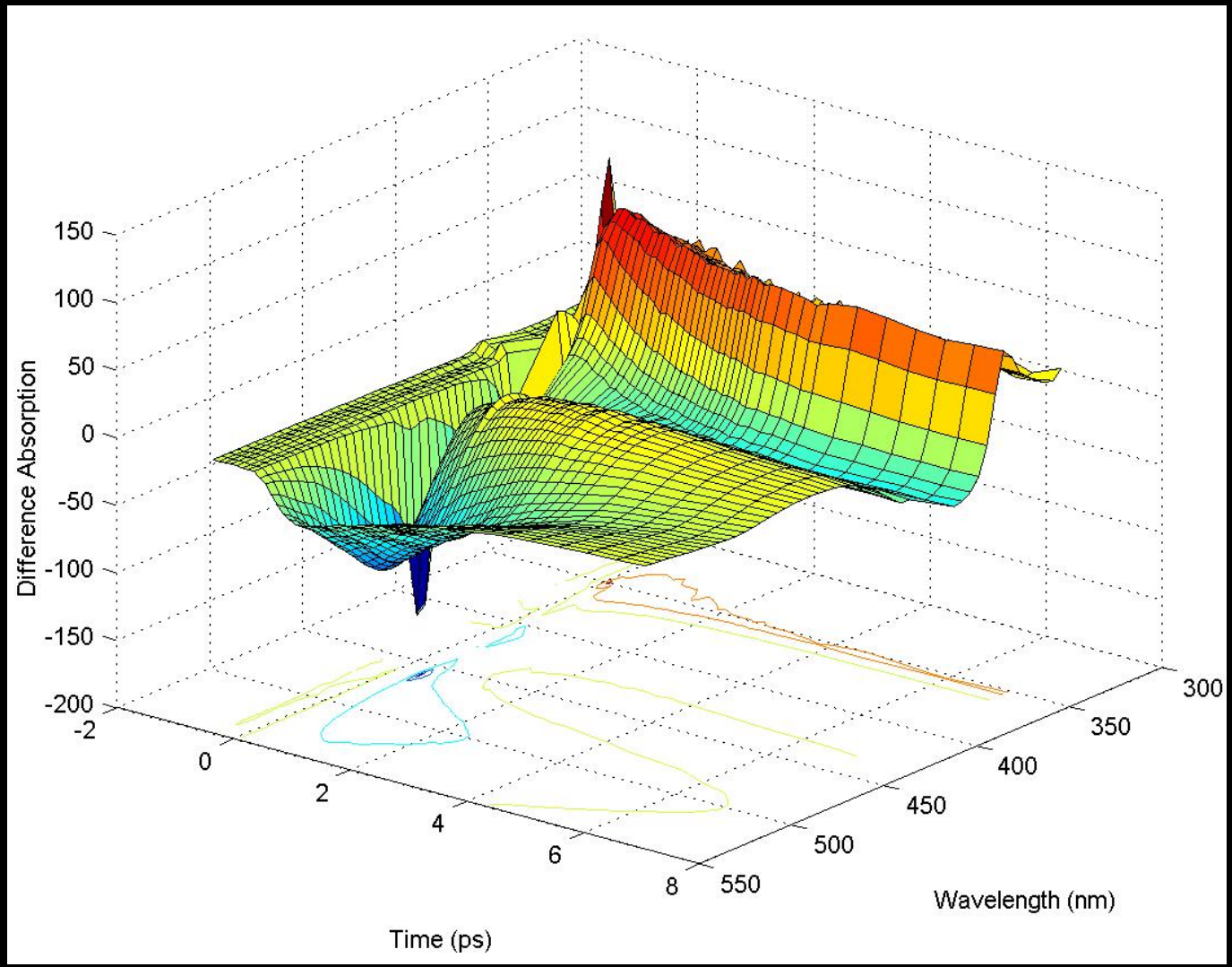

-.60ps
-.70ps
-.80ps
-.90ps
-1.00ps
-1.10ps
-1.20ps
-1.30ps
-1.39ps
-1.49ps
λ(nm)

Or…


335 nm
337 nm
339 nm
342 nm
344 nm
346 nm
349 nm
351 nm
353 nm
356 nm
358 nm
361 nm
363 nm
365 nm
368 nm
370 nm
372 nm
375 nm
377 nm
379 nm
382 nm
384 nm
386 nm
389 nm
391 nm
410 nm
412 nm
414 nm
417 nm
419 nm
421 nm
424 nm
426 nm
428 nm
431 nm
433 nm
435 nm
438 nm
440 nm
442 nm
445 nm
447 nm
449 nm
452 nm
454 nm
456 nm
459 nm
461 nm
463 nm
466 nm
468 nm
470 nm
473 nm
475 nm
478 nm
480 nm
482 nm
485 nm
487 nm
489 nm
492 nm
494 nm
496 nm
499 nm
501 nm
503 nm
506 nm
508 nm
510 nm
513 nm
515 nm
517 nm
520 nm
522 nm
524 nm
527 nm
529 nm
531 nm
534 nm
536 nm
538 nm
541 nm
543 nm
545 nm
548 nm
550 nm
552 nm
555 nm
557 nm
559 nm
562 nm
564 nm
566 nm
569 nm
571 nm
573 nm
576 nm
578 nm
580 nm
583 nm
585 nm
587 nm
590 nm
592 nm
595 nm
597 nm
599 nm
602 nm
604 nm
606 nm
609 nm
611 nm
613 nm
616 nm
618 nm
620 nm
623 nm
625 nm
627 nm
630 nm
Time(ps)

Or

Pump-probe dataset



Models are reflections of reality in our minds

Phenomenological
Intuitive
Simplistic
Good description of data

Complicated
First principles based
Meaningful
Unintuitive
Far away from data

$$R_1(t_3,t_2,t_1) = \sum_{abcd} \mathrm{P}(a)\mu_{ad}\mu_{dc}\mu_{cb}\mu_{ba} \exp\{i\bar{\omega}_{cb}t_3 + i\bar{\omega}_{db}t_2 - i\bar{\omega}_{ba}t_1 + F_{abcd}^{(1)}(t_1,t_2,t_3)\}$$

$$R_2(t_3,t_2,t_1) = \sum_{abcd} \mathrm{P}(a)\mu_{ad}\mu_{dc}\mu_{cb}\mu_{ba} \exp\{i\bar{\omega}_{cb}t_3 + i\bar{\omega}_{db}t_2 + i\bar{\omega}_{da}t_1 + F_{abcd}^{(2)}(t_1,t_2,t_3)\}$$

$$R_3(t_3,t_2,t_1) = \sum_{abcd} \mathrm{P}(a)\mu_{ad}\mu_{dc}\mu_{cb}\mu_{ba} \epsilon$$

$$+$$

$$R_4(t_3,t_2,t_1) = \sum \mathrm{P}(a)\mu \ldots \epsilon$$

$$\mathbf{P}_{3D}(\mathrm{r},t) = N\int_0^\infty dt_3 \int_0^\infty dt_2 \int_0^\infty dt_1 R_{3D}(t_3,t_2,t_1) \times \mathbf{E}(\mathrm{r},t-t_3)\mathbf{E}(\mathrm{r},t-t_3-t_2) \times \mathbf{E}(\mathbf{r},t-t_3-t_2-t_1) \quad (4)$$

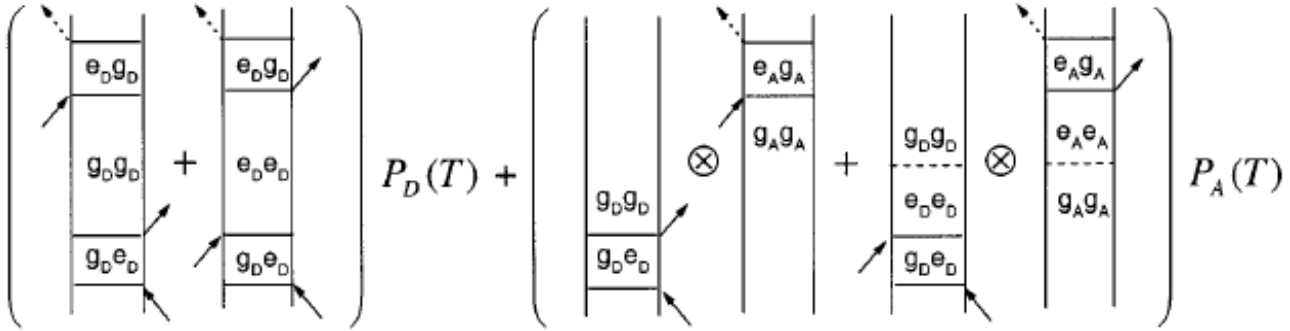
$$\tilde{R}_1^{++}(\Omega_1,t_2,\Omega_3) \equiv \int_{-\infty}^{\infty} dt_1 \int_{-\infty}^{\infty} dt_3\, R_1(t_3,t_2,t_1) e^{i\Omega_1 t_1 + i\Omega_3 t_3}$$

$$= \sum_{abcd} \mathrm{P}(a)\mu_{ad}\mu_{dc}\mu_{cb}\mu_{ba} \int_{-\infty}^{\infty} dt_3 \int_{-\infty}^{\infty} dt_1 \exp\{i(\bar{\omega}_{cb}+\Omega_3)t_3 + i\bar{\omega}_{db}t_2 - i(\bar{\omega}_{ba}-\Omega_1)t_1\}$$

$$\times \exp\left(f_1(t_2) - \frac{1}{2}\delta_1^2(t_2)t_1^2 - \frac{1}{2}\Delta_1^2(t_2)t_3^2\right.$$

$$= \sum_{abcd} \mathrm{P}(a)\mu$$

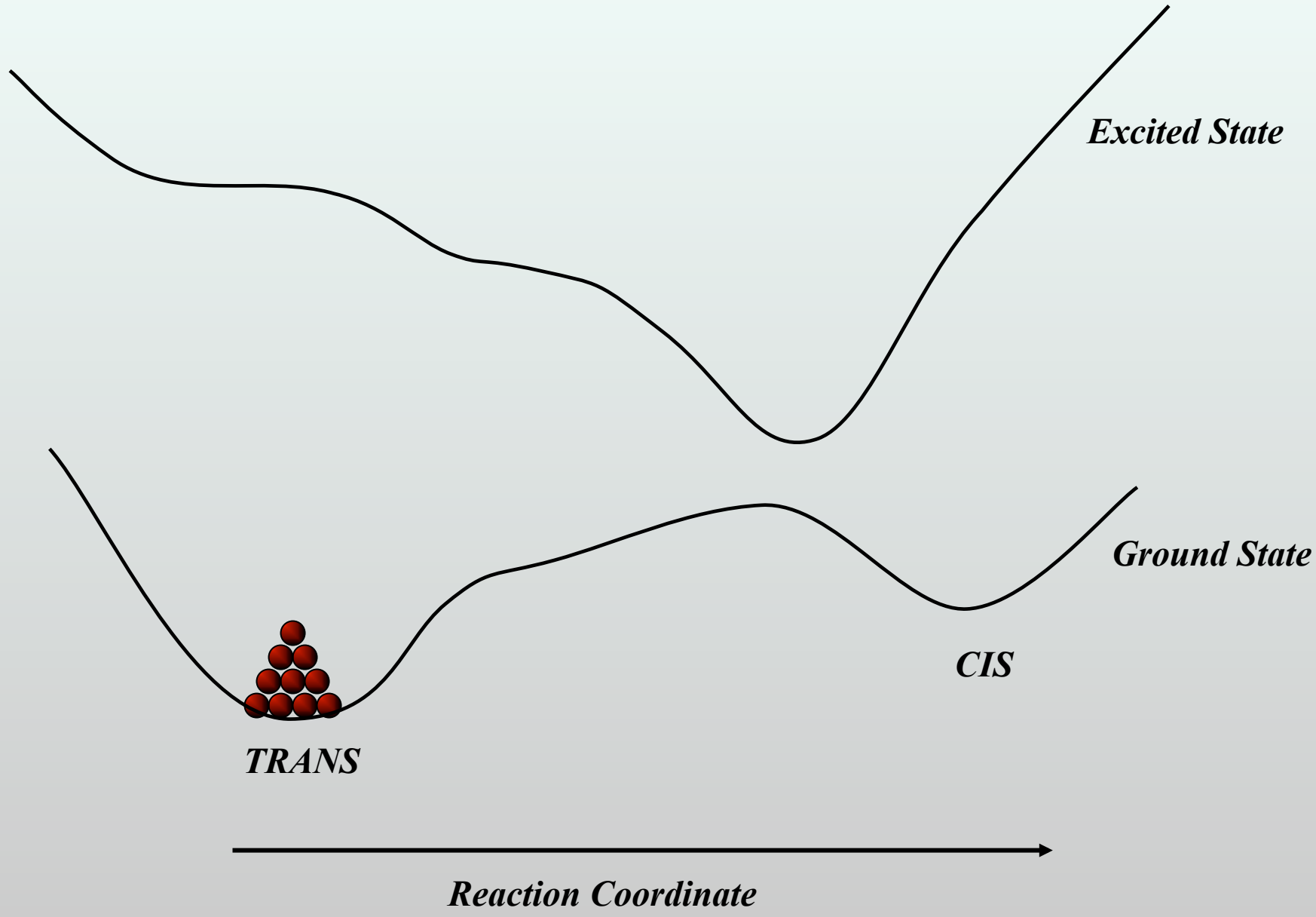
$$\times G\left\{\Omega_3 + \bar{\omega}_{cl}\right.$$



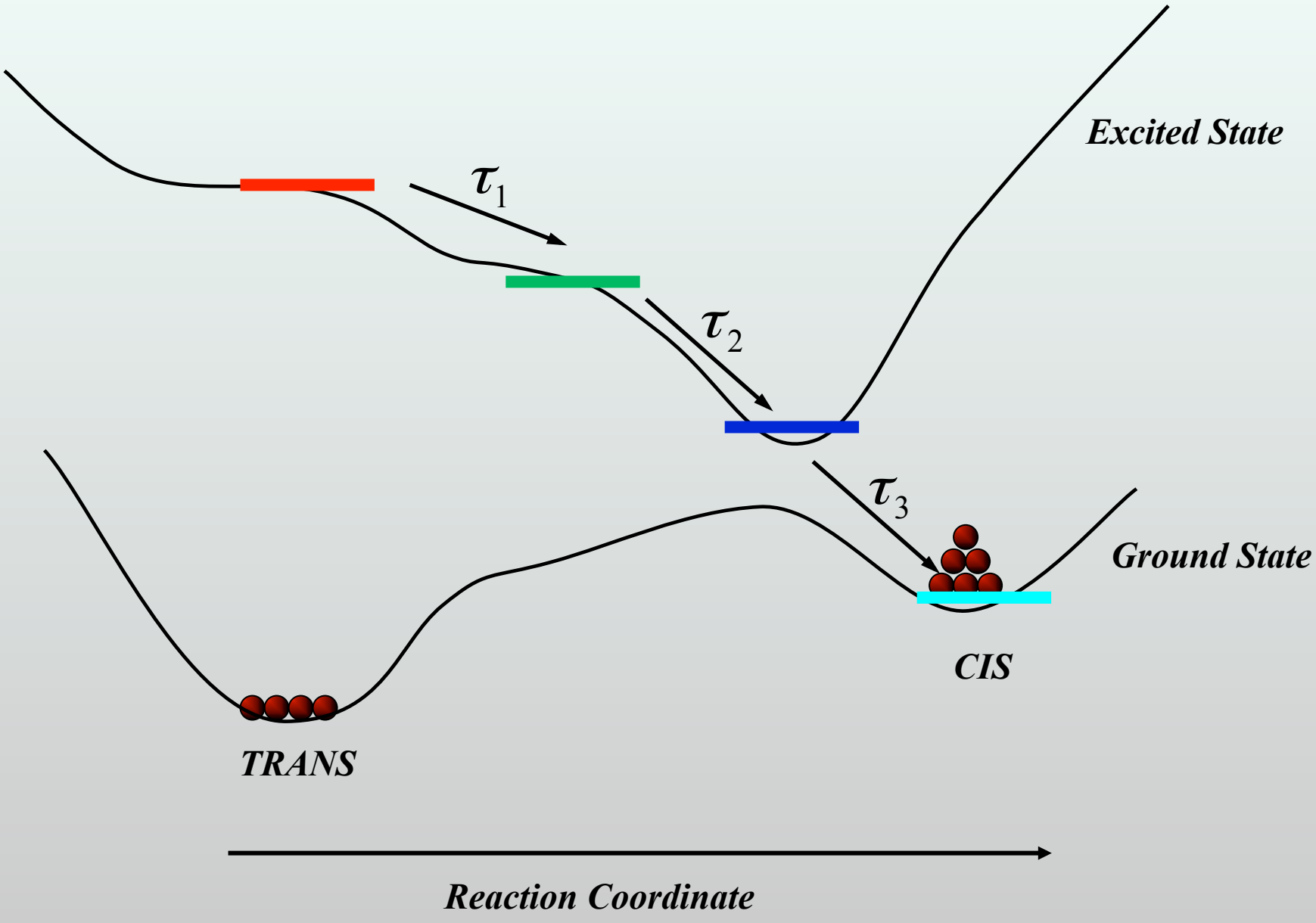
Hole Exciton Hole Exciton

$$\delta_1^2(t_2)\Big]$$

Photoinduced Dynamics



Photoinduced Dynamics

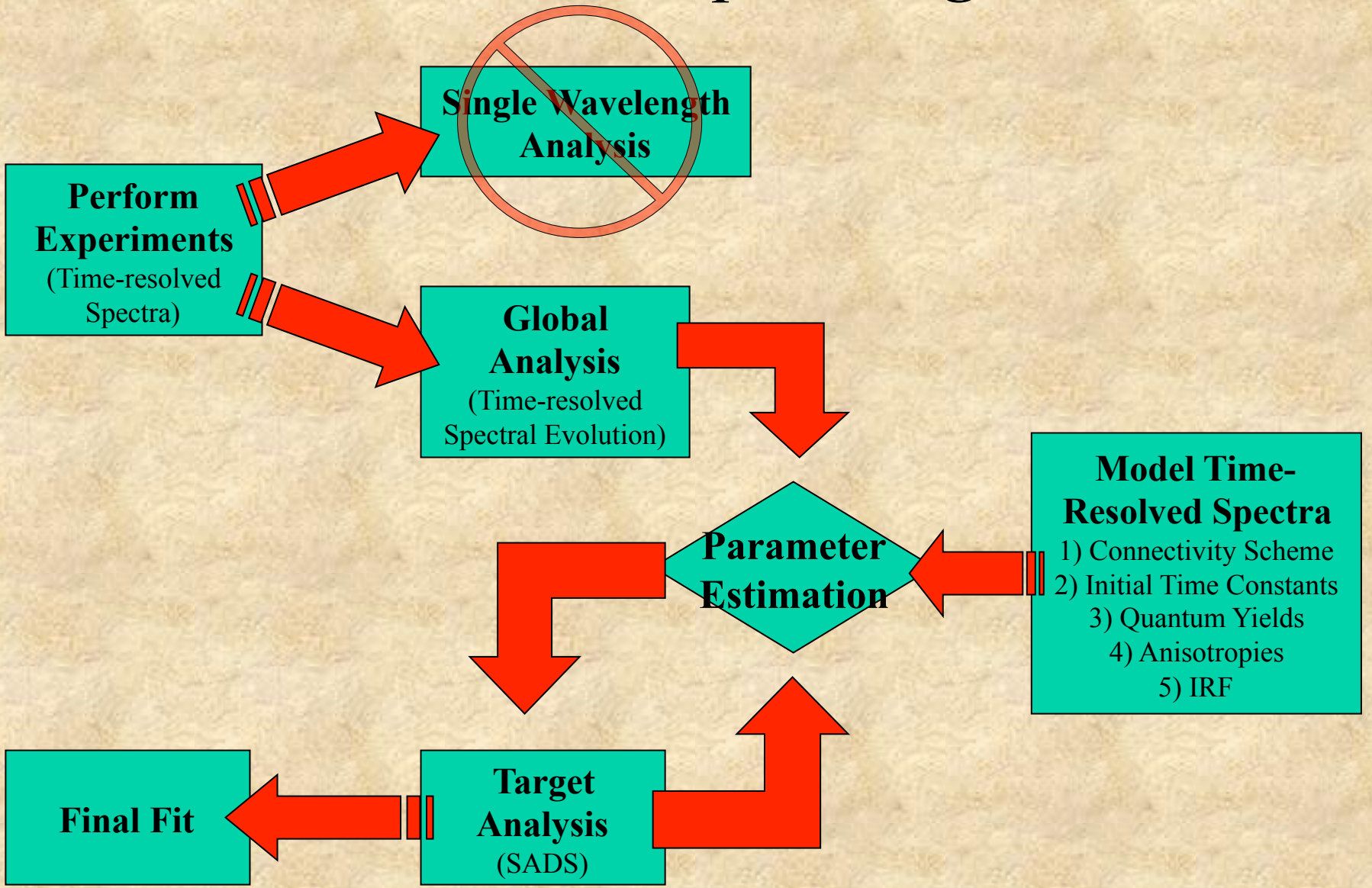


Global analysis is a ‘pinball machine’ approximation of ultrafast data

Use it when:

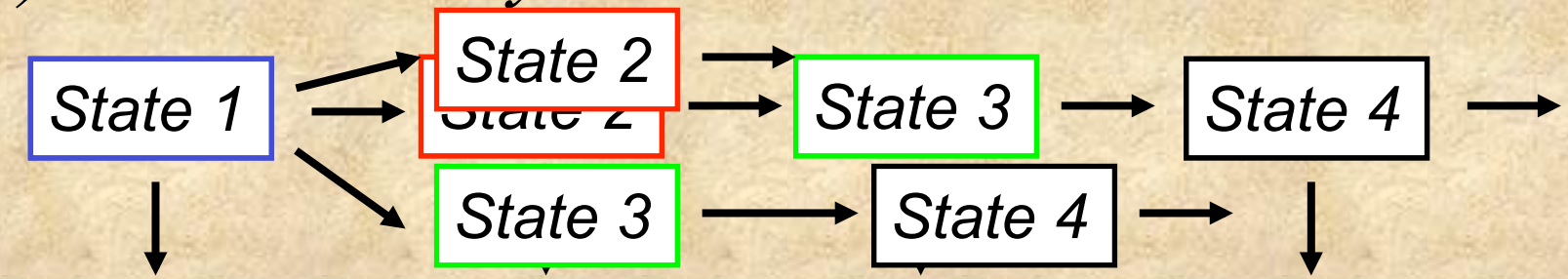
- You do not know any better.
- You need to parametrize large datasets concisely.
- You need to present and interpret the data to people without hardcore physics background.

Hierarchical Modeling of Dispersed Transient Absorption Signals



Three Principle Objectives of Global Analysis

1) Connectivity



2) Timescales

$$\dot{n}_i(t) = \sum_j k_{ij} n_j(t)$$

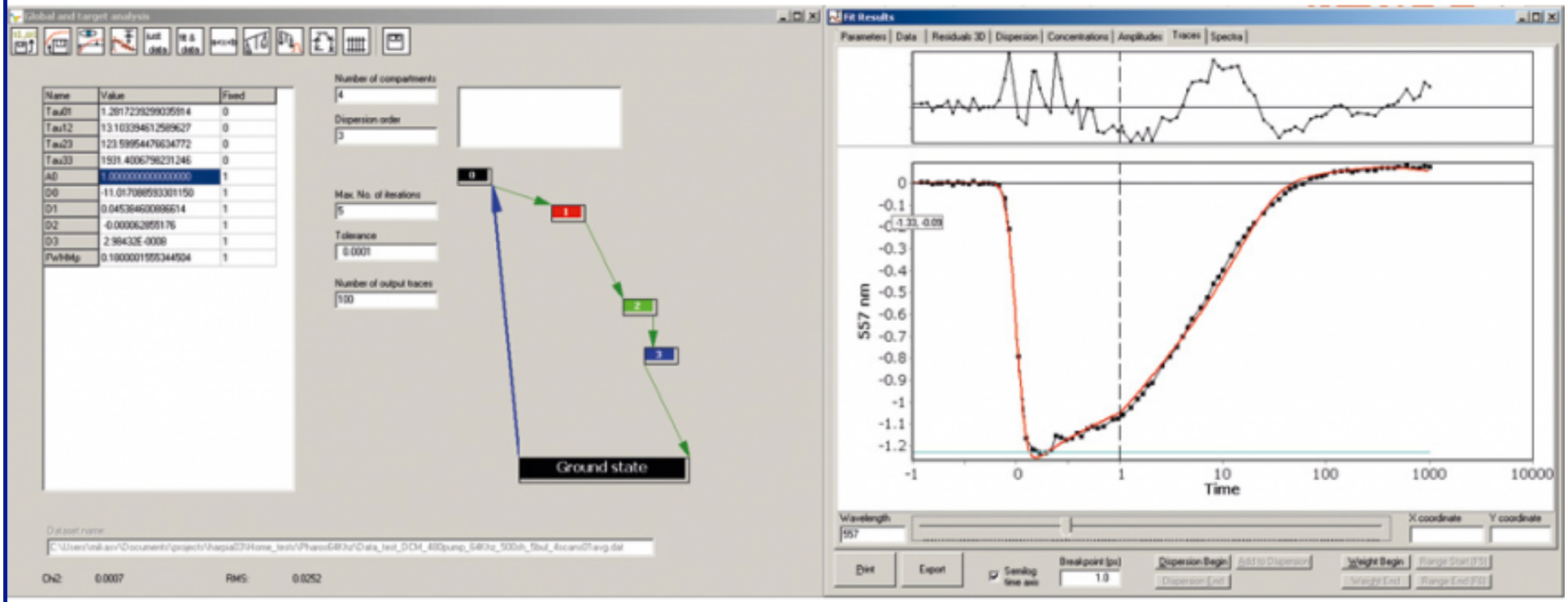
3) Spectra

$$D(\lambda, t; k_{ij}) = \sum_i A_i(\lambda) n_i(t; k_{ij})$$

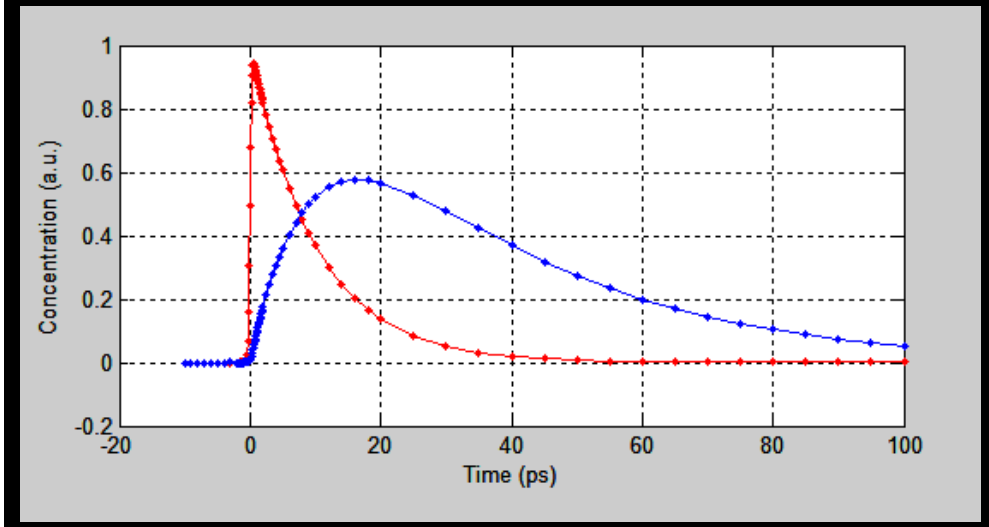
Available options:

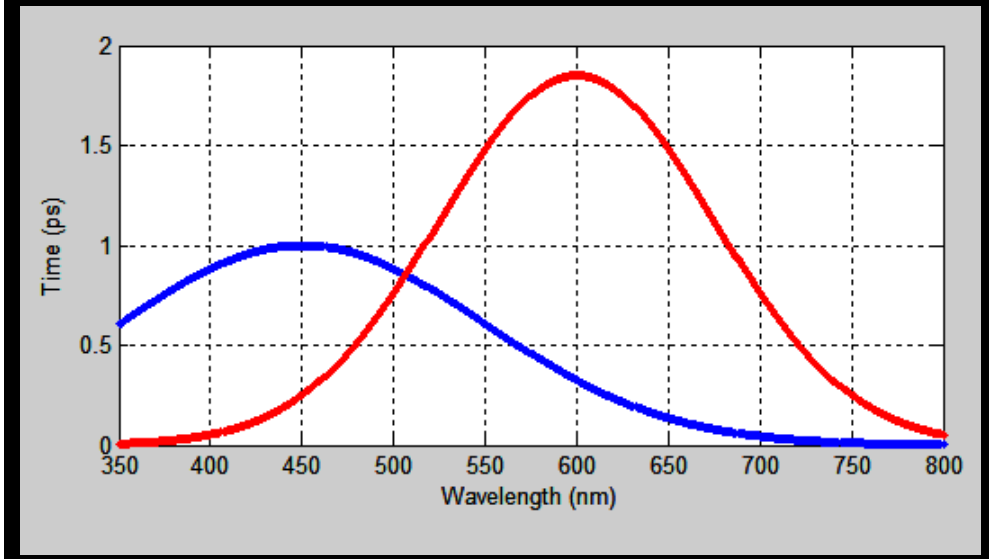
- Glotaran – VU Amsterdam
- CarpetView – 

- Jasper van Thor's Matlab® based package
- A number of groups have developed their own software

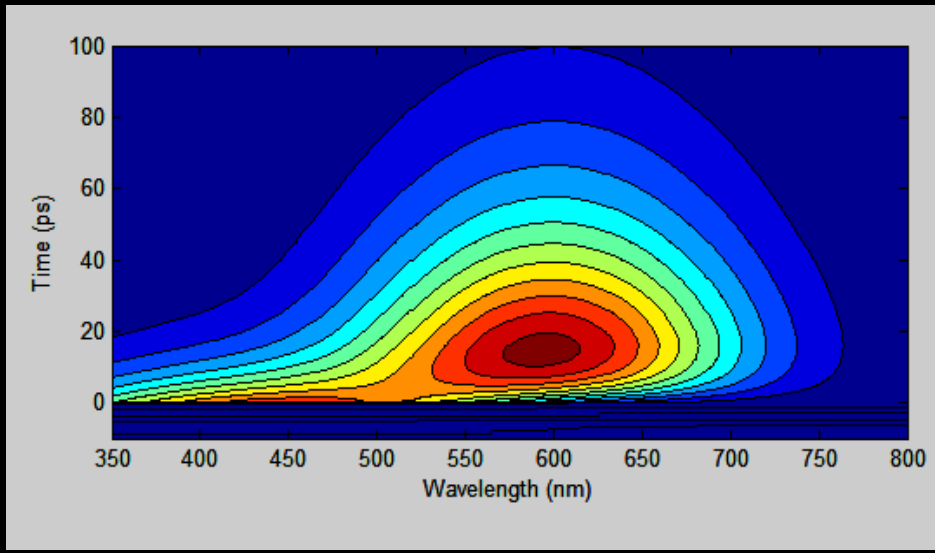

Global and target analysis
Name
Value
Fixed
Tau01
Tau12
Tau23
Tau33
A0
D0
D1
D2
D3
FWHMp
Number of compartments
Dispersion order
Max. No. of iterations
Tolerance
Number of output traces
0
1
2
3
Ground state
Dataset name:
Chi2:
0.0007
RMS:
0.0252
FR Results
Parameters
Data
Residuals 3D
Dispersion
Concentrations
Amplitudes
Traces
Spectra
557 nm
Time
Wavelength
X coordinate
Y coordinate
Print
Export
Breakpoint (px)
Dispersion Begin
Weight Begin

Time-resolved fluorescence dataset (fake data)

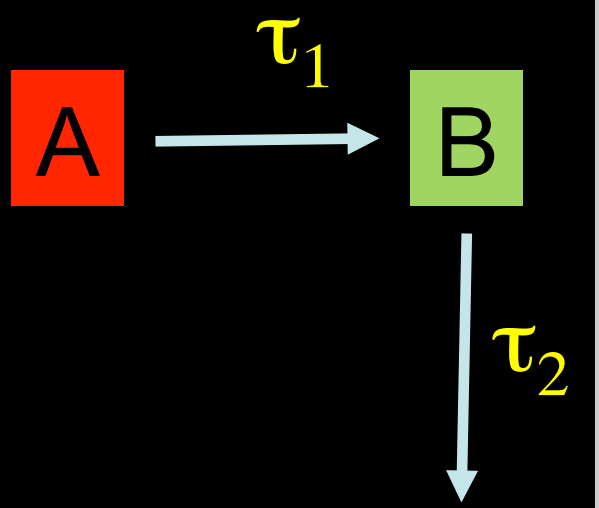


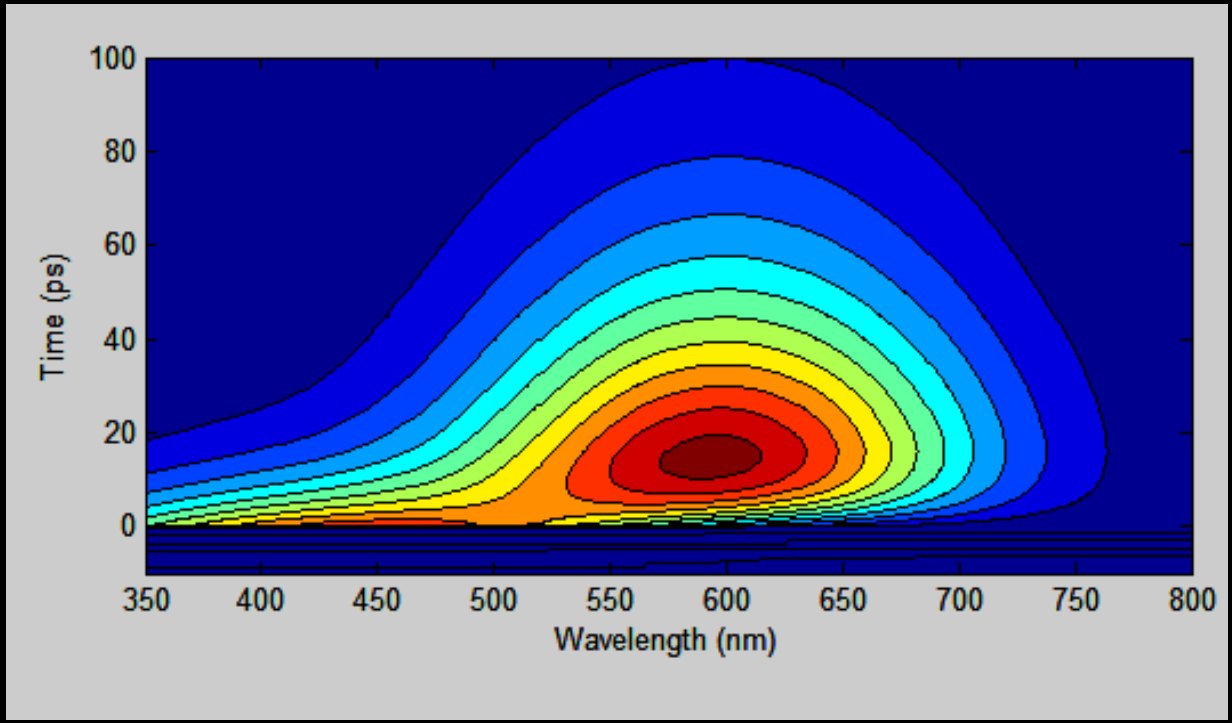


Time constants: 10 and 30 ps, IRF width: 0.2 ps

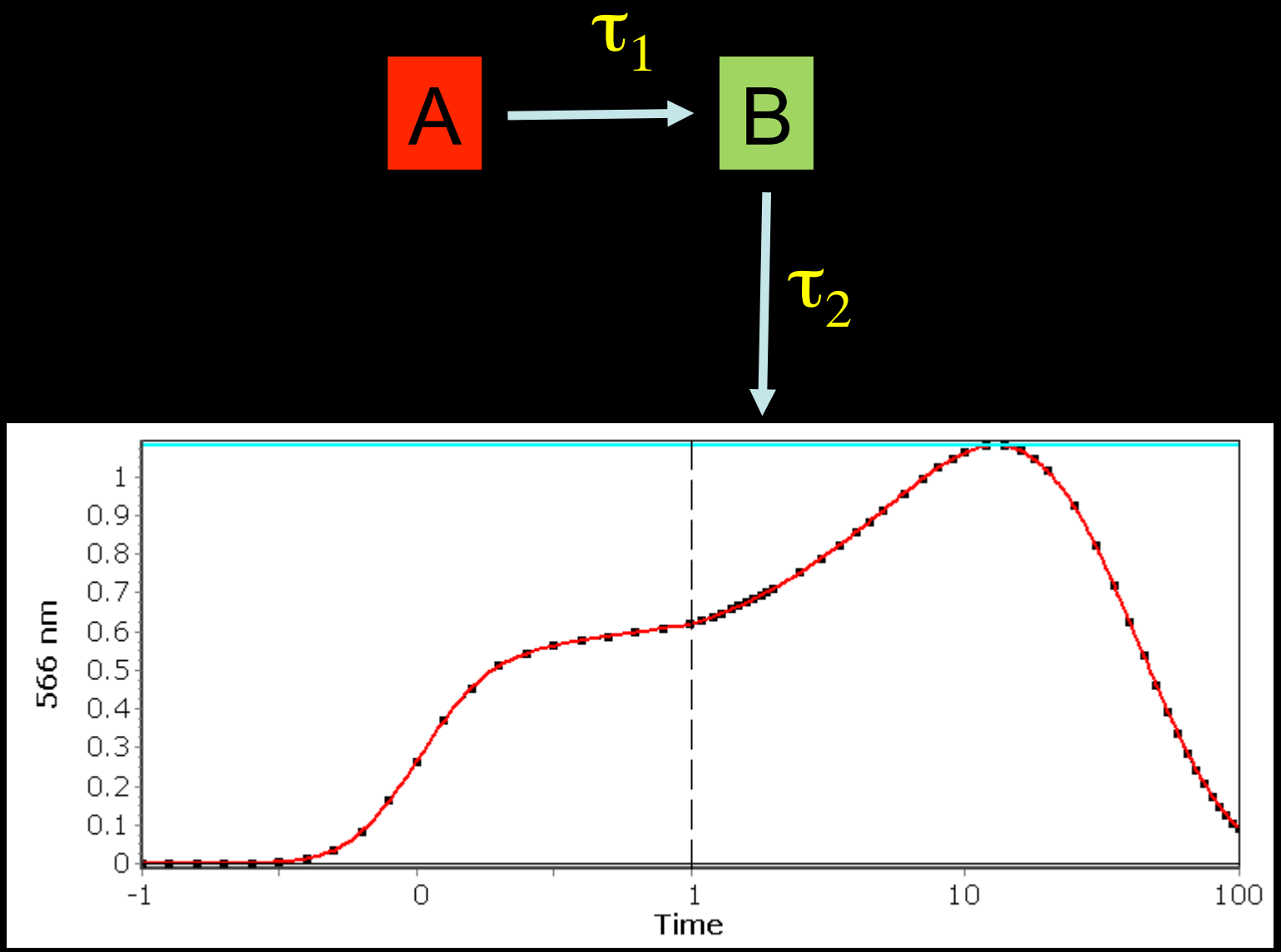


Let's fit it using sequential model…

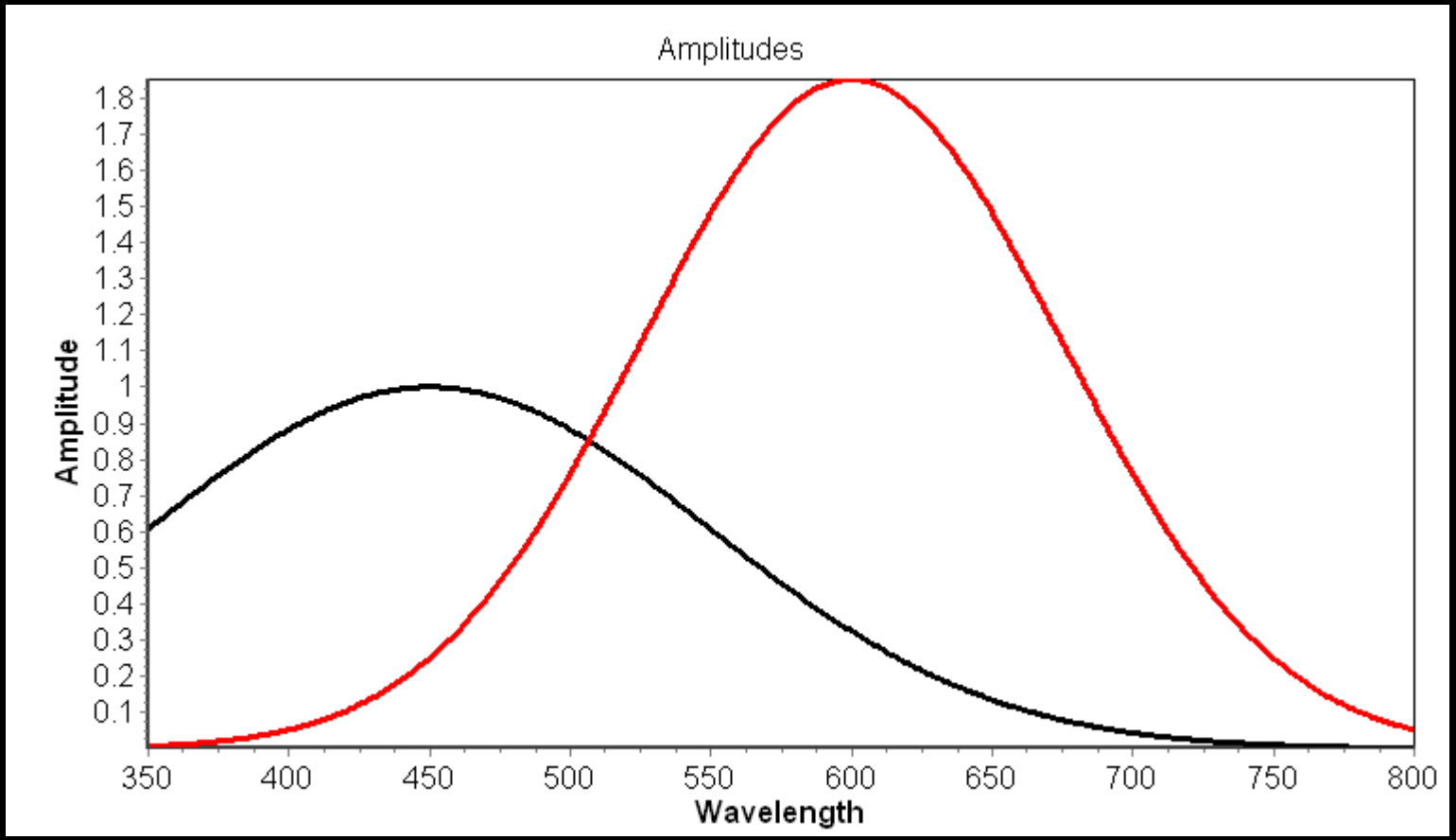




Let's fit it using sequential model…



Let's fit it using sequential model…

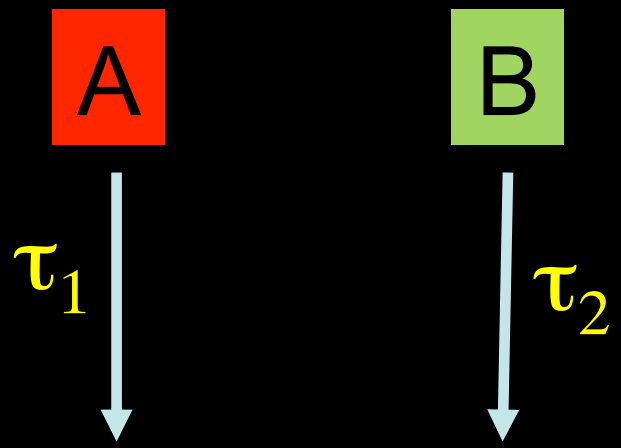


Time constants: 10 and 30 ps,
IRF width: 0.2 ps, just what we put in.

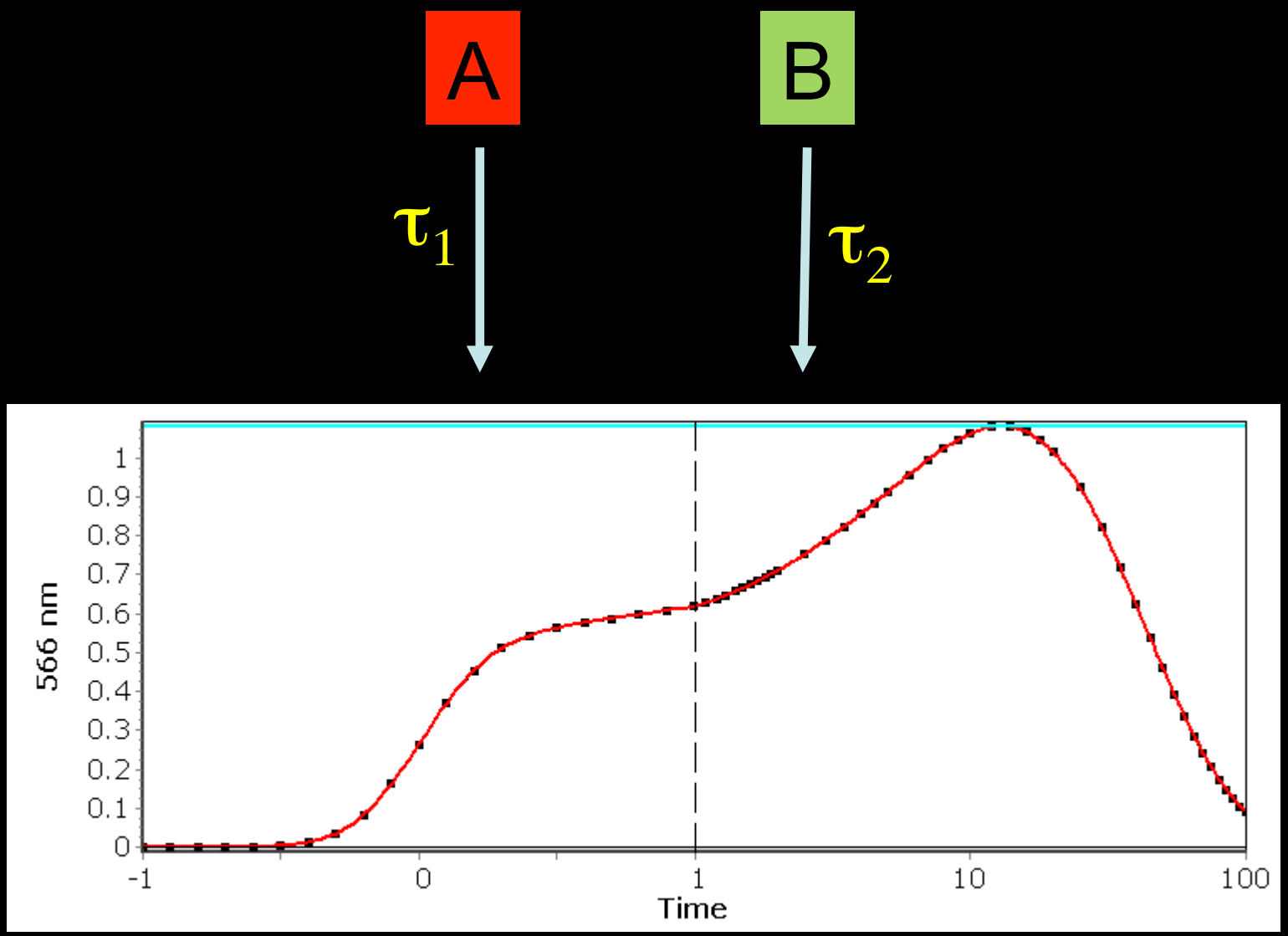
So, we nailed it, right?...

WRONG.

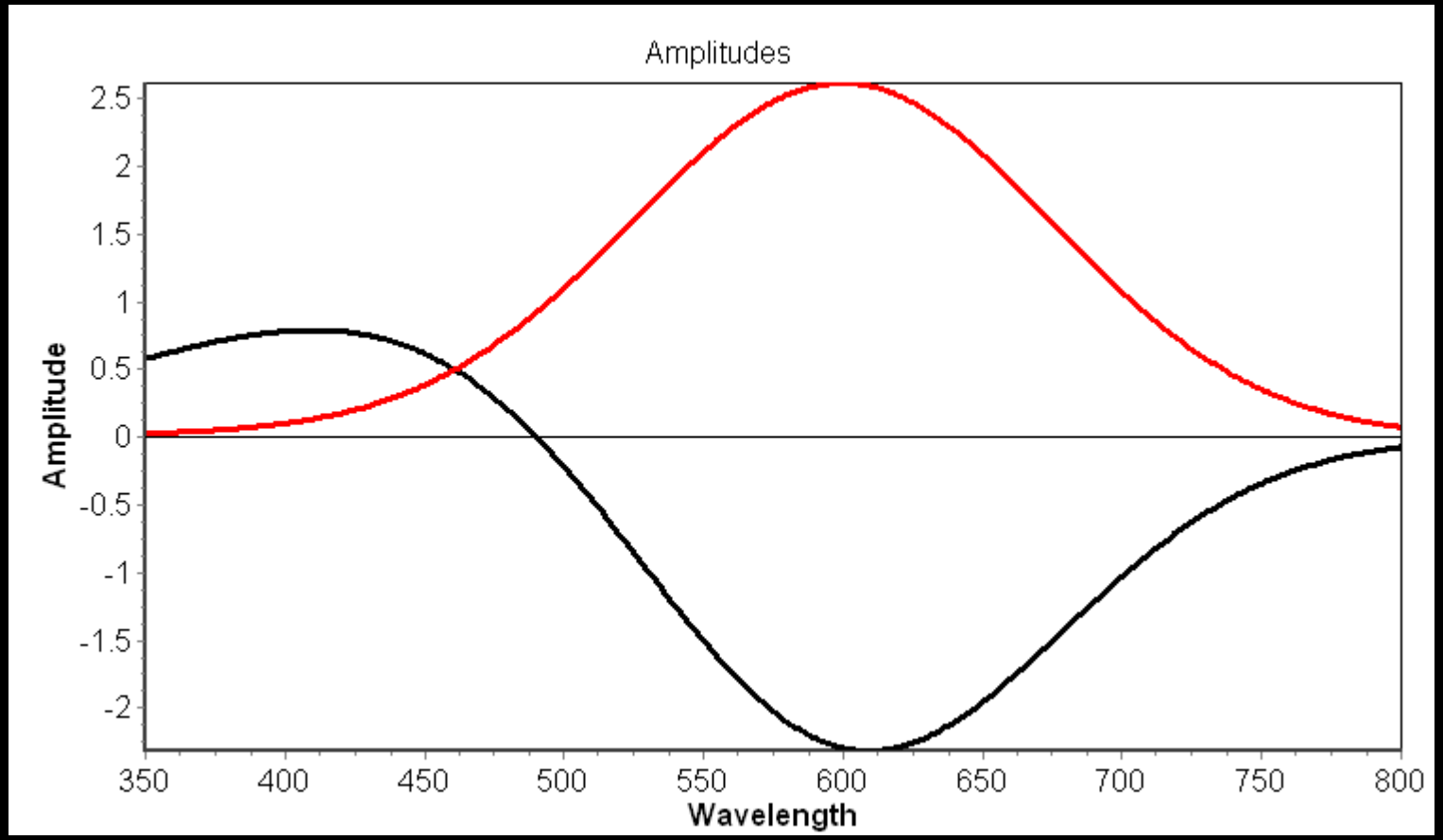
To see why, let's fit it using another model:



The fit is just as good

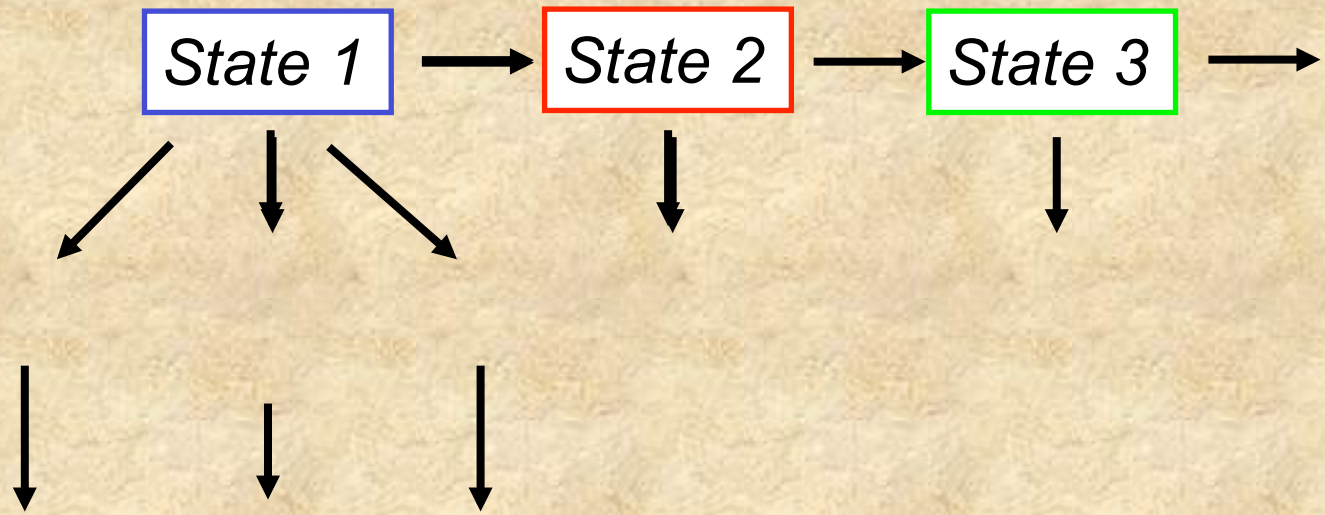


But the component spectra look different:

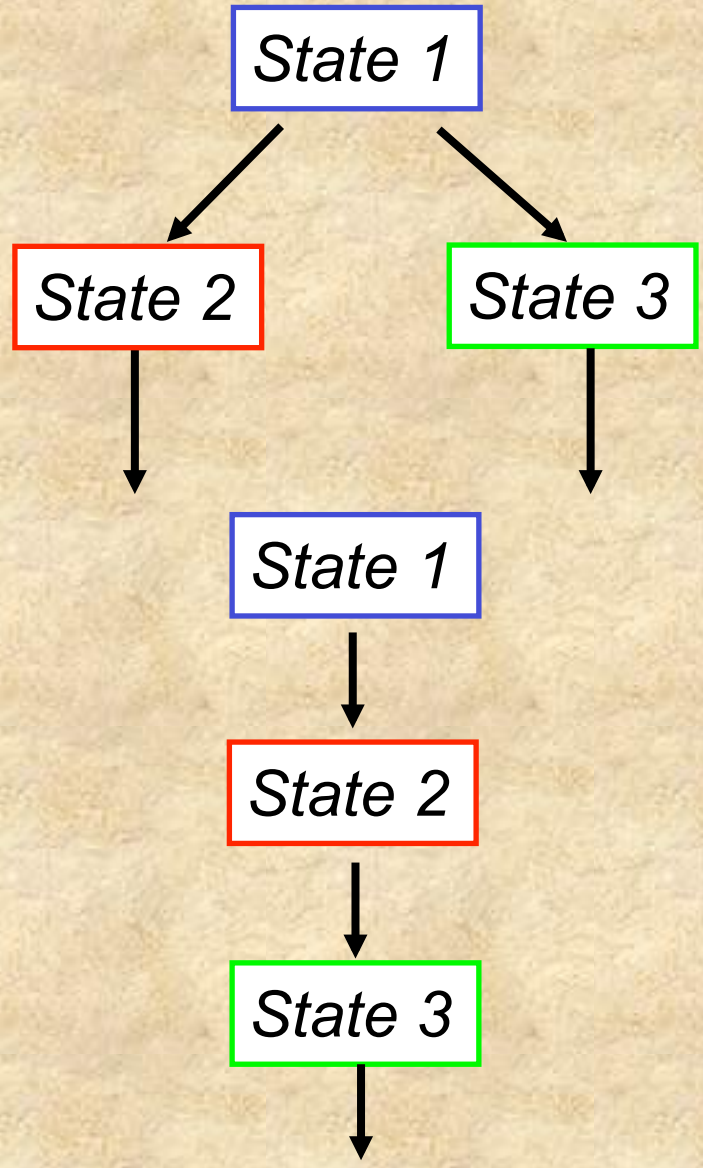


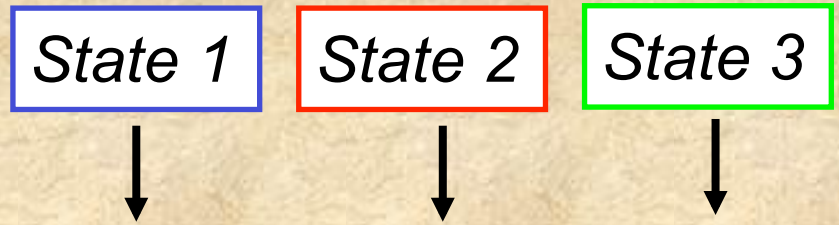
The models represent two different realities, they can't both be correct.

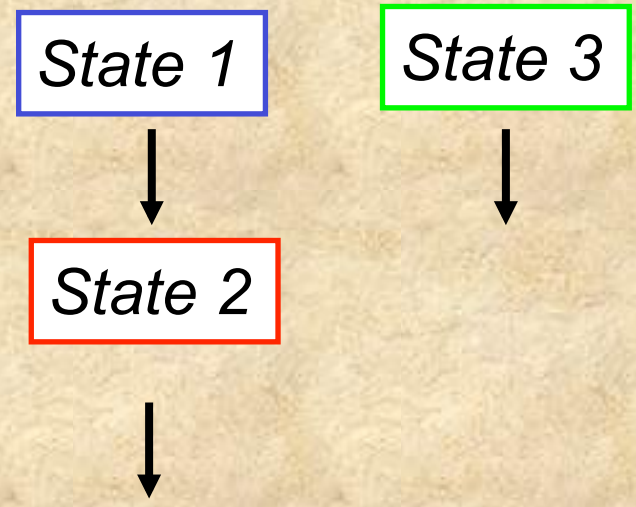
Imagine what you could do with three-component models:



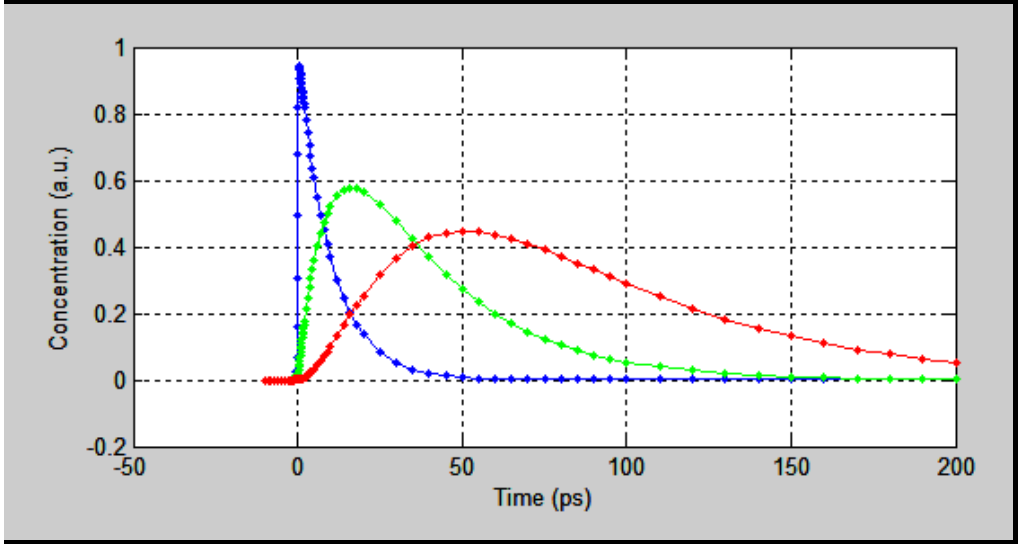
Imagine what you could do with three-component models:



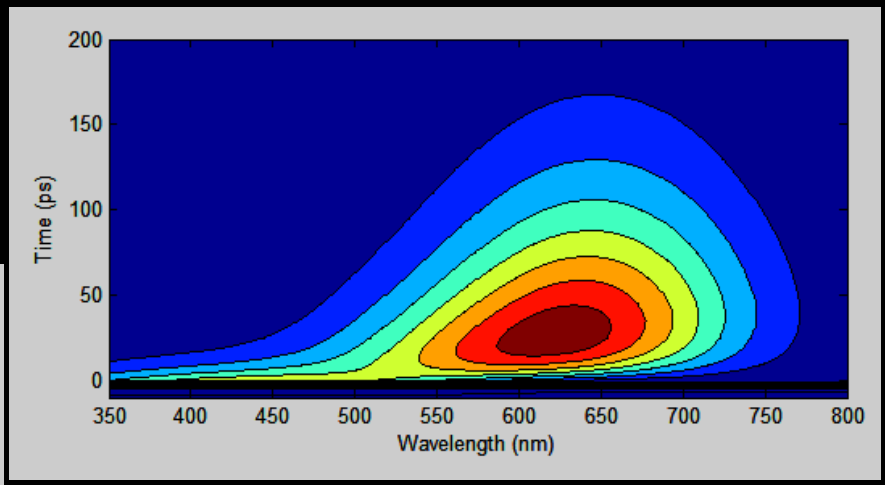


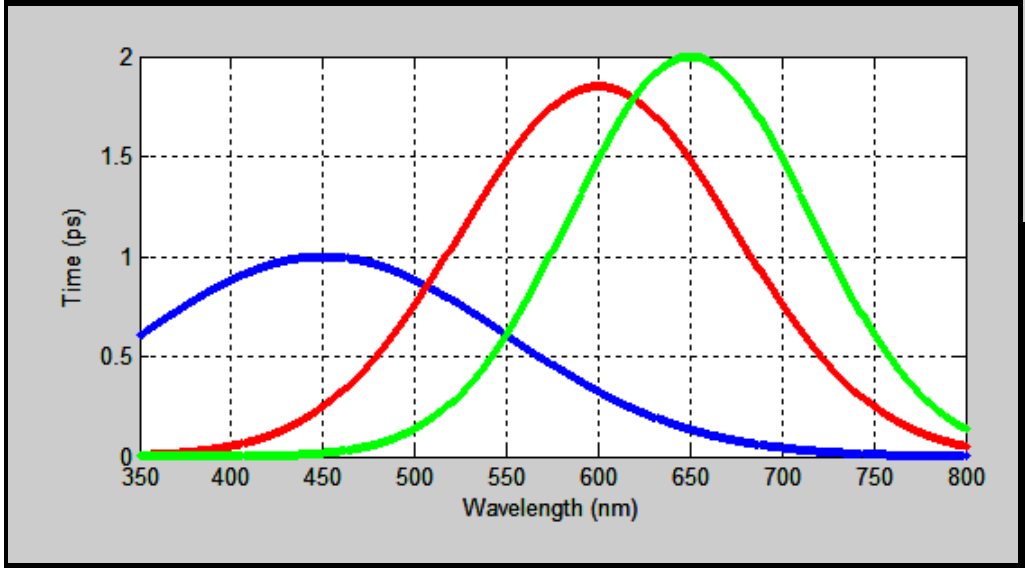


Fluorescence dataset (fake data)

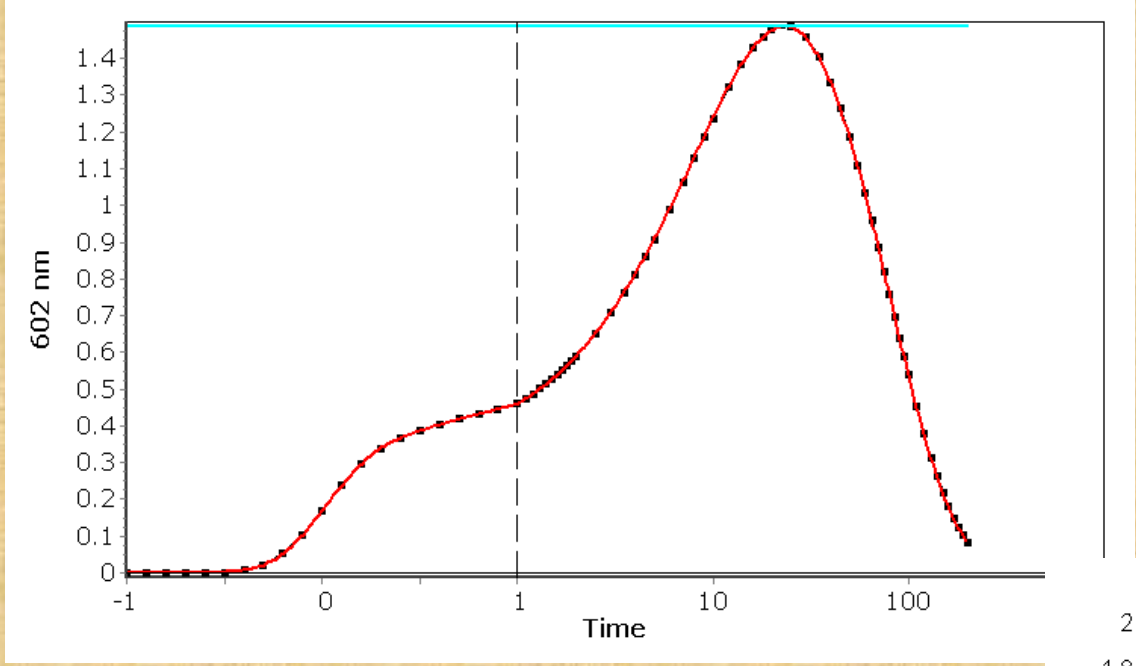


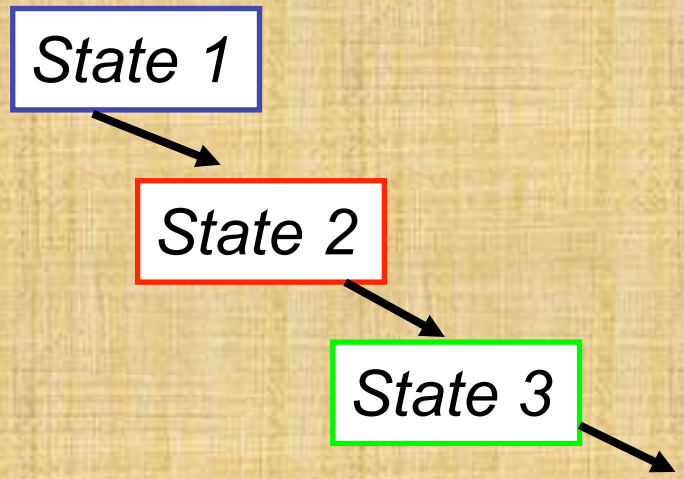
Time constants: 10, 30 and 50 ps, IRF width: 0.2 ps

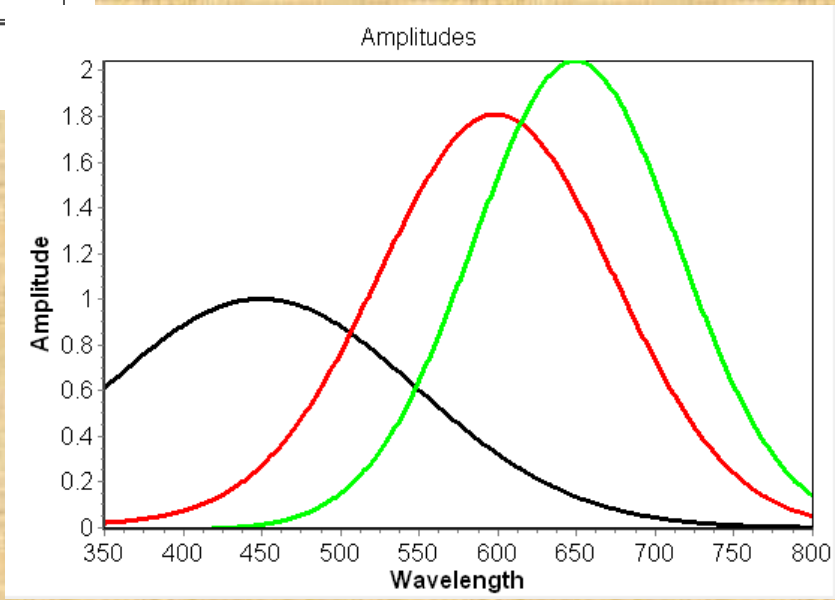




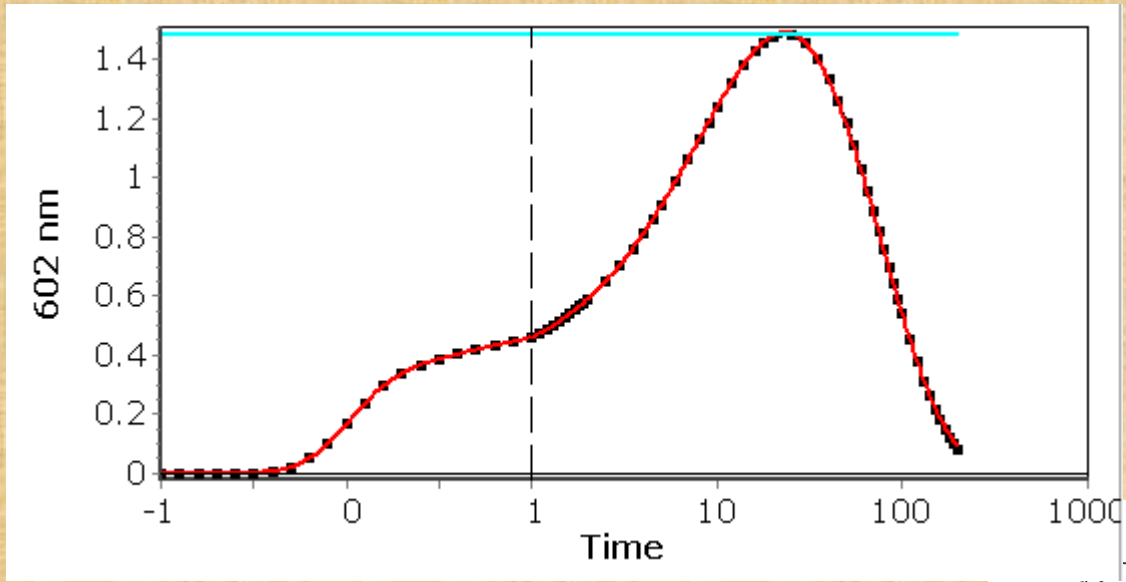
Pump-probe dataset (fake data)

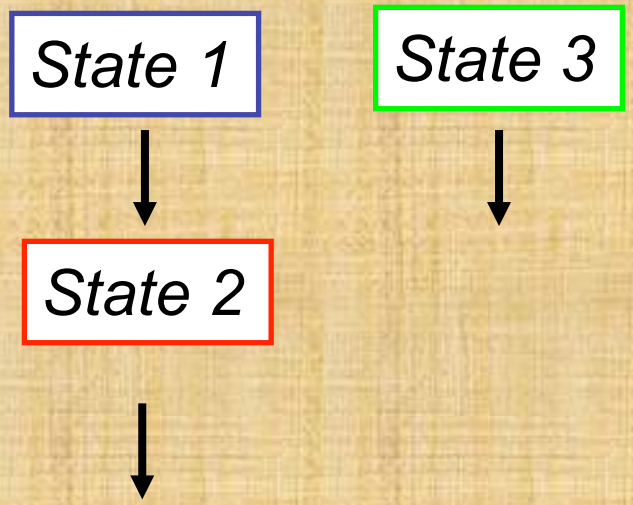


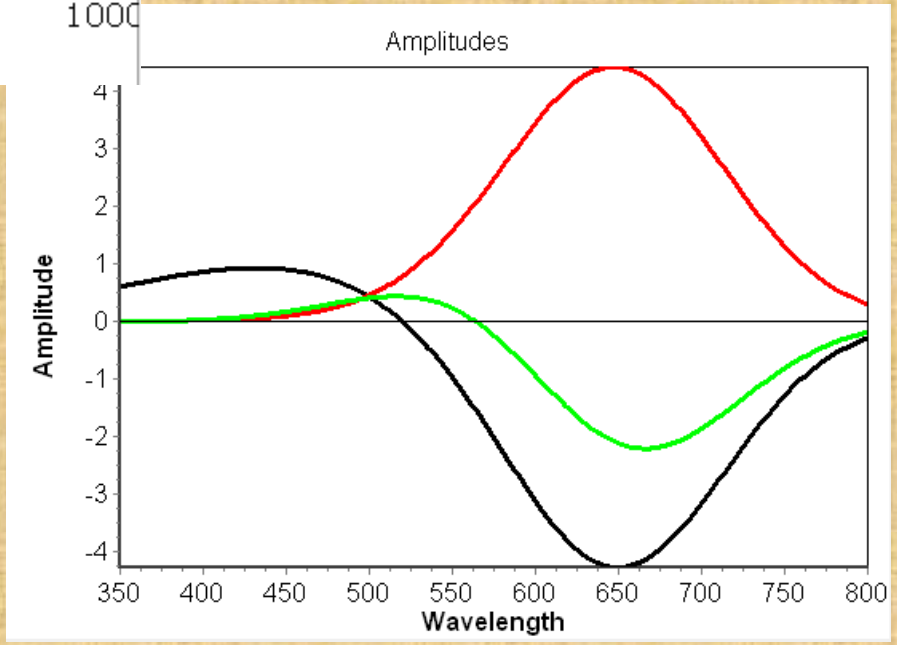




Pump-probe dataset (fake data)



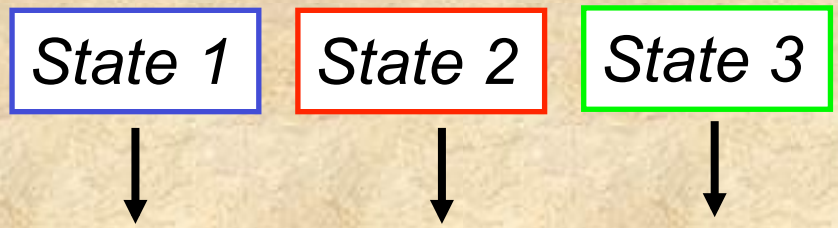




Model degeneracy

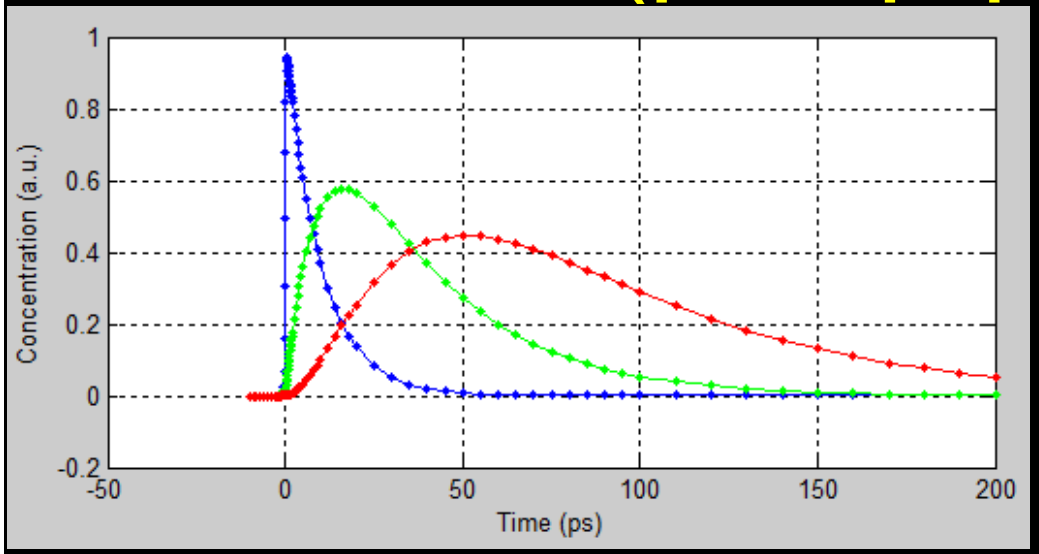
- Any model using connectivity scheme with the same rank (number of different lifetimes observed) will fit the data equally well.
- Besides the quality of the fit, the models have to be judged by the plausibility of component spectra they produce!

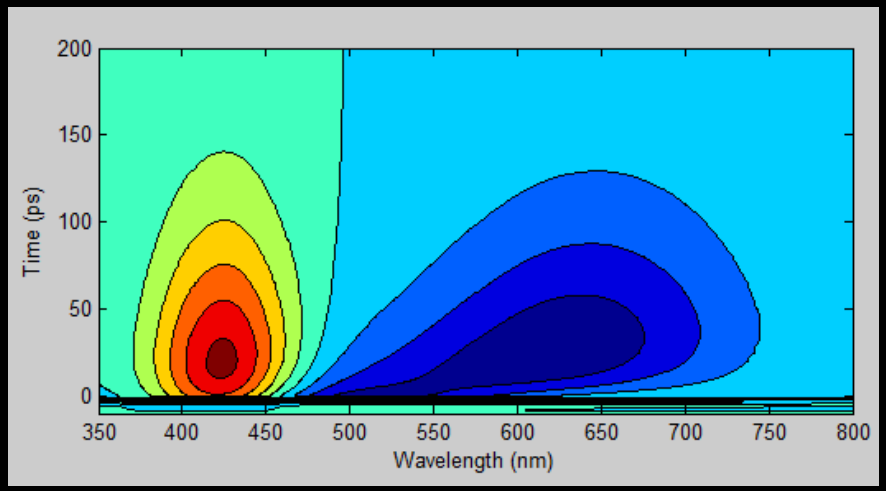
Models describing data for parametrization purposes (global analysis): parallel

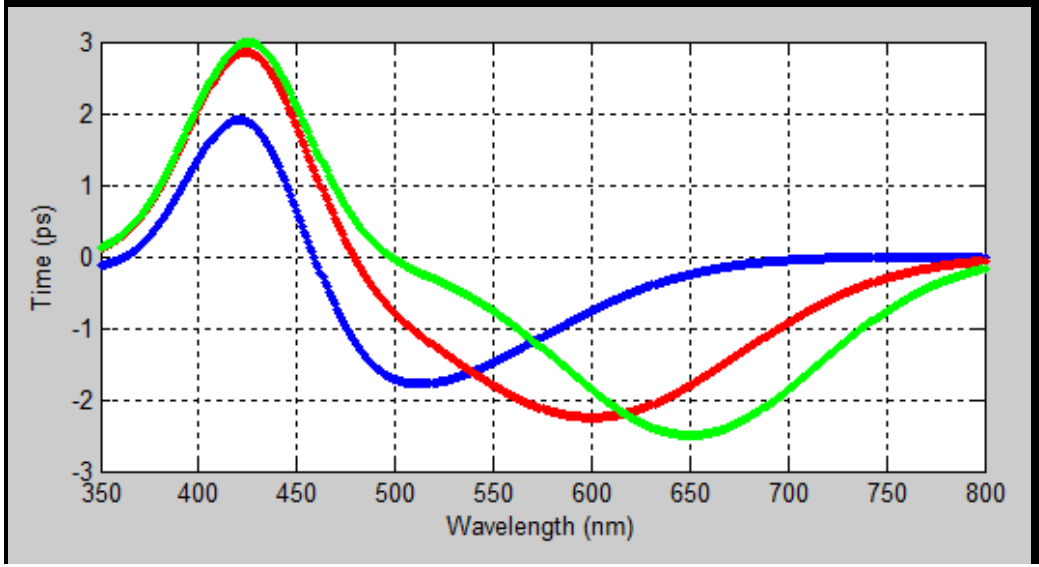


- Independent (parallel) decay model;
- Assumes independent lifetimes for different components;
- Produces *Decay-Associated Difference Spectra*, DADS (in TA) or *Decay-Associated Spectra*, DAS (in fluorescence).
- Negative amplitude means loss of (positive) signal, positive amplitude means gain (growth) of (positive signal.
- What about the signals with varying signs?

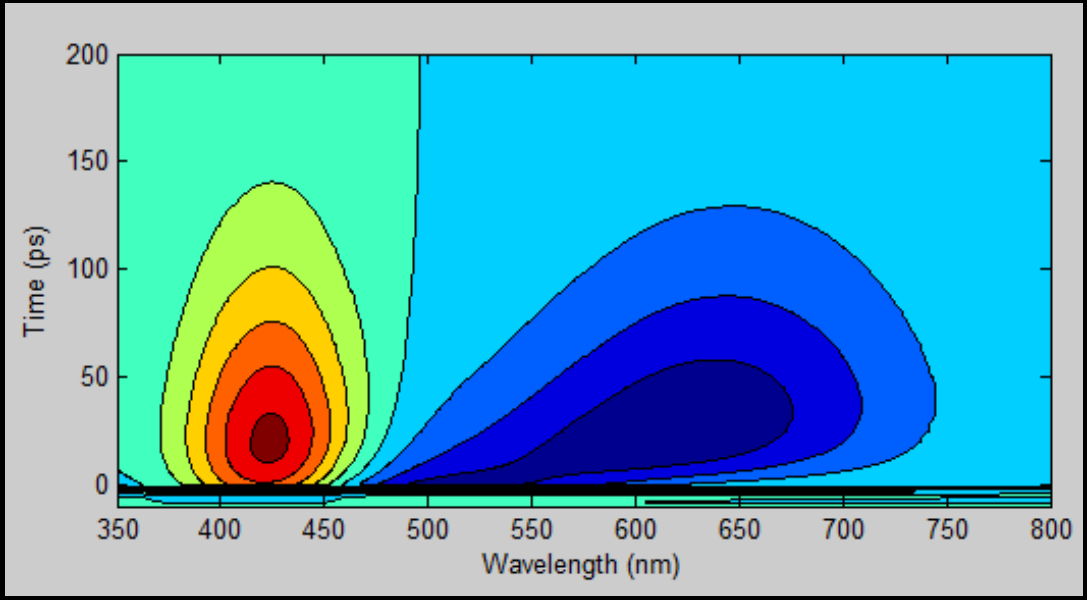
Dataset with varying signs (pump-probe)

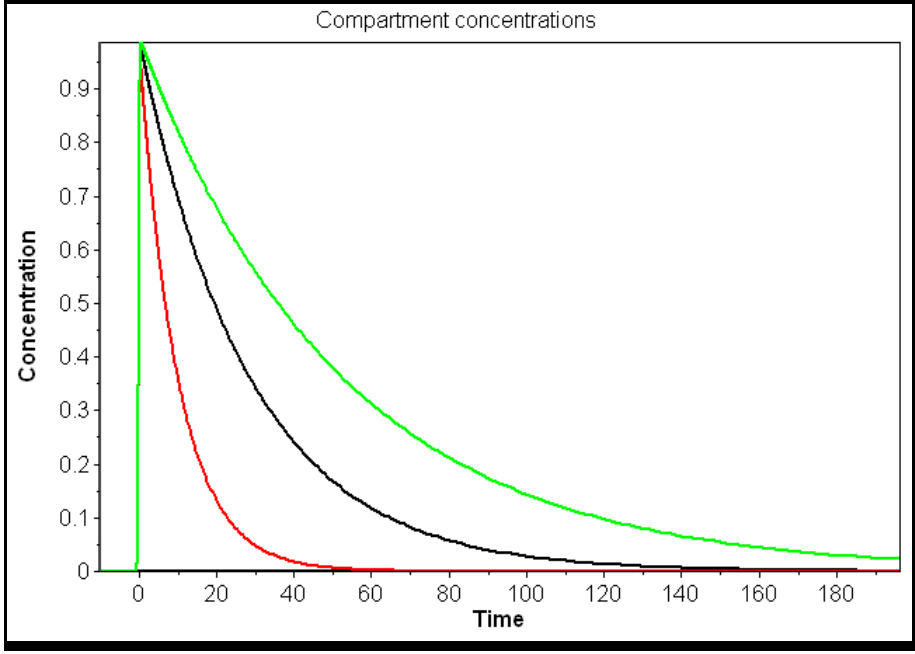


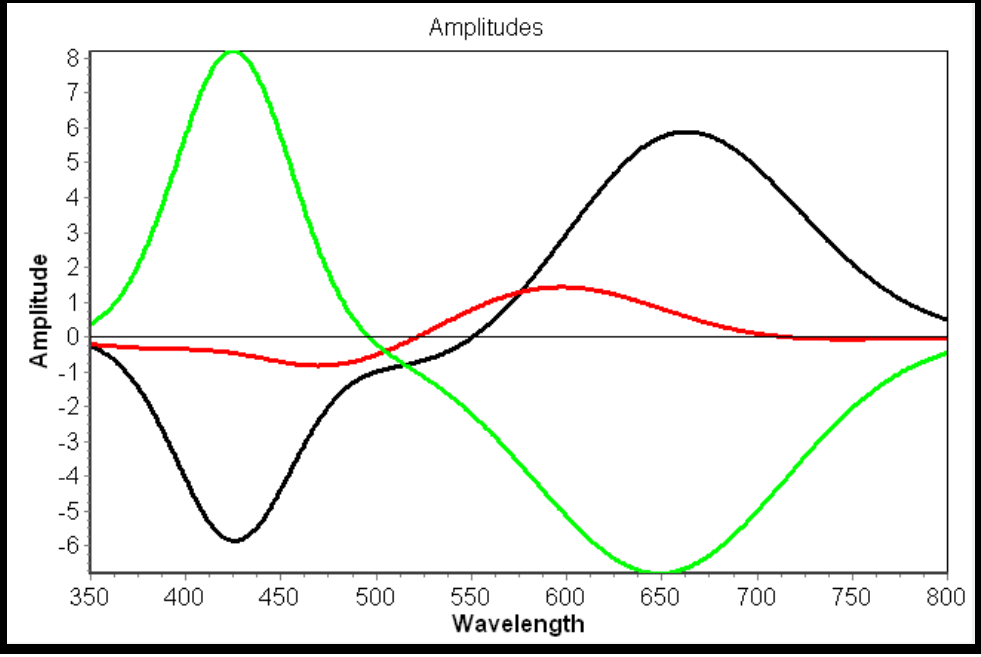




Dataset with varying signs

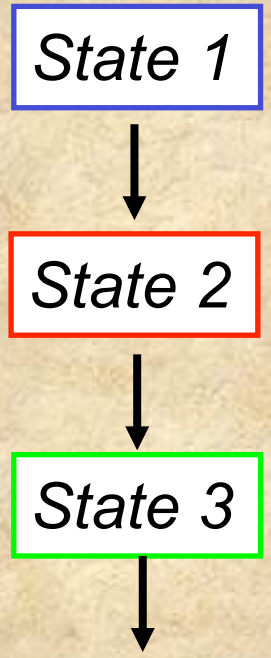




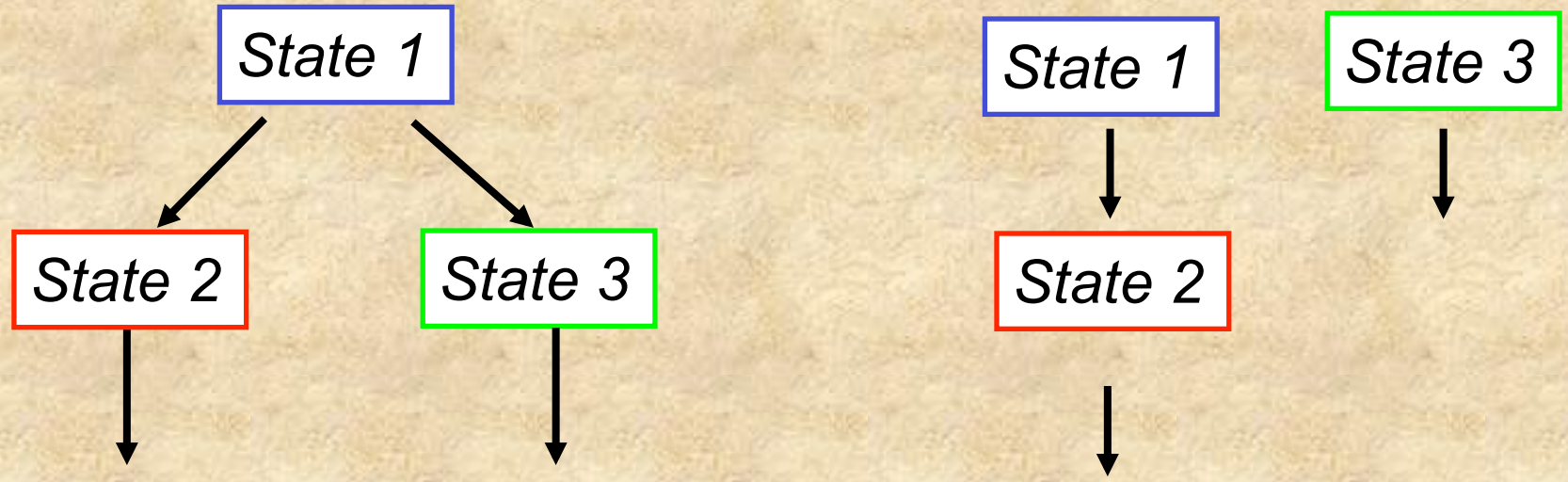


Models describing data for parametrization purposes (global analysis): sequential

- Assumes initial population put in compartment 1, and spectra evolving one into the next.
- Produces *Evolution-Associated Difference Spectra* (EADS).
- Different EADS resemble spectra observed at different times.
- Should be the first model of choice when doing preliminary analysis of TA (and probably fluorescence).



When you start to wonder…



When the different compartments are ascribed physical meanings and connectivity scheme is established using physical assumptions, you are entering the realm of *Target Analysis*.

The resulting spectra with physical meaning are called *Species-Associated Difference Spectra* (SADS)

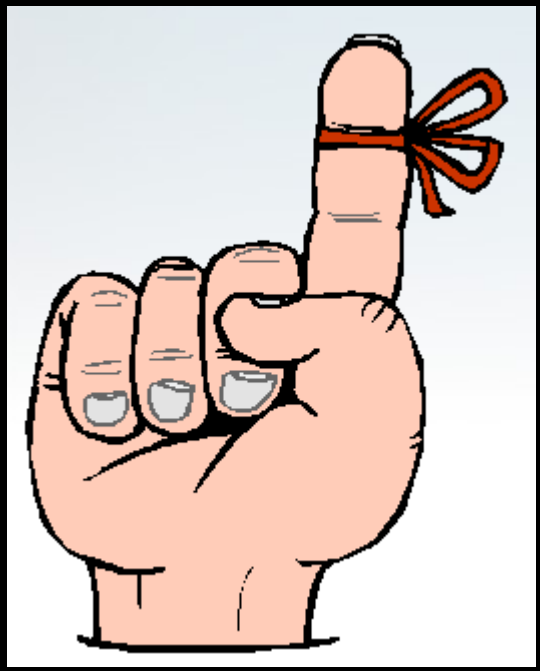
Build your intuition about SADS:

- Fluorescence SADS should be positive.
- Upon solvation, stimulated emission shifts to the red.
- Ground state SADS are negative only in the GSB region.

- Spectral changes ascribed to different physical processes match your intuition.

Important to remember:

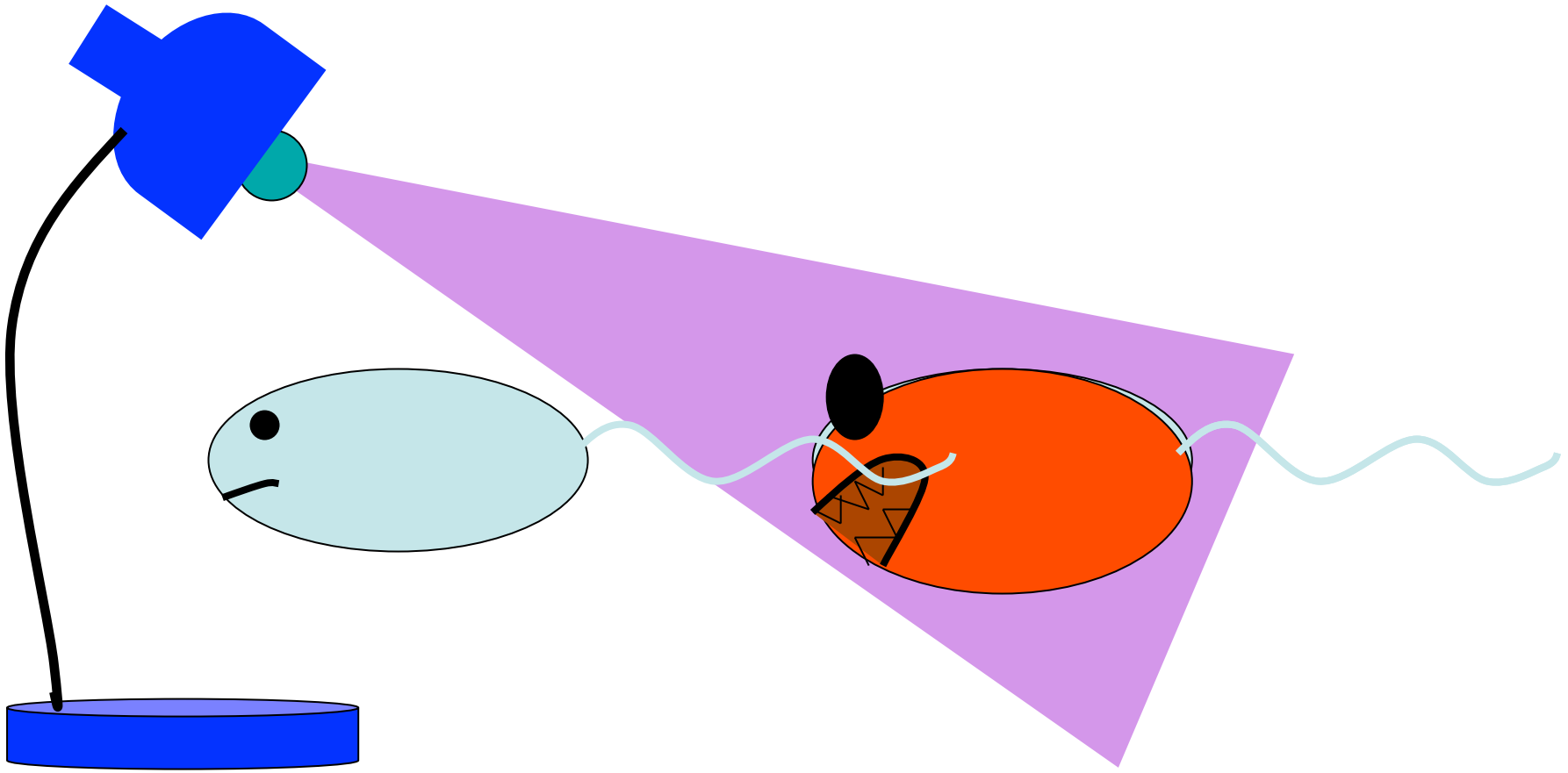
- Not all kinetics are exponential, but most of what we measure can be depicted as such.
- Worse fit and reasonable spectra is better than good fit with ridiculous spectra



The Photoactive Yellow Protein Structure

- Function: phototaxis photoreceptor in *Halorhodospira halophila*
- Water-soluble protein, suitable for genetic and chemical engineering
- High-resolution structures available (~0.85Å)

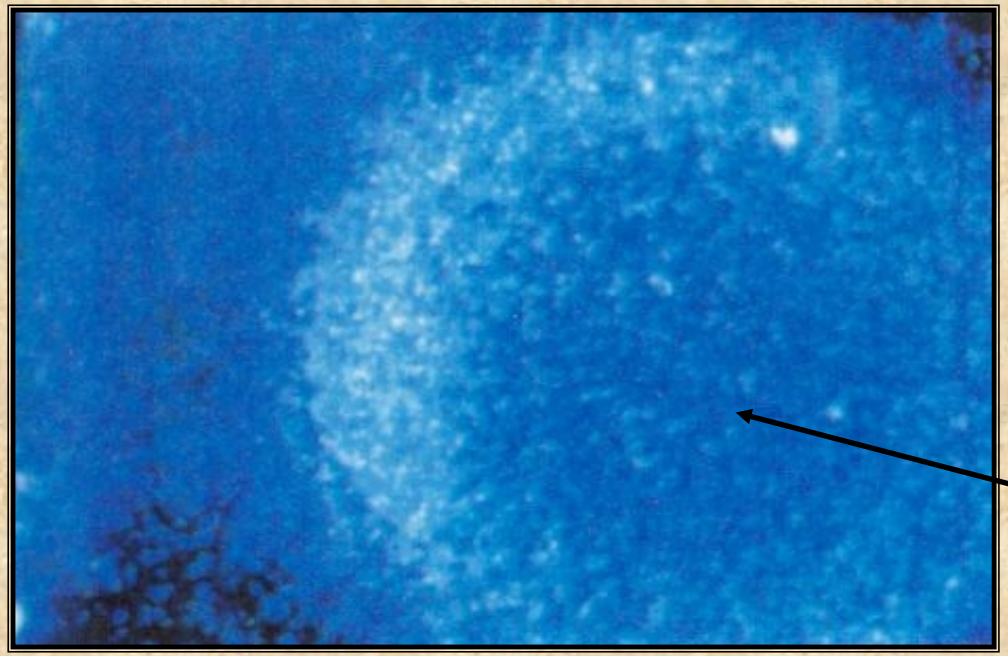
Negative phototactic response to blue light



UnHappy bacterium

Negative Phototactic Response in Halorhodospira halophila :

PYP as the signal Transducer

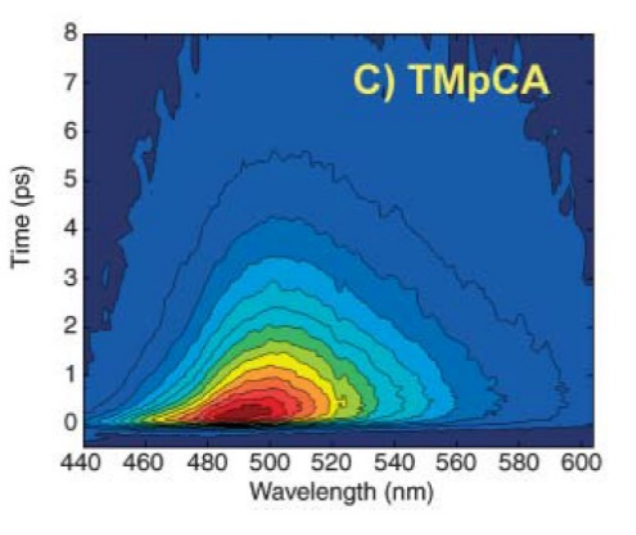


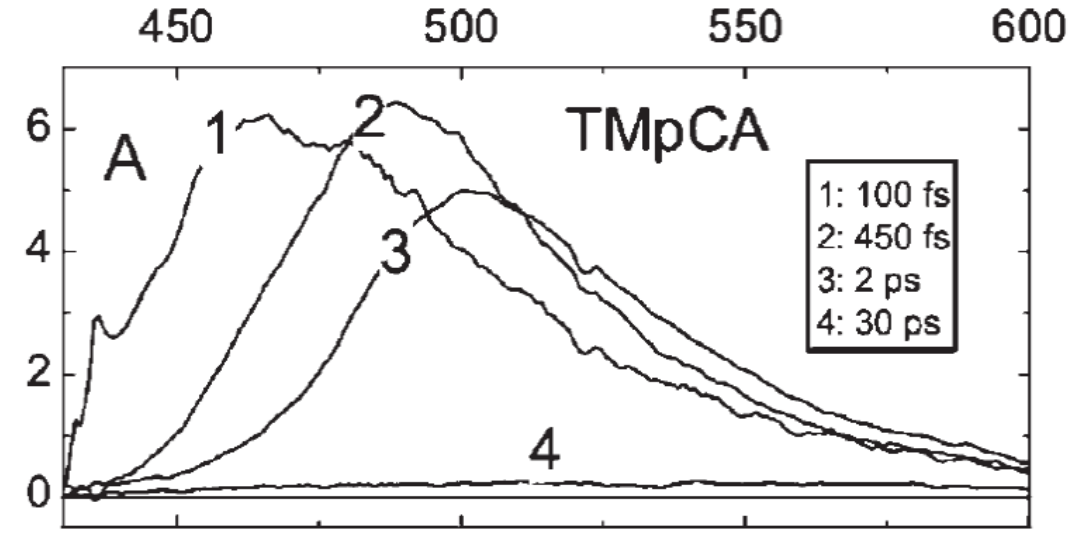
Negative Phototaxic Response to Applied Blue Light

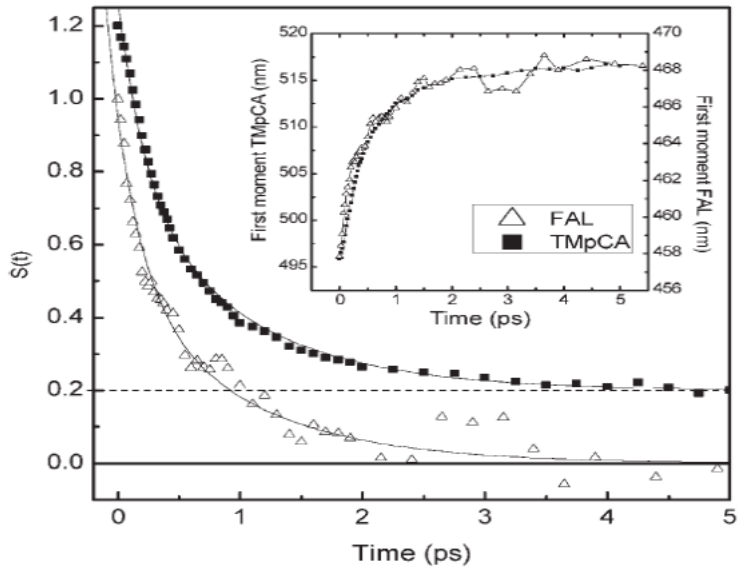
Local Light Illumination

Hellingwerf and co-workers: J Bacteriol, 1993. 175(10): p. 3096-104.

Case study: solvation +isomerization







Adding a twist...

Advanced ultrafast spectroscopies

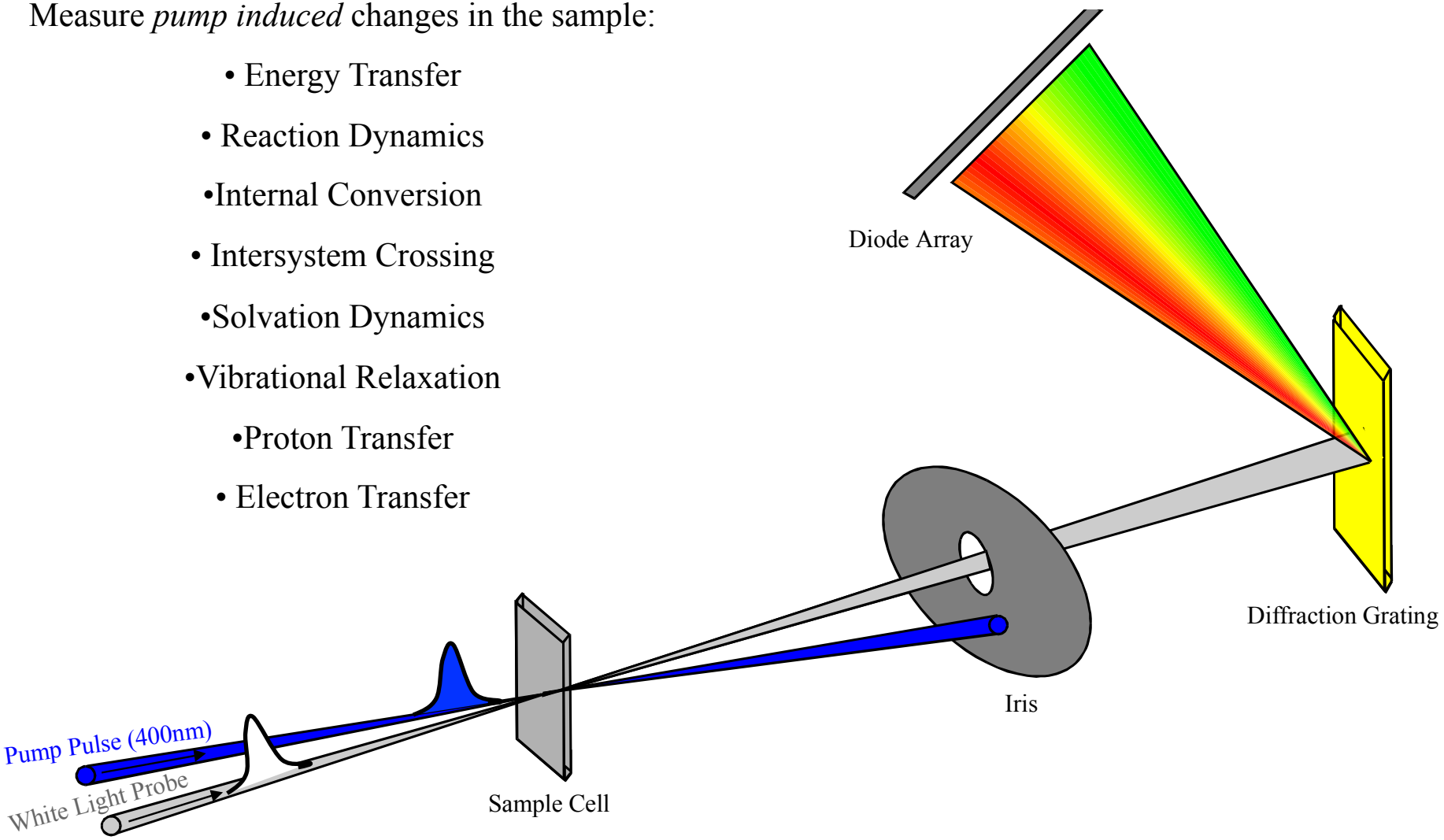
Twist #1:

Multi-pulse transient absorption

Dispersed Pump-Probe Experimental Setup

Measure *pump induced* changes in the sample:

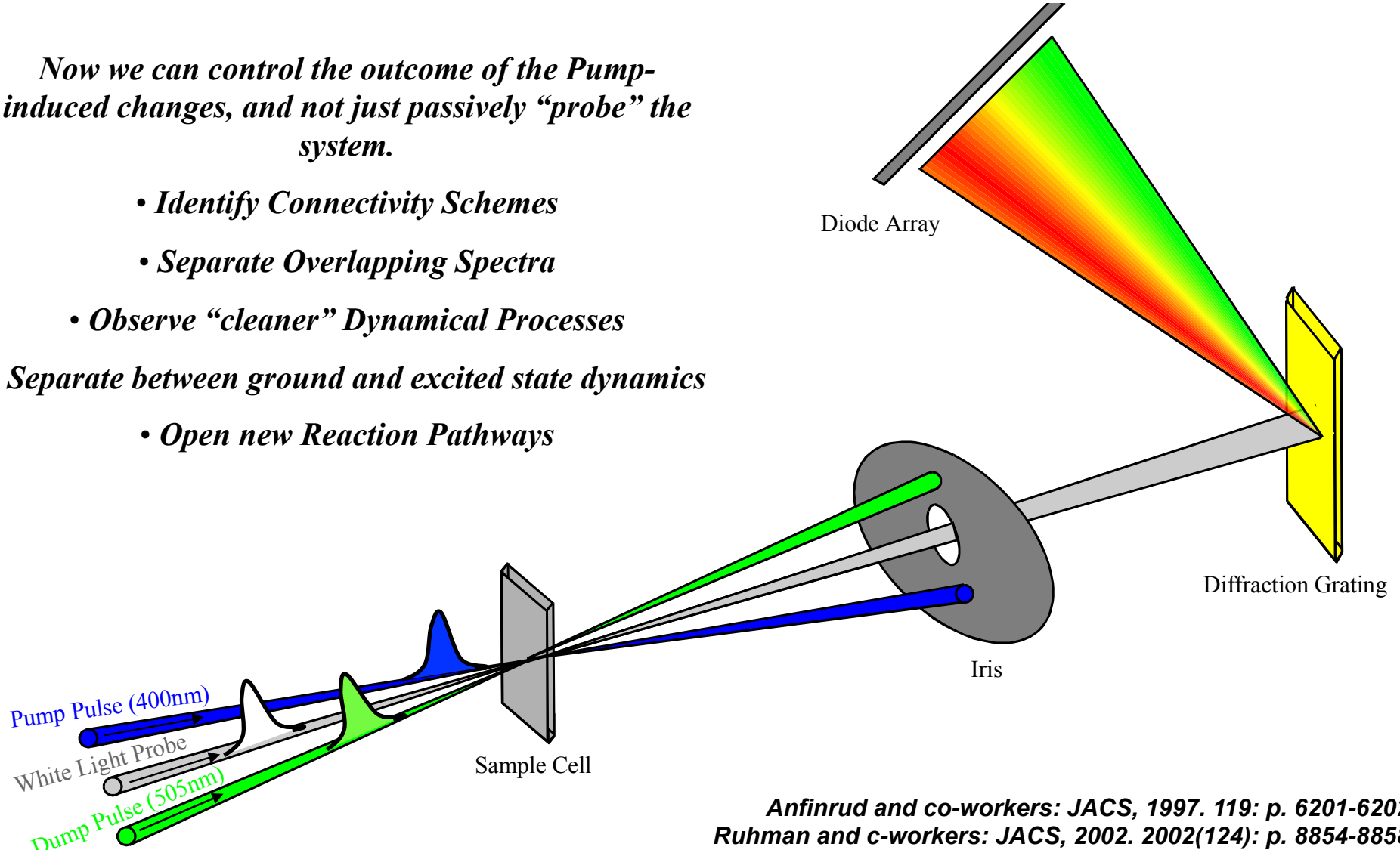
- Energy Transfer
- Reaction Dynamics
- Internal Conversion
- Intersystem Crossing
- Solvation Dynamics
- Vibrational Relaxation
- Proton Transfer
- Electron Transfer



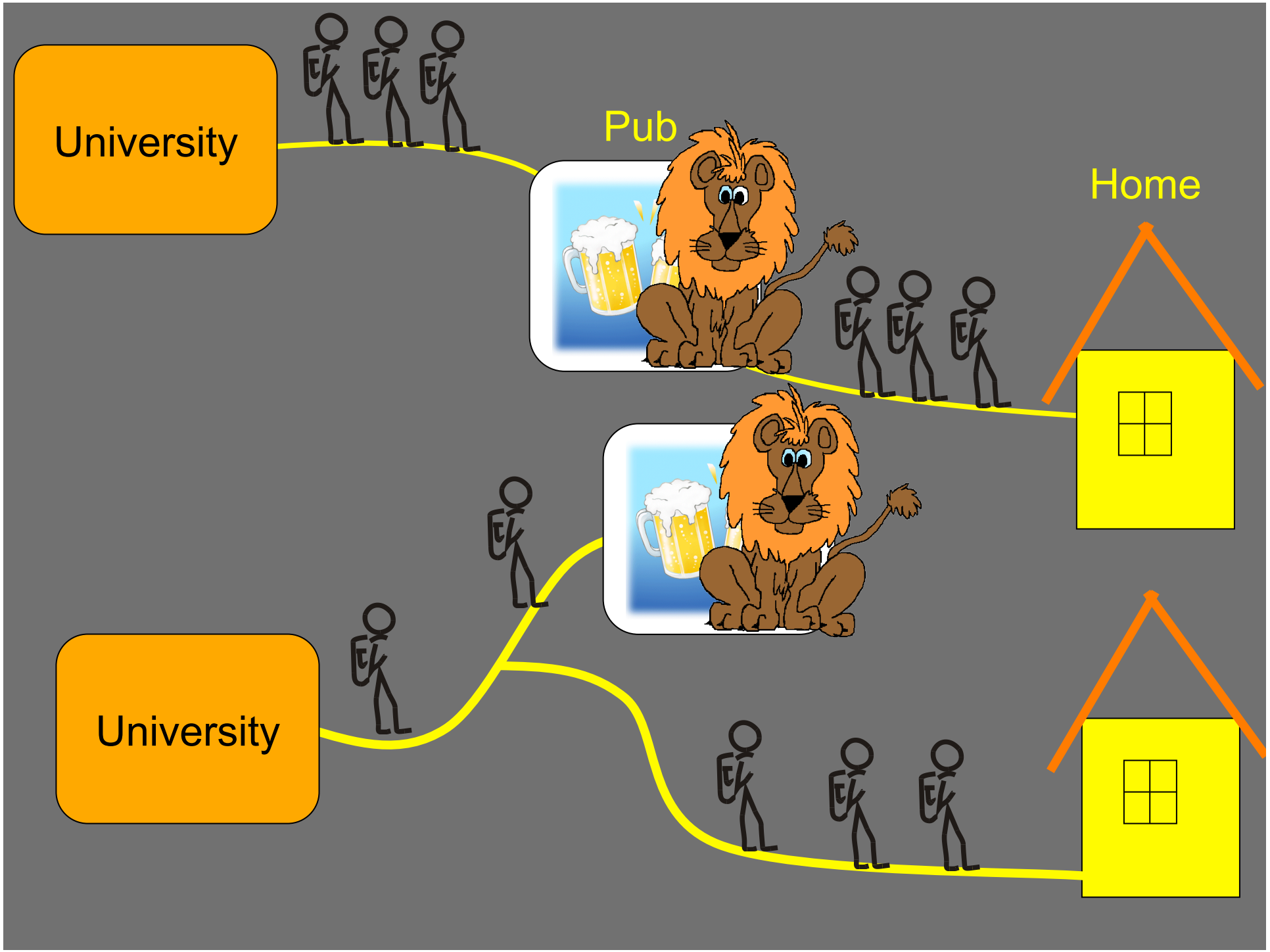
Dispersed Multi-Pulse Experimental Setup

Now we can control the outcome of the Pump-induced changes, and not just passively "probe" the system.

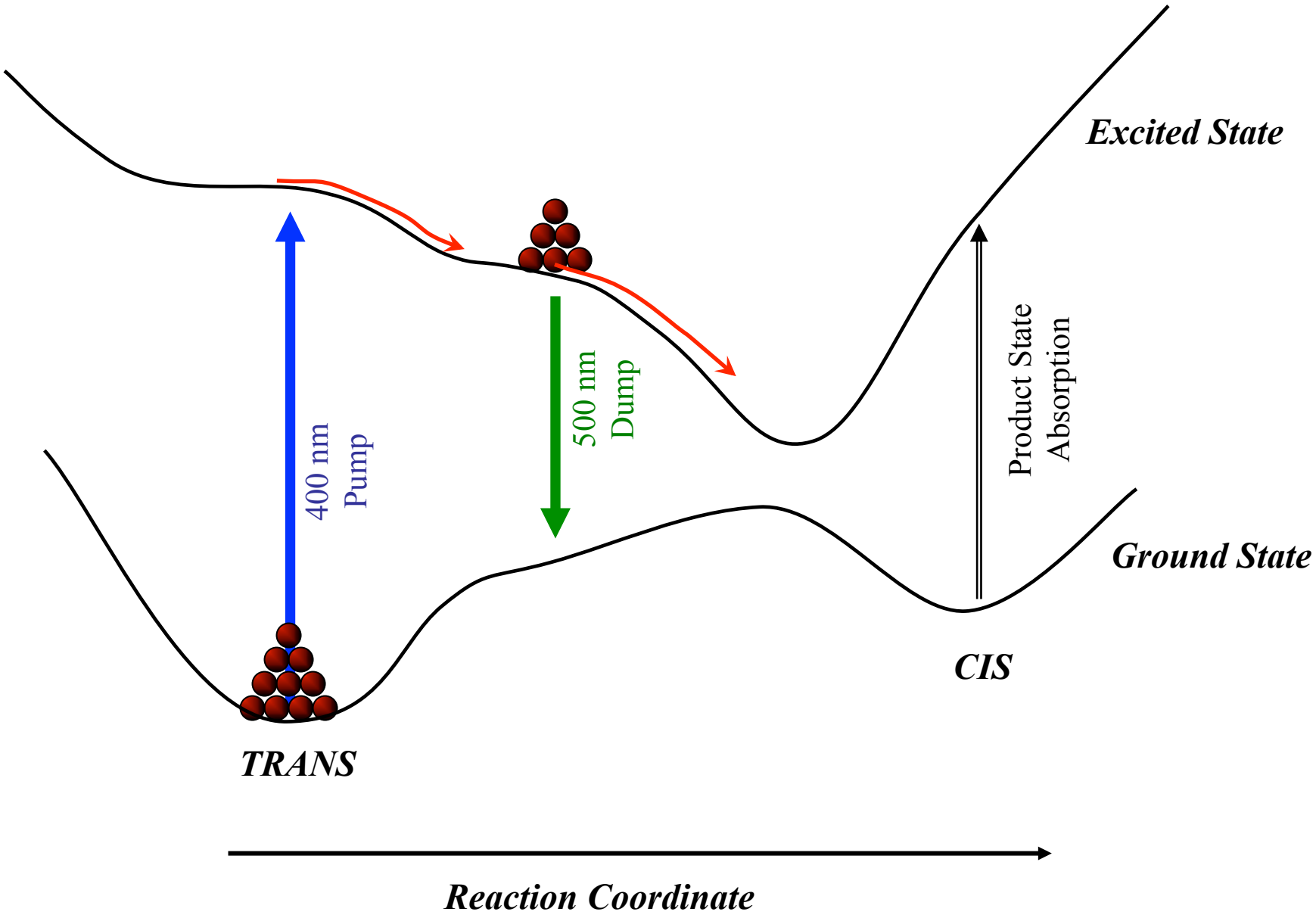
- ***Identify Connectivity Schemes***
- ***Separate Overlapping Spectra***
- ***Observe "cleaner" Dynamical Processes***
- ***Separate between ground and excited state dynamics***
- ***Open new Reaction Pathways***



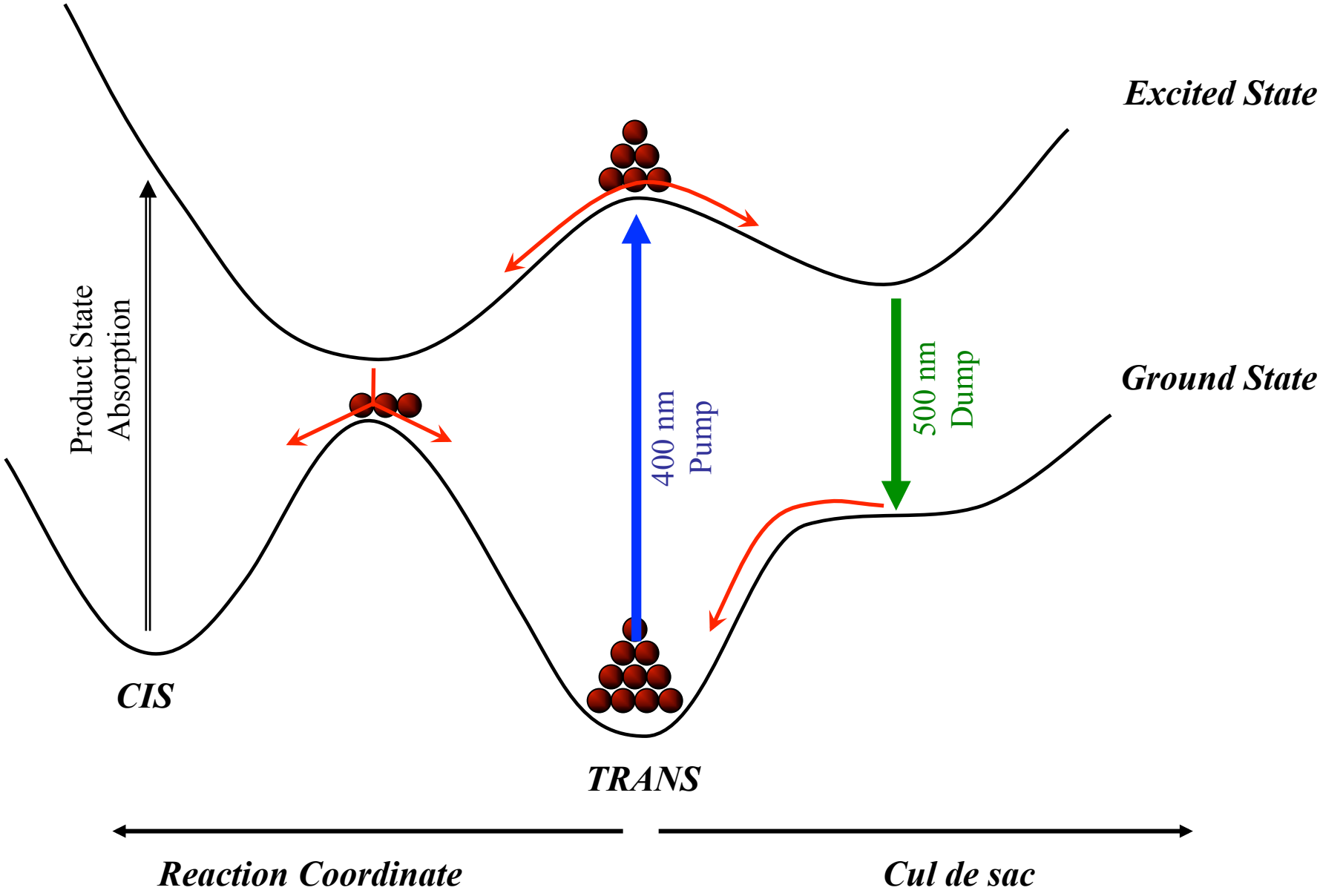
Anfinrud and co-workers: JACS, 1997. 119: p. 6201-6202
Ruhman and c-workers: JACS, 2002. 2002(124): p. 8854-8858
Field and co-workers: Journal of Chemical Physics, 1988. 88(9): p. 5972-5974


University
Pub
Home
University

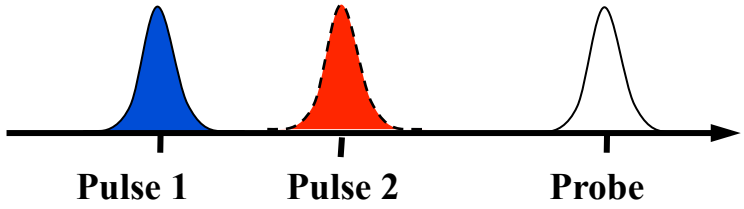
Sequential Isomerization Dynamics

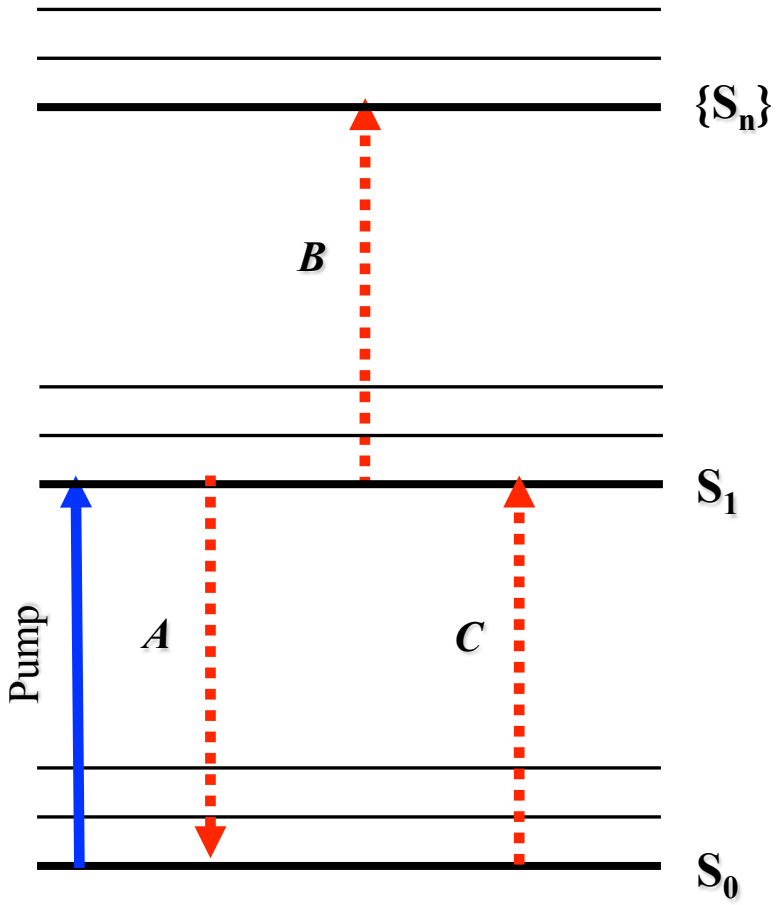


Branched Isomerization Dynamics



Multi-pulse Transient Absorption Spectroscopies





A: Pump-dump-probe (PDP)

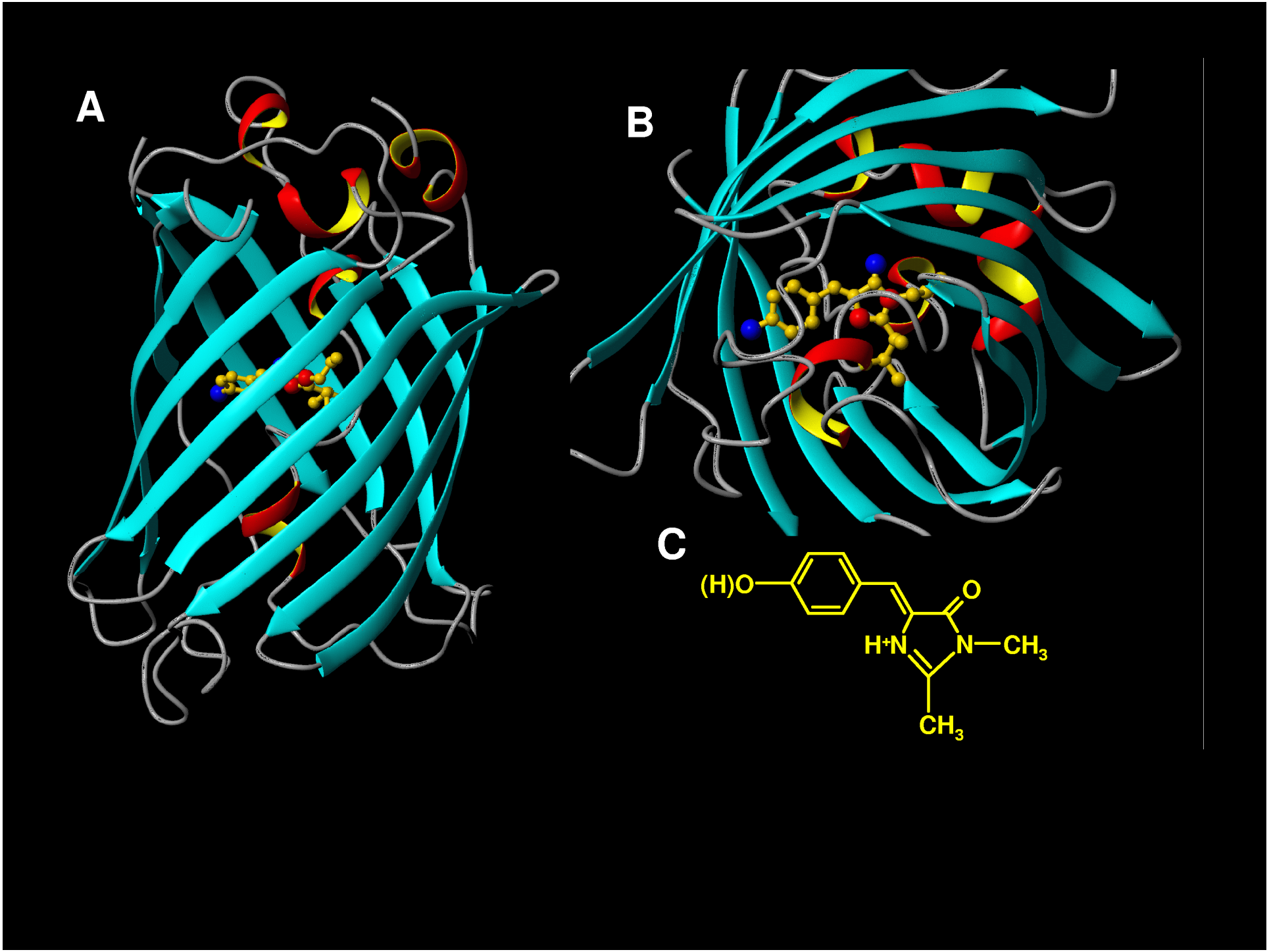
The second laser pulse is resonant with the stimulated emission band: (*loss of excited population leading to decrease of all signals*)

B: Pump-repump-probe (PrPP)

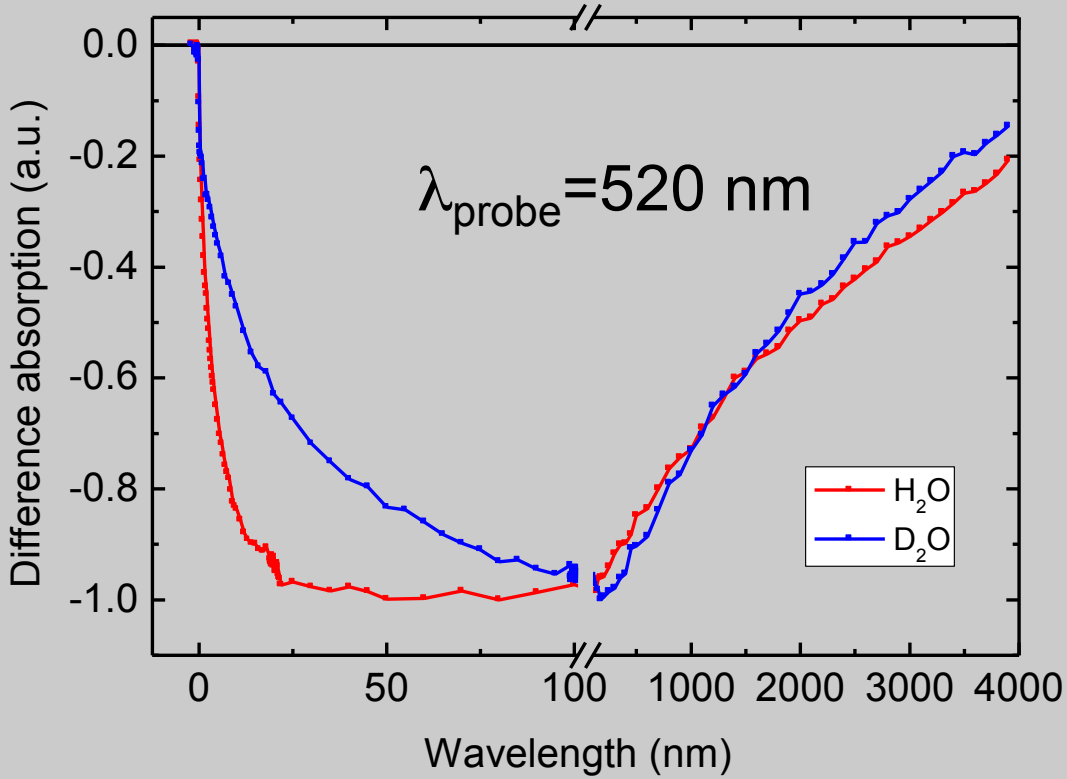
The second laser pulse is resonant with an excited state absorption: (*loss of excited state signals with no effect on the bleach*)

C: Pre-pump Pump-Probe (4P) and Double Pump-Probe (PPP)

Both actinic pulses are resonant with the ground state absorption: (*pump probe on the excited state*)

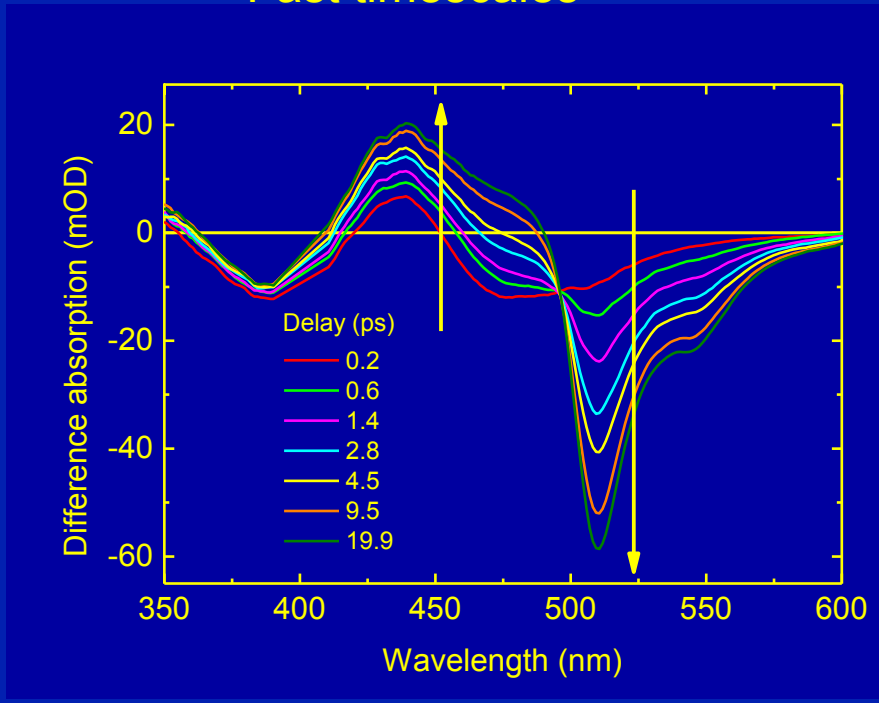

A
B
C
(H)O
O
H+N
N—CH3
CH3

Kinetic Isotope Effect: evidence for proton transfer

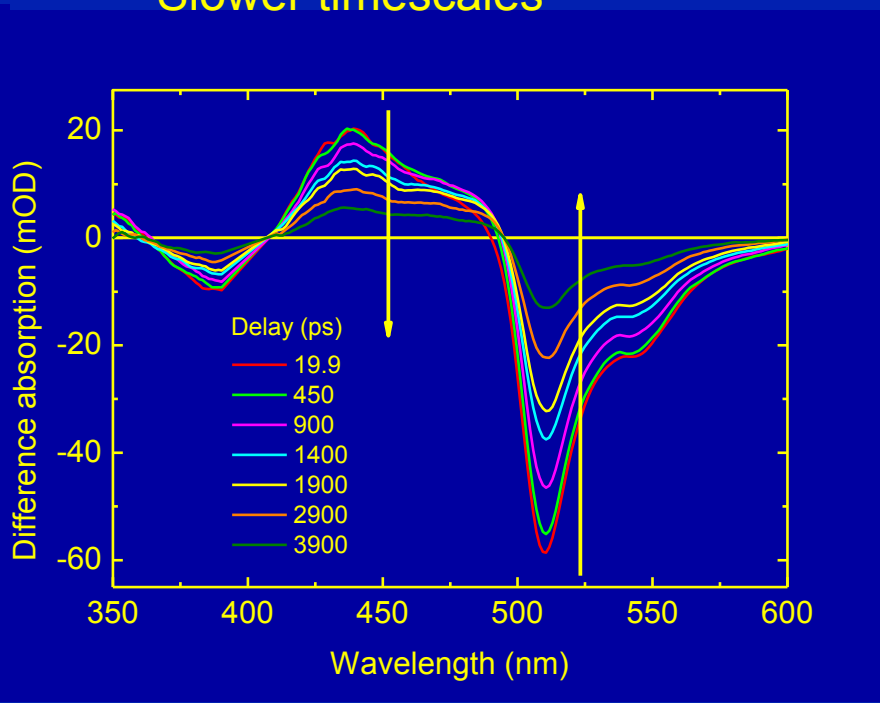


Excited state proton transfer in GFP

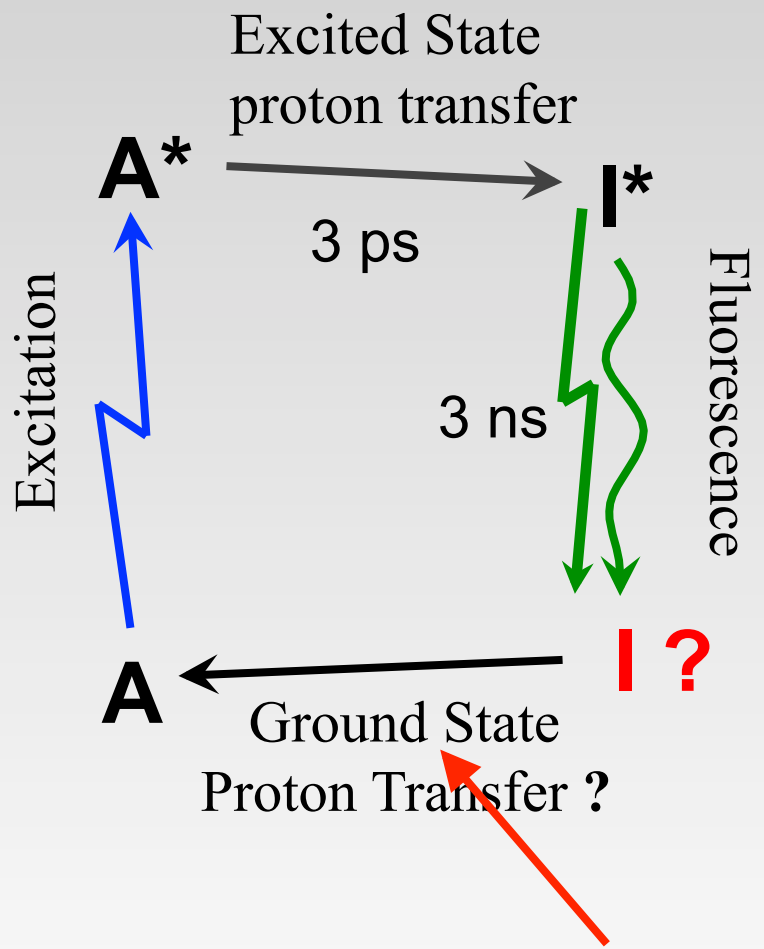
Fast timescales



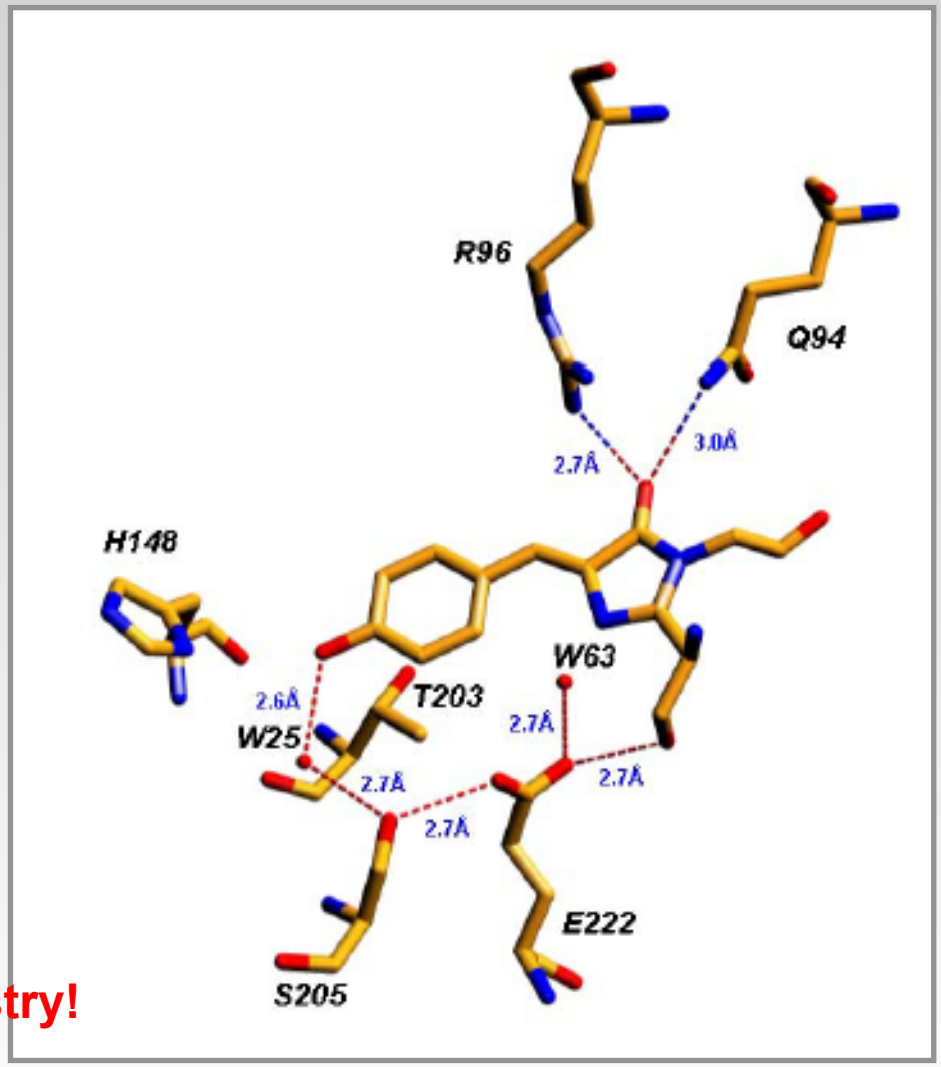
Slower timescales



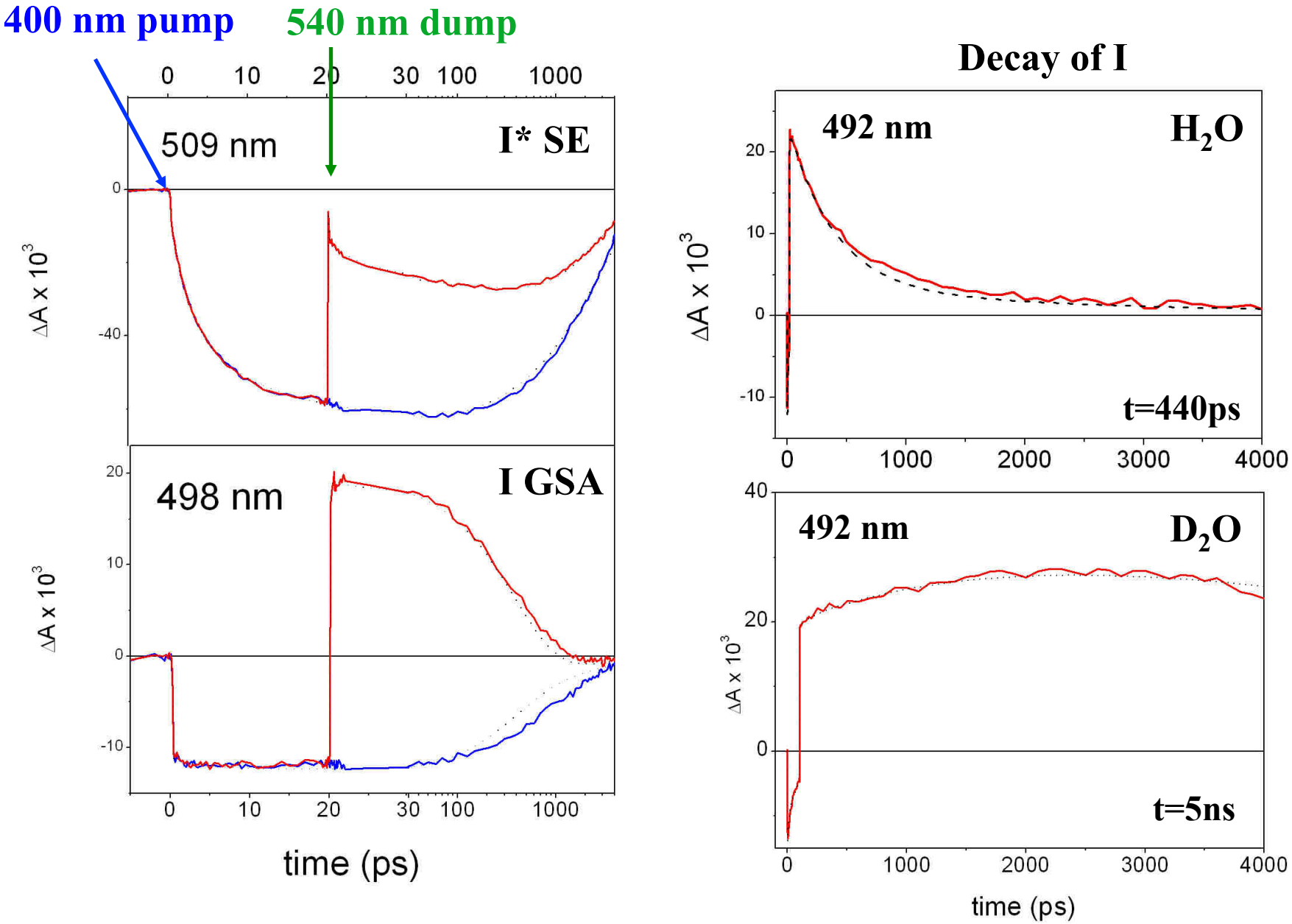
Idea: try to dump the excited state!



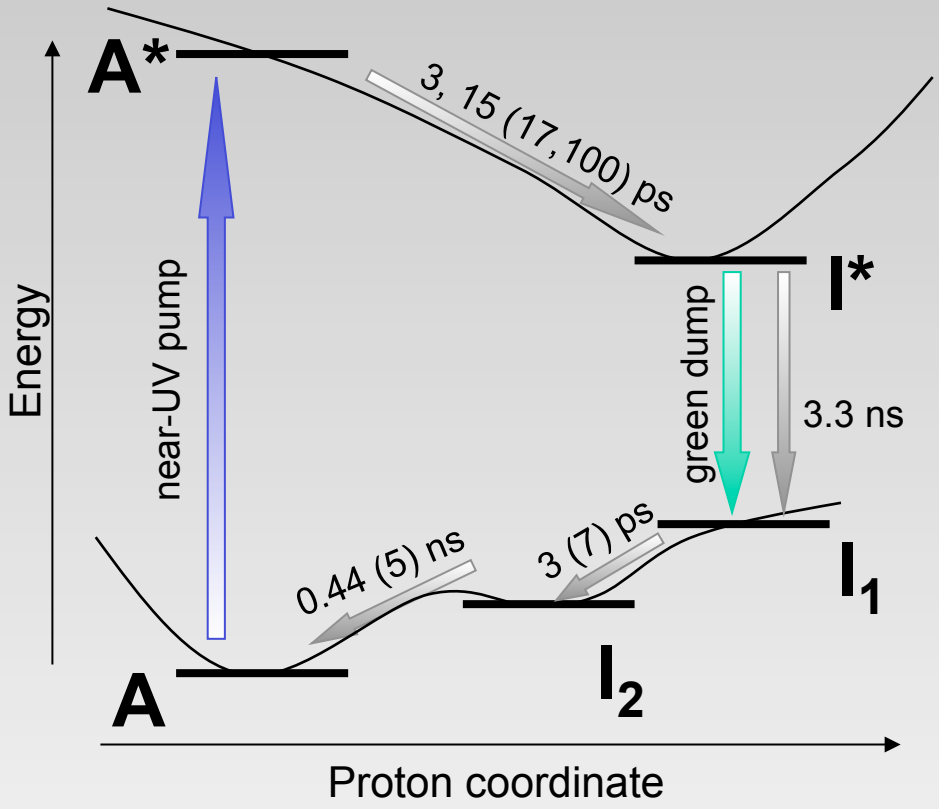
A fundamental reaction in biochemistry!

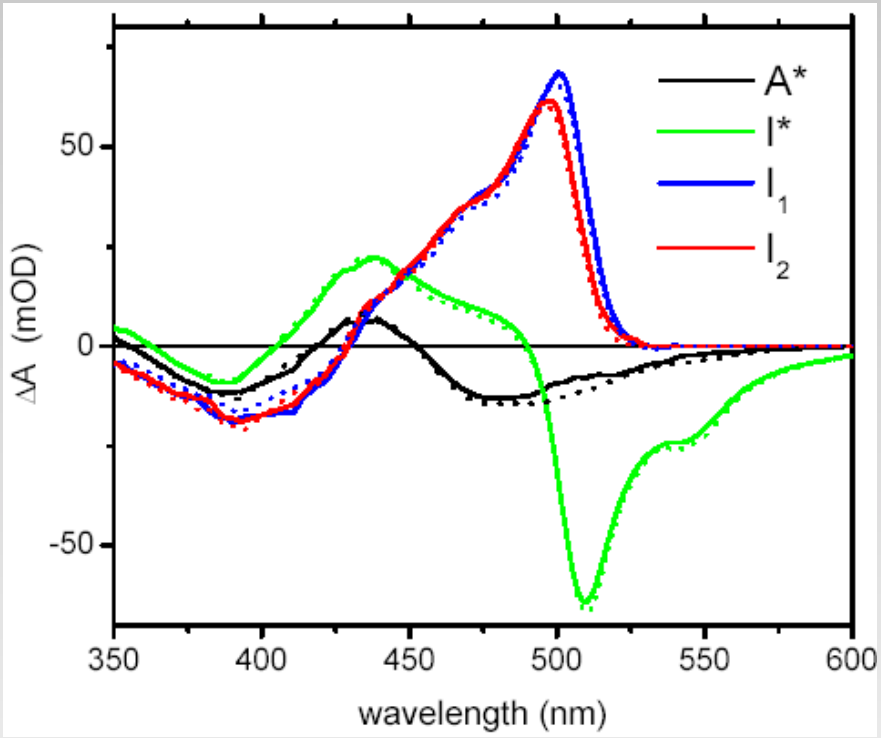


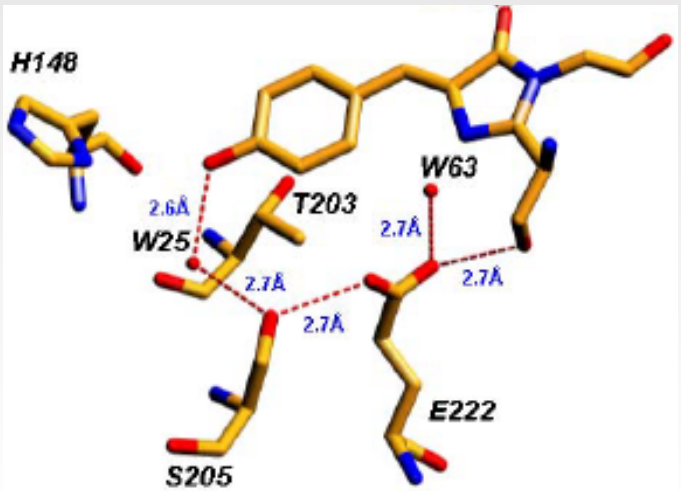
Pump-dump-probe spectroscopy on GFP



Spectra and lifetimes of Hidden Intermediates





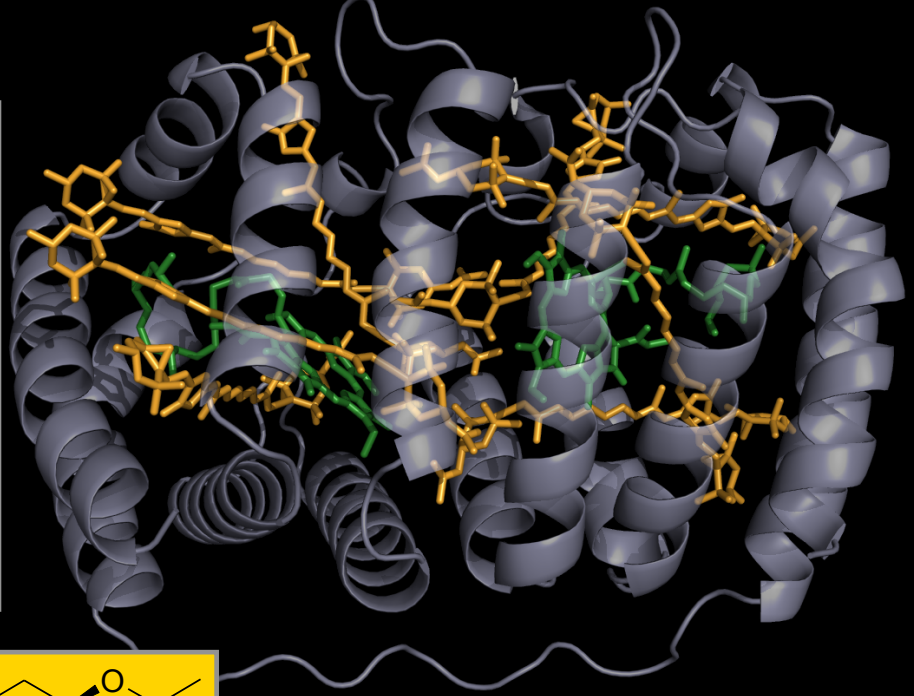


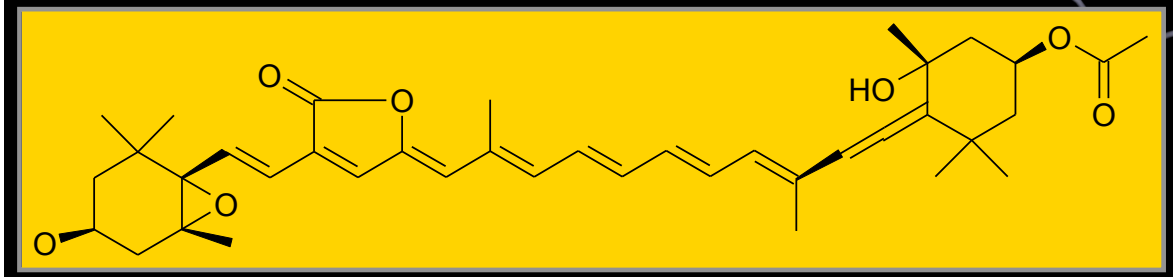
- **I_1 max: 500 nm, I_2 max: 498 nm**
- **I_1 stokes shift: 9 nm**
- **I_1 to I_2 evolution: H-bond rearrangement?**

Energy transfer in PCP: role of ICT state

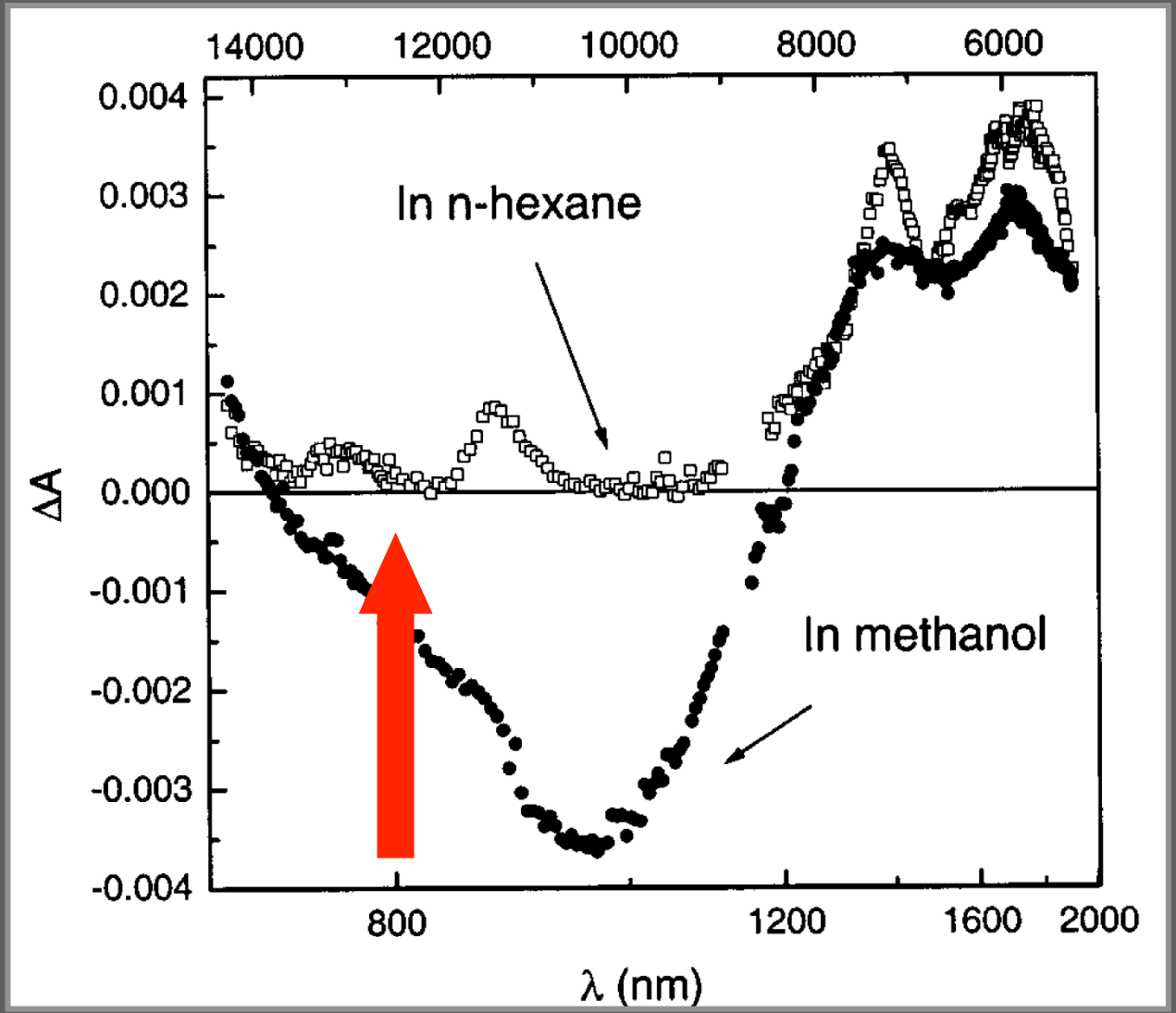
Red algae

PCP



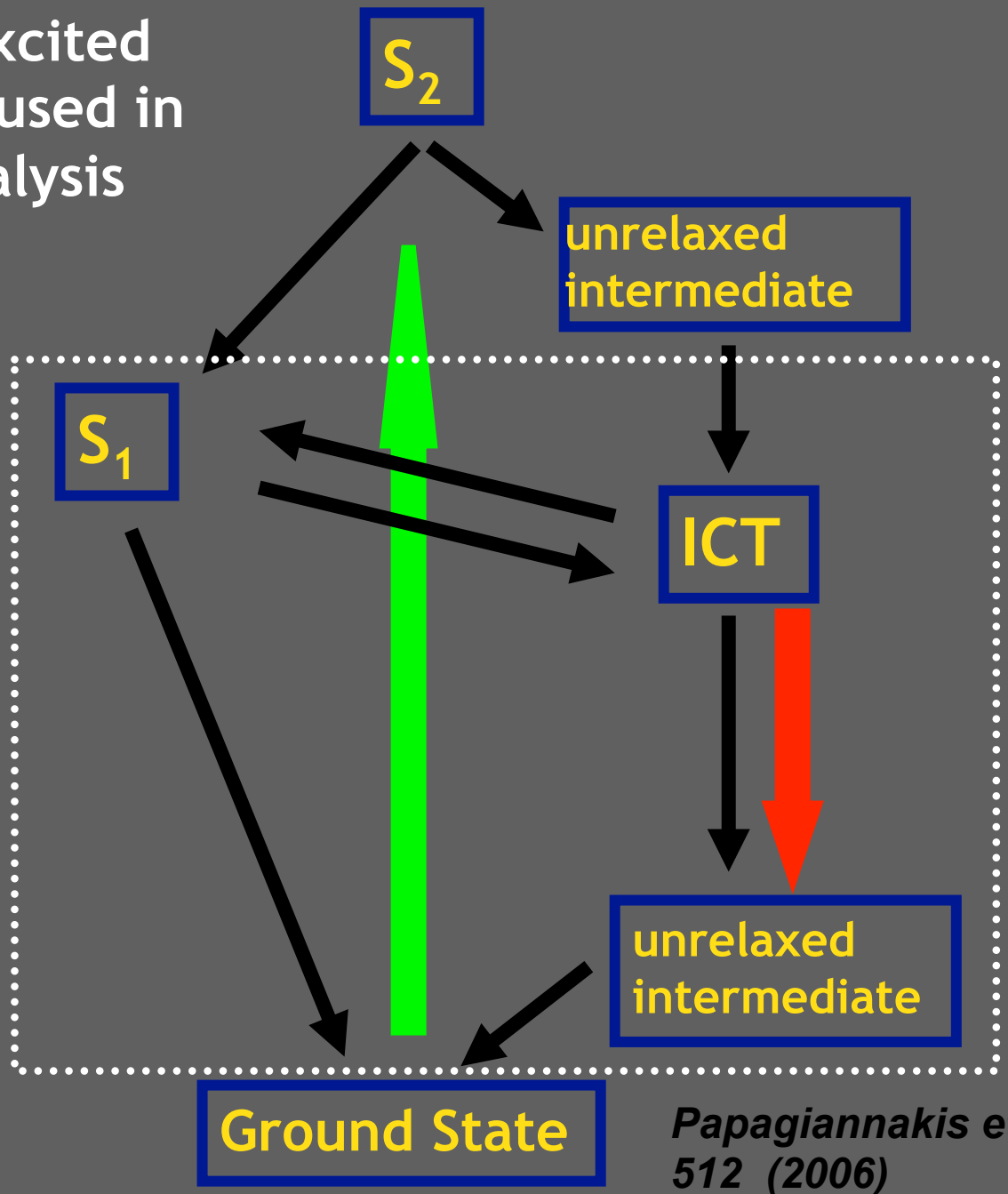


Near-IR transient Absorption spectra of peridinin



Zigmantas *et al.*, *JPC A* 105, 2001

Peridinin excited state model used in target analysis



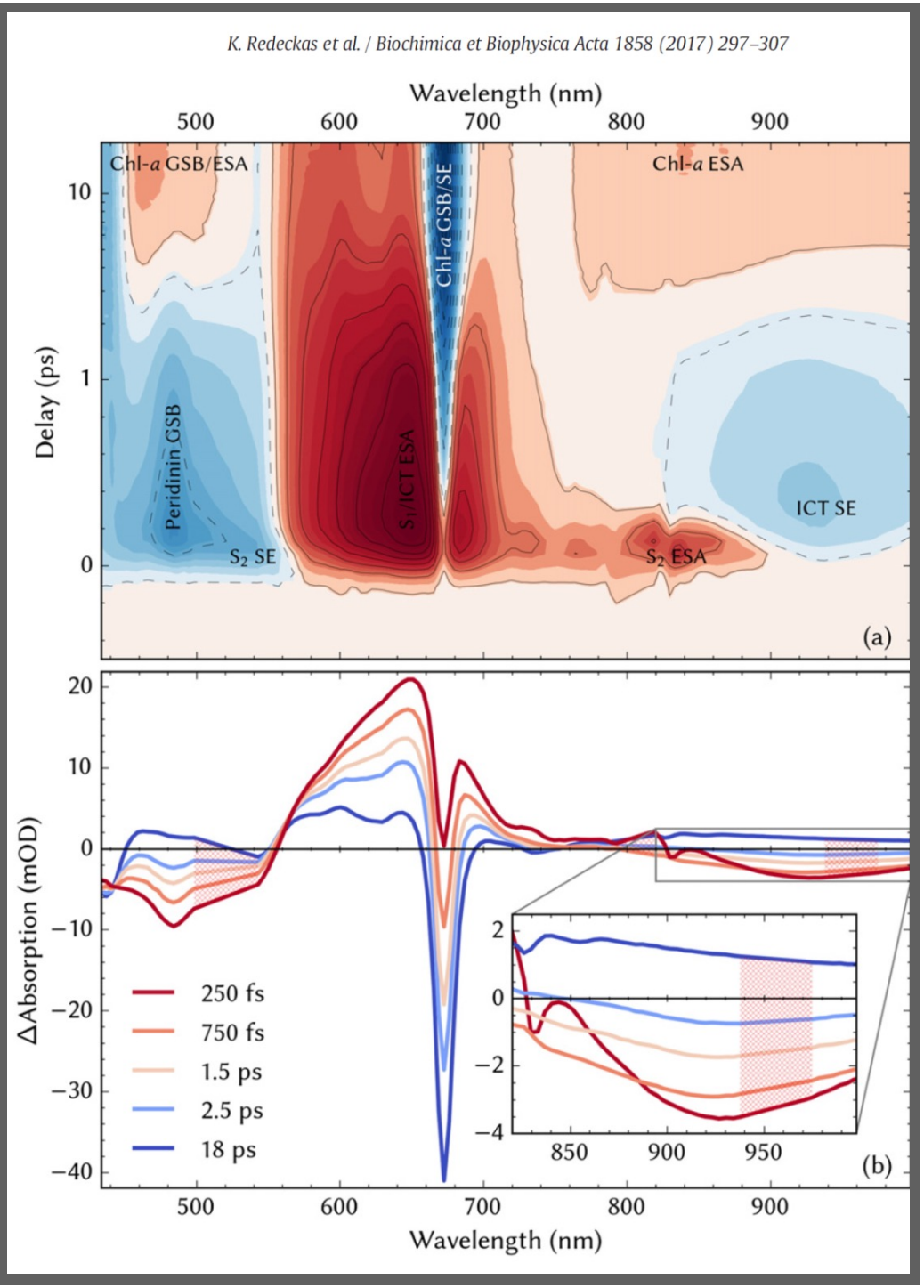
excitation

relaxation

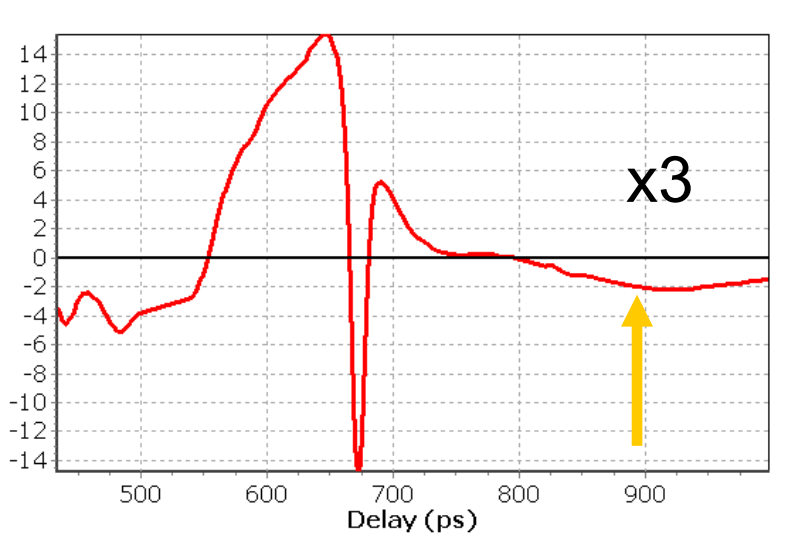
dump

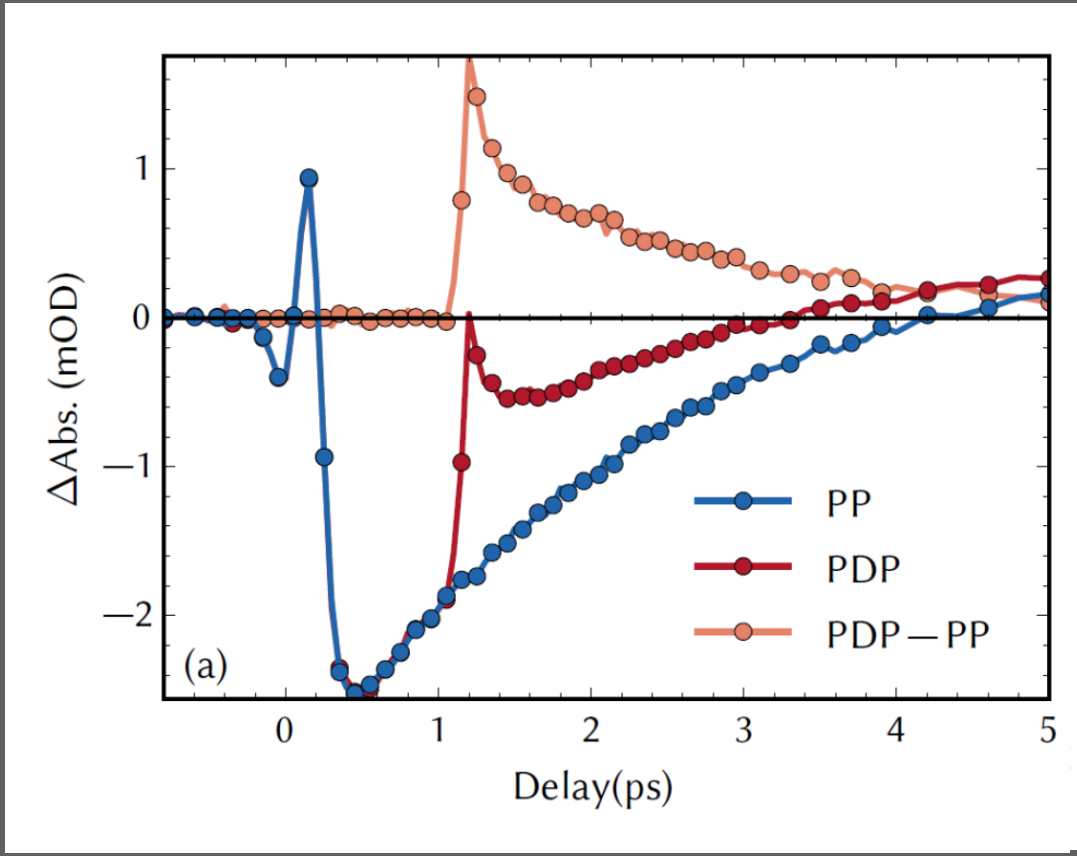
Papagiannakis et al., JPCB, 110, 512 (2006)

PCP Data: pump at 520 nm probe 440-1000 nm, dump 950 nm

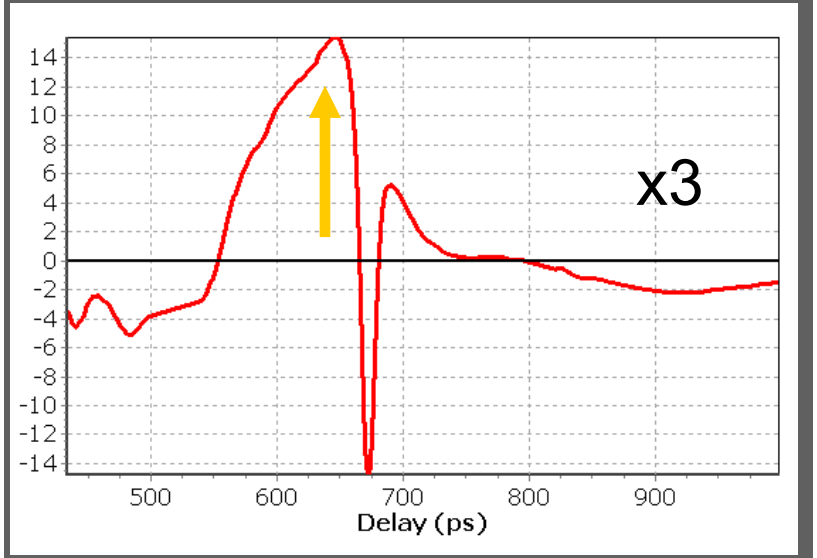


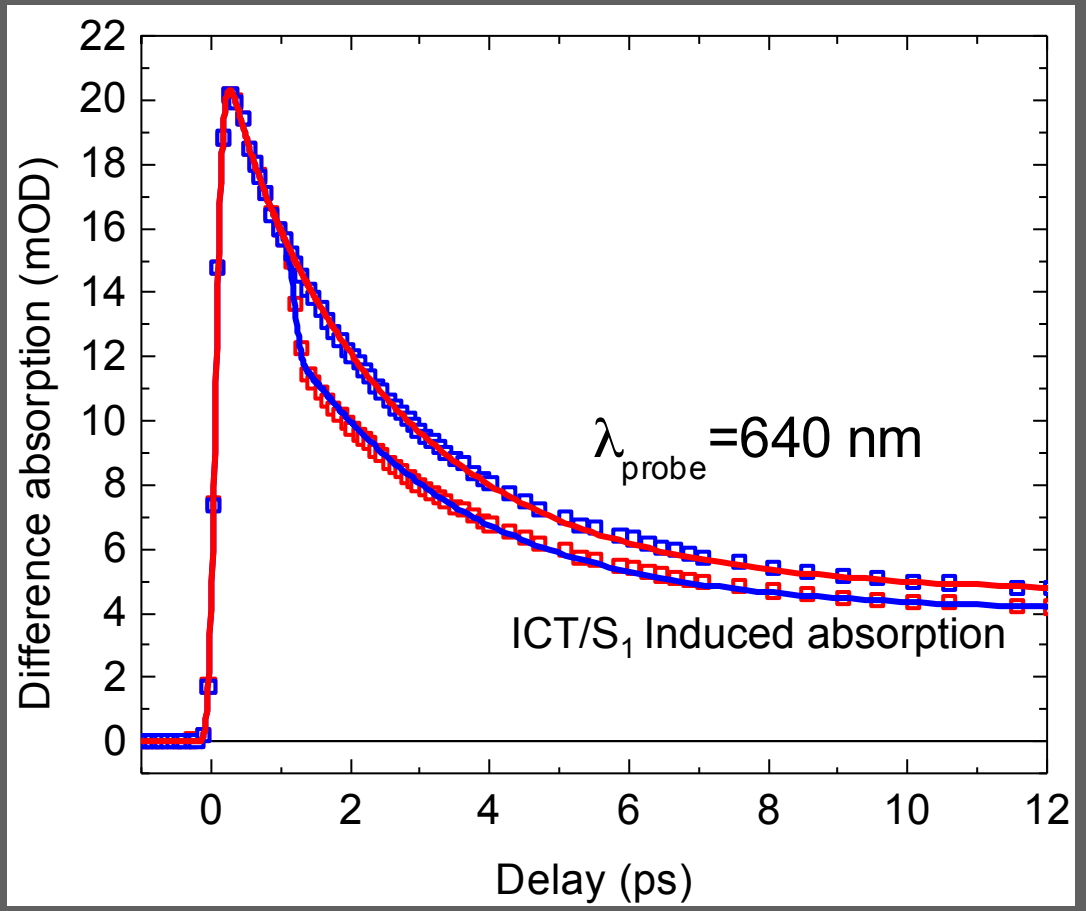
Dump effects at different wavelengths



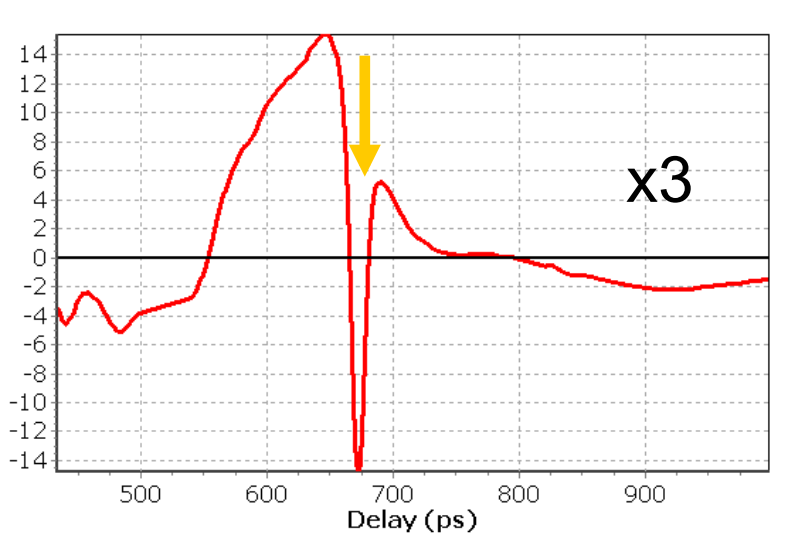


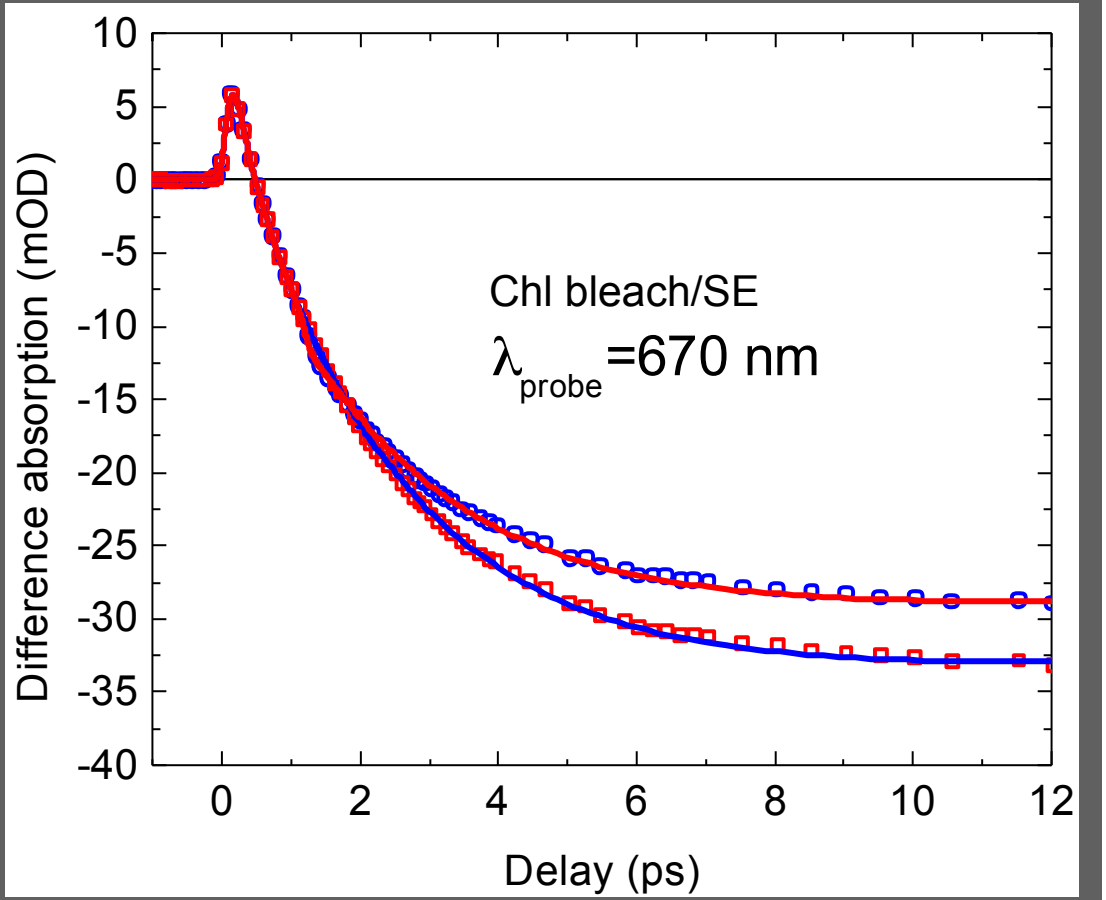
Dump effects at different wavelengths





Dump effects at different wavelengths





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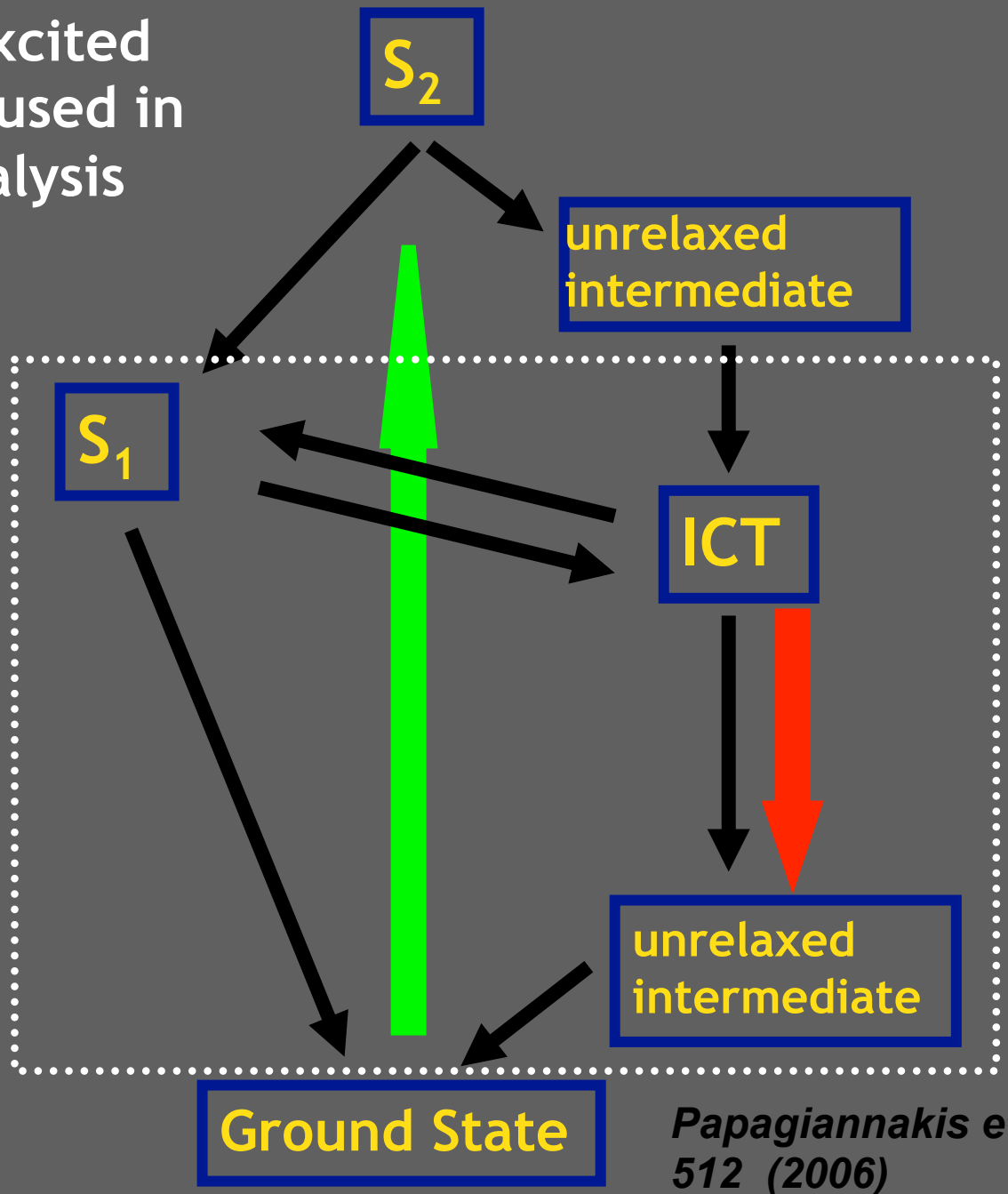
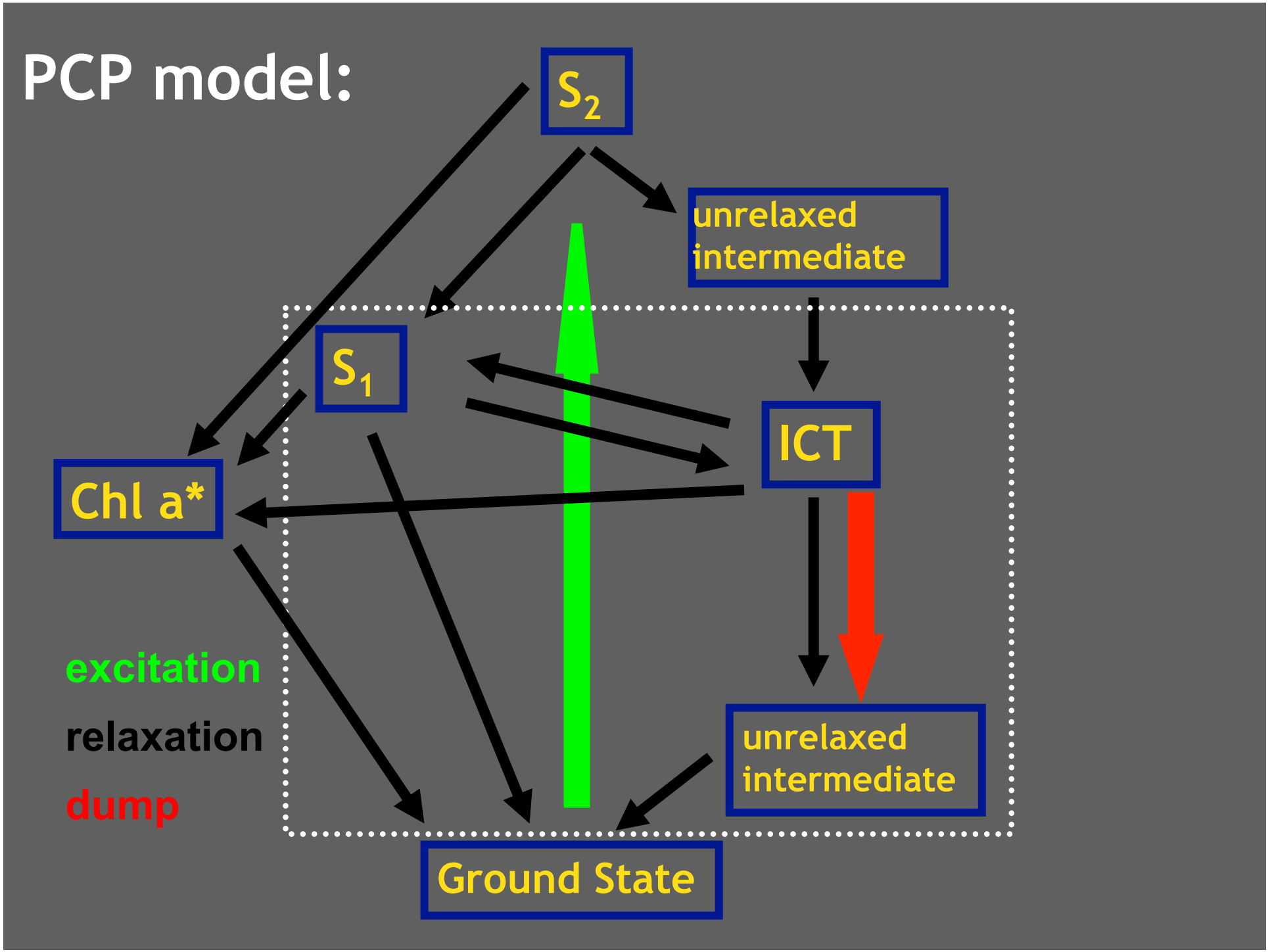


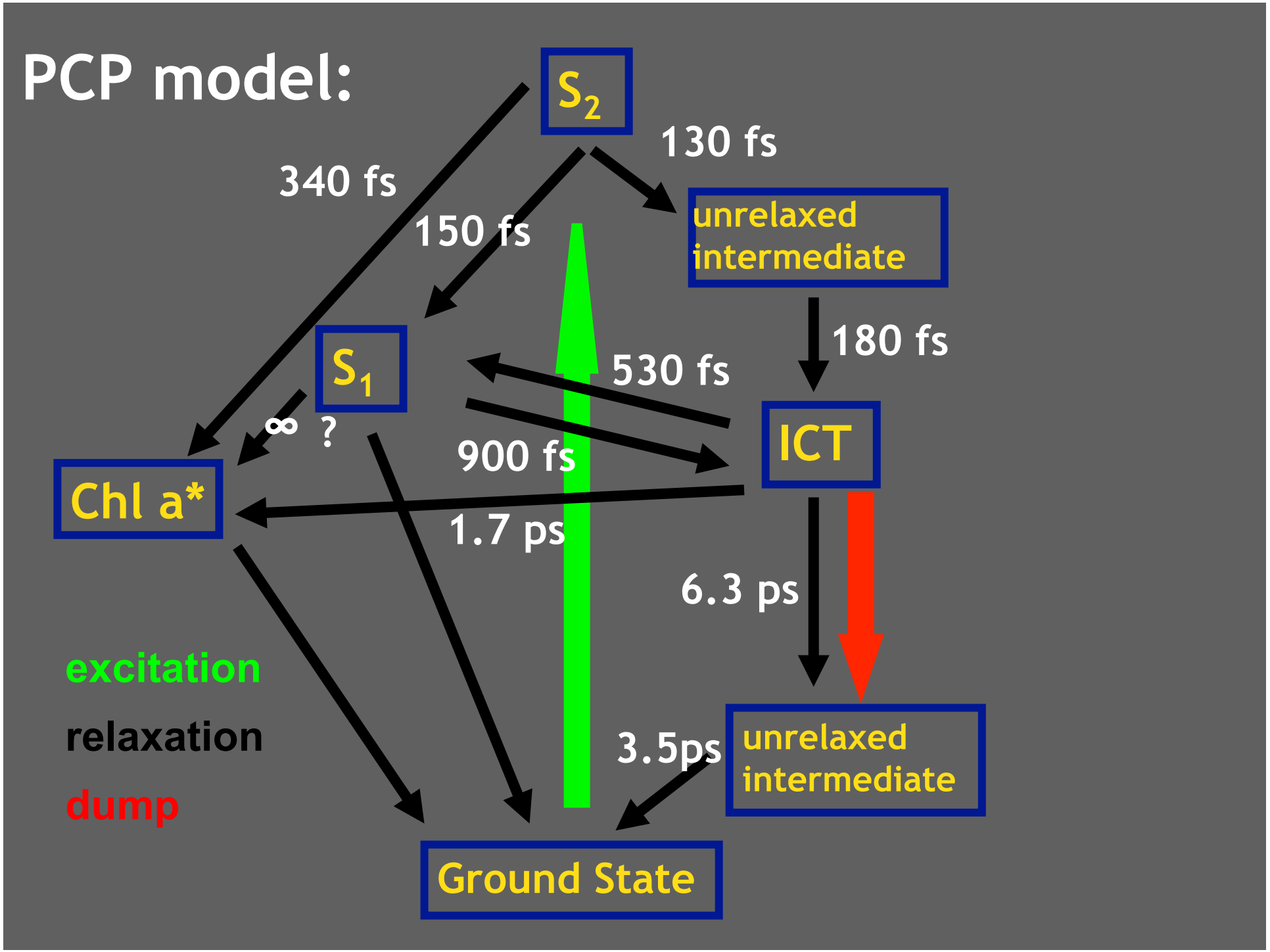
excitation

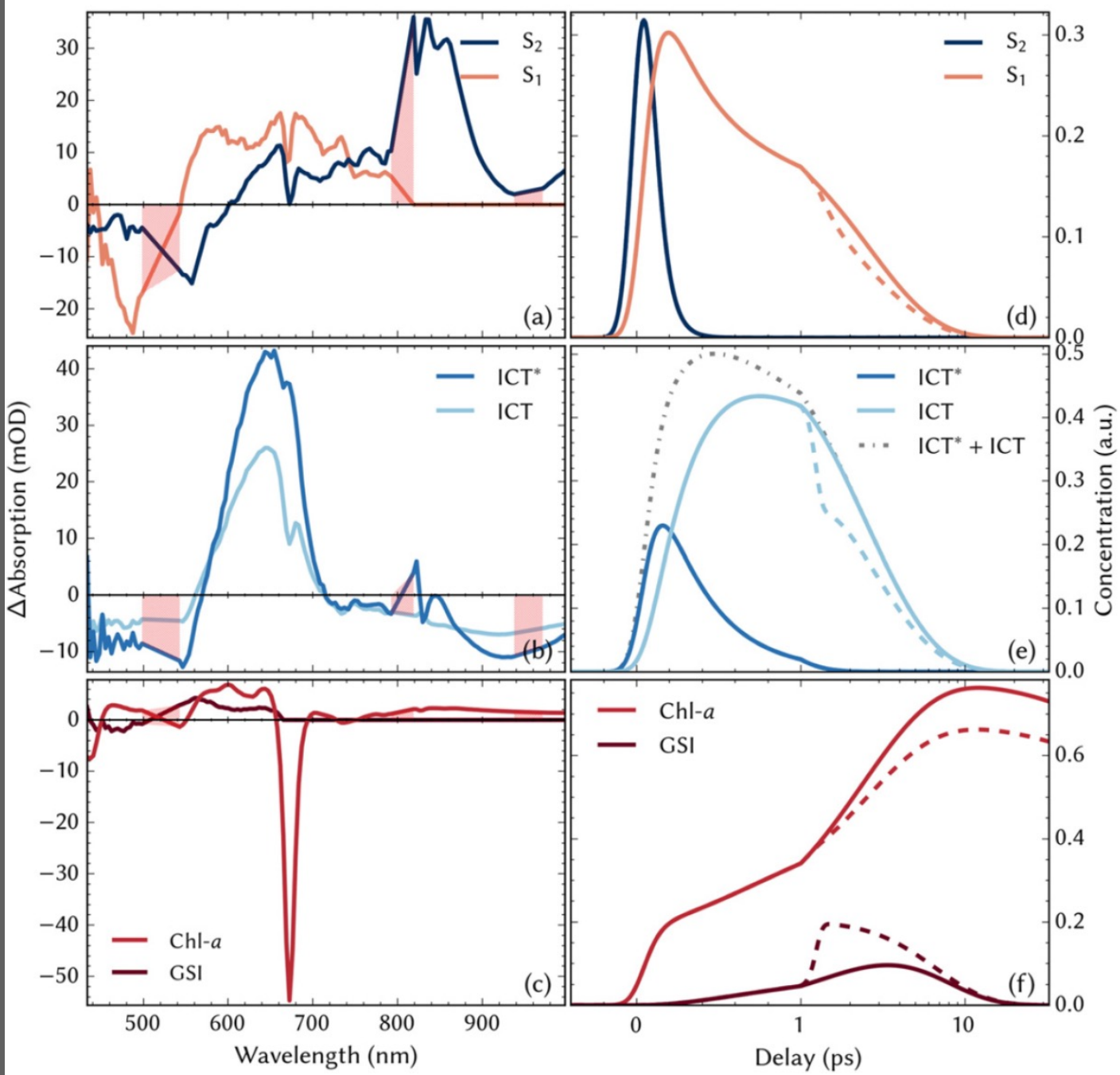
relaxation

dump

Papagiannakis et al., JPCB, 110, 512 (2006)

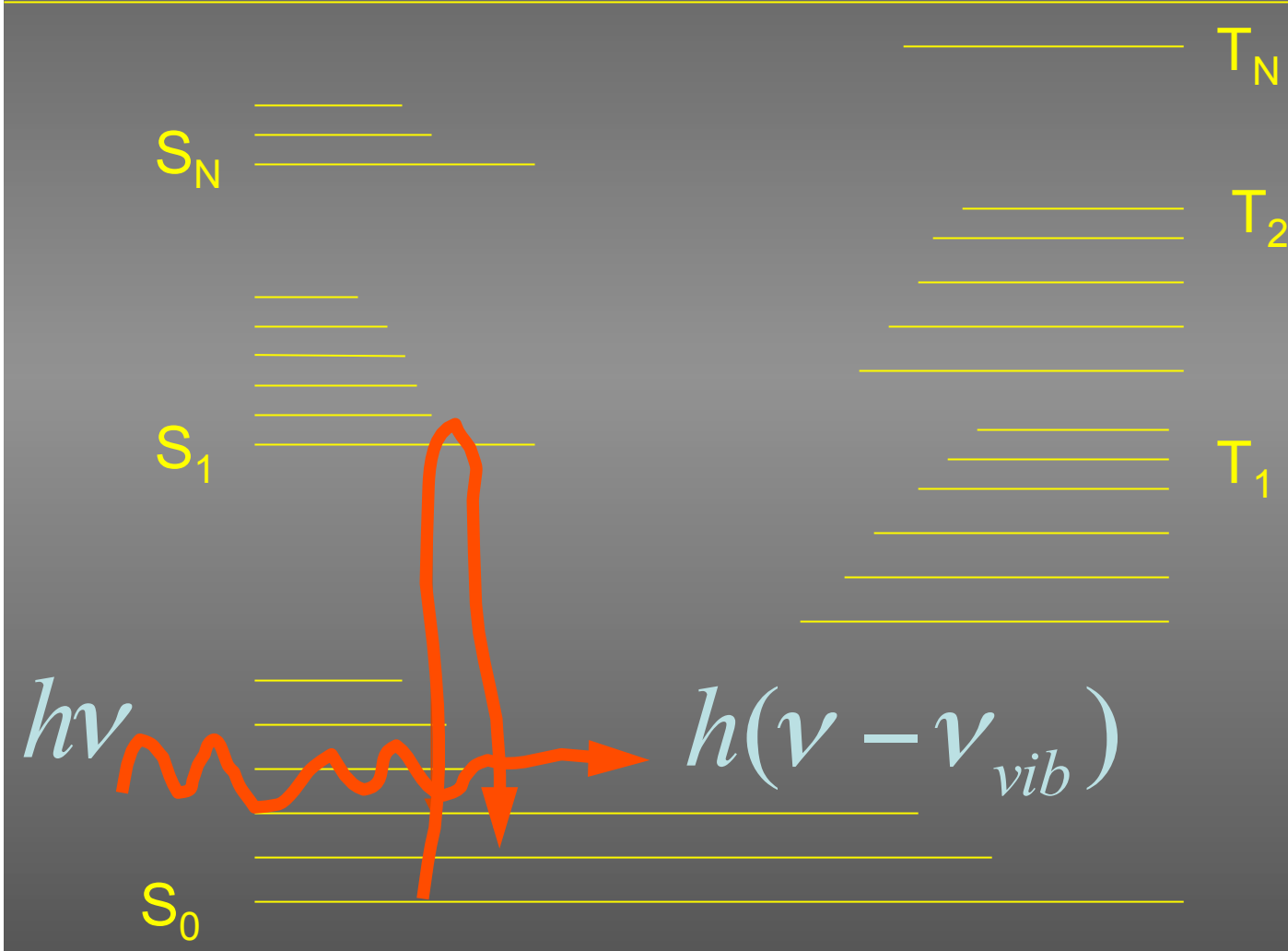

PCP model:
S_2
unrelaxed intermediate
S_1
ICT
Chl a*
excitation
relaxation
dump
unrelaxed intermediate
Ground State


PCP model:
S2
130 fs
340 fs
150 fs
unrelaxed intermediate
180 fs
S1
530 fs
∞ ?
900 fs
ICT
Chl a*
1.7 ps
6.3 ps
excitation
relaxation
dump
3.5ps
unrelaxed intermediate
Ground State


S2
S1
(a)
(d)
ICT*
ICT
ICT* + ICT
(b)
(e)
Chl-a
GSI
(c)
(f)
ΔAbsorption (mOD)
Concentration (a.u.)
Wavelength (nm)
Delay (ps)

Twist #2: Femtosecond stimulated Raman spectroscopy (FSRS)

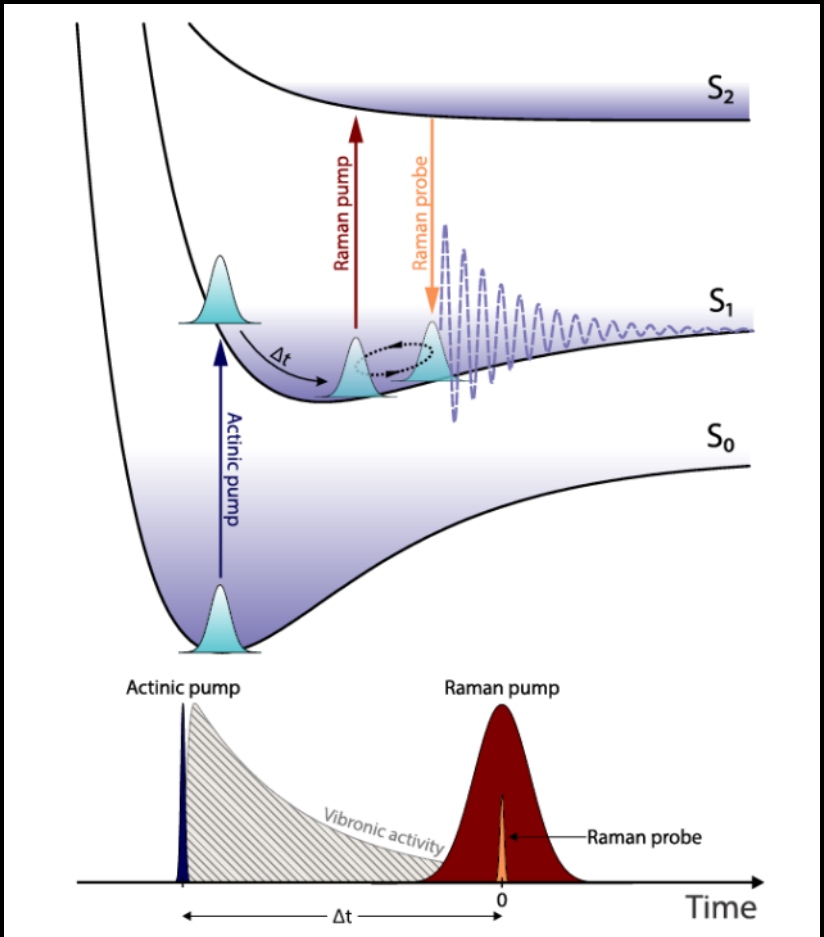
Raman scattering



Twist #2: Femtosecond stimulated Raman spectroscopy (FSRS)

- Playing around with electronic states is all good and well, however, the underlying structural changes are mostly guesswork.
- Raman spectroscopy is measuring vibrational frequencies, therefore it is directly sensitive to conformational changes in molecules.
- However, to resolve narrow vibrational lines require narrow Raman Pump spectra (poor time resolution ~3 ps).
- Enter FSRS.

Twist #2: Femtosecond stimulated Raman spectroscopy (FSRS)

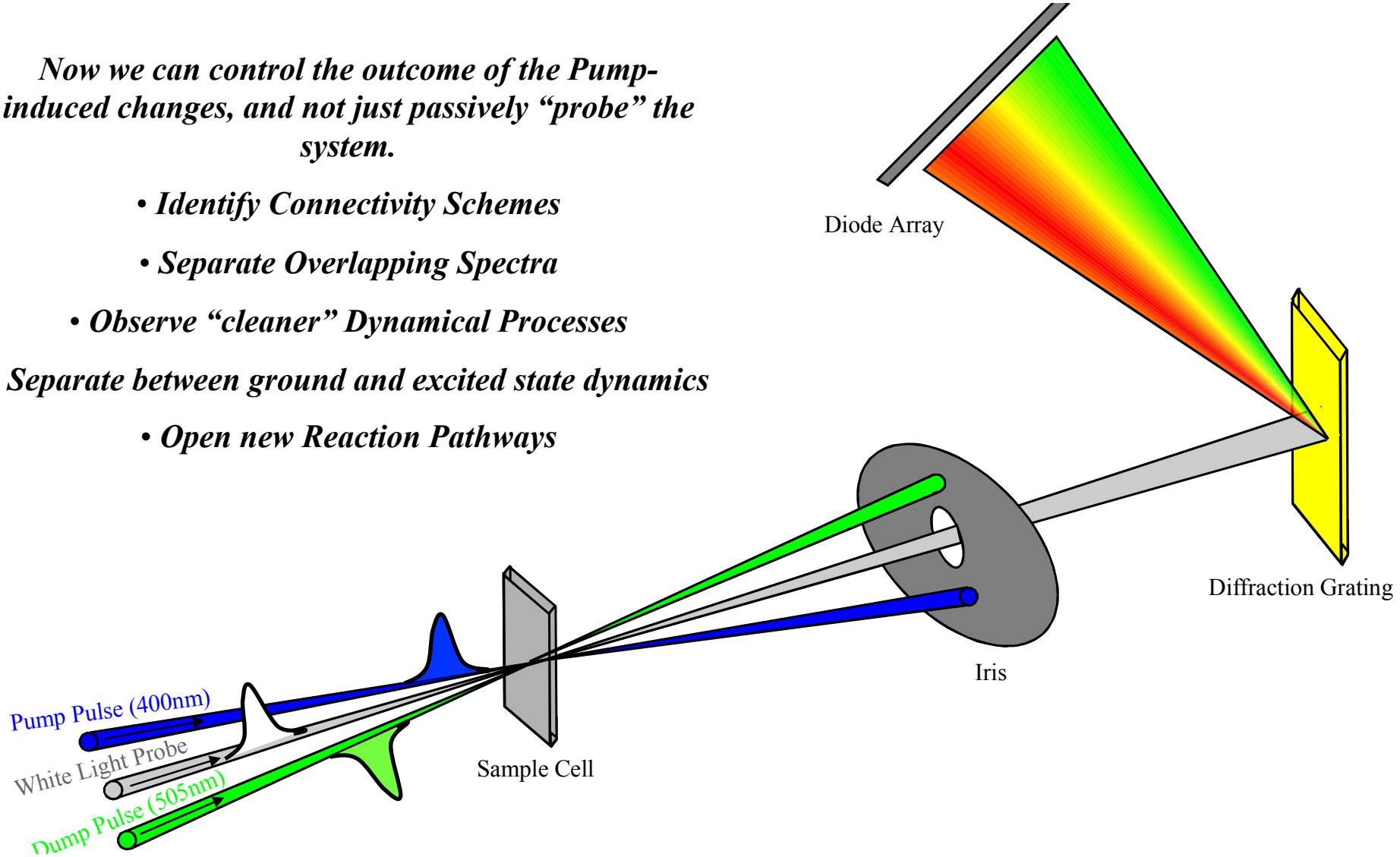


Pump-probe with a pair of probe pulses: one long (for narrow spectrum and good spectral resolution), and one short (for femtosecond time resolution).

Dispersed Multi-Pulse Experimental Setup

Now we can control the outcome of the Pump-induced changes, and not just passively "probe" the system.

- ***Identify Connectivity Schemes***
- ***Separate Overlapping Spectra***
- ***Observe "cleaner" Dynamical Processes***
- ***Separate between ground and excited state dynamics***
- ***Open new Reaction Pathways***



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- ***Identify Connectivity Schemes***
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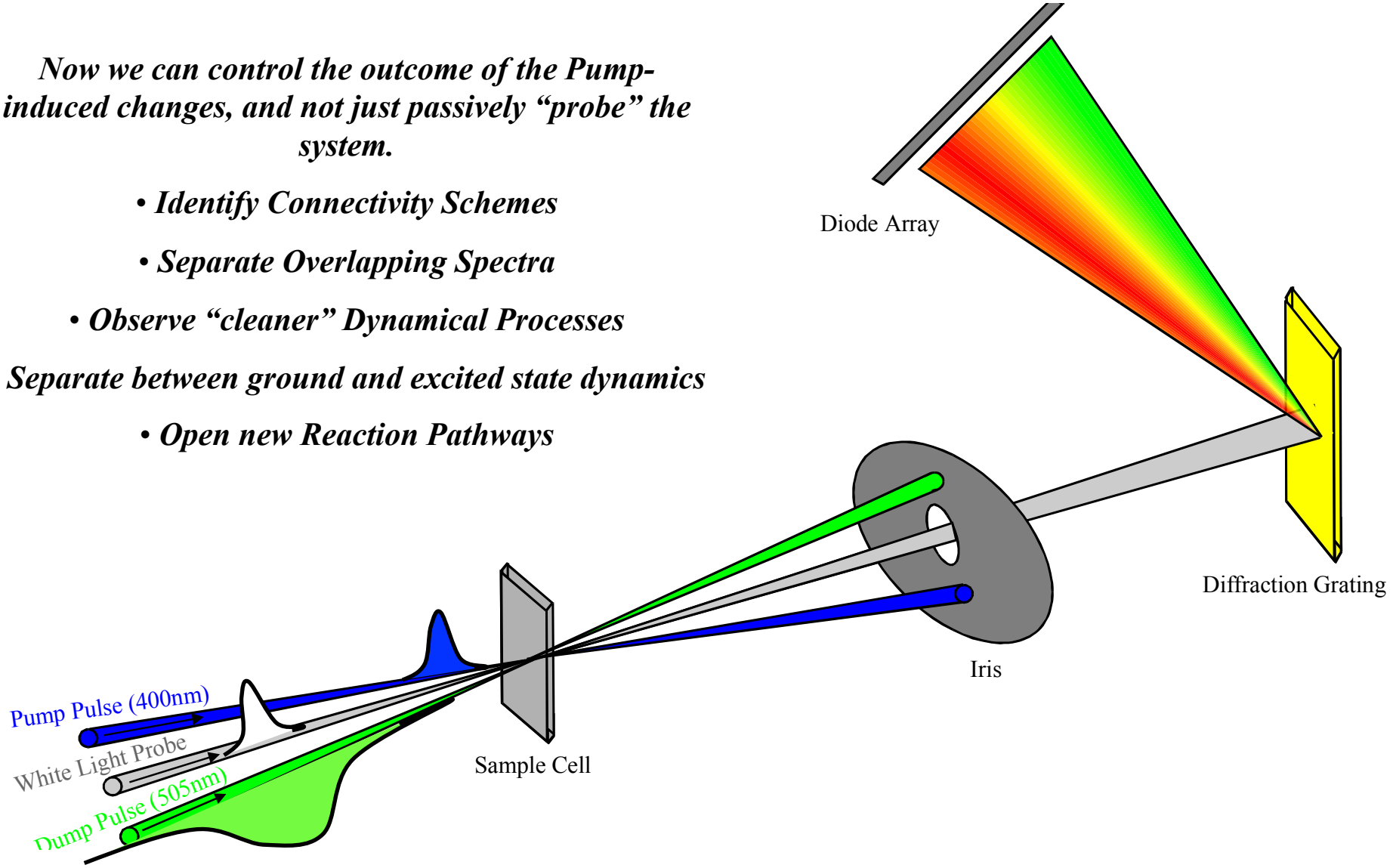
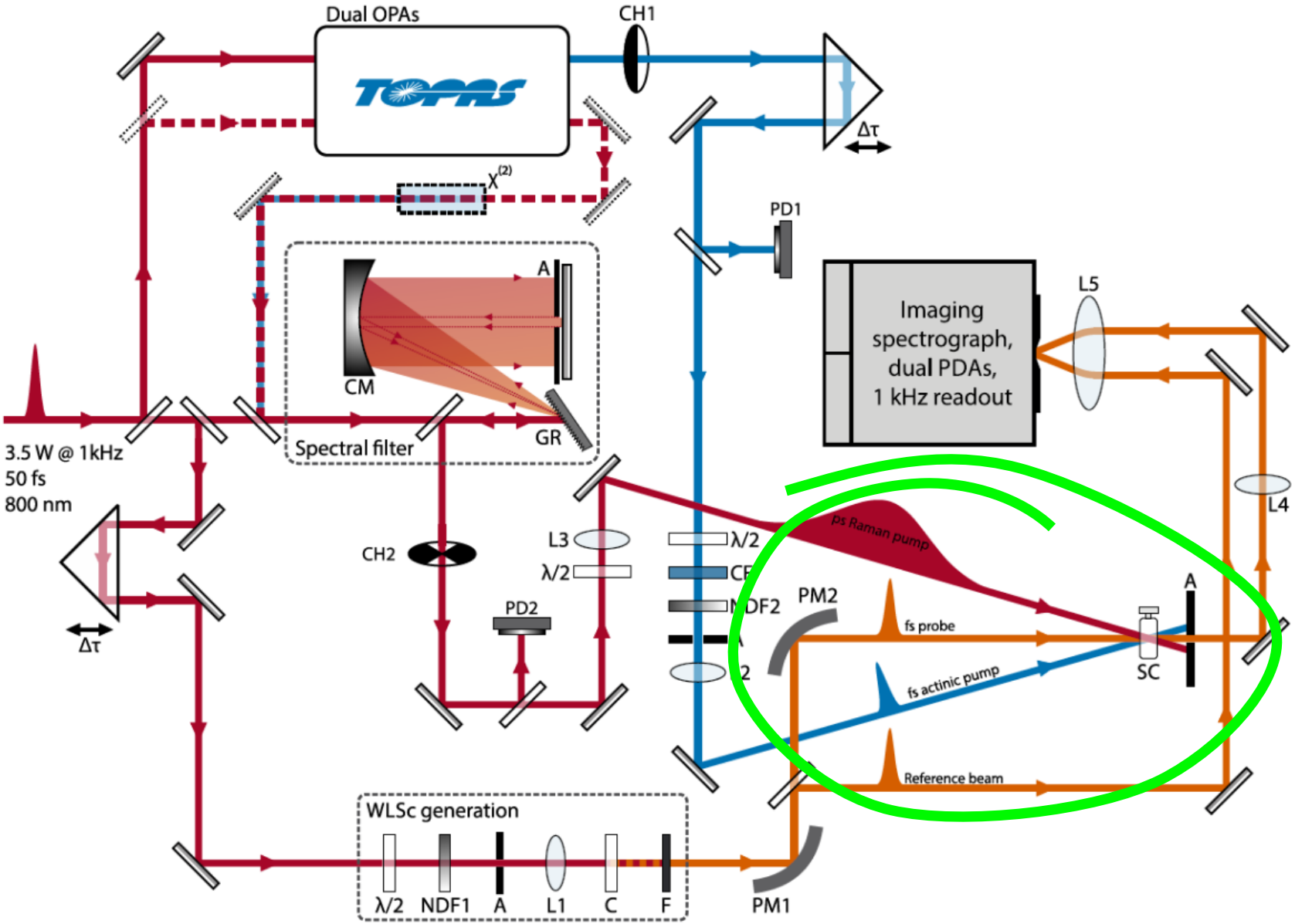
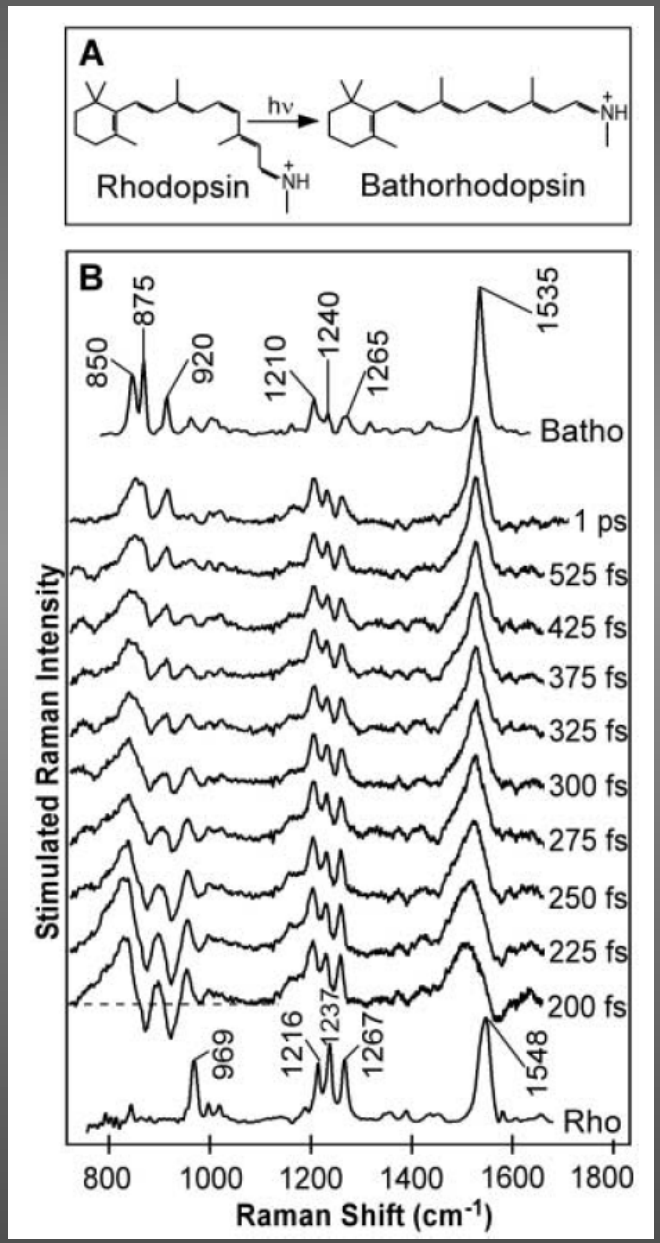
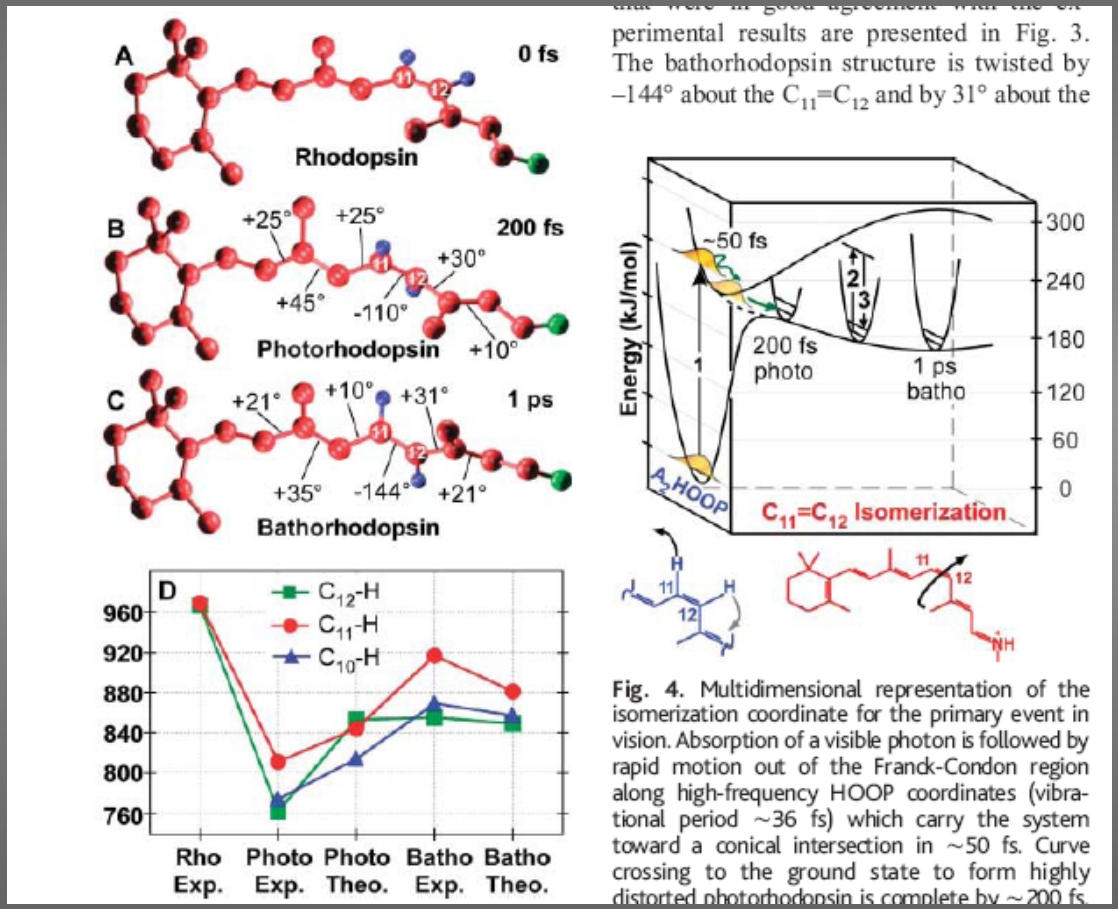


Setup for FSRS



Rhodopsin: vibrational signatures of isomerization





perimental results are presented in Fig. 3. The bathorhodopsin structure is twisted by –144° about the C_{11}=C_{12} and by 31° about the

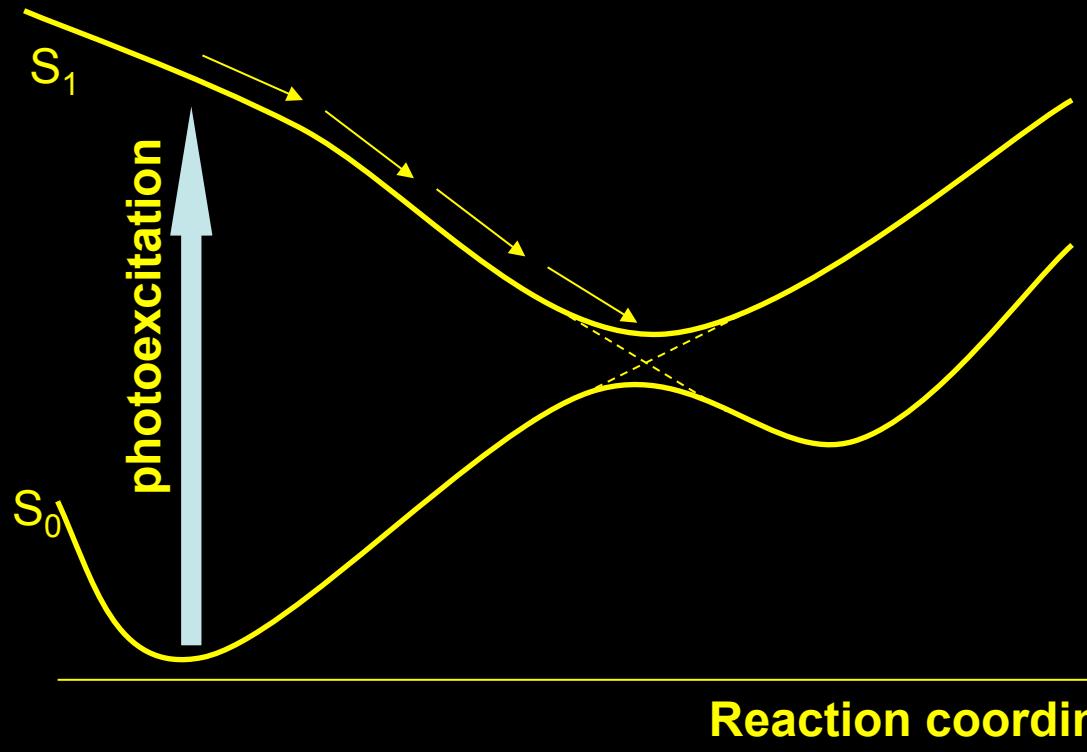
Fig. 4. Multidimensional representation of the isomerization coordinate for the primary event in vision. Absorption of a visible photon is followed by rapid motion out of the Franck-Condon region along high-frequency HOOP coordinates (vibrational period ~36 fs) which carry the system toward a conical intersection in ~50 fs. Curve crossing to the ground state to form highly distorted photorhodopsin is complete by ~200 fs

Kukura et al. Science 2005

Application: photochromism of indolo-benzoxazines

(spoiler alert: it’s non-existent)

Bistable ground-state: sensitive to everything

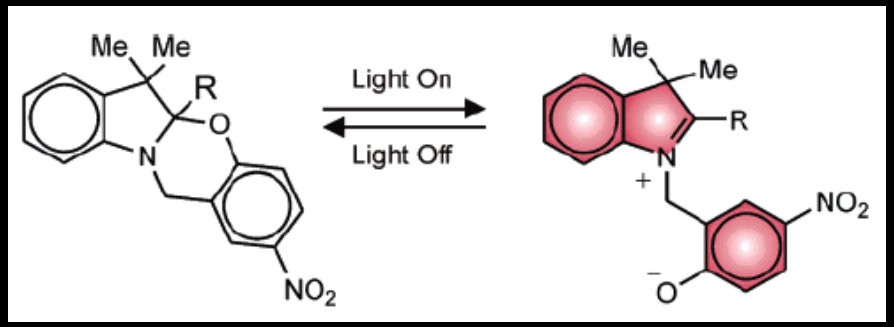


- Solvatochromism
- Electrochromism
- Acidochromism
- Thermochromism
- etc.

Indolo-benzoxazines: new generation photochromic switches

Tomasulo, M.; Sortino, S.; Raymo, F. i. M. *Organic Letters* **2005**, *7*, 1109.
Tomasulo, M.; Sortino, S.; White, A. J. P.; Raymo, F. M. *Journal of Organic Chemistry* **2005**, *70*, 8180. and at least 10 more papers on the same subject

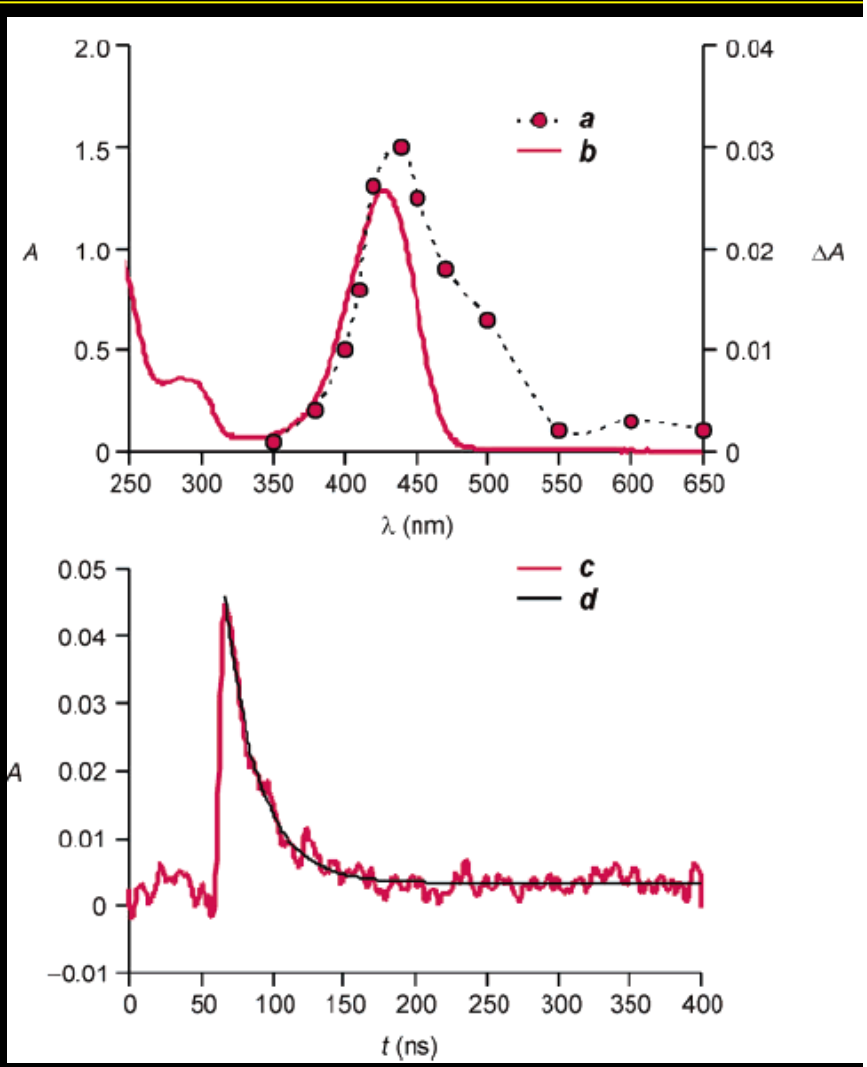
Shachkus, A. A.; Degutis, J. A.; Urbonavichyus, A. G. *Khim. Geterotsikl. Soed.* **1989**, *5*, 672.



- Structure similar to spiropyrans;
- No triplet state – stable in aerobic conditions
- Fast thermal recyclization (25 ns)

Indolo-benzoxazines: ns photodynamics

Photoinduced absorption spectrum similar to that induced by the addition of strong base (Bu_4NOH)



Photochromism of indolo-benzoxazines

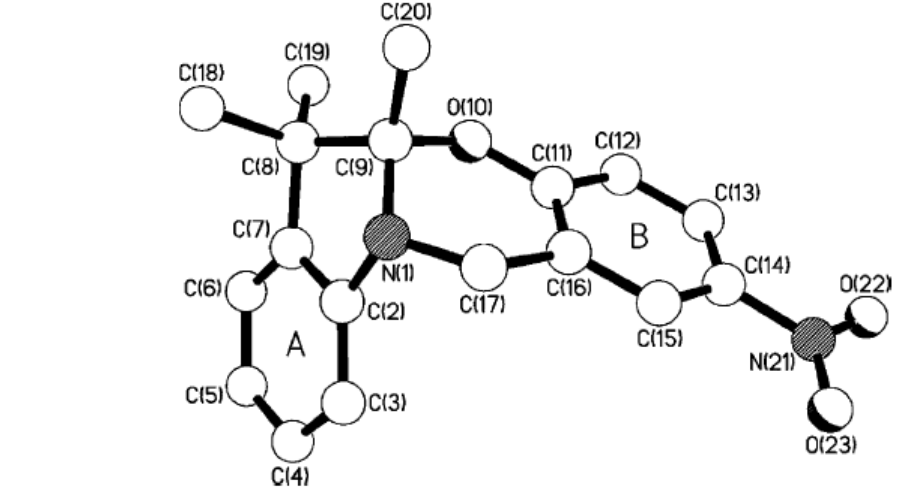
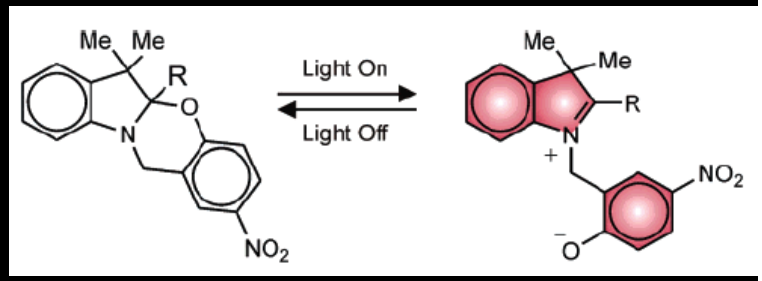


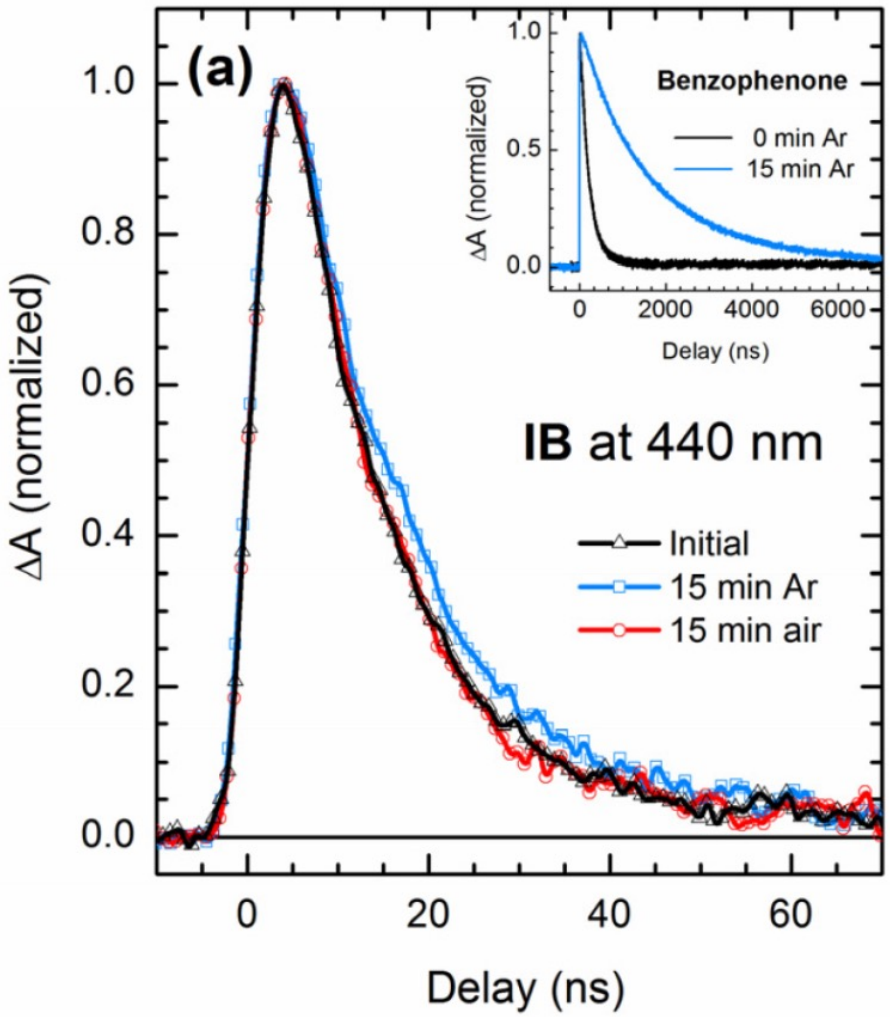
FIGURE 5. Single-crystal X-ray structure of **3a**.

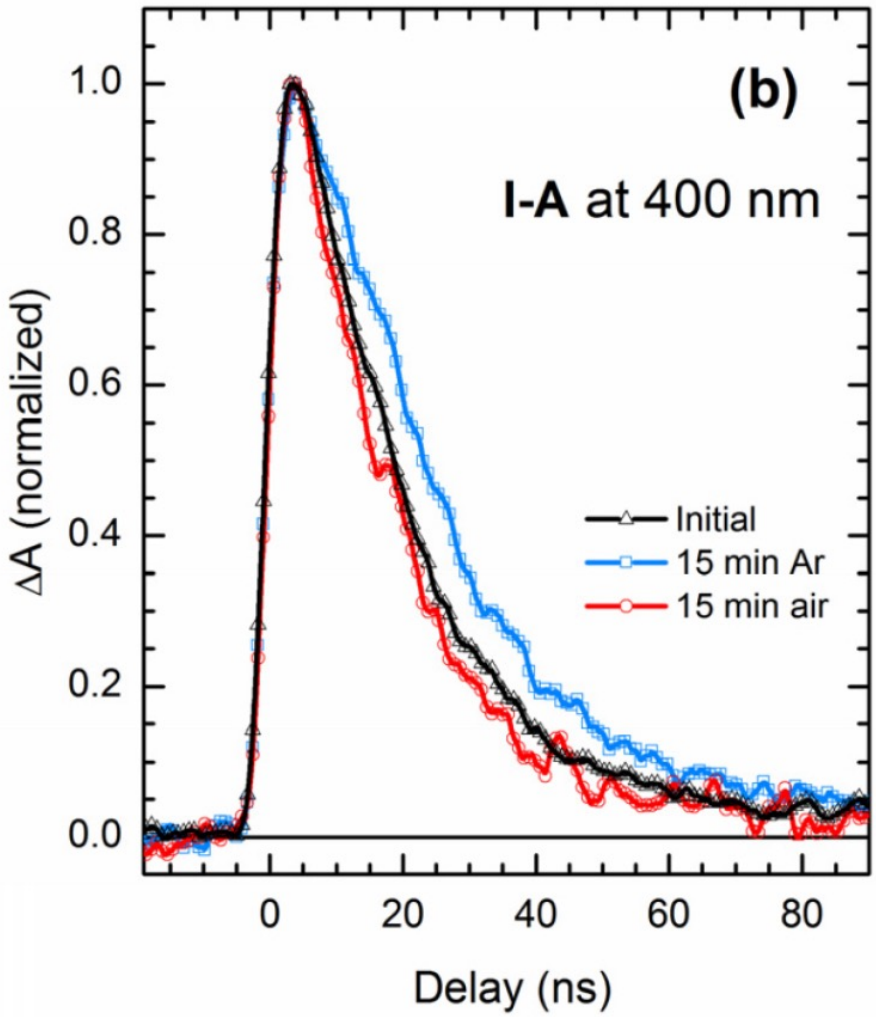


Prevaililng view: UV light induces bond cleavage, and produces a p-nitrophenolate chromophore responsible for the visible absorption.

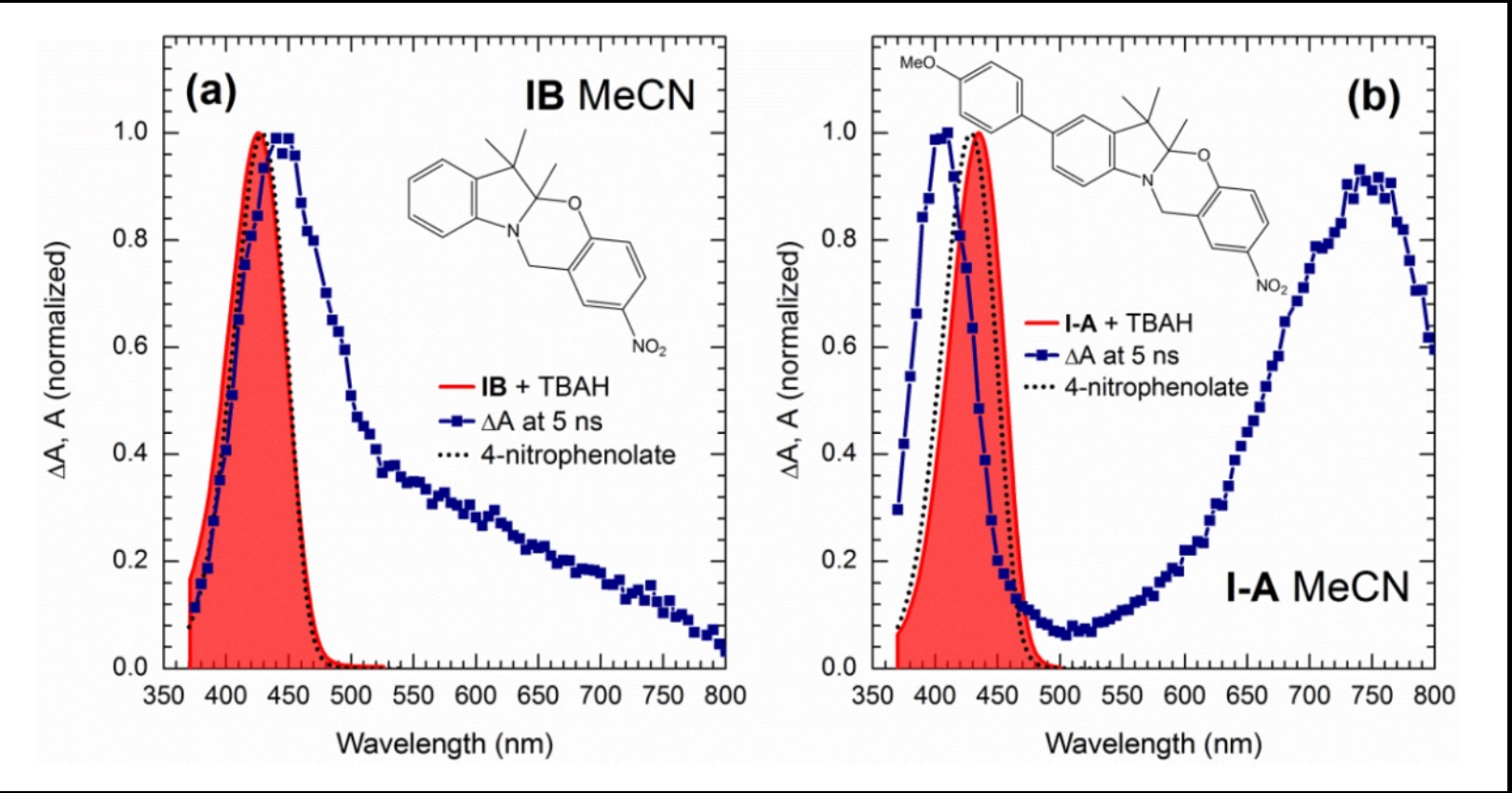
Or is it?..

Oxygen effect on lifetime of "isomer"

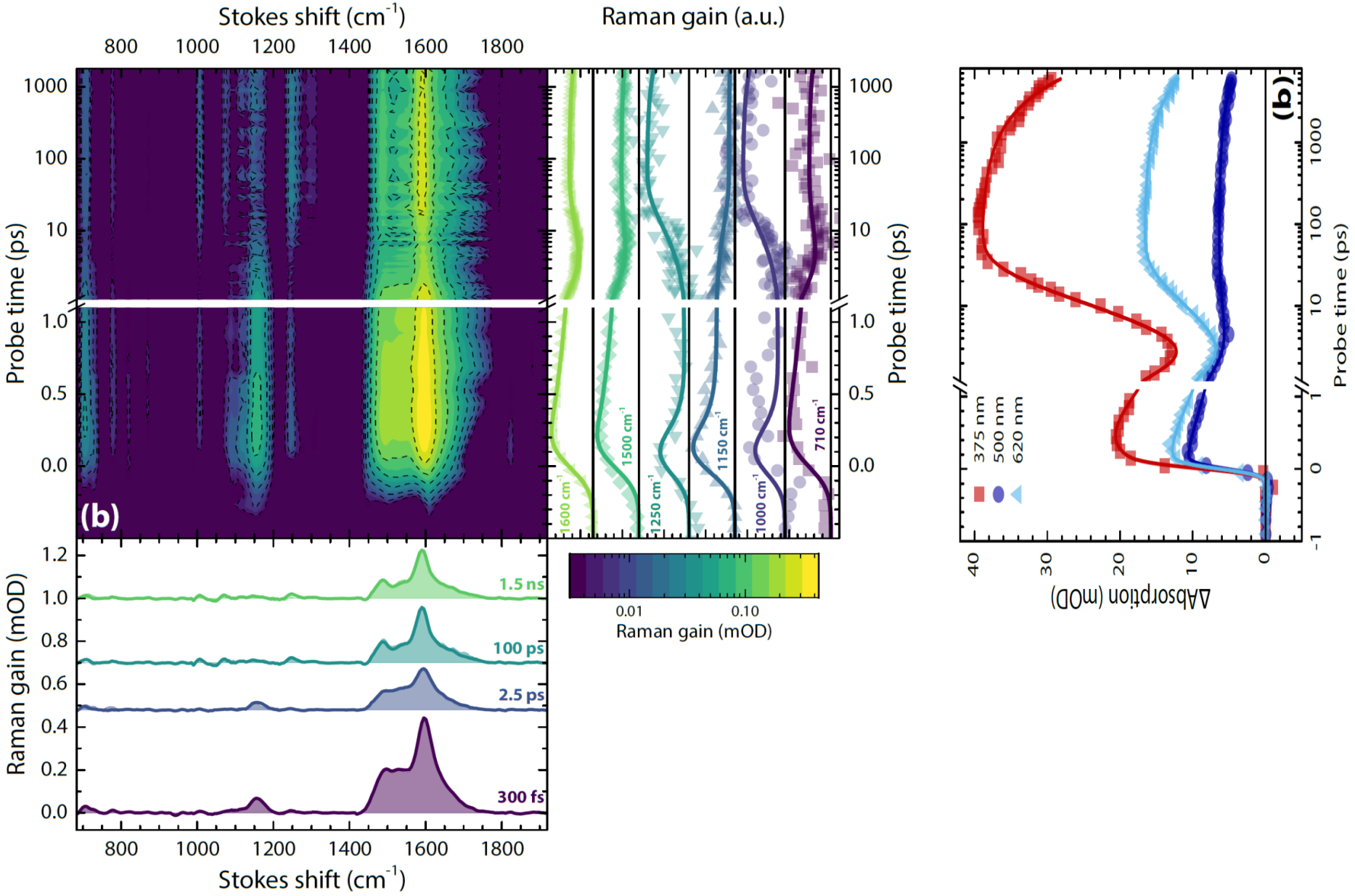




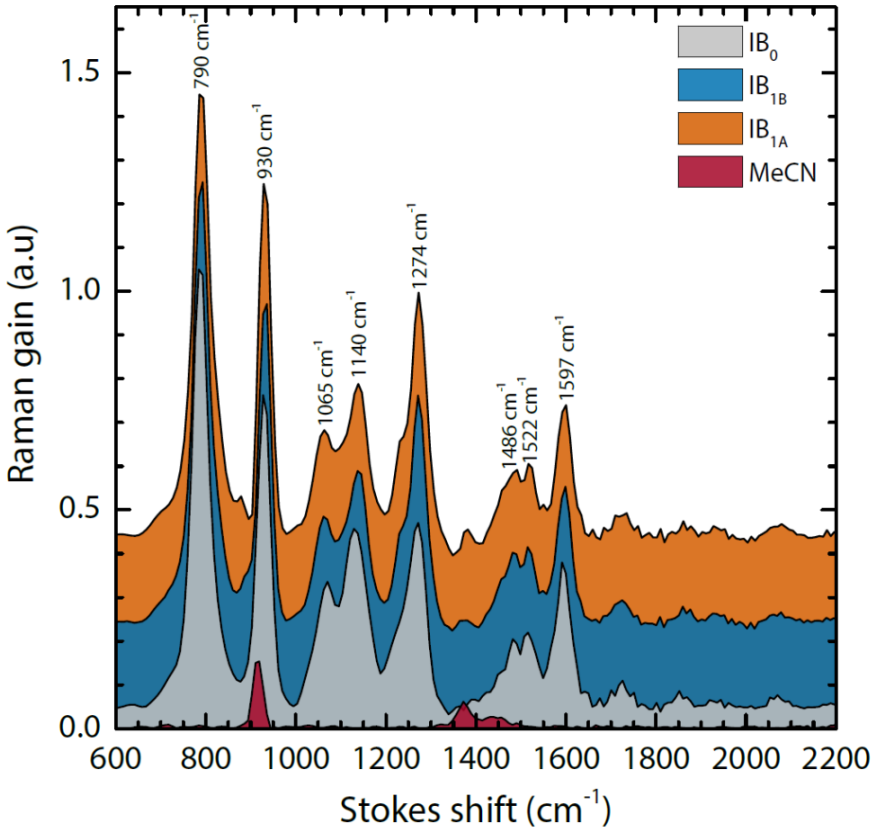
Absorption of open and closed forms

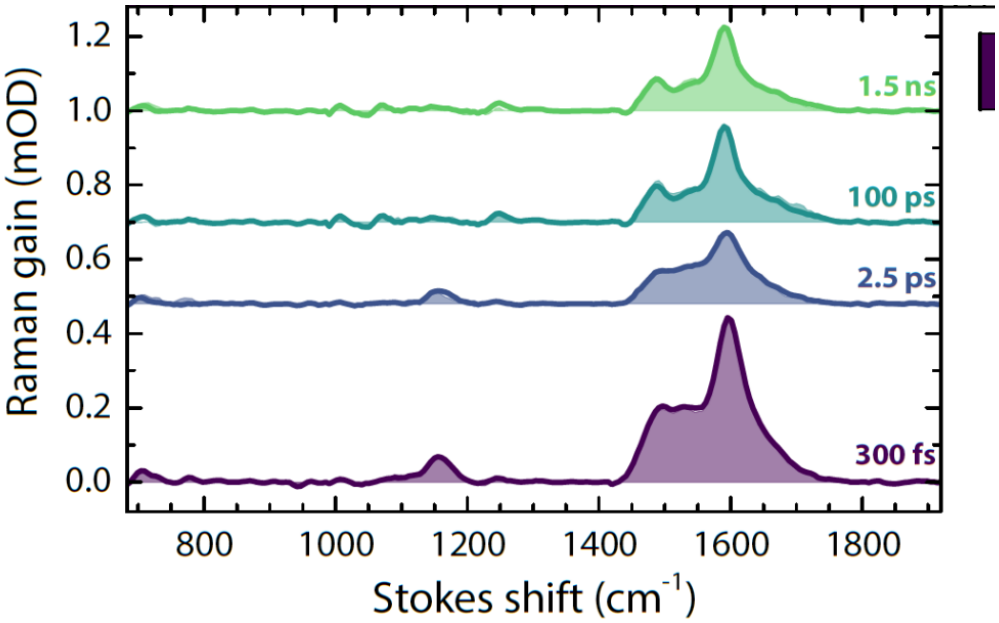


FSRS and optical pump-probe data



No match between optically and chemically induced Raman spectra!



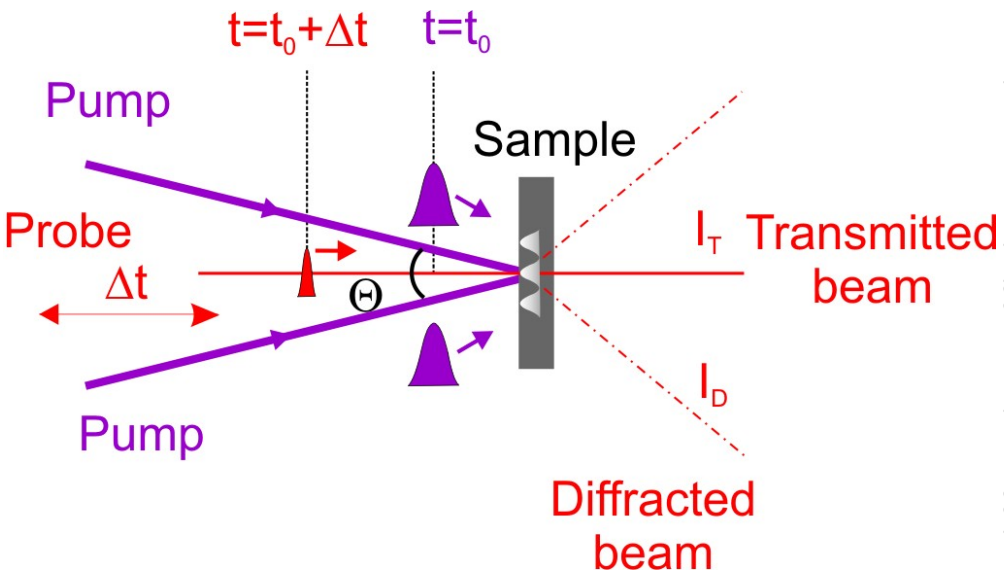


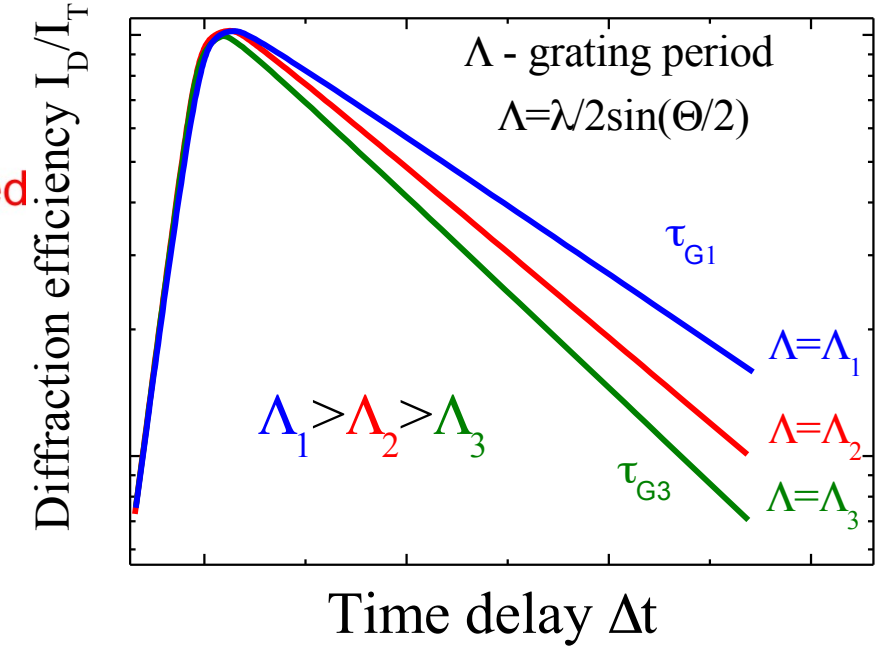
Therefore, definitely not photochromic. Pity.

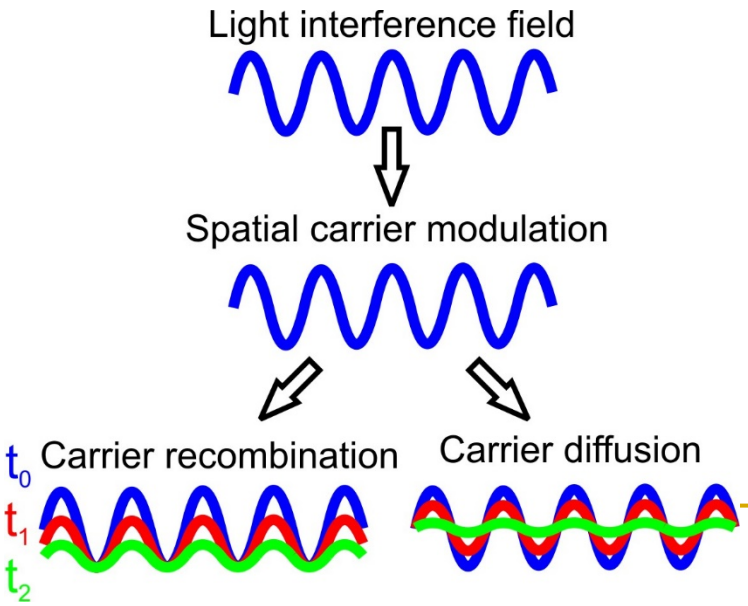
Twist #3:

Coherent spectroscopies

Transient grating for diffusion length determination in semiconductors



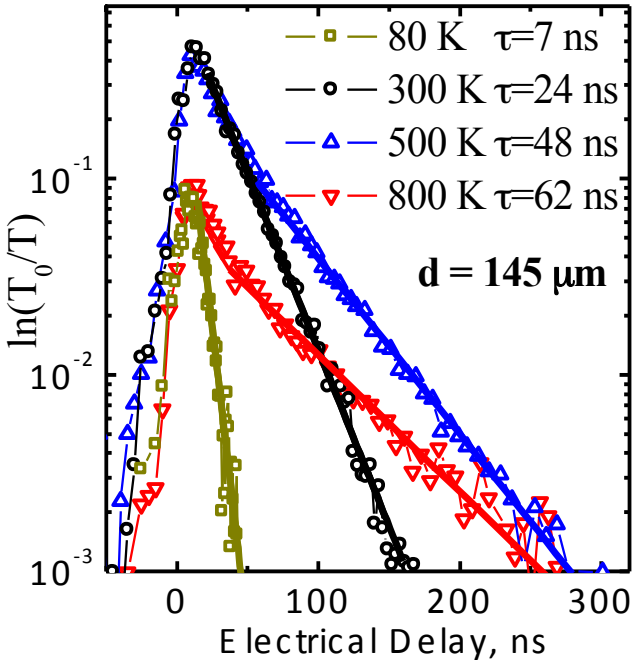




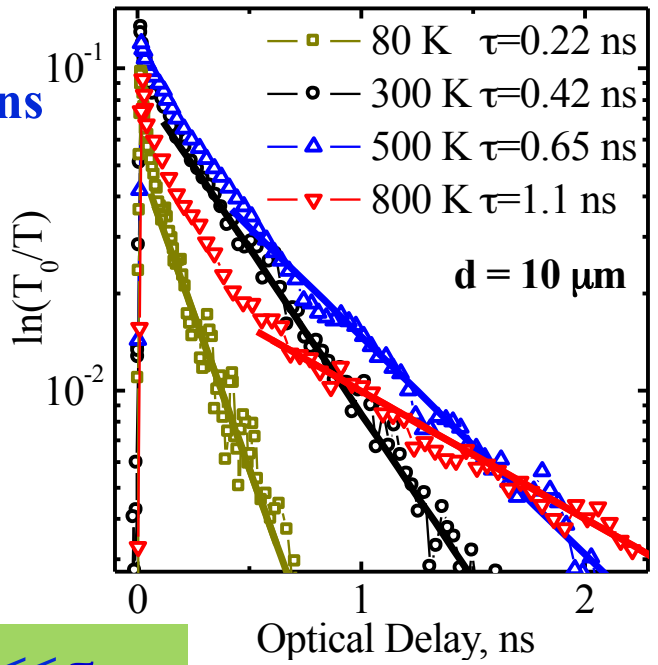
$$\frac{1}{\tau_G} = \frac{1}{\tau_R} + \frac{1}{\tau_D} = \frac{1}{\tau_R} + \frac{4\pi^2 D}{\Lambda^2}$$

$$\tau_G^{-1} = f(\Lambda^{-2}) \Rightarrow \tau_R, \mathrm{D} \Rightarrow L_D = \sqrt{\tau_R \mathrm{D}}$$

Non-radiative recombination time in GaN



$\tau_{Rad} = 60$ ns



$\tau_{Rad} = 10$ ns

$\tau_{NonRad} \ll \tau_{Rad}$

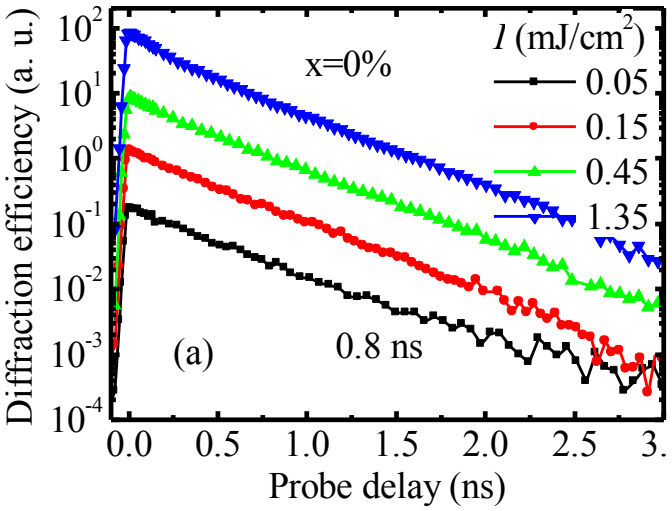
$$\frac{1}{\tau_R} = \frac{1}{\tau_{Rad}} + \frac{1}{\tau_{NonRad}}$$

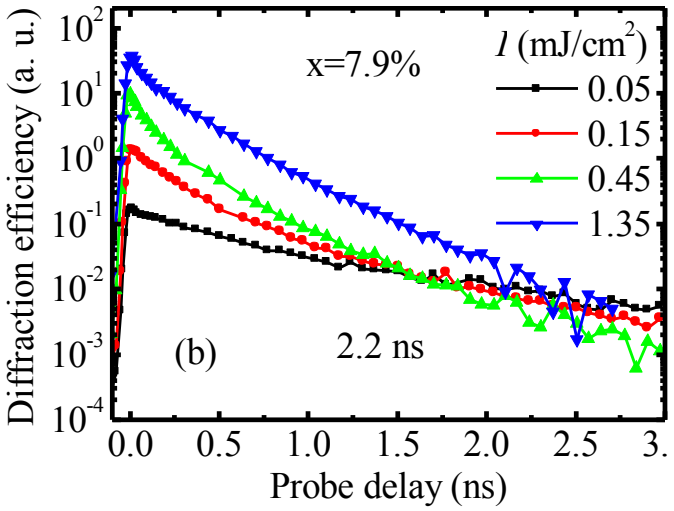
$$\tau_{Rad}(T, \Delta N) = \frac{1}{B(T)\Delta N}$$

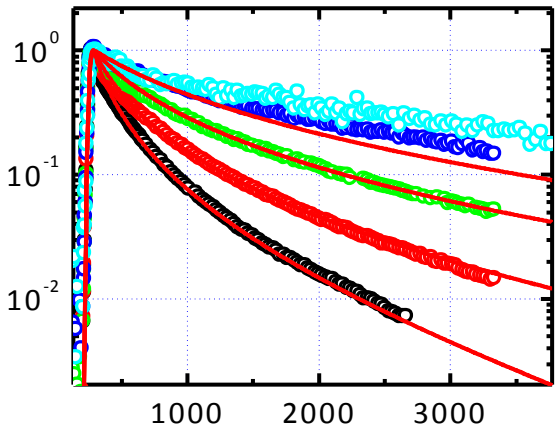
$$B(T) = 2 \times 10^{-11} \left(\frac{T}{300K}\right)^{-1.5}$$

- Non-radiative decay time τ_{NonRad} can be determined at low injection and/or after long decay time, when ΔN becomes small.

Recombination in $In_xGa_{1-x}N$ MQWs







Non-radiative **Auger**

$$\frac{\partial N(z,t)}{\partial t} = D\frac{\partial^2 N(z,t)}{\partial z^2} - AN(z,t) - BN^2(z,t) - CN^3(z,t) + G(z,t)$$

Band-to-band

- **A** and **B** coefficients are determined from fitting to carrier continuity equation. ***D*** and N_0 other parameters in the equation are determined experimentally.

Transient grating

- Combined with temperature dependence, can reveal the difusion coeffcients and recombination rates and modes in semiconductors.

More general case: three pulse echo

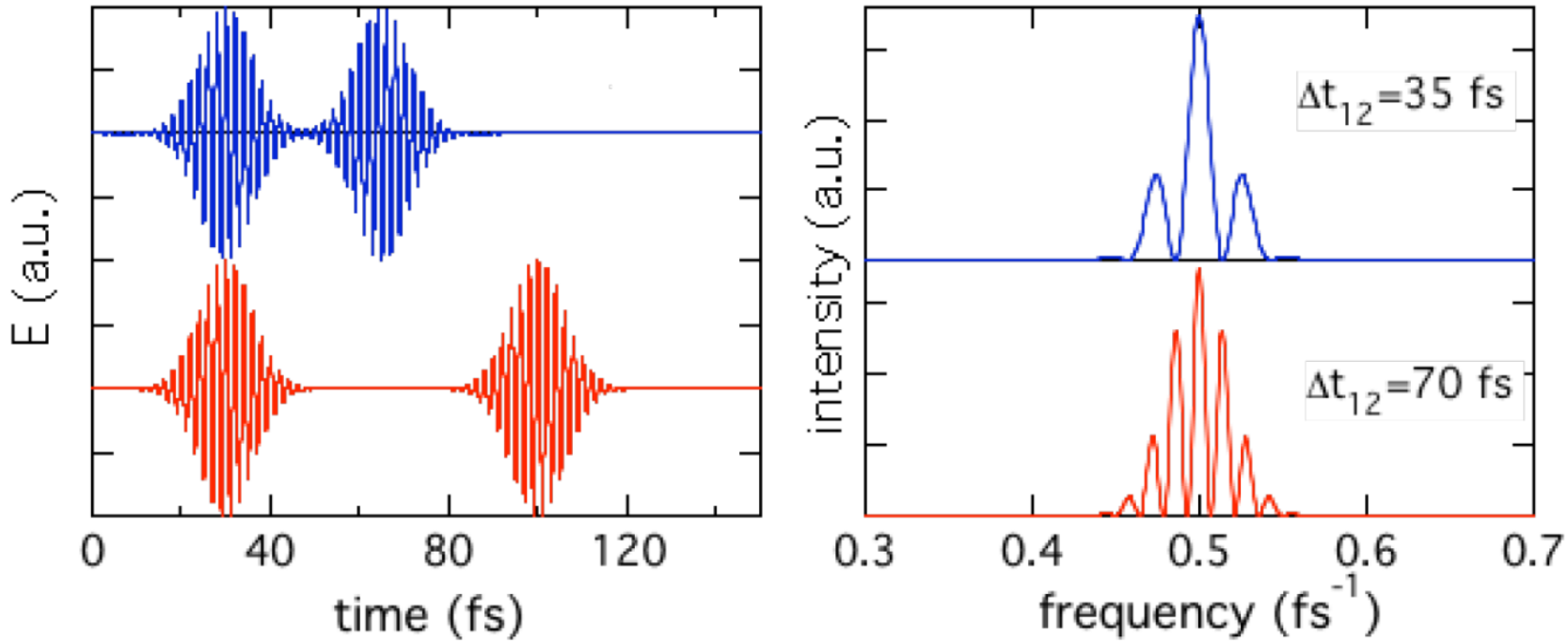
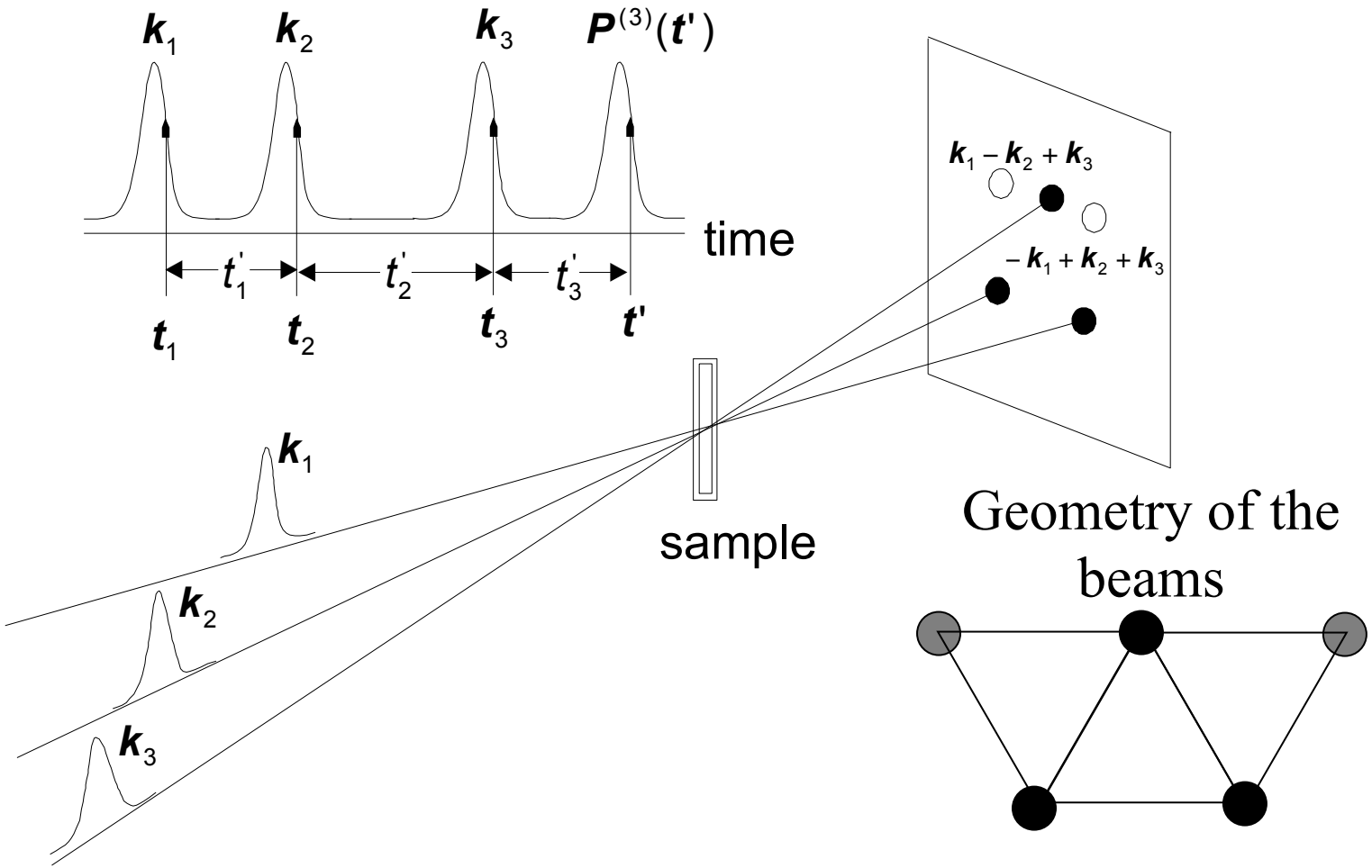
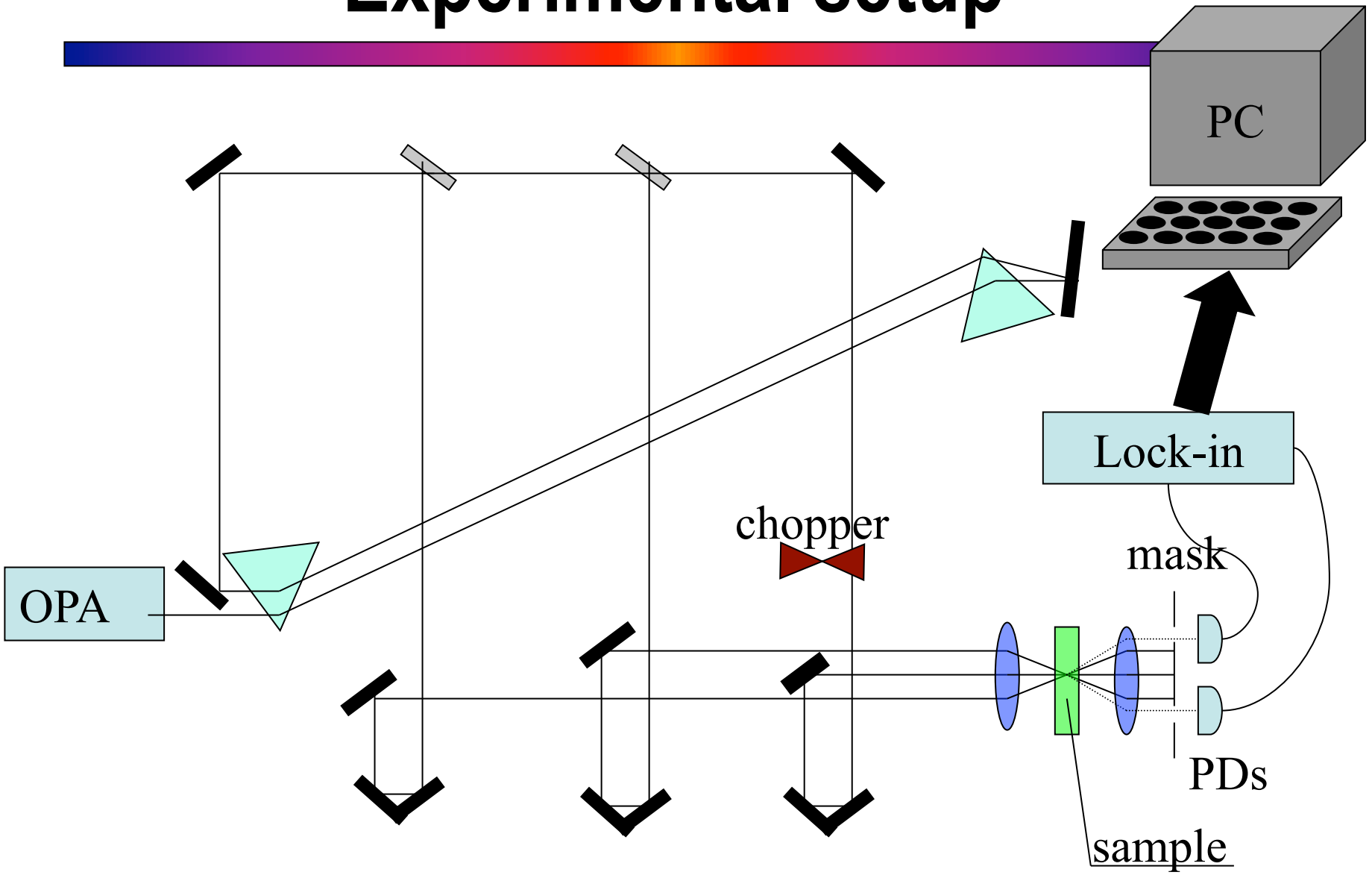


Figure 31: Electric field (left) and spectral intensity (right) associated with two 10 fs pulses separated by 35 and 70 fs.

Experiment scheme



Experimental setup



Three pulse echo

- The „writing“ pulses are separated in time, and their spectra are as wide as the absorption band of the sample. Shifted pulses result in frequency beating or *frequency grating.*
- The further the pulses – the finer the grating is.
- When too fine, it is very sensitive to spectral diffusion and signal quickly disappears;
- When too coarse, diffraction is weak produced
- Therefore, signal maximum is observed when the writing pulses are *SLIGHTLY* separated in time.

Frequency gratings

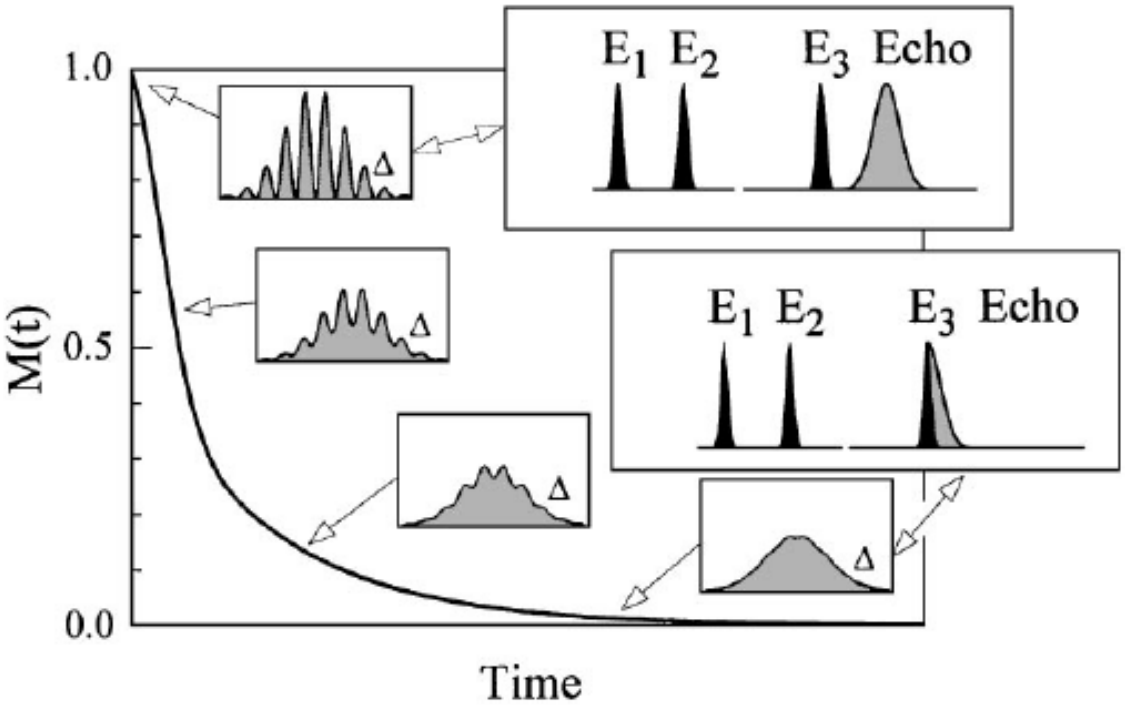
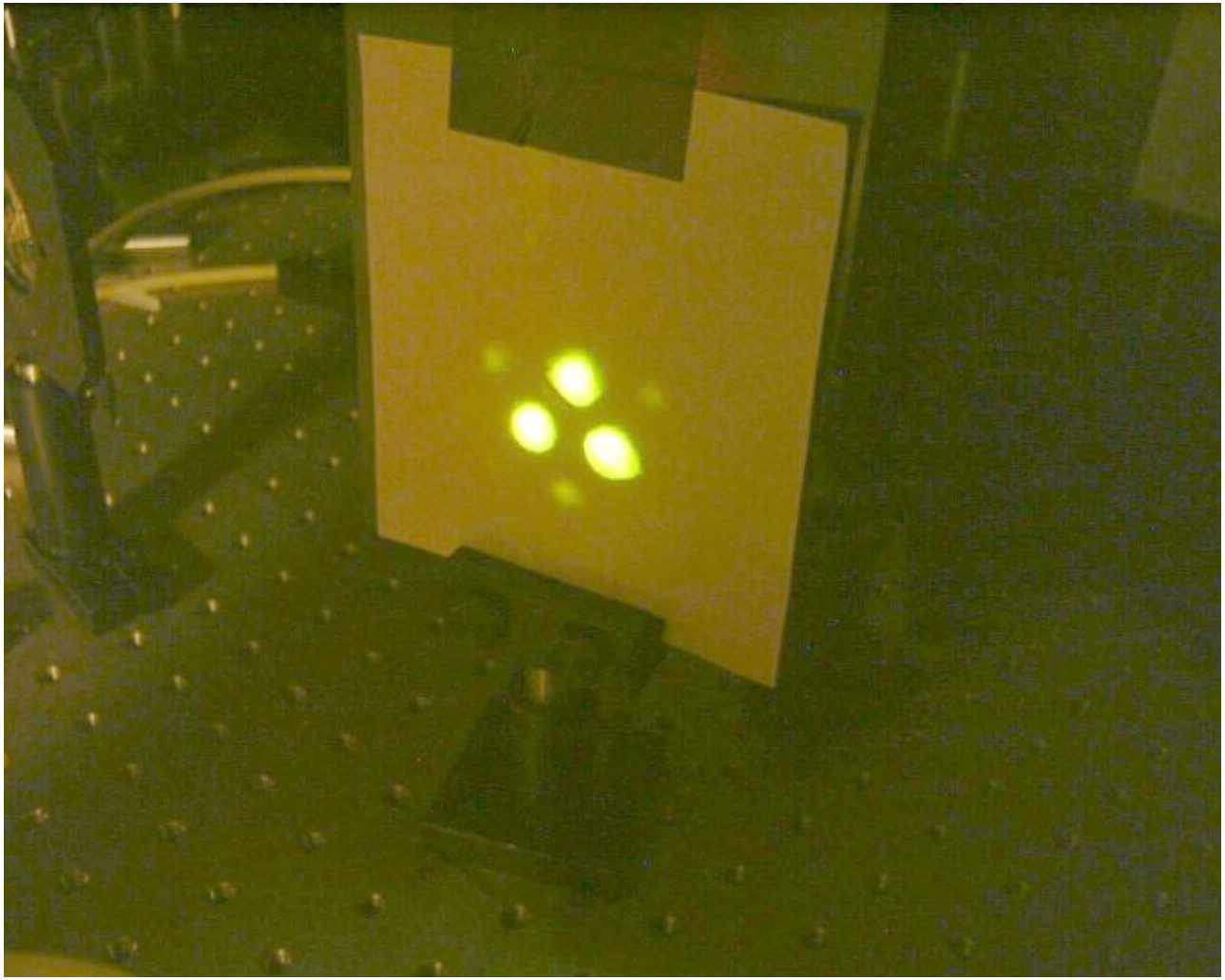
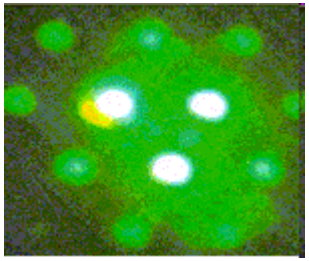


Figure 2 System-bath correlation function and associated decays of the frequency grating formed in the ground state. Δ denotes the frequency detuning from the optical transition. The excitation pulse sequence E_1-E_3 as well as the emitted signals are depicted for $M(t) = 1$ (inhomogeneous broadening) and $M(t) = 0$ (homogeneous broadening). Note that in the former case, the signal is delayed with respect to the last excitation pulse (photon echo), whereas in the latter case, the signal maximum coincides with the last excitation pulse (free induction decay).

How does it look?





What do we measure?

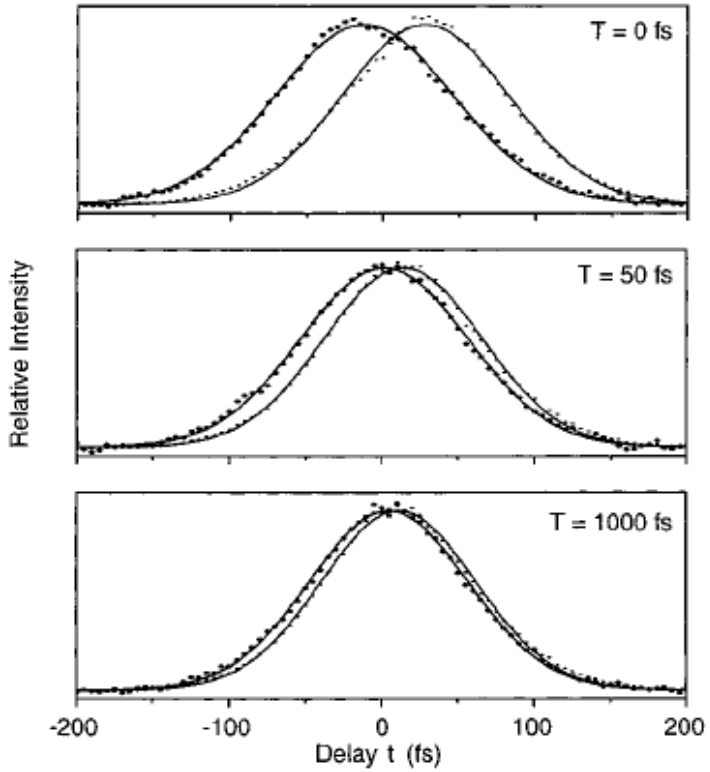
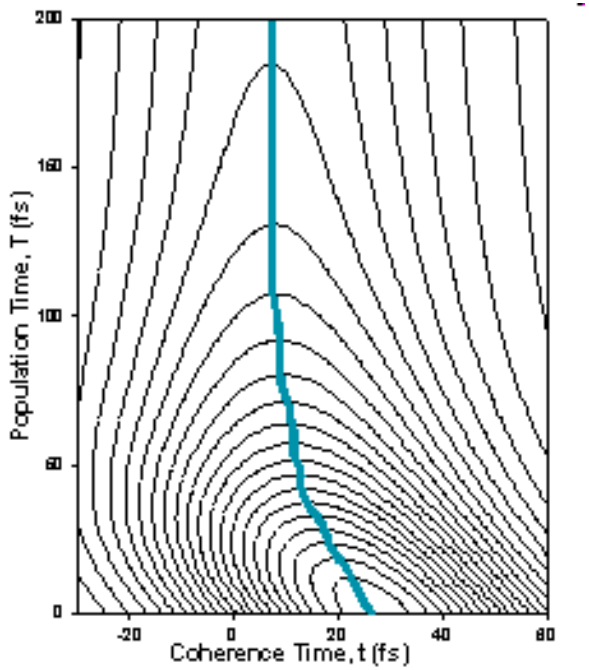


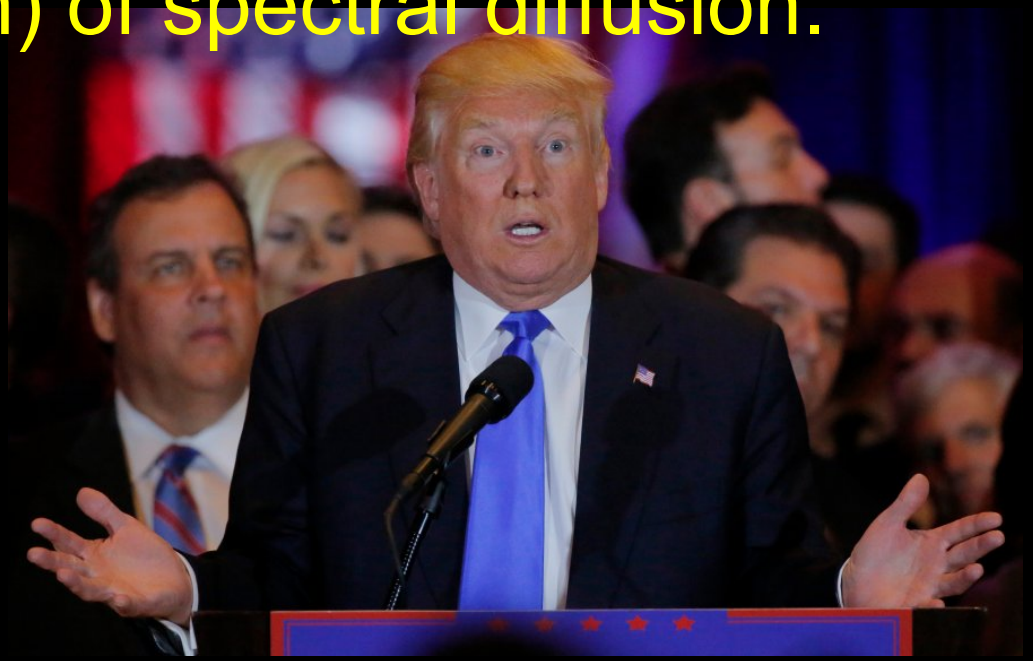
Figure 4. Examples of three 3PEPS scans (in the *t* dimension) obtained with α-subunit preparations at room temperature at T = 0, 50, and 1000 fs. The two traces represent the echo signals obtained from the two phase-matched directions. The traces are superimposed with fits to Gaussian line shapes, as determined by a nonlinear least-squares regression routine.



Peak shift decay follows the memory function of the bath coupled system

Problems

- After all this work, just one decaying curve is measured. Cannot produce too much science with just one curve ☹.
- Data interpretation requires the microscopic model (hamiltonian) of spectral diffusion.



2D electronic spectroscopy

- What if we measure not just the intensity of the photon echo, but the time dependence of the radiated EM field?
- We get 2D NMR analogue in optics, called two dimensional electronic spectroscopy (2DES).

JOURNAL OF CHEMICAL PHYSICS VOLUME 121, NUMBER 9 1 SEPTEMBER 2004

Phase-stabilized two-dimensional electronic spectroscopy

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Department of Chemistry, University of California, Berkeley and Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, California 94720

2D spectroscopy: plethora of pulses

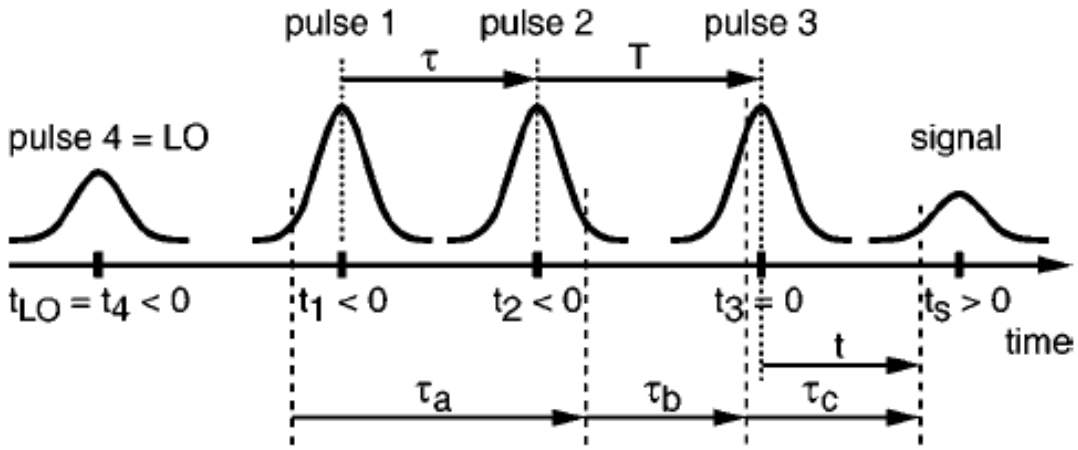


FIG. 1. Definition of time variables. Time zero is defined at the center of the third excitation pulse. The first two excitation pulses arrive at times $t_1 < 0$ and $t_2 < 0$, separated by the coherence time τ which is positive for the shown pulse order, and negative if pulse 2 arrives first. The population time $T > 0$ is the separation between the second and third excitation pulse at $t_3 = 0$. Nonlinear third-order polarization at time t is induced by field interactions at times $\tau_a + \tau_b + \tau_c$, $\tau_b + \tau_c$, and τ_c earlier, which may occur somewhere under the excitation pulse envelopes. This leads to a free-induction decay and for inhomogeneously broadened systems, an additional photon echo signal is observed with an average arrival time t_s that is similar to the coherence time. The local oscillator (LO) used for heterodyned signal detection always arrives first at time t_4.

Experimental implementation

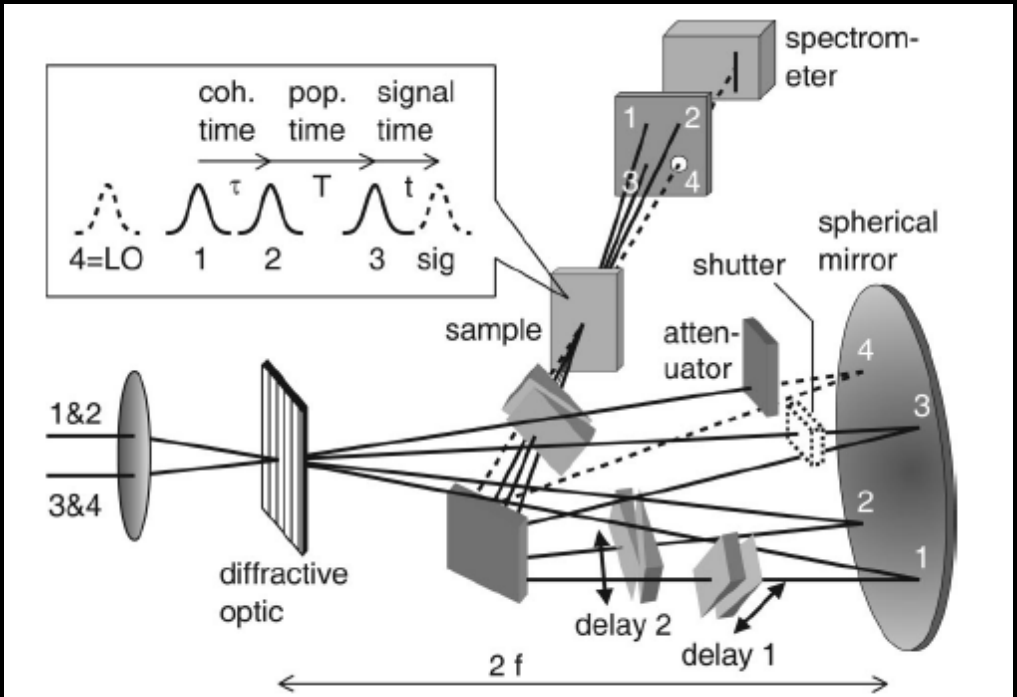


FIG. 2. Experimental setup. Two parallel beams of femtosecond laser pulses in the visible spectral region are focused by a lens onto a grating. The first diffraction orders emerge with high efficiency and provide the excitation pulses 1–3 as well as a local oscillator (4=LO) for heterodyne-detected three-pulse photon-echo electronic spectroscopy. A spherical mirror ($2f$ =50 cm) creates an image of the pulse overlap in the sample cell via a plane folding mirror. The required time delays are provided with subwavelength precision by motor-controlled movable glass wedges. Full characterization of the nonlinear phase-matched signal field is carried out by spectral interferometry with the attenuated LO. An automated beam shutter is used for subtraction of scattering contributions. This diffractive-optics based setup is inherently phase-stabilized.

You can calibrate your λ/20 delays

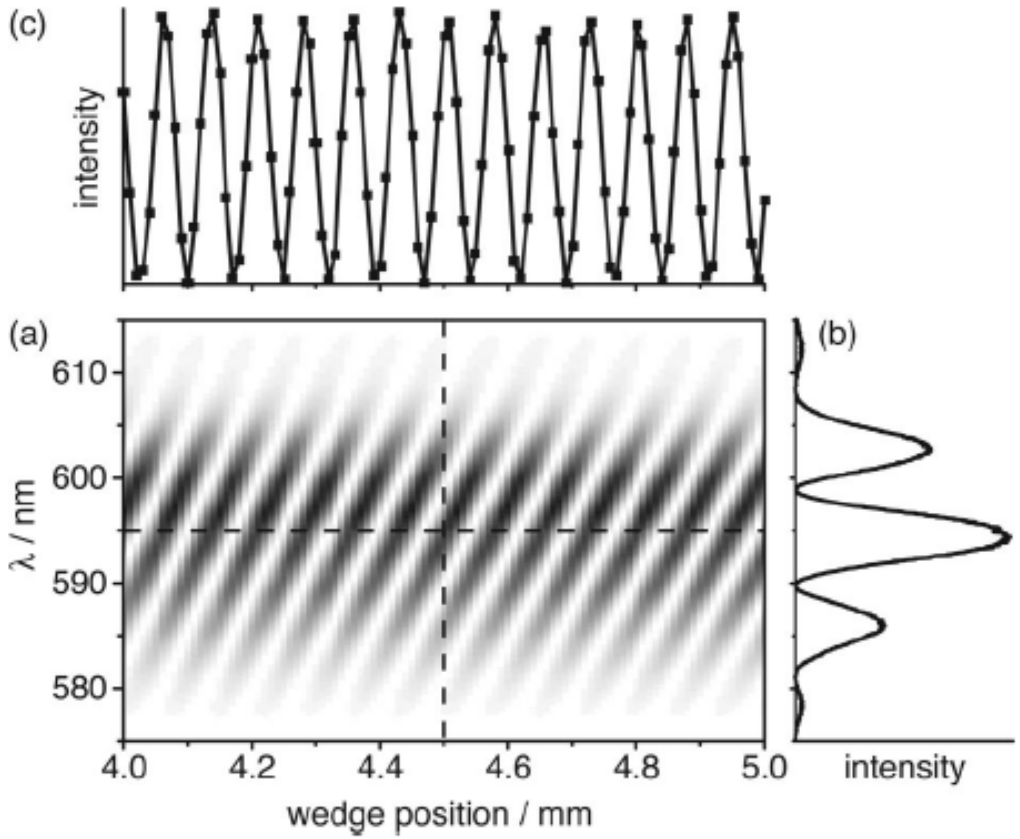


FIG. 3. Delay calibration by spectral interferometry. (a) Spectral interference patterns between pulses 1 and 2 are recorded in 10 μm steps by moving the glass wedge in arm 1. The cross section along the vertical dashed line shows (b) the spectral interference outside of the temporal pulse overlap, whereas the cross section along the horizontal dashed line delivers (c) the temporal oscillation for one particular wavelength. Counting these oscillations gives a precise calibration factor of wedge position vs time delay.

2D Electronic spectroscopy

- Can distinguish homogeneous and inhomogeneous broadening;
- Time resolution is not limited by spectral resolution on excitation scale;
- It's like many pump-probe experiments in one go!

2D electronic spectroscopy

The multiplication $E(t-\tau_a-\tau_b-\tau_c)E(t-\tau_b-\tau_c)E(t-\tau_c)$ in Eq. (1) using the field of Eq. (2) yields $6\times6\times6=216$ terms of the form of multiplication of three envelope functions $\tilde{A}(t)$ or $\tilde{A}^*(t)$ and corresponding phase factors determining their directions and frequencies. Each term of this sum corresponds to a specific time order of interaction contributions. For example, the contribution

$$\tilde{A}(t-t_2-\tau_a-\tau_b-\tau_c)\tilde{A}^*(t-t_1-\tau_b-\tau_c)\times\tilde{A}(t-t_3-\tau_c)e^{-i\omega_0(t-t_2-\tau_a-\tau_b-\tau_c)}\times e^{i\omega_0(t-t_1-\tau_b-\tau_c)}e^{-i\omega_0(t-t_3-\tau_c)}e^{i(\vec{k}_2-\vec{k}_1+\vec{k}_3)\cdot\vec{r}}, \quad (3)$$

corresponds to the case when the system first interacts with the pulse going along the $\vec{k}_2$ direction at the time $t-\tau_a-\tau_b-\tau_c$, then it interacts with the pulse characterized by the direction $-\vec{k}_1$ at time $t-\tau_b-\tau_c$ and finally with the pulse traveling along $\vec{k}_3$ at time $t-\tau_c$. The star denotes complex conjugation. Only six of the 216 terms generate a signal in the direction of $-\vec{k}_1+\vec{k}_2+\vec{k}_3$ along which we measure. All terms possess a common phase factor $e^{-i\omega_0 t+i\omega_0\tau}$, and in addition they contain one of the phase factors that can be either $e^{i\omega_0(\tau_a+\tau_c)}$, $e^{-i\omega_0(\tau_c-\tau_a)}$, or $e^{i\omega_0(\tau_a+2\tau_b+\tau_c)}$.

Depending on the system in question, the response function $S^{(3)}(\tau_a,\tau_b,\tau_c)$ contains a sum of contributions with similar phase factors. If the laser frequency ω_0 approximately matches the electronic transition frequencies in the system, some of the phase factors originating from the response function may cancel with those originating from the laser field. Thus, under the integration in Eq. (1) we would have slowly varying terms (where oscillatory factors canceled) and fast oscillating terms (where phase factors added). After the integration, oscillatory terms result in a much smaller contribution than slowly varying ones, and we can neglect them. This is usually referred to as rotating-wave approximation (RWA). Thus, taking into account only the signal contribution emitted into the phase-matched direction $\vec{k}_s=-\vec{k}_1+\vec{k}_2+\vec{k}_3$ under the RWA, one arrives at

$$\begin{aligned}P^{(3)}_{rw}(\tau,T,t)=\exp[-i\omega_0 t+i\omega_0\tau]\int_0^\infty\int_0^\infty\int_0^\infty d\tau_a d\tau_b d\tau_c\\ \times\{S^{(3)}_{R,rw}(\tau_a,\tau_b,\tau_c)e^{-i\omega_0(\tau_c-\tau_a)}[\tilde{A}^*(t-t_1-\tau_a-\tau_b-\tau_c)\tilde{A}(t-t_2-\tau_b-\tau_c)\tilde{A}(t-t_3-\tau_c)\\ +\tilde{A}^*(t-t_1-\tau_a-\tau_b-\tau_c)\tilde{A}(t-t_3-\tau_b-\tau_c)\tilde{A}(t-t_2-\tau_c)]\\ +S^{(3)}_{NR,rw}(\tau_a,\tau_b,\tau_c)e^{i\omega_0(\tau_a+\tau_c)}[\tilde{A}(t-t_2-\tau_a-\tau_b-\tau_c)\tilde{A}^*(t-t_1-\tau_b-\tau_c)\tilde{A}(t-t_3-\tau_c)\\ +\tilde{A}(t-t_3-\tau_a-\tau_b-\tau_c)\tilde{A}^*(t-t_1-\tau_b-\tau_c)\tilde{A}(t-t_2-\tau_c)]\\ +S^{(3)}_{DC,rw}(\tau_a,\tau_b,\tau_c)e^{i\omega_0(\tau_a+2\tau_b+\tau_c)}[\tilde{A}(t-t_2-\tau_a-\tau_b-\tau_c)\tilde{A}(t-t_3-\tau_b-\tau_c)\tilde{A}^*(t-t_1-\tau_c)\\ +\tilde{A}(t-t_3-\tau_a-\tau_b-\tau_c)\tilde{A}(t-t_2-\tau_b-\tau_c)\tilde{A}^*(t-t_1-\tau_c)]\}. \quad (4)\end{aligned}$$

Herein the response functions $S^{(3)}_{R,rw}$, $S^{(3)}_{NR,rw}$, and $S^{(3)}_{DC,rw}$ are sums of Liouville pathways surviving the rotating-wave approximation, i.e., those which contain phase factors that approximately cancel with the corresponding electric field factors. We use t_1, t_2, and t_3 in Eq. (4) as an abbreviation for $-\tau-T$, $-T$, and 0 (compare Fig. 1) to elucidate that each $\tilde{A}$ represents an envelope of the first, second, or third pulse, respectively. The exponential factor in front of the integral indicates that the polarization oscillates at frequencies within a certain interval around $+\omega_0$ for the t coordinate and at frequencies within an interval around $-\omega_0$ for the τ coordinate. (This is the reason why the 2D spectra shown below, e.g., Fig. 6, have a negative ω_τ frequency axis and a positive ω_t frequency axis.) The integrand of Eq. (4) contains only slowly oscillating factors from the complex pulse envelopes. The details of the response functions S depend on the molecular system under study and will be given later in Sec. IV.

A two-dimensional Fourier transformation of $P^{(3)}_{rw}(\tau,T,t)$ with respect to τ and t then delivers two-dimensional (2D) spectra (one for each population time T) with frequency axes ω_τ and ω_t. However, experimentally one does not detect the polarization of Eq. (4) directly but rather the phase-matched signal field, which is in turn not observed as a function of t but instead with a spectrometer as a function of the conjugate frequency ω_s. Using Maxwell's equations, it can be seen that under ideal circumstances this frequency-domain signal field E_s is related to the polarization by

$$E_s(\tau,T,\omega_t)\sim\frac{i\omega_t}{n(\omega_t)}P^{(3)}(\tau,T,\omega_t) \quad (5)$$

with linear refractive index $n(\omega_t)$. Since one of the Fourier transformations is therefore already implicit in the frequency-domain detection technique, it is necessary to carry out only the transformation along the coherence time $\tau=t_2-t_1$ (for each fixed population time T), which finally leads to the 2D correlation spectra,

$$S_{2D}(\omega_\tau,T,\omega_t)=\int_{-\infty}^{\infty}iP^{(3)}(\tau,T,\omega_t)\exp(i\omega_\tau\tau)d\tau \quad (6)$$

- Requires detailed QM theory
- High coolness factor

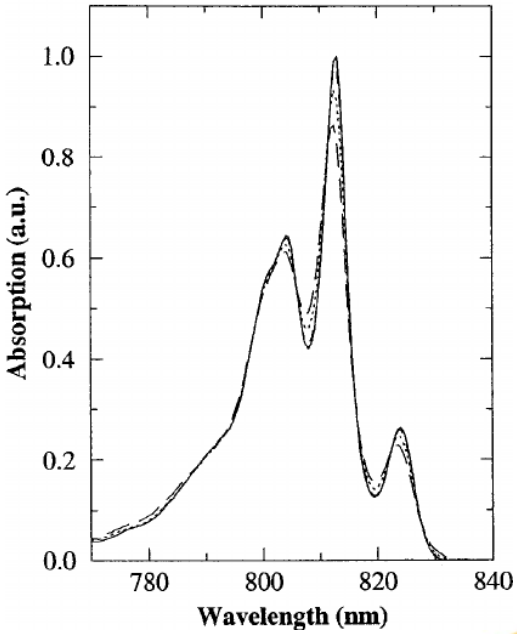
If you are from Berkeley, you can publish anything you like!

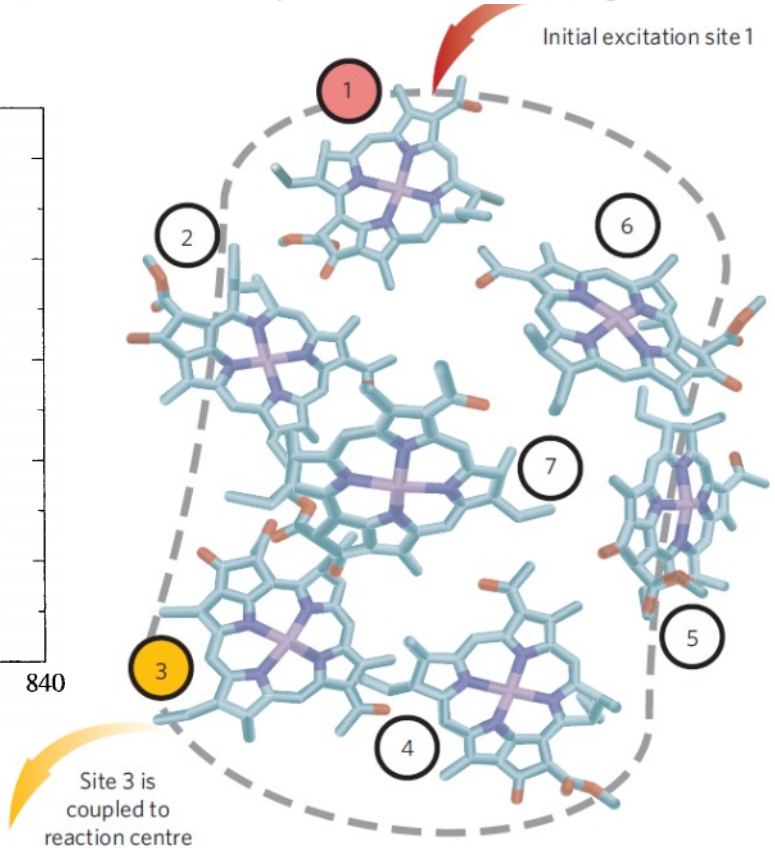
nature Vol 446|12 April 2007|doi:10.1038/nature05678

LETTERS

Evidence for wavelike energy transfer through quantum coherence in photosynthetic systems

Gregory S. Engel[1,2], Tessa R. Calhoun[1,2], Elizabeth L. Read[1,2], Tae-Kyu Ahn[1,2], Tomáš Mančal[1,2]†, Yuan-Chung Cheng[1,2], Robert E. Blankenship[3,4] & Graham R. Fleming[1,2]

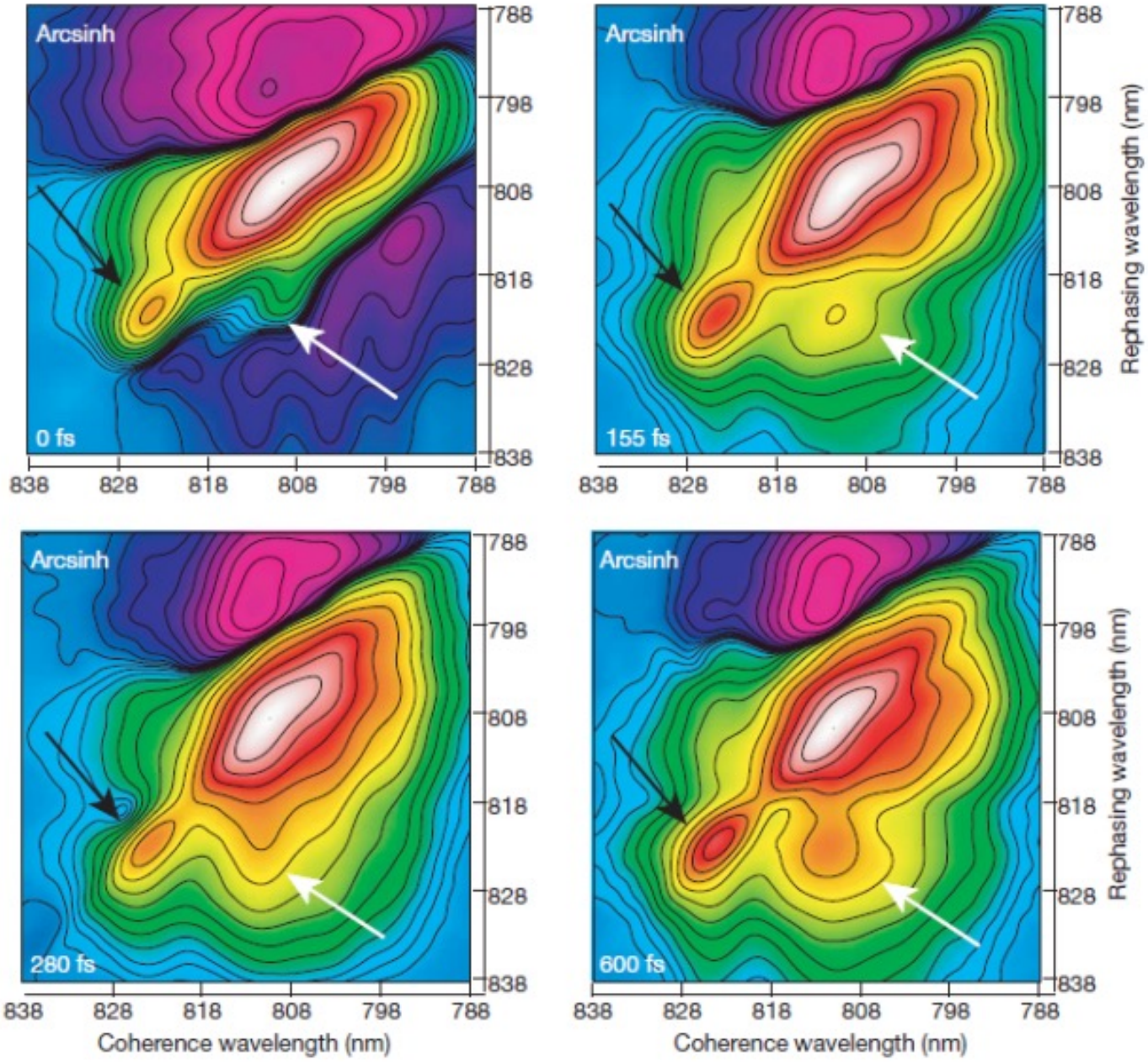




FMO complex, another light harvesting pigment protein complex.

Not much more than a bag of bacteriochlorophyll molecules with tight couplings.

Off-diagonal peak is oscillating!



Off-diagonal peak is oscillating!

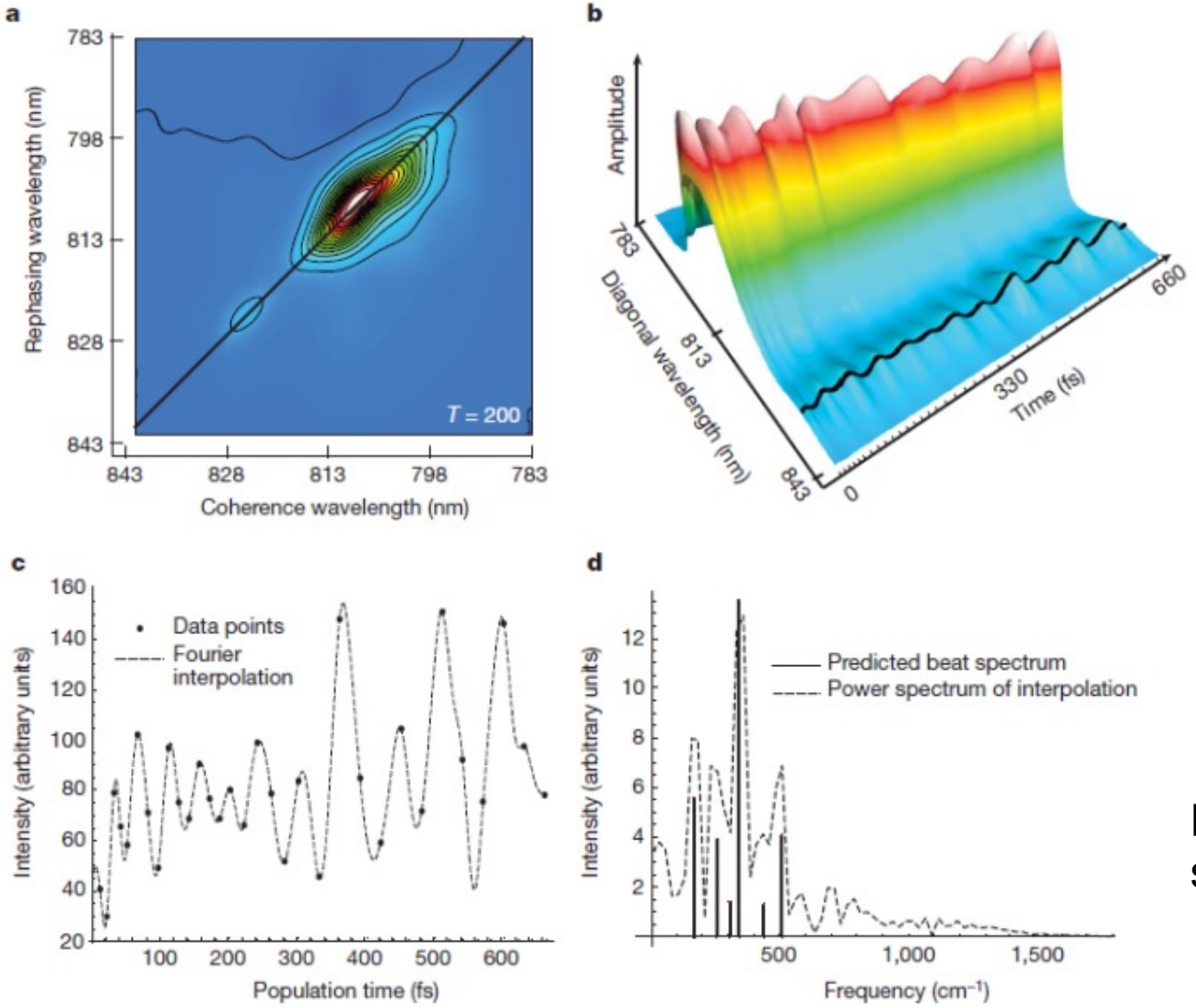


Figure 2 | Electronic coherence beating. **a**, A representative two-dimensional electronic spectrum with a line across the main diagonal peak. The amplitude along this diagonal line is plotted against population time in **b** with a black line covering the exciton 1 peak amplitude; the data are scaled by a smooth function effectively normalizing the data without affecting oscillations. A spline interpolation is used to connect the spectra; the times at which spectra were taken are denoted by tick marks along the time axis. **c**, The amplitude of the peak corresponding to exciton 1 shown with a dotted Fourier interpolation. **d**, The power spectrum of the Fourier interpolation in **c** is plotted with the theoretical spectrum showing beats between exciton 1 and excitons 2–7.

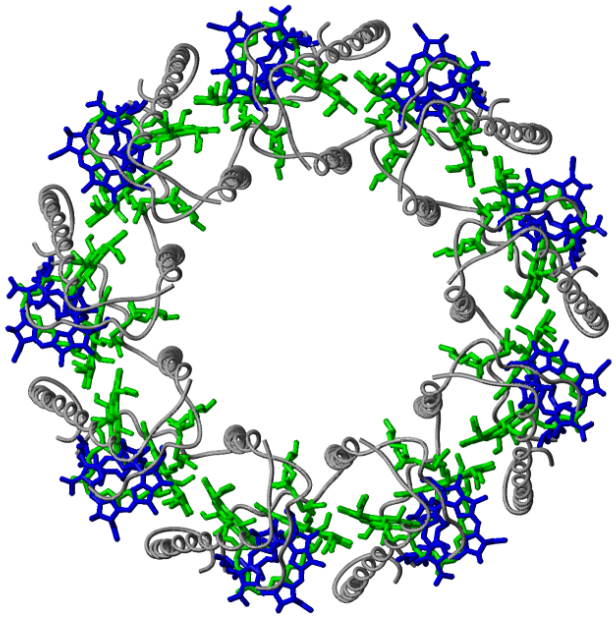
Electronic coherences should dephase in <50 fs

This ‘wavelike’ energy transfer started the now fashionable (hopefully, not for long) field of quantum biology…

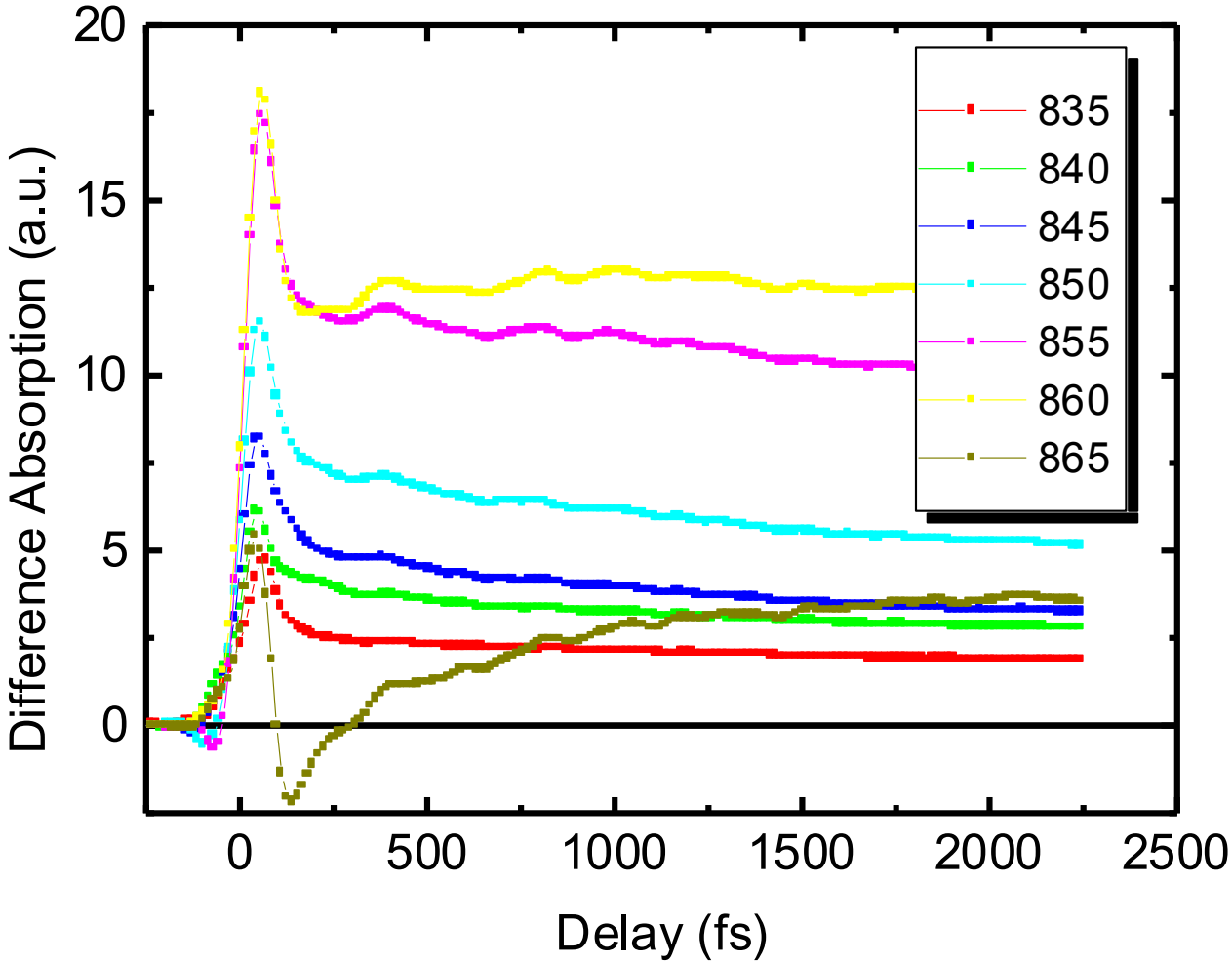
My never published data from 2000

Coherent response of nucleic subsystem to the excitation.

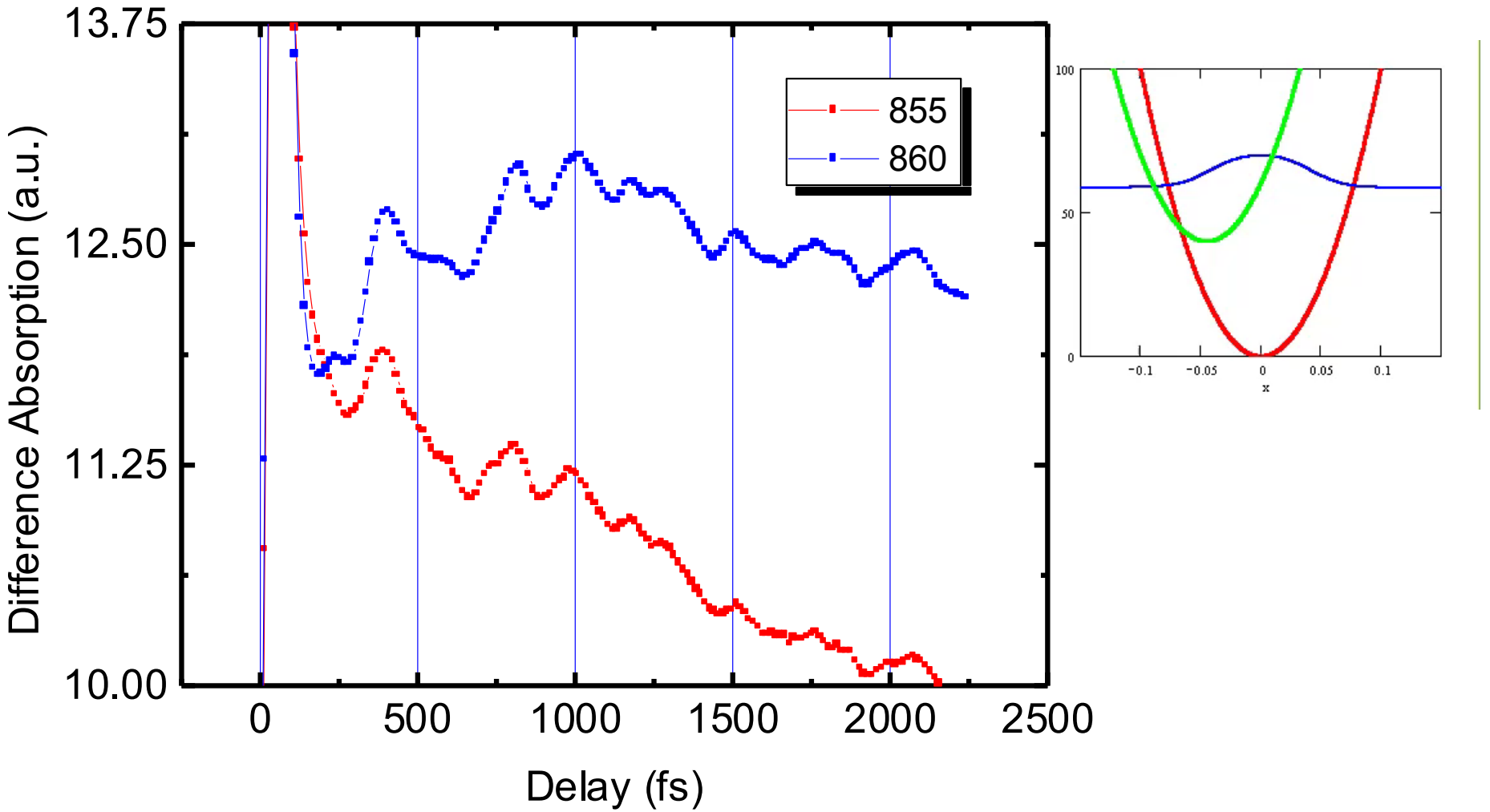
- Good old Rps. Acidophila;
- Pump-probe at 77K;
- Purpose: observe how the nuclei of BChl molecules respond to the electronic excitation.



Kinetics at various wavelengths (77K)



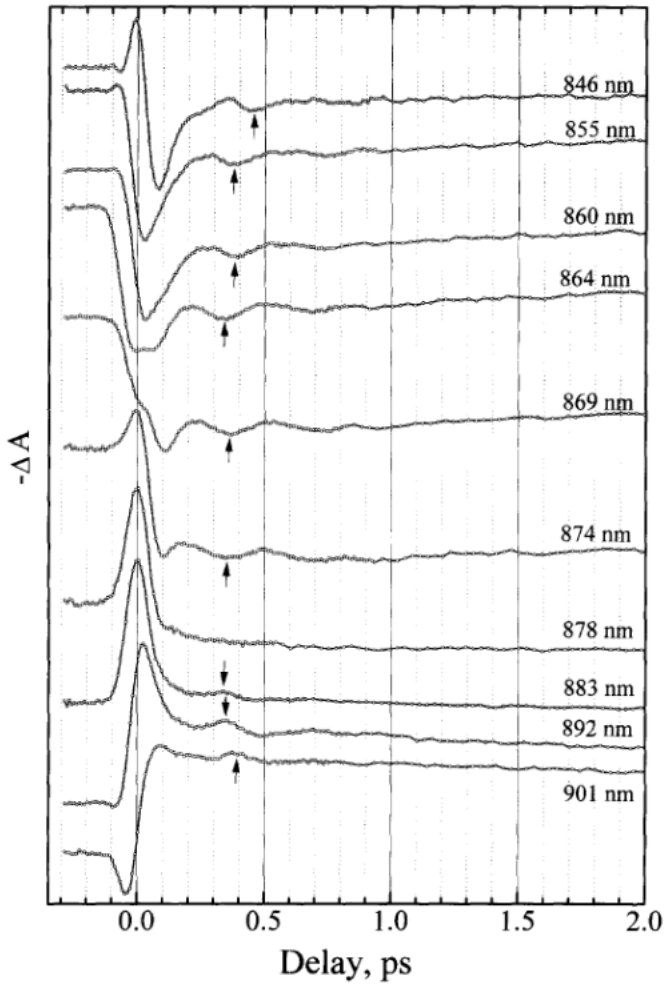
It is not noise!



4 years earlier (1996)

vation of the wavelength dependent phase shifts further confirms the vibrational origin of the oscillatory features.

In this Letter we will concentrate on the nuclear motions in the LH1 complex by describing it with a model system consisting of three electronic states and one nuclear degree of freedom. We tentatively assign the above three states to the ground, singly excited and doubly excited electronic manifolds of a BChl *a* exciton coupled aggregate, thereby assuming



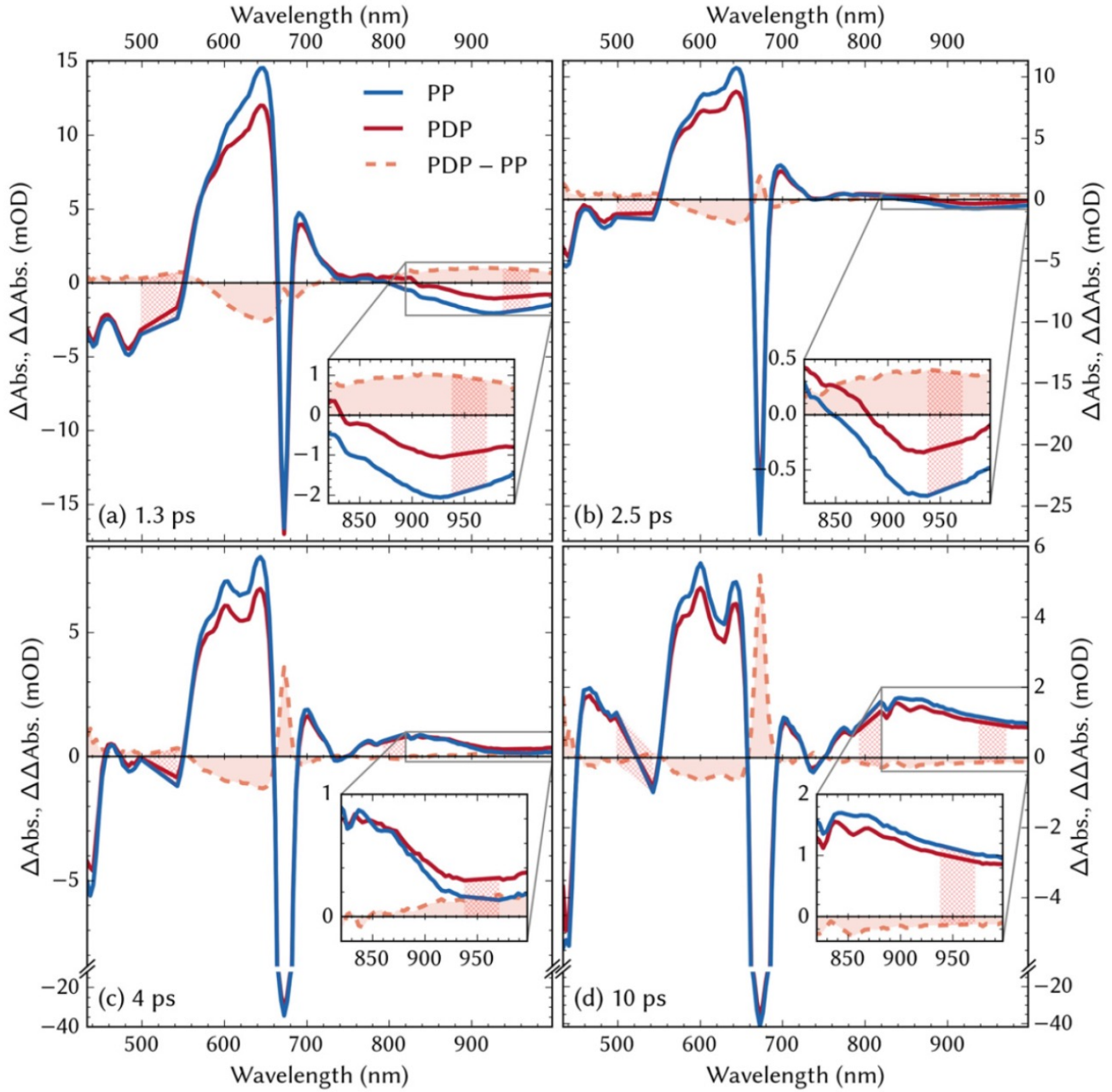
Instead of conclusions…

Time-resolved spectroscopy comes in a lot of different guises and is used in different fields for understanding the quantum-mechanical functioning of light sensitive matter.

Used wisely, it is a powerful box of tools for investigating nature.

Used bravely, it gets you papers in Nature (sometimes even despite the science being wrong).

Time to relax…


Wavelength (nm)
ΔAbs., ΔΔAbs. (mOD)
PP
PDP
PDP – PP
(a) 1.3 ps
(b) 2.5 ps
(c) 4 ps
(d) 10 ps