Spectroscopy on photoactive proteins

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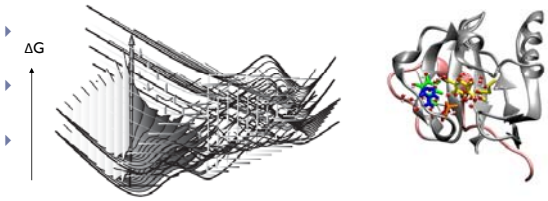
Theoretical Chemistry and Spectroscopy Graduate Course
Domaine des Masures, Han-sur-Lesse, Belgium
December 12-16, 2011

Outline

1. What do we want to know?
2. Techniques:
   a. Why ultrafast spectroscopy
   b. How to measure this
   c. Lasers and nonlinear optics
3. Data analysis
4. Examples:
   photosynthetic reaction centers, protochlorophyllide oxidoreductase, photoactive yellow protein

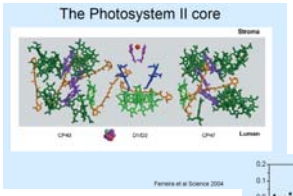
1. What do we want to know?

Understand with atomic detail how:

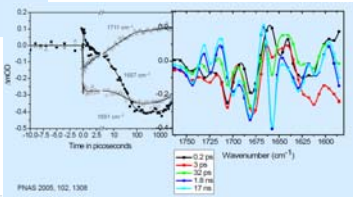


→ Relation between structure – dynamic – functionality

1. Identifying electron donor and acceptors in photosynthesis



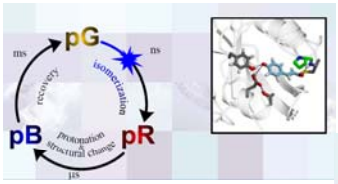
Initial electron donor and acceptor in isolated Photosystem II reaction centers identified with femtosecond mid-IR spectroscopy
Proc. Nat. Acad. Sci. USA, 2005 ,102, 37, 13087-13092

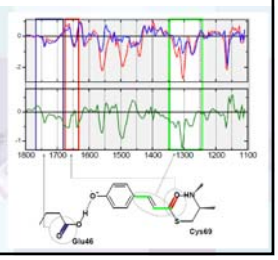


'Cofactors Involved in Light-Driven Charge Separation in Photosystem I Identified by Subpicosecond Infrared Spectroscopy', **Biochemistry** 50, 2011

New work on bacterial RCs

1. Isomerization in Photoactive Yellow Protein





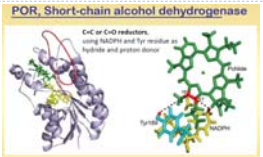
Groot et al *'Initial steps of signal generation in photoactive yellow protein revealed with femtosecond mid-infrared spectroscopy'*, **Biochem** 2003

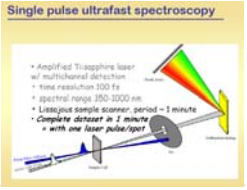
Wilderen et al, *'Ultrafast infrared spectroscopy reveals key step for successful entry into photocycle for Photoactive Yellow Protein'* **Proc. Natl. Acad. Sci. USA**, 2006,

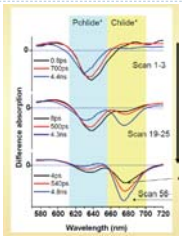
Rupenyan et al, *'Proline 68 Enhances Photoisomerization Yield in Photoactive Yellow Protein'* **J Phys Chem B**. 2011

Stahl et al, *On the Involvement of Single-Bond Rotation in the Primary Photochemistry of Photoactive Yellow Protein* **Bioph. J.** 2011

1. Light-dependent enzyme activation and catalysis







Sytina et al
- *Conformational changes in an ultrafast light-driven enzyme determine catalytic activity* **Nature** 456, 1001-1008, 2008
- *Enzyme activation and catalysis: characterisation of the vibrational modes of substrate and product in protochlorophyllide oxidoreductase* **PCCP** *2011*
- *Single and Multi-Exciton Dynamics in Aqueous Protochlorophyllide Aggregates* **JPhysChem** *2011*
- *Modeling of Multi-Exciton Transient Absorption Spectra of Protochlorophyllide Aggregates in Aqueous Solution,* **JPhysChem** *2011*
- *Protochlorophyllide Excited-State Dynamics in Organic Solvents Studied by Time-Resolved Visible and Mid-Infrared Spectroscopy* **JPhysChem** *2011*

2a. Techniques: why *ultrafast* spectroscopy

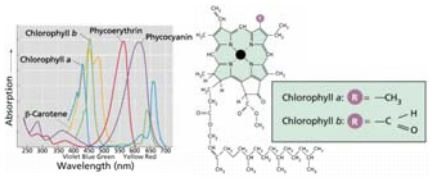
- Molecules move with respect to each other on a time scale of 10 fs -1 ps (= 10.10^{-15}-10^{-12}s)

O⟷H

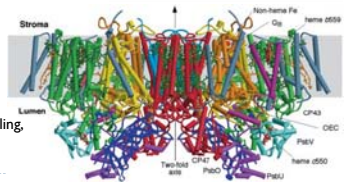
Stretch frequency ~3500 cm^{-1}
period of 10 fs

- So reactions which constitute the transfer or movement of a proton or electron or C-atoms will occur on these time scales
- → An ultrafast technique is necessary

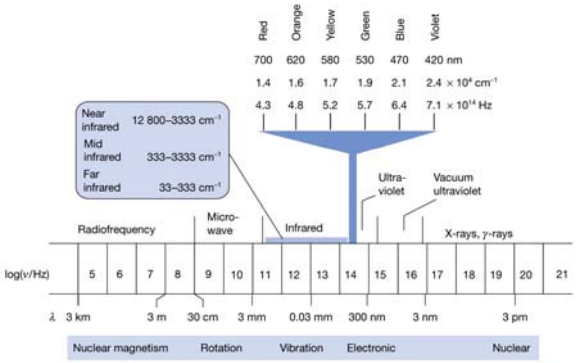
2a. Techniques: why ultrafast *spectroscopy*



Spectroscopy
↓
Electronic transitions
↓
Nature of the pigment
↓
Environment of the pigment (coupling, dephasing, hydrogen bonds, …)

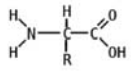


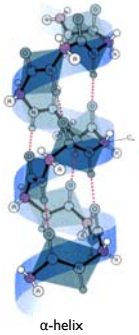
2a. Techniques: why ultrafast *spectroscopy*

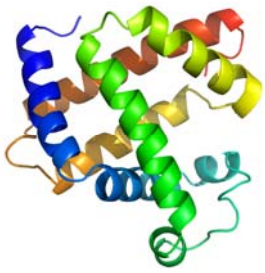


2a. Techniques: why ultrafast *spectroscopy*

C = carbon
N = nitrogen
O = oxygen
H = proton
R = amino acid

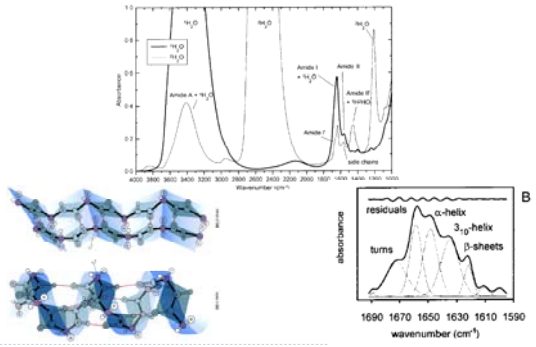






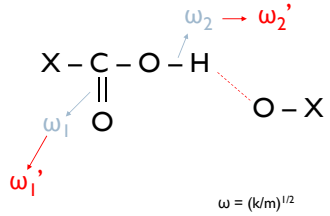
Peptide α-helix Protein

2a. Techniques: why ultrafast *spectroscopy*



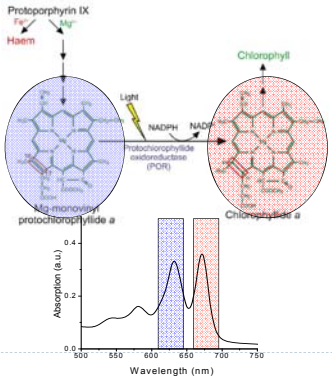
2a. Techniques: why ultrafast *spectroscopy*

Infrared spectroscopy, measuring the frequency of vibrational modes, is structure sensitive.
For example: H-bond lengths can be determined with sub-Å resolution



$\omega = (k/m)^{1/2}$

2b. Techniques: how to measure this?

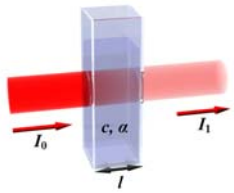


2b. Techniques: how to measure this?

Lambert-Beer's law: transmission of light through a cell

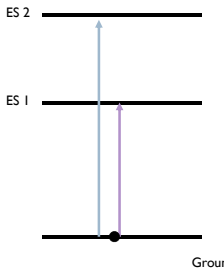
$T = I/I_0 = 10^{-\varepsilon c L}$ where ε (or α) is the molar extinction coefficient

$OD(\lambda) \equiv \mathrm{Log}(I_0/I) = c\,\varepsilon(\lambda)L$

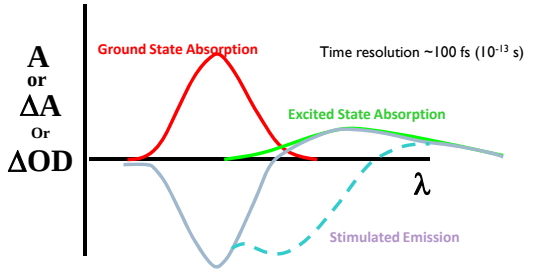


2b. Techniques: how to measure this?

Electronic transitions in molecules



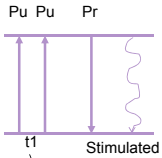
2b. Techniques: how to measure this?



Difference Absorption Spectrum: A(t)-A(t=0)

2b. Techniques: how to measure this?

- But formally, pump-probe spectroscopy is a self-heterodyned third order spectroscopy



May be important around time zero: different time orderings give different signals.
Independent control over the fields and time delays t1 and t2 can be used to gain information on coherences.

Time to absorb a photon, either determined by pulse length of pump, or by the dephasing time of the optical coherence i.e. ħ/absorption bandwidth

$$\frac{\partial}{\partial z}E(z,t) = i\frac{2\pi\omega_j}{cn(\omega_j)}P(z,t)$$

2b. Techniques: how to measure this?

Heterodyne detection, observation of superposition of 'local oscillator' field (= probe field) and signal field:

$I(t) = n(\omega_s)c/4\pi\,|E_{lo}(t) + E_s(t)|^2 = I_{LO}(t) + I_S(t) + 2\,n(\omega_s)c/4\pi\,\mathrm{Re}[E^*_{LO}(t).E_S(t)]$

And solve $\frac{\partial}{\partial z}E(z,t) = i\frac{2\pi\omega_j}{cn(\omega_j)}P(z,t)$ to get

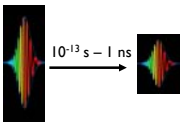
$$\frac{\partial I}{\partial z} = -I\frac{4\pi\omega_j}{cn(\omega_j)}\mathrm{Im}[P_j(z,t)/E_j(z,t)]$$ ← absorption coefficient

Here is used that $\mathrm{Im}[E^*_j(t)P(t) = |E(t)|^2\mathrm{Im}[P(t)/E(t)]$

The probe absorption is related to the out-of-phase component of the polarization
Signal is quadratic in both pump and probe field: $S \sim |E_{pu}|^2.|E_{pr}|^2$
And linear rather than quadratic in the weak nonlinear polarization P

2b. Techniques: how to measure this?

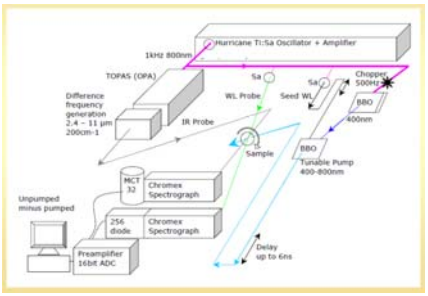
- In summary, we need a short laser pulse to start a reaction and we need probe light in a large as possible spectral region



10^{-13} s – 1 ns

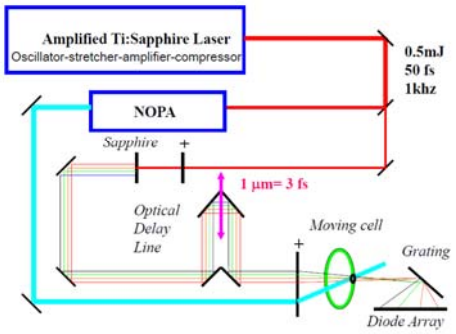
Volg e^-, H^+ transfer via absorptieveranderingen in elektronische overgangen 400 – 1100 nm, en vibrationele overgangen: 3 – 10 µm = struktuur info

2c. Lasers and nonlinear optics



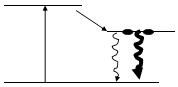
Probe/excite from 350nm – 10 µm, $100 .10^{-15}$ s – 1 ns

2c. Lasers and nonlinear optics



2c. Lasers and nonlinear optics

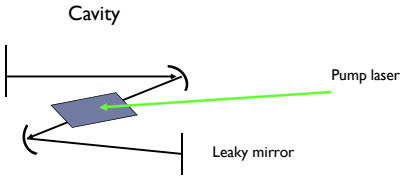
Light **A**mplification by **S**timulated **E**mission **R**adiation:



- Population inversion
- Cavity
- Gain medium -> Titanium:sapphire

Single mode → CW laser
Many modes with phase relation → short pulse in the cavity

Oscillator



For Ti:sapphire oscillators
λ = 800 nm,
Rep. rate = 80 MHz
Low power ~10 nJ
Pulses can be as short as ~10 fs

Regenerative amplification

Amplify from nanoJ to milliJoules
→ peak power 20 fs pulse if focussed to 100 micrometer = 10^{12}W/cm^{-2}
=1000 times damage treshold most materials!

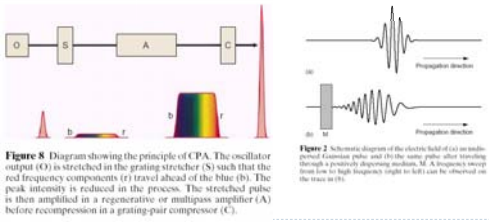


Figure 8 Diagram showing the principle of CPA. The oscillator output (O) is stretched in the grating stretcher (S) such that the red frequency components (r) travel ahead of the blue (b). The peak intensity is reduced in the process. The stretched pulse is then amplified in a regenerative or multipass amplifier (A) before recompression in a grating-pair compressor (C).

Regenerative amplification

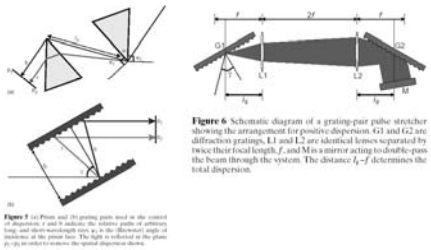


Figure 6 Schematic diagram of a grating-pair pulse stretcher showing the arrangement for positive dispersion. G1 and G2 are diffraction gratings, L1 and L2 are identical lenses separated by twice their focal length, f, and M is a mirror acting to double-pass the beam through the system. The distance $l_g - f$ determines the total dispersion.

Regenerative amplification

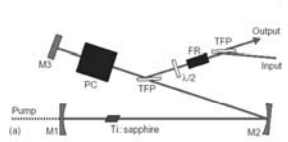
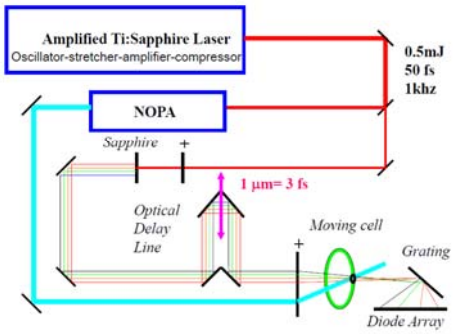


Figure 9 Two schemes for regenerative amplification. The design in (a) is often used for kilohertz repetition rate amplifiers and that in (b) at a 10–20-Hz repetition rate. The Ti:sapphire rod is usually ca. 20 mm long and doped for 90% absorption. **TFP**, thin-film polarizing beamsplitter; **PC**, Pockels cell; **FR**, Faraday rotator; $\lambda/2$, half-wave plate. In (a), M1 is 150-mm radius of curvature, M2 is 1 m and M3 is flat. In (b) M1 is −20 m and M2 is +10 m.

Setup



White light generation by Self Phase Modulation

The index of refraction n is in general intensity dependent
$n(I) = n_0 + n_2 I + \ldots$

The propagation of an electrical laser field depends on the index of refraction of the material:

$E(x,t) = E(t)\cos(\omega t - kx)$

$\varphi = \omega t - kx = \omega t - \omega n x/c$
$= \omega(t - n_0 x/c) - n_2\, \omega x/c\, I(t)$

$\omega = d\varphi/dt = \omega - A\, dI/dt$

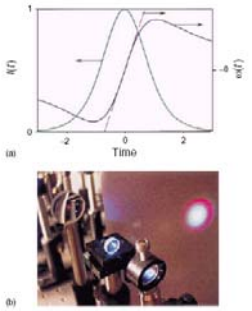
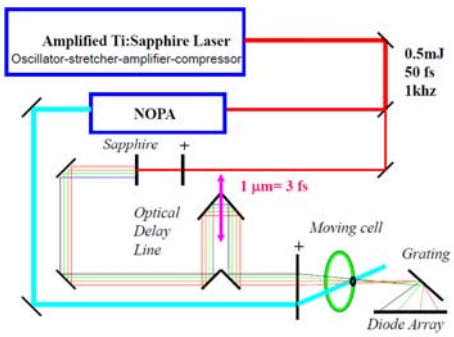


Figure 12 (a) Chirp on an ultrashort pulse induced by the nonlinear refractive index of a dielectric medium. The input pulse has a sech^2 envelope and the figure shows the instantaneous frequency within the pulse. A Taylor expansion around the peak of the pulse shows that the frequency sweep is approximately linear around time zero. (b) Photograph of a femtosecond white-light continuum beam generated in a piece of sapphire. (Picture courtesy of the Center for Ultrafast Optical Science, University of Michigan.)

Setup



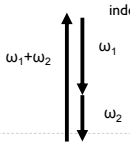
Parametric generation or amplification

The splitting of one photon in two:
$\omega_{pump} = \omega_{signal} + \omega_{idler}$

Phase matching condition = Conservation of momentum:
$k_{pump} = k_{signal} + k_{idler}$

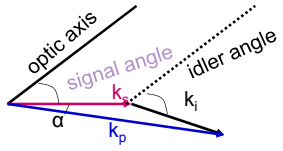
Or: $n_p\, \omega_{pump}/c = n_s\, \omega_{signal}/c + n_i\, \omega_{idler}/c$

This can be done in nonlinear, birefringent crystals were the index of refraction depends on the polarization



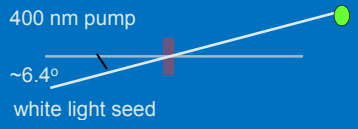
Noncollinear optical parametric amplification

- When using a non-collinear phase matching angle in BBO pumped at 400 nm, the phase matching angle becomes independent of wavelength over a large part of the spectrum,
  for an angle of a 3.7° between pump and signal (Gale,Hache 1994)
  ⇒ large bandwidth
- The spatial walk-off (from the extraordinary pump beam) is 4.0°, with P_p farther from optical axis than k_p.This is coincidently close to the noncollinear angle a!
  ⇒ high gain
- Sub-10 fs with μJ energies can be obtained (efficiency 10-30%)



Optimize bandwidth by matching the signal and idler group velocities (=degeneracy for collinear beams).

Expressed in terms of α and θ and solved for large bandwidths, one finds α = 3.7° and θ = 32°



Shorter pulses by
- minimizing dispersion of white light (no dispersive optics)
- or even lengthening pump pulse
- optimal compression (small apex angle prisms or gratings)

NOPA

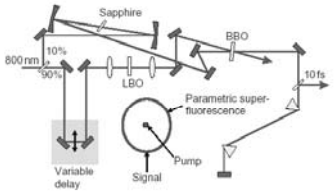
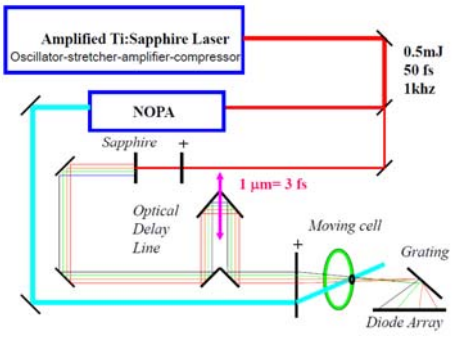
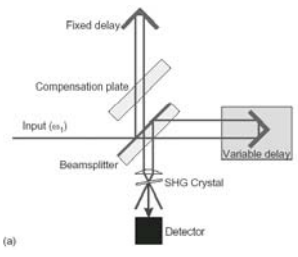


Figure 14 Schematic diagram of a noncollinear OPA. The inset shows the arrangement of the seed beam (the signal) relative to the pump and the generated superfluorescence.

Setup

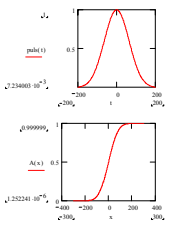


The instrument response function



The cross- or auto correlation is given by

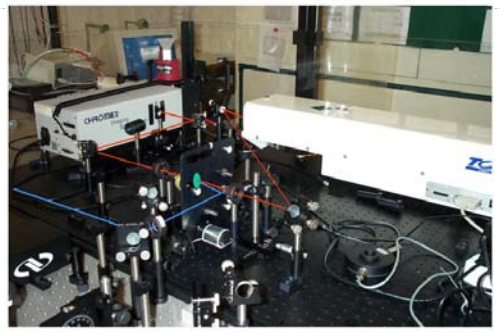
$$I_{cross}(\tau) \propto |\chi^{(2)}|^2 \int I_{w_1}(t) I_{w_2}(t-\tau)\,dt$$

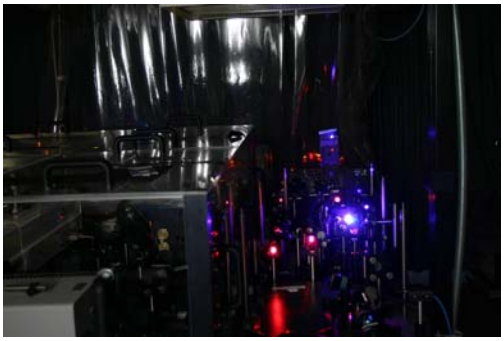


Rule of thumb: time from 10-90% ~ τ_{cross}

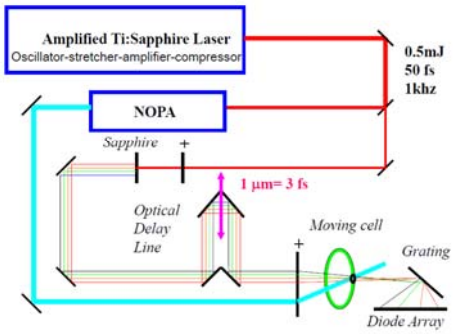
Important experimental aspects:

- Repetition rate of laser must be slower than photocycle, or sample must be refreshed for every shot
- Excitation density must be low, only when less than 10% of complexes are excited you are in a linear regime -> annihilation, saturation due to stimulated emission, orientational saturation
- Population dynamics are measured under the 'magic' angle 54.7°, at other angles orientational dynamics are measured anisotropy = $r = (\Delta OD_{//} - \Delta OD_{\perp}) / (\Delta OD_{//} + 2\Delta OD_{\perp})$



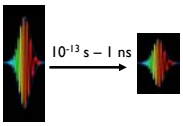


Setup



2b. Techniques: how to measure this?

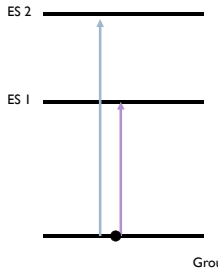
- In summary, we need a short laser pulse to start a reaction and we need probe light in a large as possible spectral region



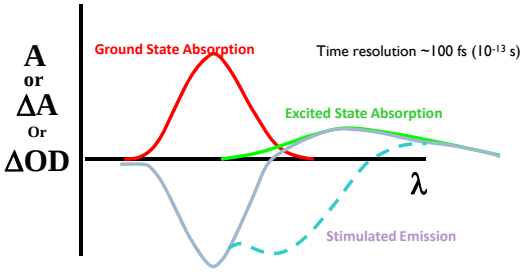
Volg e^-, H^+ transfer via absorptieveranderingen in elektronische overgangen 400 – 1100 nm, en vibrationele overgangen: 3 – 10 μm = struktuur info

2b. Techniques: how to measure this?

Electronic transitions in molecules



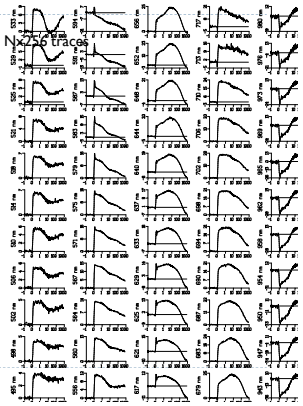
2b. Techniques: how to measure this?

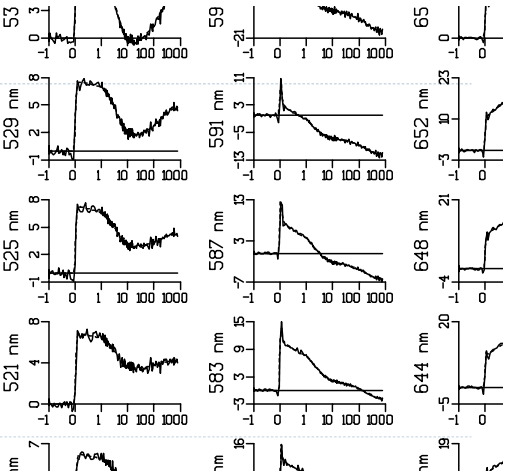


Difference Absorption Spectrum: A(t)-A(t=0)

Data: example

Typically 256 traces per experiment, so N x 256 traces





Global analysis

$$\psi(t,\lambda) = \sum_{i=1}^{n_{comp}} (\exp(-k_i t) \otimes i(t)) DAS_i(\lambda)$$

Without any a priori knowledge, fit your data to a minimum number of exponential decays, convoluted with instrument response function i(t):

$$i(t) = \frac{1}{\Delta\sqrt{2\pi}} \exp(-\log(2)(2(t-\mu)/\Delta)^2)$$

Amplitudes: Decay Associated (Difference) Spectra

Expert: dr Ivo van Stokkum

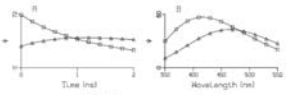
Global analysis

Or, if there is some a priori knowledge, set up a compartmental model.
For instance, assume that the first compartment, representing component 1, is excited. Component 1 irreversibly decays with rate k1 thereby forming component 2 with fractional yield Φ1. Component 2, which is represented by the second compartment, decays with rate k2, which is smaller than k1.

Target analysis

Time traces at 400 and 500 nm

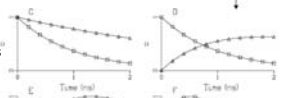
Time-gated spectra at 0.4 ns (squares) and 1.6 ns (triangles)



Model 1

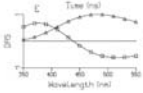
Model 2

Concentration profiles

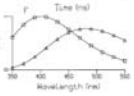


k_1 = 1 ns, k_2 = 4 ns

DADS



SADS



You can set up a 'physical realistic' linear compartmental model, where transitions between compartments are described by microscopic rate constants which constitute the off-diagonal elements of the transfer matrix K.
The diagonal elements of *K* contain the total decay rates of each compartment.
With the concentration of the compartments are described by the vector

$$c(t) = \left[c_1(t) \ \ldots \ c_{n_{comp}}(t)\right]^T$$

Thus, a linear compartmental model with n_{comp} compartments is described by a differential equation for these concentrations:

$$\frac{d}{dt}c(t) = Kc(t) + j(t)$$

Where $j(t) = i(t)\left[1 \ x_2 \ \ldots \ x_{n_{comp}}\right]^T$

Look at residuals etc

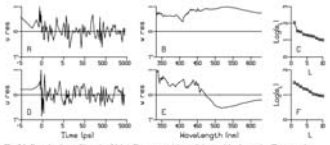
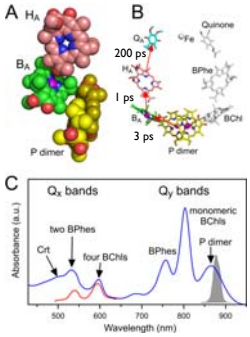
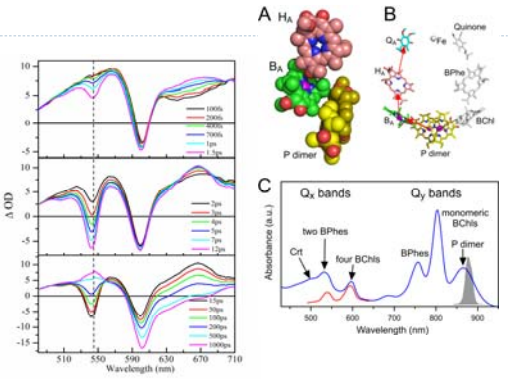
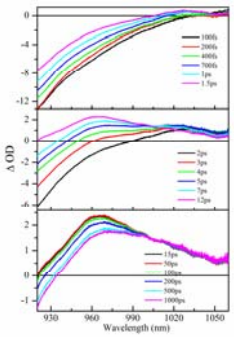


Fig.29. Results from Singular Value Decomposition of the residual matrix. Top panels: uncorrected data. Bottom panels: residuals from data after correction for baseline fluctuations. (A,D): first left singular vector, showing dominant temporal structure. (B,E): first right singular vector, showing dominant spectral structure. (C,F): Singular values on logarithmic scale.

Real data: the bacterial reaction center



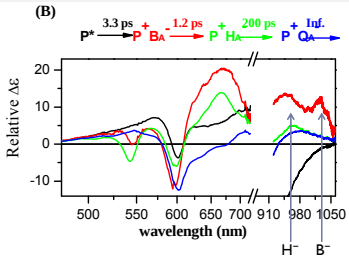




H and B anion absorption region

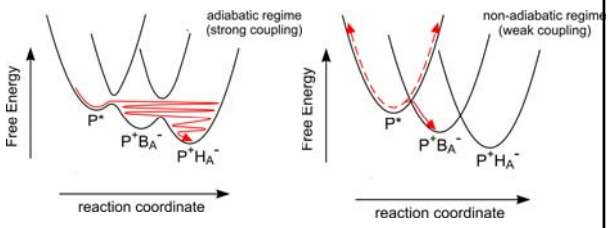
According to chemically reduced solvent spectra

Interpretation…



The first radical pair 'P+B⁻' contains
- anion bands of both B and H!
- H bleaching!

→ First RP is actually a mixture. Coherent or incoherent?



Coherent or incoherent fast equilibrium between B^- and H^-?

Coherence between H and B

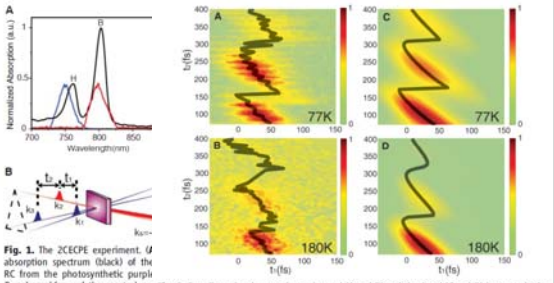


Fig. 1. The 2CECPE experiment. (A absorption spectrum (black) of the RC from the photosynthetic purple *R. sphaeroides* and the spectral pr ~40-fs laser pulses (blue, 750 nm; n used in the experiment. (B) The pul for the 2CECPE experiment. We de tegrated intensity in the phase-ma tion $k_s = -k_1 + k_2 + k_3$. a.u., arbitr...

Fig. 2. Two-dimensional maps of experimental (**A** and **B**) and simulated (**C** and **D**) integrated echo signals as a function of the two delay times, t_1 and t_2, from the RC. The black lines follow the maximum of the echo signal at a given t_2. The data at $t_2 < 75$ fs are not shown because the conventional two-color three-pulse photon echo signal (750-750-800 nm) overwhelms the 2CECPE signal in this region, due to the pulse overlap effect.

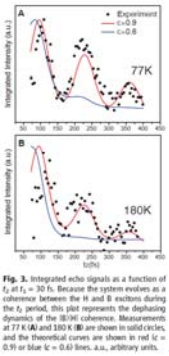


Fig. 3. Integrated echo signals as a function of t_2 at $t_1 = 30$ fs. Because the system evolves as a coherence between the H and B excitons during the t_2 period, this plot represents the dephasing dynamics of the |B⟩⟨H| coherence. Measurements at 77 K (**A**) and 180 K (**B**) are shown in solid circles, and the theoretical curves are shown in red (λ_c = 0.9) or blue (λ_c = 0.6) lines. a.u., arbitrary units.

Gaussian decay time of 440 and 310 fs at 77 and 180 K

The decay of the |g⟩⟨H| coherence is much faster than the decay of the |B⟩⟨H| coherence. Considering that the dephasing of the |g⟩⟨H| coherence is caused by the transition energy fluctuations on H, whereas the dephasing of the |B⟩⟨H| coherence is due to the fluctuations on the gap between H and B transition energies, the transition energy fluctuations on B and H must be strongly correlated, because in-phase energy fluctuations do not destroy coherence.
Such a strong correlation can arise for two possible reasons: strong electronic coupling between B and H and/or strong correlation between nuclear modes that modulate transition frequency fluctuations of localized BChl and BPhy excitations. Our theoretical analysis found that strong electronic coupling alone cannot reproduce the sawtooth pattern and a dephasing time as long as that observed (11). Instead, crosscorrelation between nuclear modes modulating the energy levels of localized BChl and Bphy excitations is required.

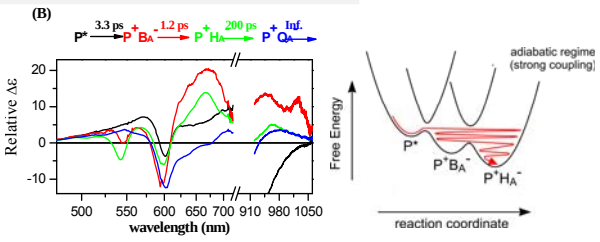
Modeling: the electronic coupling J=220 cm-1 and the gap between excitonic H and B states is 680 cm-1

Does this put us in the adiabatic electron transfer regime?
Yes, according to:

Transmission Coefficients for Chemical Reactions with Multiple States: Role of Quantum Decoherence
Aurelien de la Lande,†,* Jan Rezac,‡ Bernard Levy,† Barry C. Sanders,§ and Dennis R. Salahub||
J. Am. Chem. Soc. 2011, 133, 3883–3894:

'In principle, an adiabatic regime is achievable even with moderate electronic coupling provided T_m is large enough. In that regard it is important to mention recent experimental findings that have revealed cases of electronic coherences persisting over tens or hundreds of femtoseconds.'

Electron transfer in BRC is adiabatic



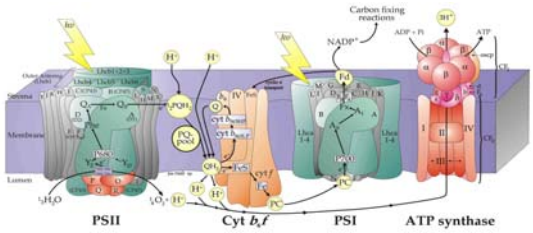
Marloes Groot

Mariangela Didonato (now at Lens, Florence)
Andy Stahl
Ivo van Stokkum

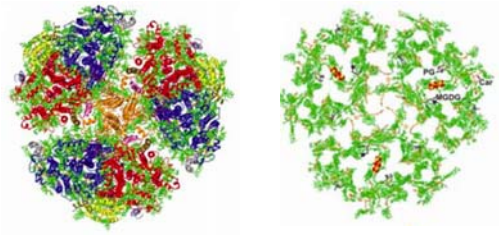
Charge separation in Photosystem I

vrije Universiteit amsterdam

Plant Photosynthesis

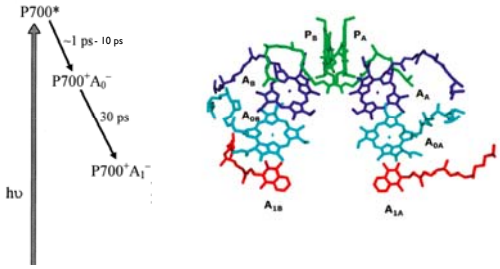


Structure of Photosystem I



Jordan, P., Fromme, P., Klukas, O., Witt, H. T., Saenger, W. and Krauß, N., Three dimensional structure of cyanobacterial photosystem I at 2.5 Å resolution. *Nature*, 2001, **411**, 909–917.

Reaction center: Cofactors and charge separation



Renger, review in Current Science, 2010

Understanding primary charge separation

- Curiosity

Biochemistry **2010,** *49,* 404–414
DOI: 10.1021/bi901704v

BIOCHEMISTRY
Current Topic

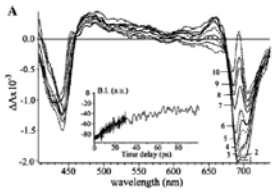
Wiring Photosystem I for Direct Solar Hydrogen Production†

Carolyn E. Lubner,‡ Rebecca Grimme,‡ Donald A. Bryant,§ and John H. Golbeck*,‡,∥

- Template for artificial systems

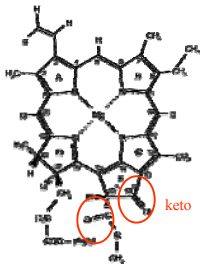
Difficult…

- ~ 100 pigments/complex
- All absorbing between 670-710 nm



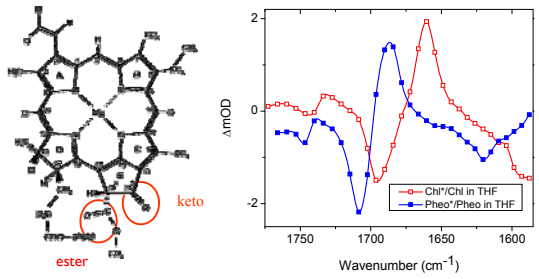
Shuvalov et al BBA 2010

Keto and ester C=O as redox probe



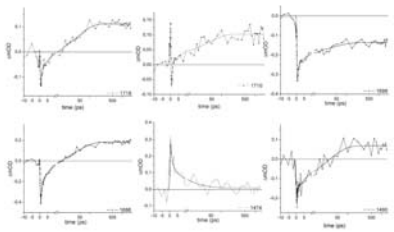
Look at changes in C=O absorption between 1800 and 1600 cm^{-1}; exact frequency depends on environment, presence of H-bonds, dimers etc, and there are very specific changes for Chl/Chl^{+} and Chl/Chl^{-}.

Excited state difference spectra

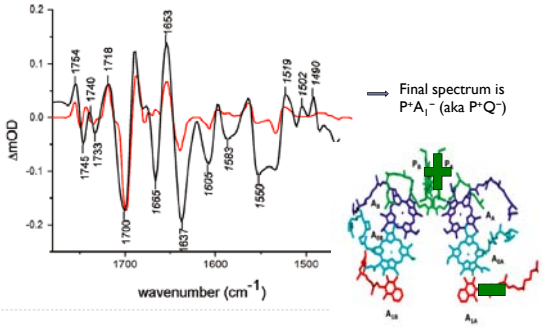


Vis-pump/IR probe on Photosystem I

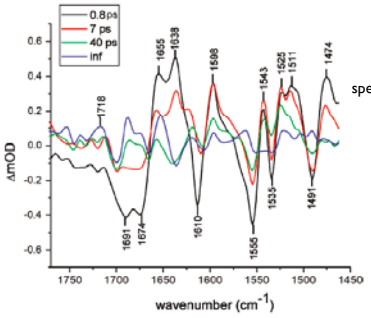
- Synechococus elongatus trimers
- Excite at 700 nm (~30% direct RC excitation), P=100 nJ,
- Probe between 1750-1450 cm^{-1}
- Sodium ascorbate & phenozine methosulfate to keep RCs open
- Sample re-excitation after 1 minute or more



Final spectrum compared with FTIR P^+/P_{700}



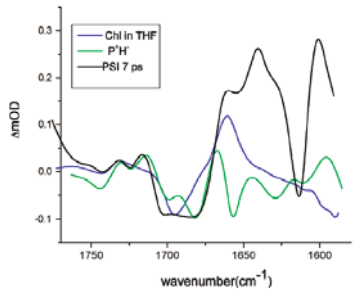
Spectral evolution in time



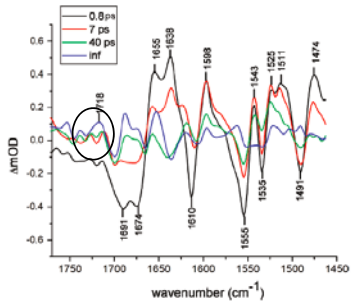
specA → specB → specC → specD →
K1 k2 k3

~ time-gated spectra

Comparison with P^+H^- of Photosystem 2



Spectral evolution in time



- Early RP formation
- Different electron donors

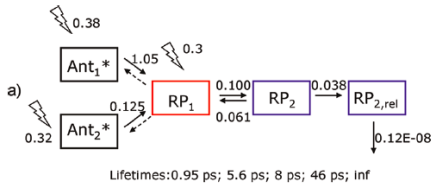
- 7 ps: mixture of excited States and RP

- 40 ps: RP, but different from

- Inf: $P^+A_1^-$ (aka P^+Q^-)

Can we model this?

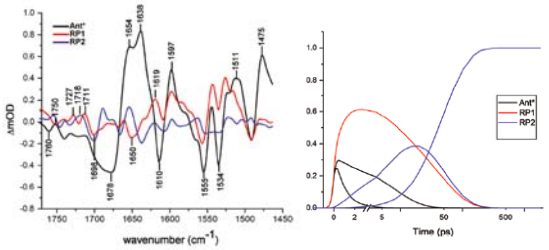
- Energy transfer, also from the red pigments



Lifetimes:0.95 ps; 5.6 ps; 8 ps; 46 ps; inf

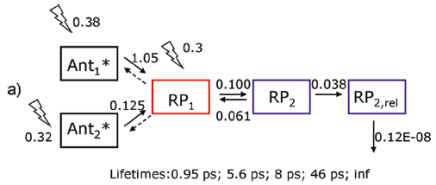
Rates free fit parameters

Spectra of these states



Can we do better?

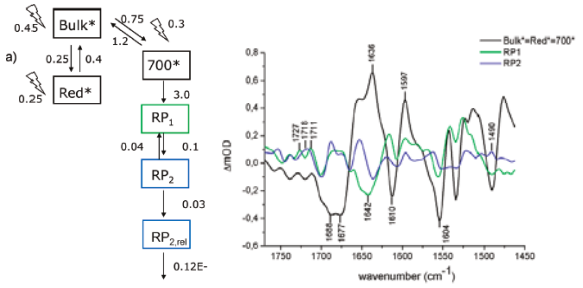
- Energy transfer, also from the red pigments



Lifetimes:0.95 ps; 5.6 ps; 8 ps; 46 ps; inf

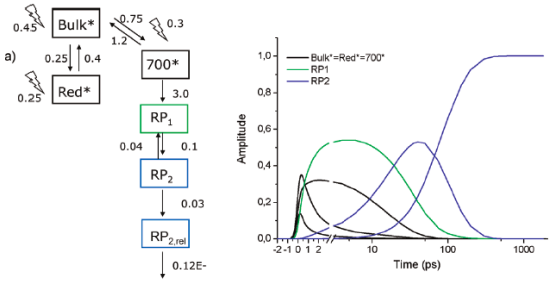
Rates free fit parameters

More complicated, but more realistic:



Lifetimes: 0.2 ps; 1.2 ps; 6.6 ps; 13 ps; 50 ps; inf

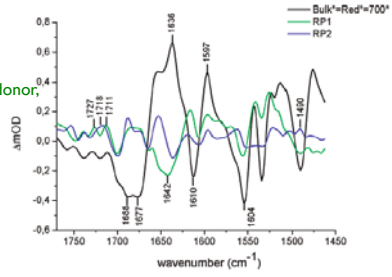
More complicated, but more realistic:



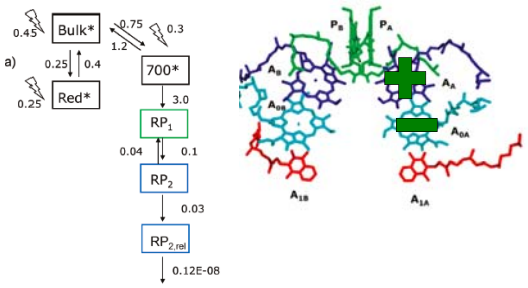
Lifetimes: 0.2 ps; 1.2 ps; 6.6 ps; 13 ps; 50 ps; inf

The radical pairs

- RP2: $P^+A_1^-$ (aka P^+Q^-)
- RP1: different electron donor, & different acceptor!

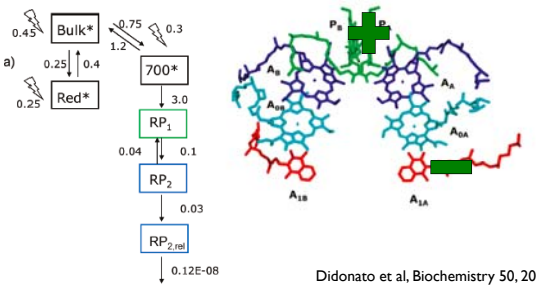


Charge separation: $A_A^+A_{0A}^-$



Lifetimes: 0.2 ps; 1.2 ps; 6.6 ps; 13 ps; 50 ps; inf

Second radical pair: $P^+A_1^-$ (aka P^+Q^-)



Didonato et al, Biochemistry 50, 2011

Lifetimes: 0.2 ps; 1.2 ps; 6.6 ps; 13 ps; 50 ps; inf

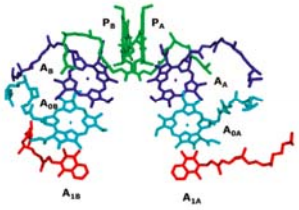
Kinetics

$A^+A_0^-$ formation: 0.3 ps

Equilibrium $A^+A_0^-$ and $P^+A_1^-$: 6 ps

Full population of $P^+A_1^-$: 40 ps (known)

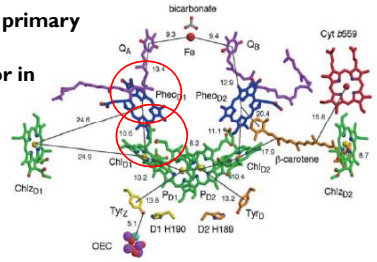
Antenna decay: 1 and 10 ps (known)



In Photosystem 2:

$Pheophytin_{D1}$ is the primary electron acceptor,

Chl_{D1} electron donor in Photosystem II

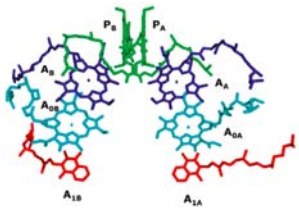


In 0.6-0.8 ps an electron is transferred from Chl_{D1} to H

Groot et al, PNAS 102, 2005

Conclusions

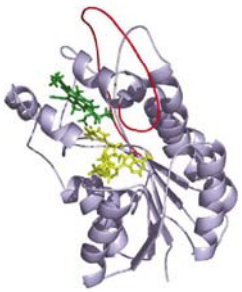
- In both plant reaction centers, P is not the primary electron donor
- Electron and hole transfer occur to create the radical pair $P_{700}^+A^-$ in PS1
- *Different* from situation in bacterial RCs



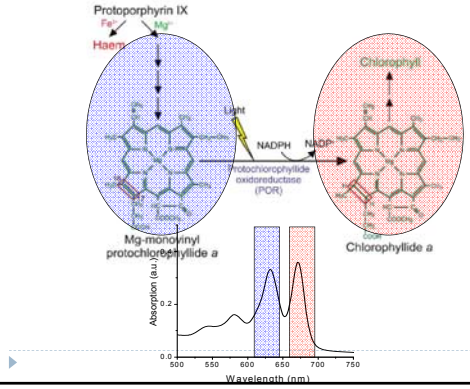
Eliminating diffusional events

Problem: Protein dynamics and conformational changes in enzyme catalysis ('slow') are almost impossible to distinguish from the actual reaction involving proton and hydride transfer ('fast') in most enzymes.

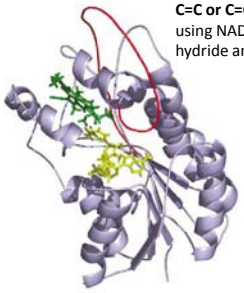
→ Look at catalytic events in the light-driven enzyme NADPH:protochlorophyllide oxidoreductase (POR).



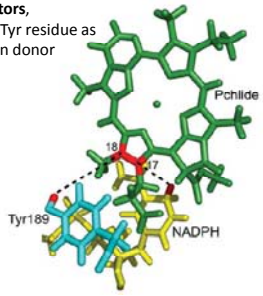
Light-driven step in the biosynthesis pathway of chlorophyll



POR, Short-chain alcohol dehydrogenase

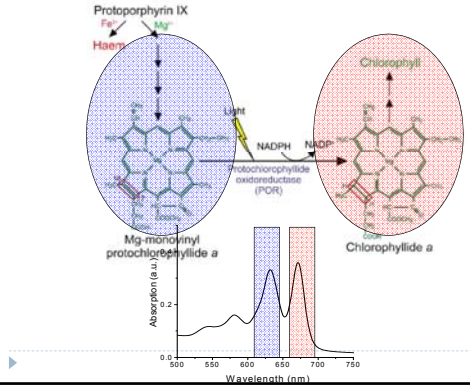


C=C or C=O reductors, using NADPH and Tyr residue as hydride and proton donor

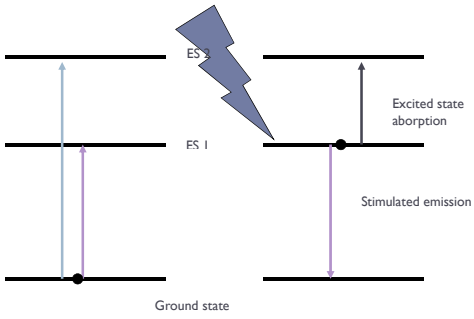


Homology model of *protochlorophyllide oxidoreductase (POR)*
From Townley et al, Proteins: Structure, Function and Genetics, 44: 329-335 (2001)

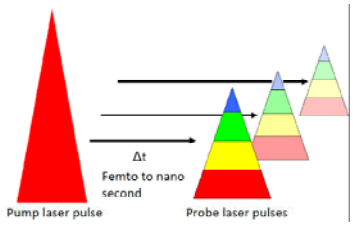
Tracking PChlide to Chlide conversion



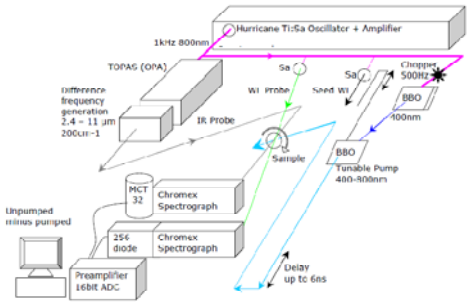
Elektronic transitions in molecules



Pump-probe experiments

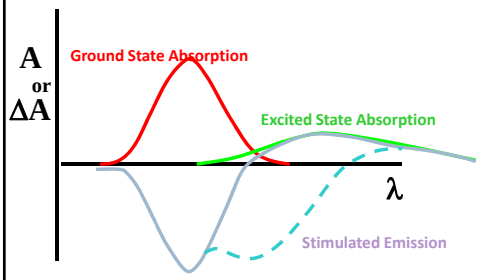


Experimental setup



Measure ultrafast dynamics via absorption difference spectroscopy

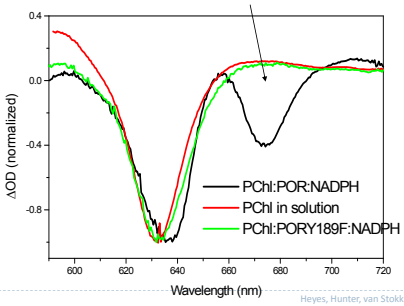
Time resolution ~100 fs (10^{-13} s)



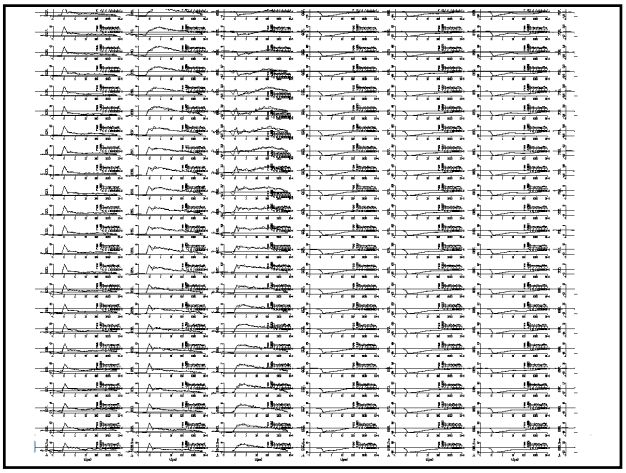
Difference Absorption Spectrum: A(t)-A(t=0)

Ultrafast spectroscopy on POR

PChlide absorption difference spectra 2 ns after excitation: formation of red **excited** state **I675**



Heyes, Hunter, van Stokkum, van Grondelle, Groot, (2003) *Nature Structural Biology* 10, 491-492



Global analysis

After averaging, typically 20.000 data points.
Analyze time traces at all 256 wavelengths with the same set of exponential decays, and obtain evolution-associated-difference spectra:

$$S(\omega,t) = \Sigma A_i(\omega) e^{-t.k_i}$$

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C$$

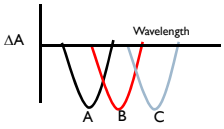
$dA(t)/dt = -k_1 * A(t)$

$dB(t)/dt = k_1 * A(t) - k_2 B(t)$

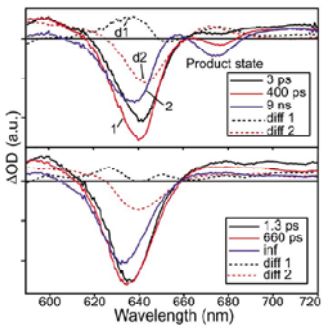
$dC(t)/dt = k_2 * B(t)$,

with $A(0) = 1$, $B(0)=0$ and $C(0) = 0$

Or more complicated but physically realistic model.....



Intermediate I675 formed in 3 and 400 ps

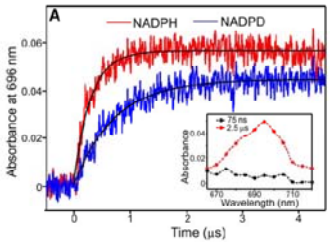


WT

Y189F mutant

Heyes, Hunter, van Stokkum, van Grondelle, Groot, (2003) *Nature Structural Biology* 10, 491-492

Nature of I675?

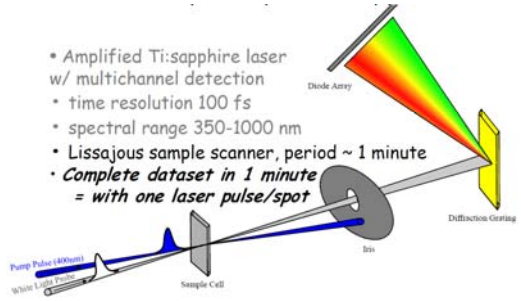


Hydride transfer is slower,
Heyes et al JBC2009

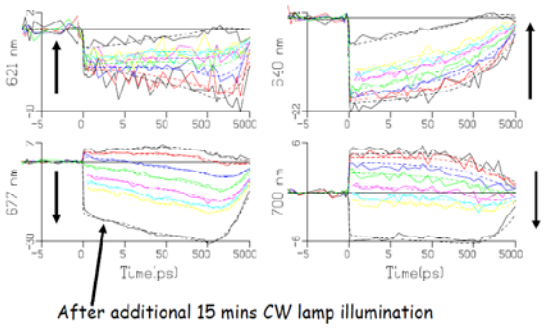
→3 ps & 400 ps:
proton transfer or formation of very strong H-bonded intermediate …

Single pulse ultrafast spectroscopy

- Amplified Ti:sapphire laser w/ multichannel detection
- time resolution 100 fs
- spectral range 350-1000 nm
- Lissajous sample scanner, period ~ 1 minute
- *Complete dataset in 1 minute = with one laser pulse/spot*

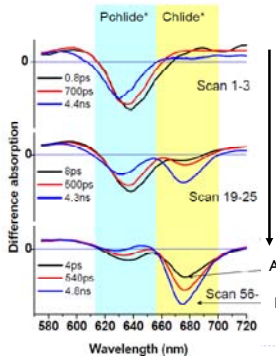


POR transient traces as function of scan nr



After additional 15 mins CW lamp illumination

POR product depends on illumination history



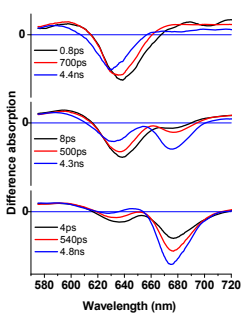
Number of photons / cycles through excited state

Reaction rate and product yield ***increase***

Accumulated Chlide

I675*

How to model this?



Inactive fraction(n)

PChlide*I → PChlide*II → PChlide*III

Active fraction(n)

PChlide*I → PChlide*II → PChlide*III

I675*

Product(n)

Chlide*$_{accumulated}$

Not, Single, and Double excited enzymes

Inactive → Active → (I_{675}) Chlide

e e x QY

Where

- e is the excitation rate per pulse and
- QY is the quantum yield of Chlide formation

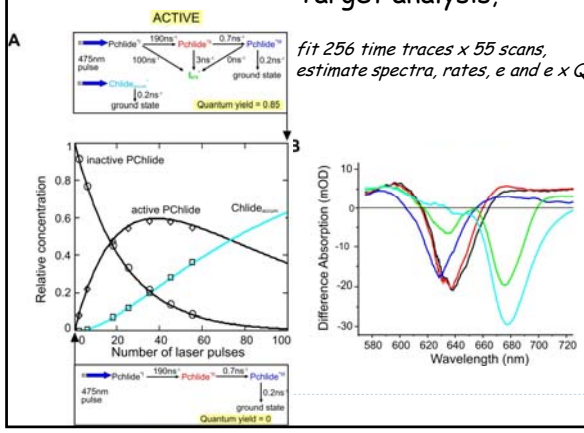
Solve the coupled differential equations and fit to the individual scans

→ e = 0.045 per pulse & QY = 0.3±0.1

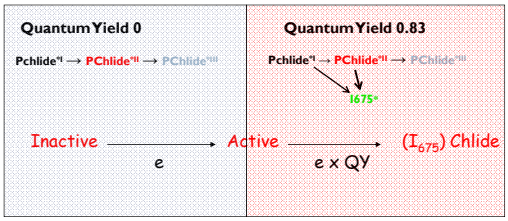
(estimated excitation density = 0.03)

Target analysis,

fit 256 time traces x 55 scans, estimate spectra, rates, e and e x QY



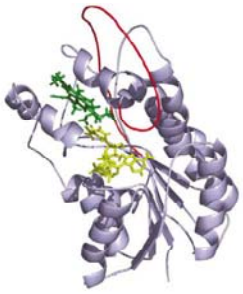
Activation of POR



Active enzymes = enzymes that have been excited once!

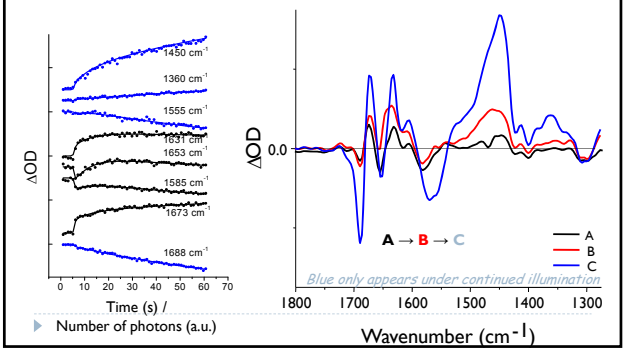
First photon turns the enzyme 'on', whereas the second photon induces catalysis

Is activation conformational change?

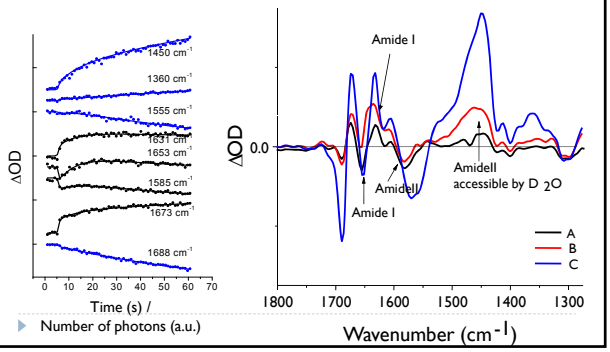


Record absorption difference spectra in the midIR under similar illumination conditions, using 5-ns laser pulses at a 20 Hz repetition rate to excite and measure, while flashing, light-induced minus dark-difference spectra every second, with rapid scan FTIR

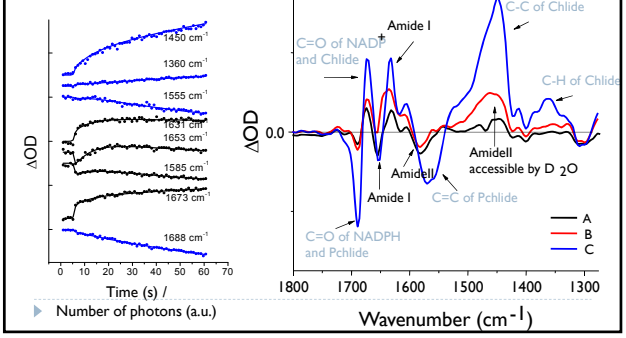
Spectral changes in midIR as a function of illumination



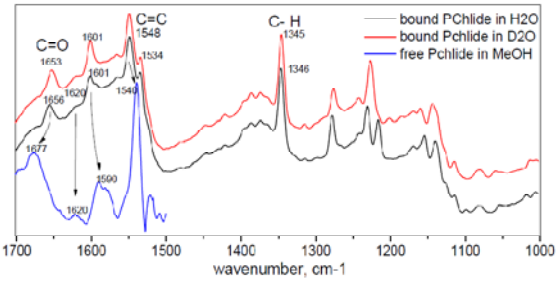
Spectral changes in midIR as a function of illumination



Spectral changes in midIR as a function of illumination

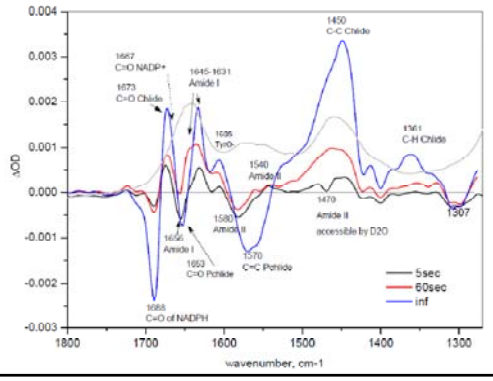


PChlide keto: FLN spectroscopy



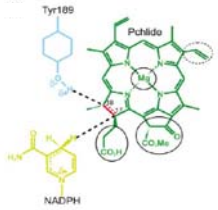
Fluorescence line narrowing experiments at 4K

Refinement

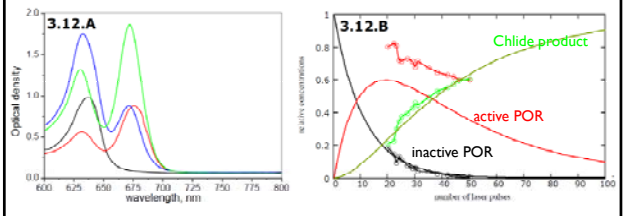


Summary so far:

- First photon induces a conformational change
- Only after absorption of a second photon can ultrafast coupled proton and hydride transfers proceed.
- Conformational change is highly efficient (1 photon) and directive (results in high QY)
- Nature of conformational change: What is the lifetime? Does it survive turnover?



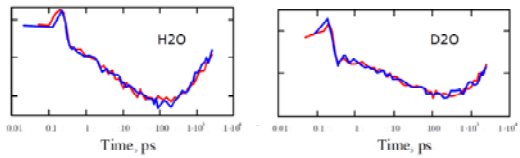
Mixing single turnover enzyme with fresh PChlide



Dark POR → Illuminated → PChlide added → after experiment

Activity survives turnover

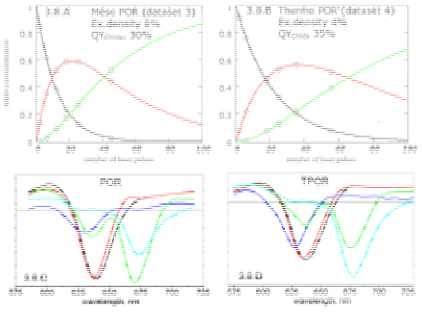
Putting an activated enzyme in the dark



Blue: POR illuminated for 30 s
Red: after being kept in the dark for 24 hrs

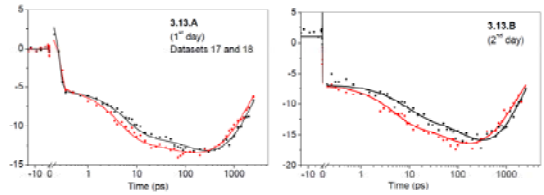
Lifetime active conformation > 24 hrs

Comparing mesophilic and thermophilic POR



Estimated excitation density consistently 2x lower in thermophilic POR
→ Quantum yield of activation is lower

Nature of I675 state



Measured in D2O the appearance of I675 is slower than in H2O: kinetic isotope effect

Pchlide*I → I675* in 3.5 ps with KIE = 2 ,
Pchlide*II →I675*, in 180 ps, with KIE = 1.5☐

Ultrafast 3 ps and 180 ps proton transfer from Tyr189 to PChlide

Conclusions

Protein motions control activity:

POR can be switched by a first photon in an active conformation, see midIR & thermophilic POR

In the activated state proton transfer occurs on picosecond time scale, hydride on microsecond time scale.

Thanks to

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Neil Hunter
Derren Heyes

University of Bristol
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