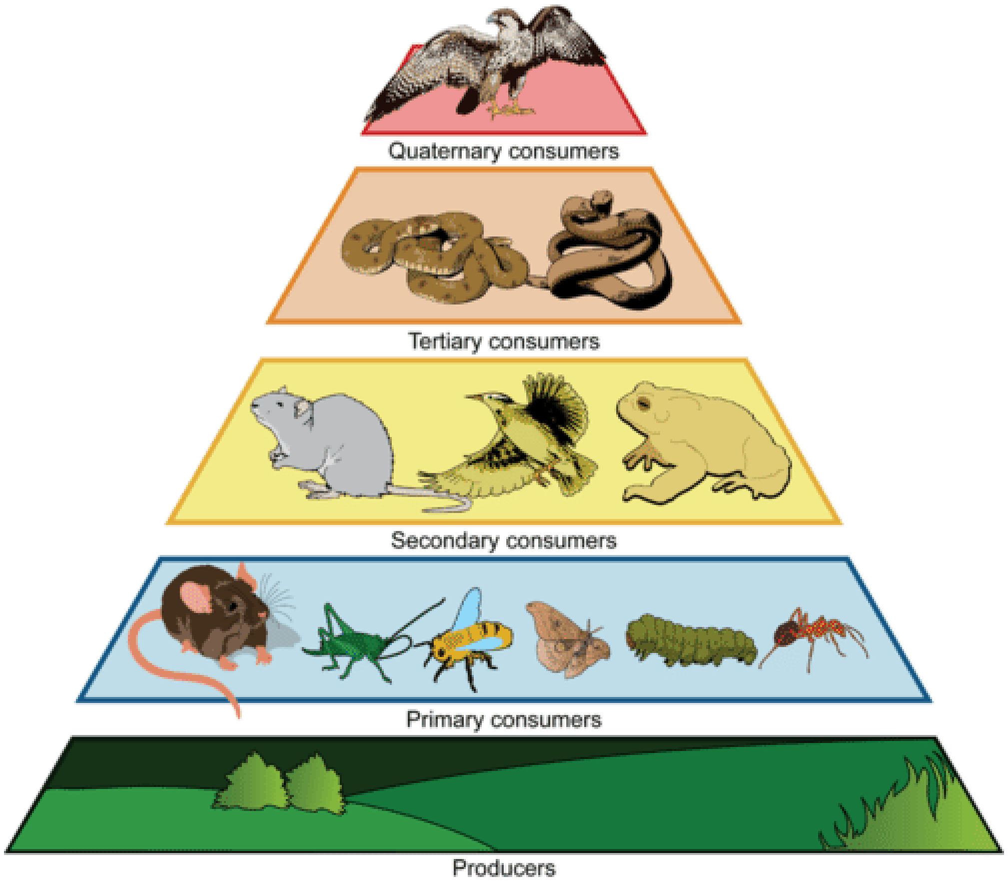
LAMELIS 2017

Lasers in Medicine and Life Sciences

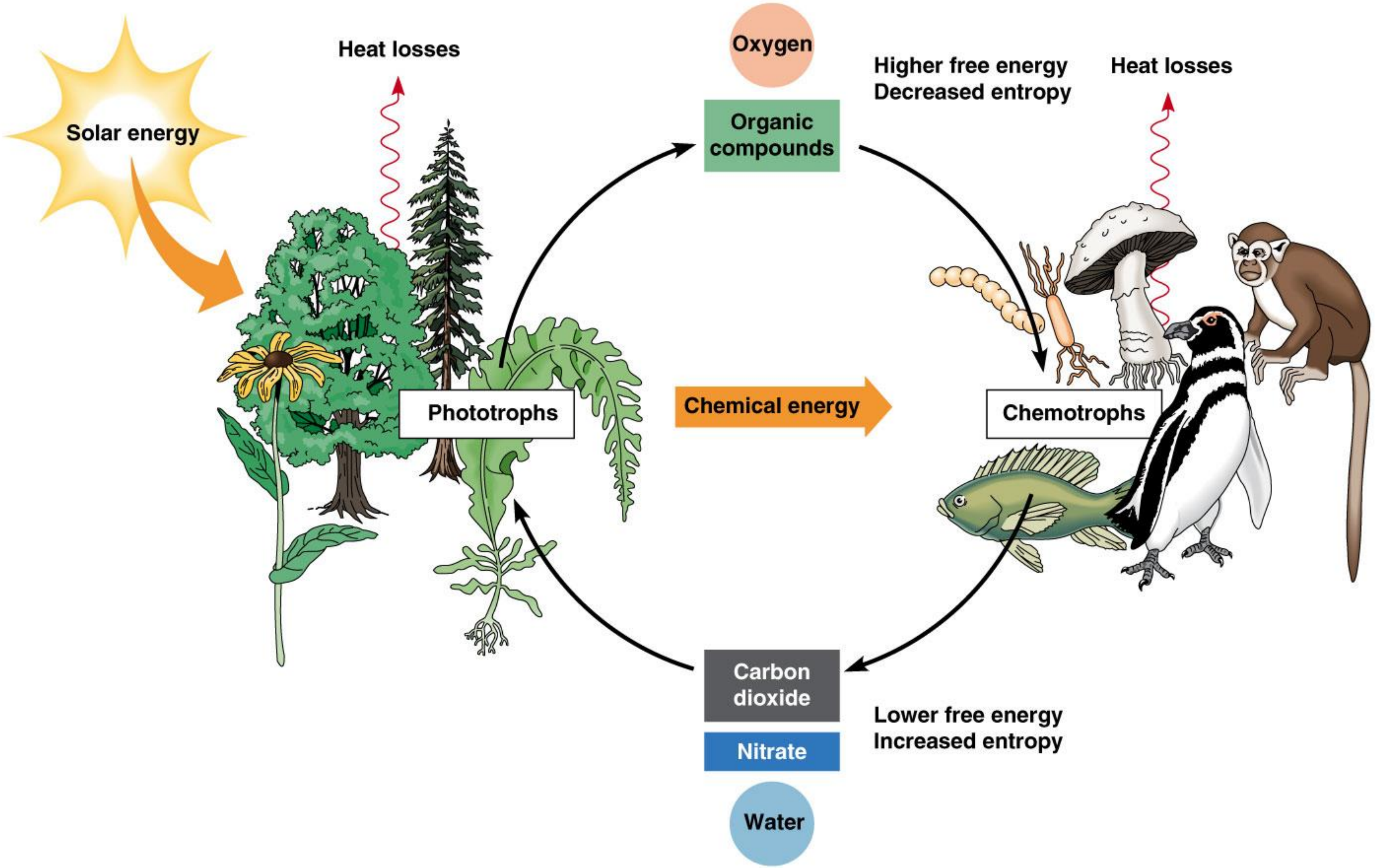
Ultrafast 2D Spectroscopy of Photosynthetic Light-Harvesting Complexes

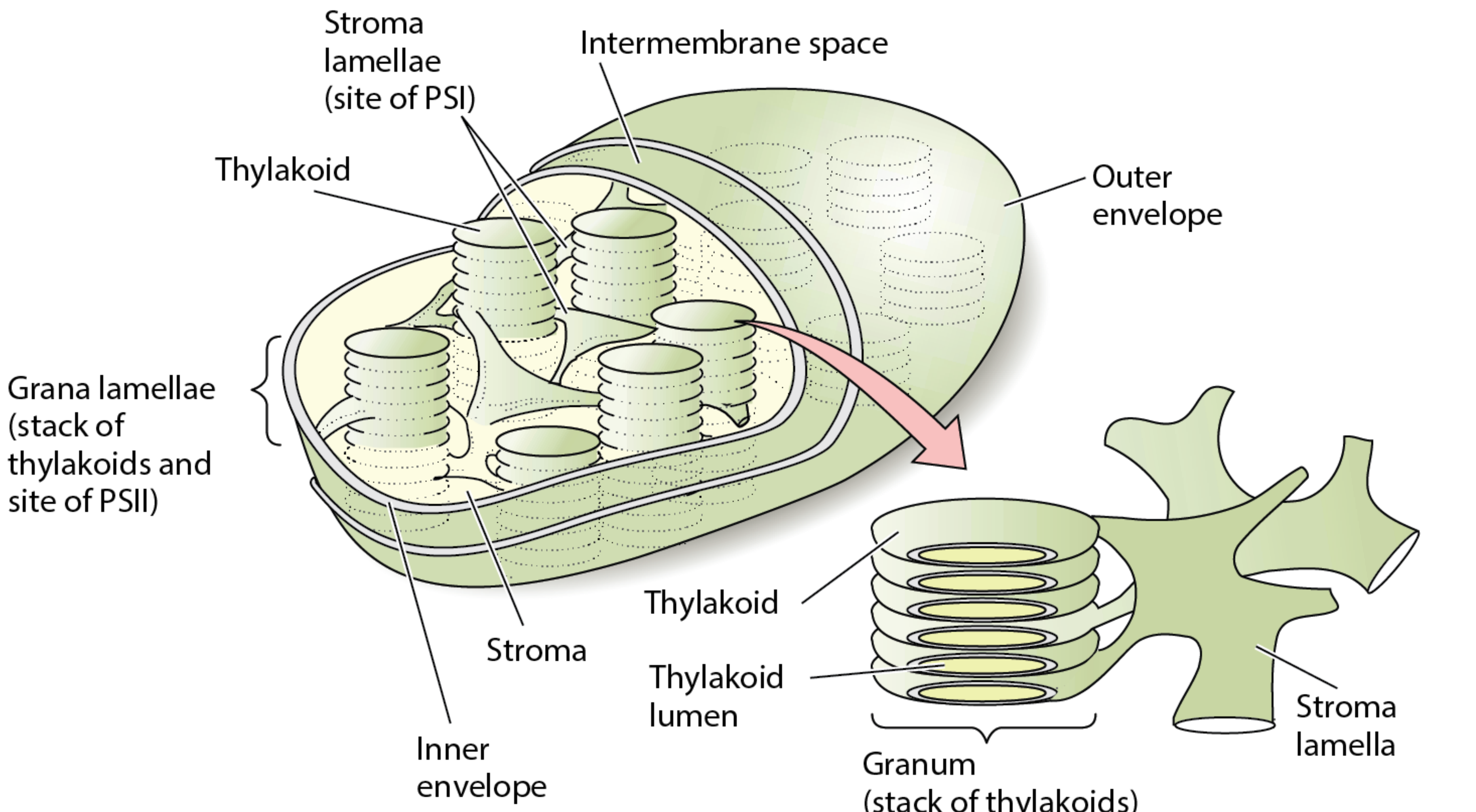
PETAR LAMBREV

LASERS IN LIFE SCIENCE



Preamble


Solar energy
Heat losses
Oxygen
Organic compounds
Higher free energy
Decreased entropy
Heat losses
Phototrophs
Chemical energy
Chemotrophs
Carbon dioxide
Nitrate
Water
Lower free energy
Increased entropy



Teiz & Zeiger, Plant Physiology, Sinauer Assoc., Inc.

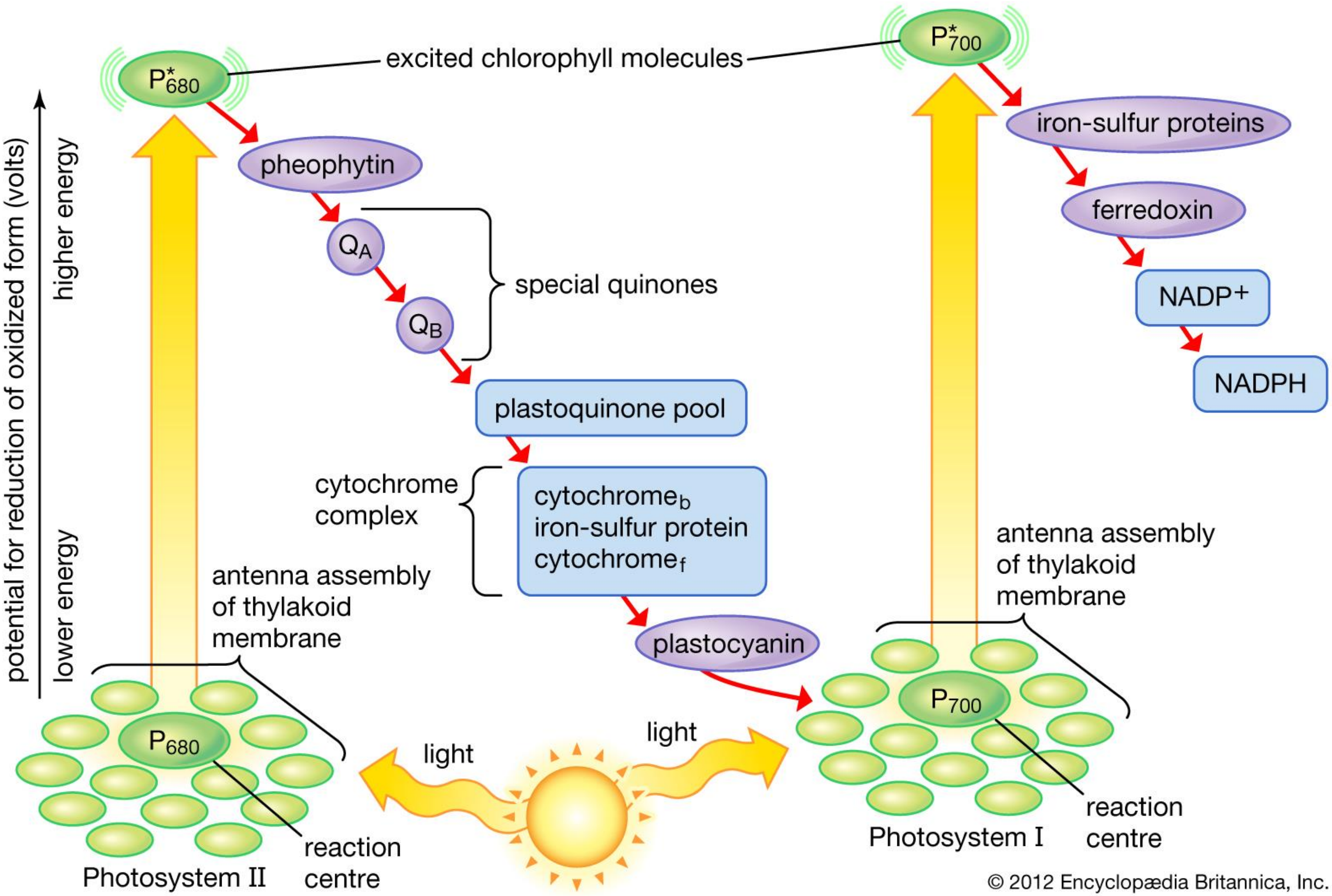
Preamble

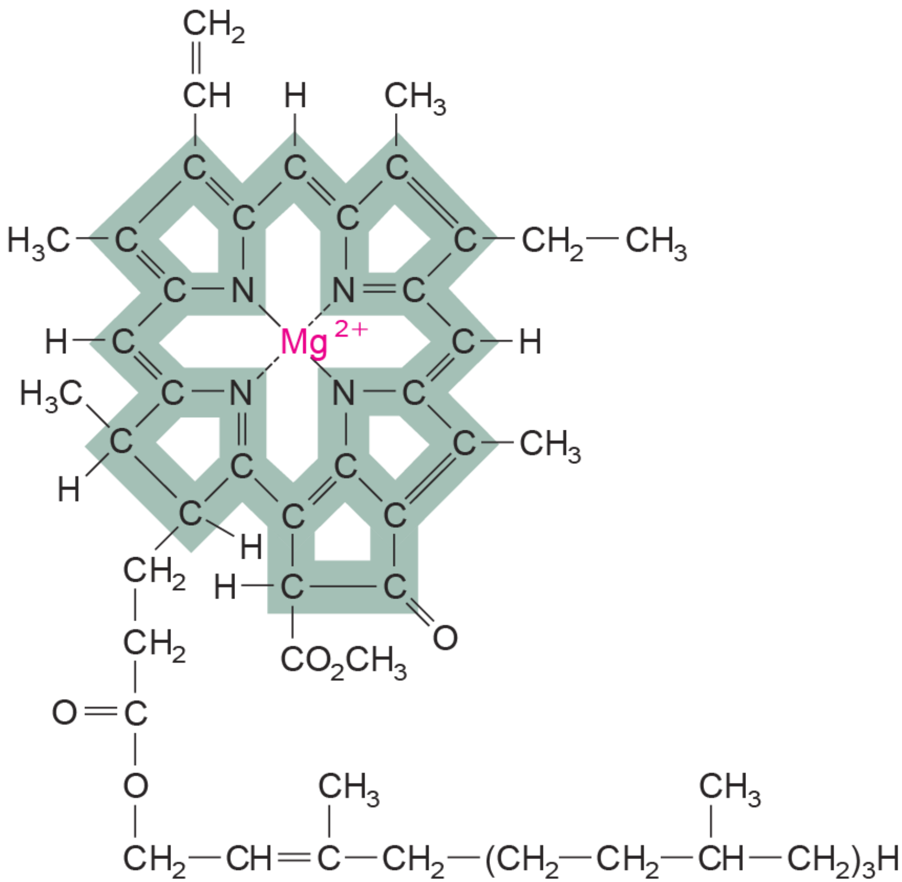
"It seems likely that in various biological systems energy consuming chemical reactions are coupled to delocalized states of energy"
Avery, Bay & Szent-Györgyi, 1961

Outline

1. The photosynthetic apparatus
2. The plant light-harvesting complex II
3. Principle of 2D electronic spectroscopy
4. 2DES spectroscopy of light-harvesting complex II

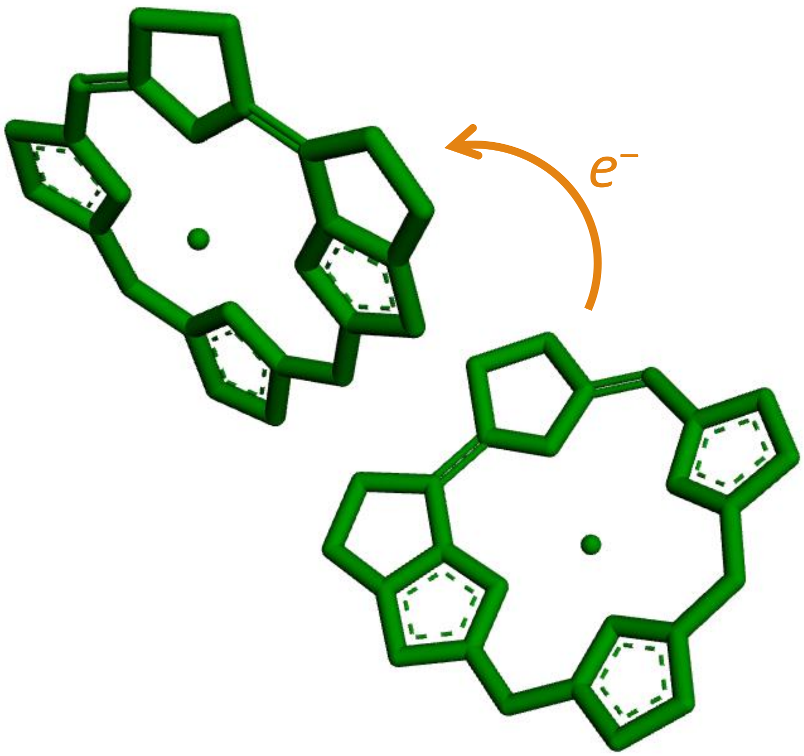
Electron Flow in Photosynthesis





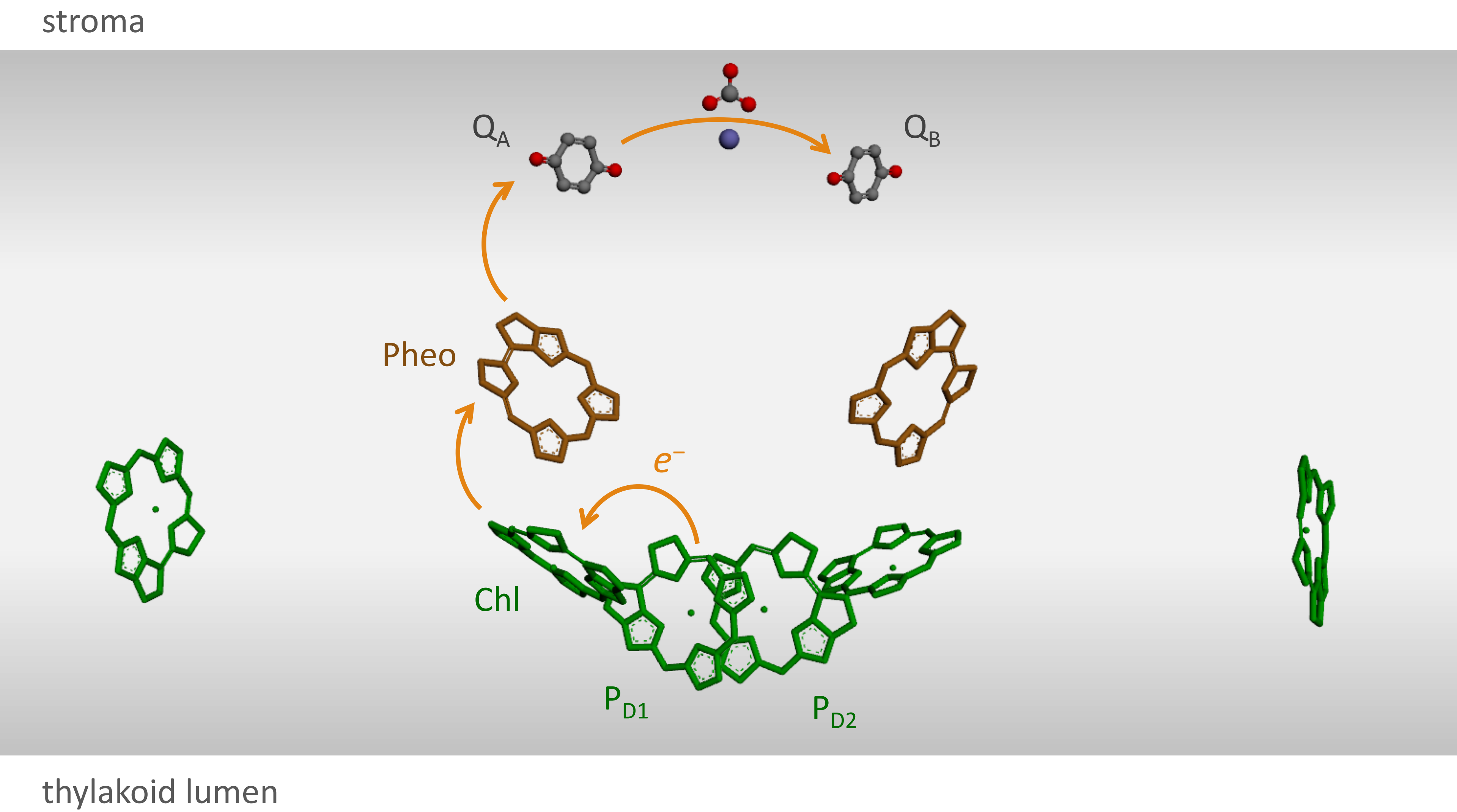
Chlorophyll *a*

Reaction Centres



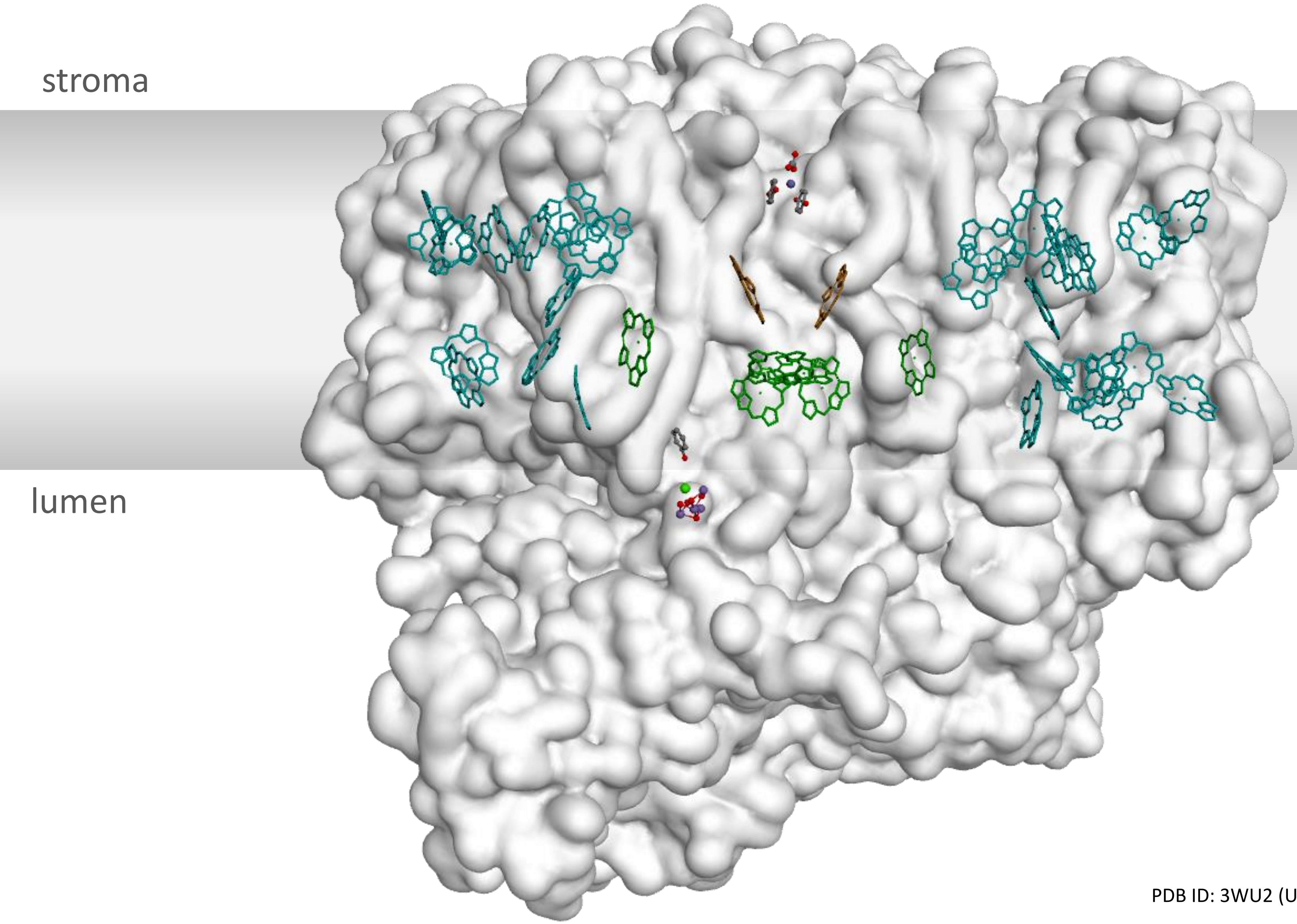
Charge separation

Reaction Centres



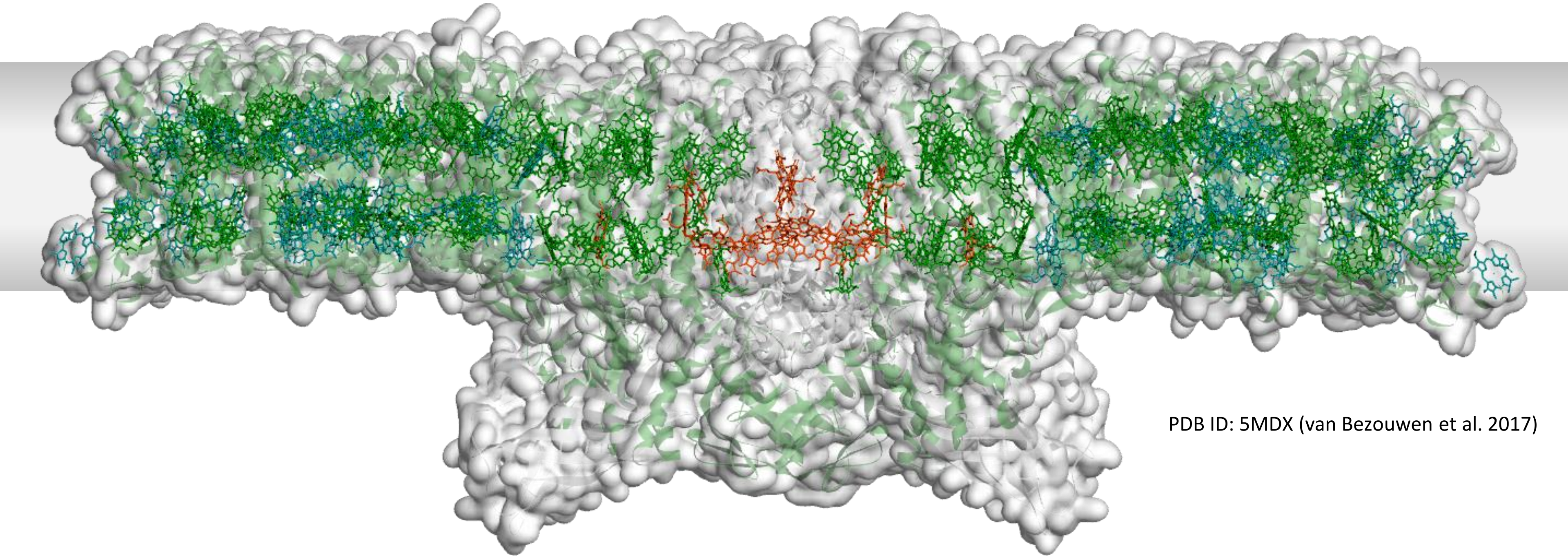
Photosystem II reaction centre

Photosystem II Core



PDB ID: 3WU2 (Umena et al. 2011)

PSII-LHCII Supercomplex



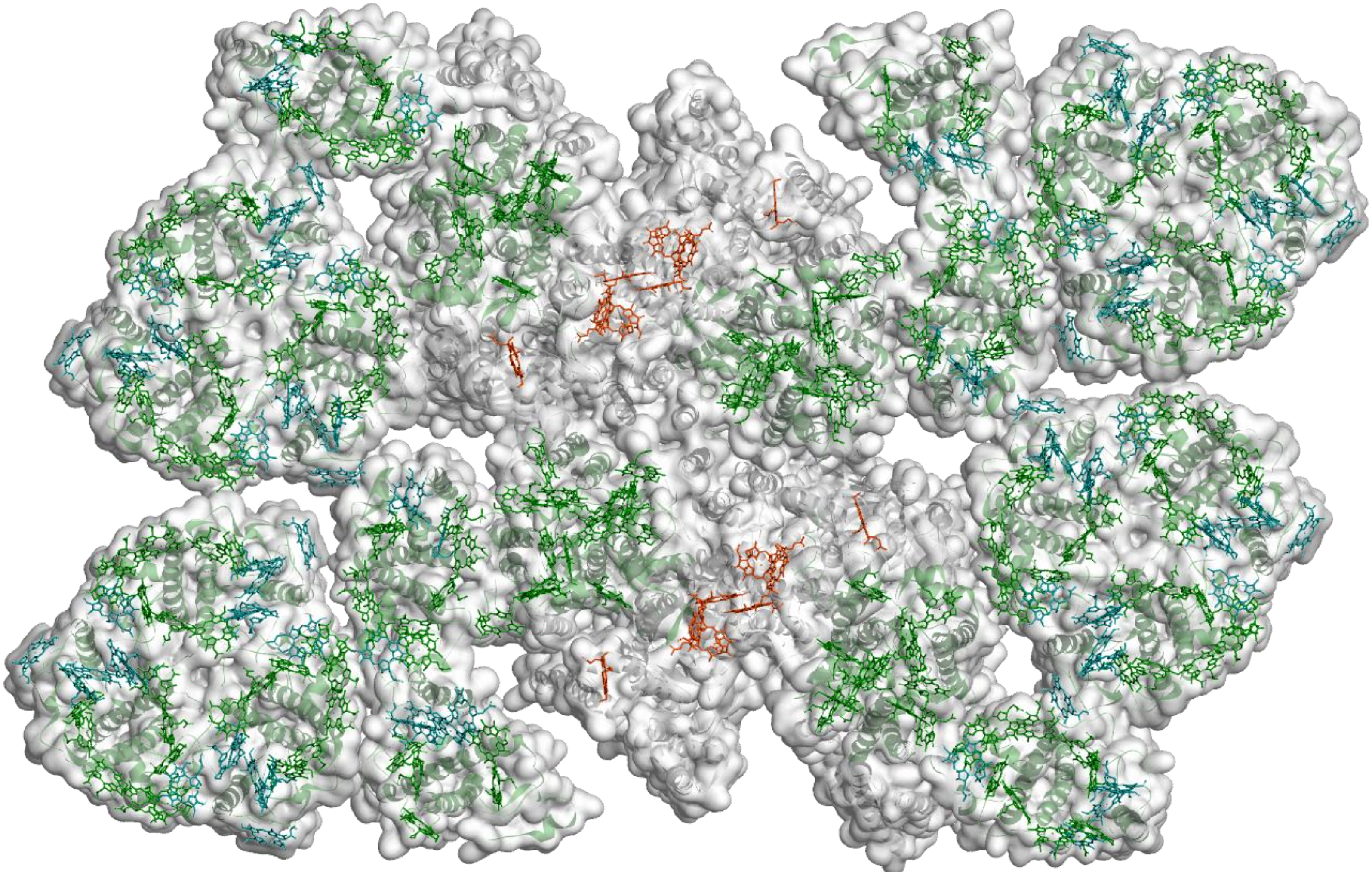
PDB ID: 5MDX (van Bezouwen et al. 2017)

light-harvesting complexes (LHCII)

dimeric core complex

light-harvesting complexes (LHCII)

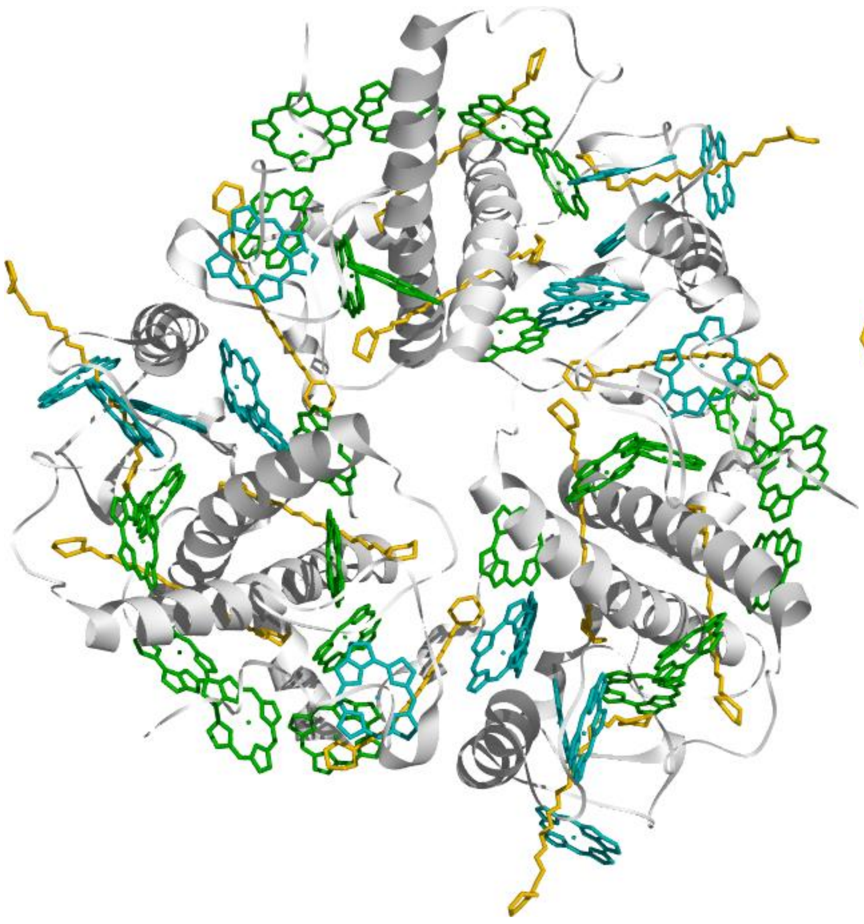
PSII-LHCII Supercomplex



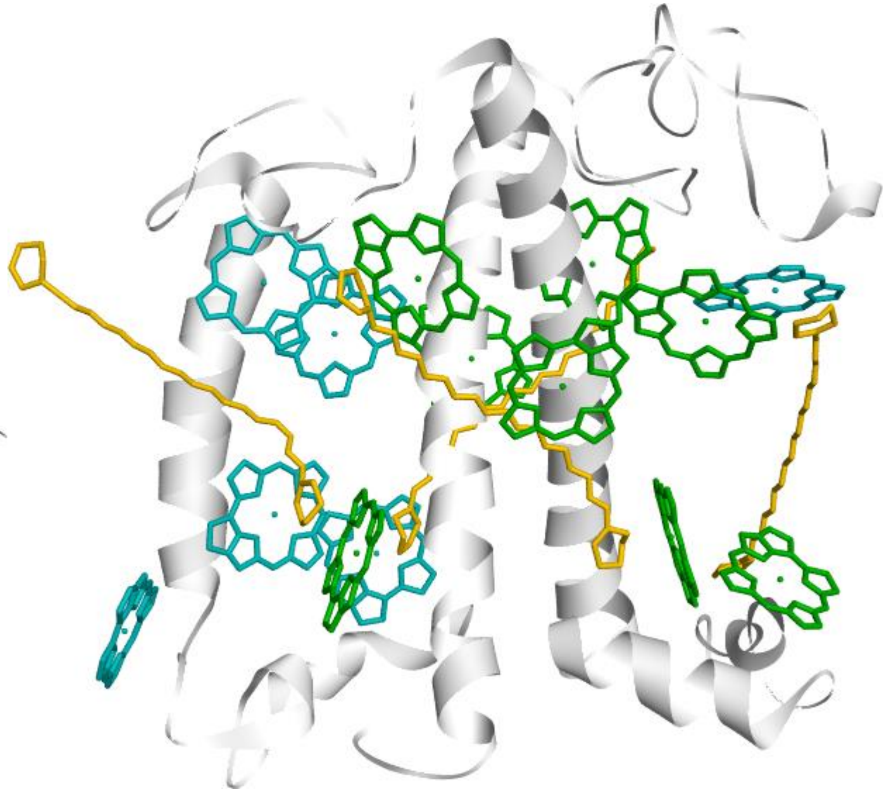
light-harvesting complexes (LHCII) | dimeric core complex | light-harvesting complexes (LHCII)

Light-Harvesting Complex II

PDB ID: 2BHW (Standfuss et al. 2005)

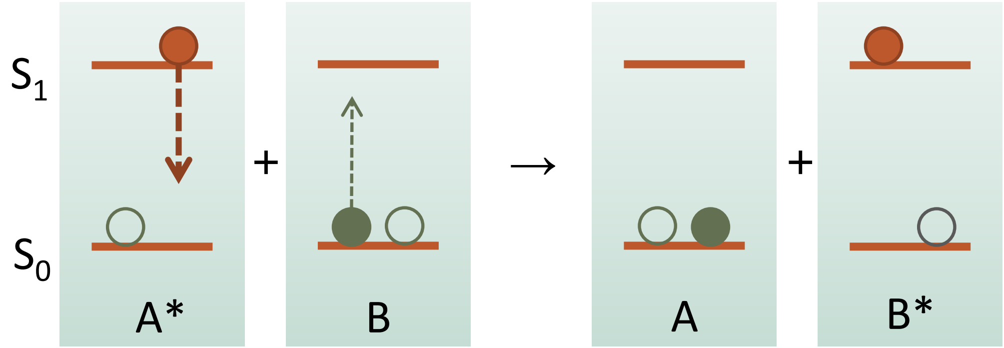


trimer top view



monomer side view

Excitation Energy Transfer



Förster energy transfer

$$k_{AB} = \frac{9\kappa^2 c^4}{8\pi\tau_{A^*} n^4 R^6} \int F_A(\omega)\sigma_B(\omega)\frac{\mathrm{d}\omega}{\omega^4}$$

Decreases with the sixth power of the distance

Is proportional to the overlap of the donor fluorescence spectrum and acceptor absorption spectrum

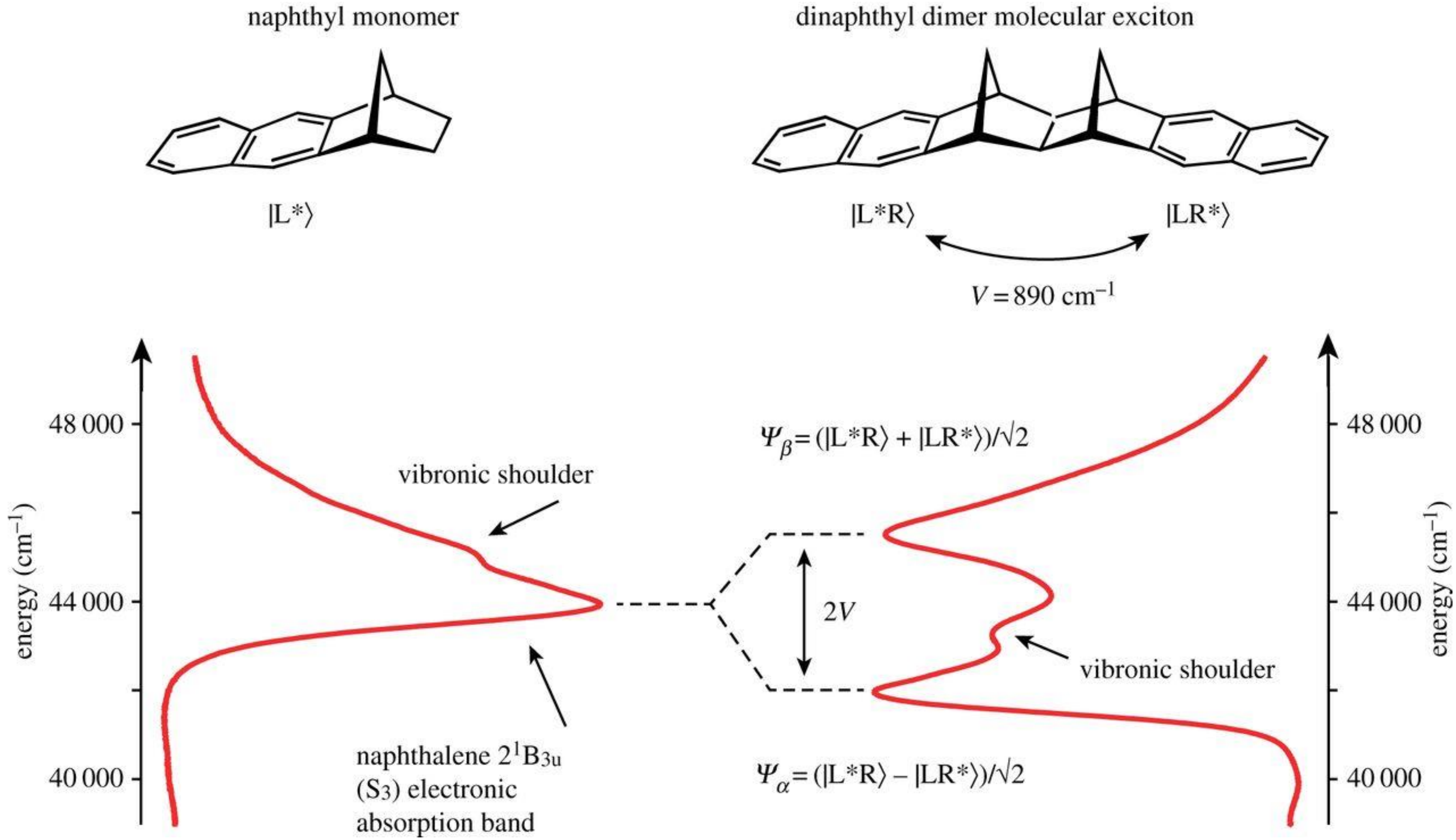
Depends on the mutual orientation of the donor and acceptor

$$\kappa = (\hat{\boldsymbol{\mu}}_A \cdot \hat{\boldsymbol{\mu}}_B) - 3(\hat{\mathbf{R}} \cdot \hat{\boldsymbol{\mu}}_A)(\mathbf{R} \cdot \hat{\boldsymbol{\mu}}_B)$$

Master equation for a system of many pigments:

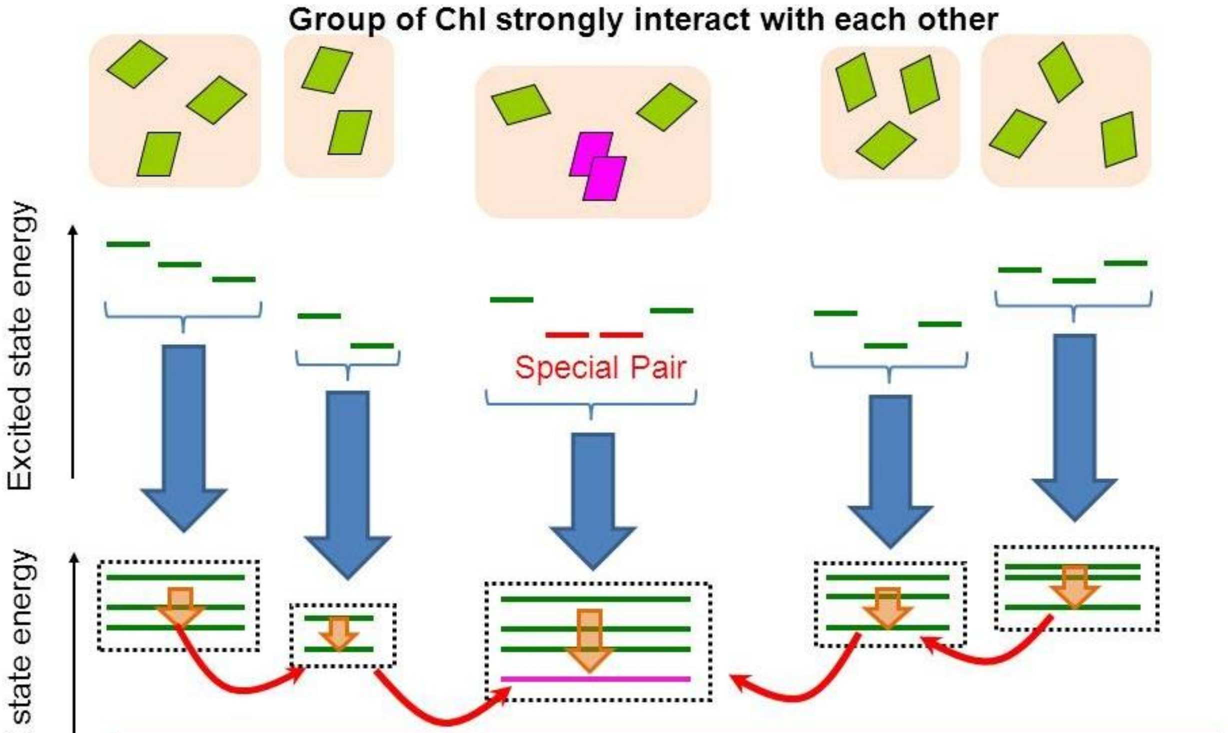
$$\dot{p}(t) = -\sum_{j \neq i}^{N} k_{ij} p_i + \sum_{j \neq i}^{N} k_{ij} p_j$$

Molecular Excitons



Fassioli *et al.* (2014) *JRS Interface*

Coherent and Incoherent energy transfer

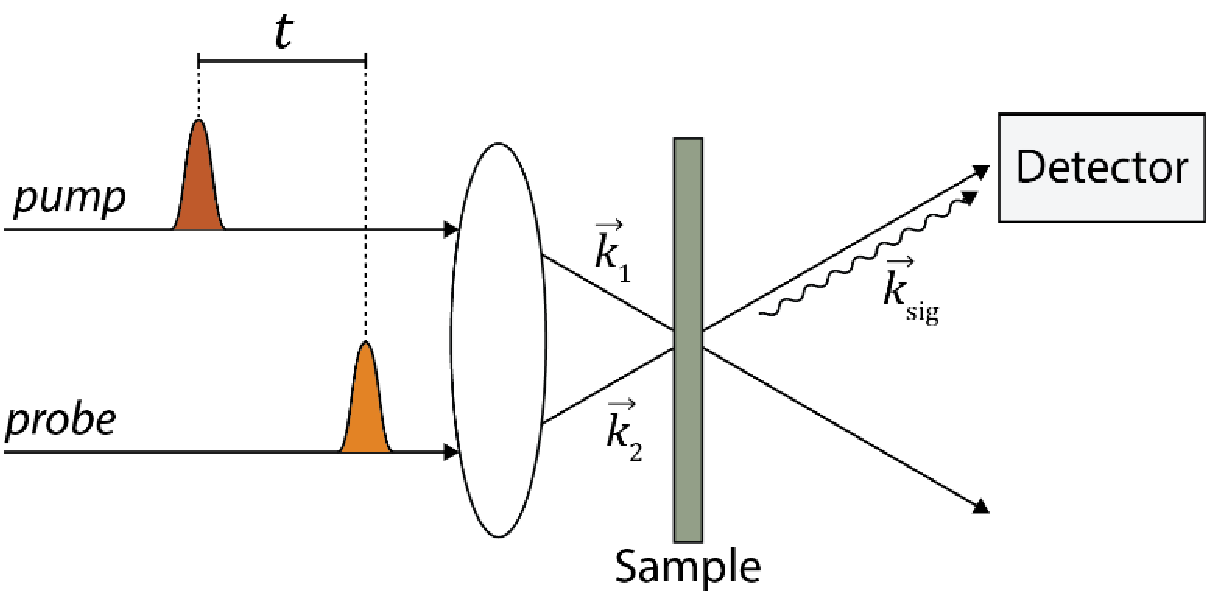


- ☐ **Delocalization of excitation over strongly interacting Chl's.**
- ☐ **Energy transfer from a molecule to another molecule ⇒ Energy transfer by the relaxation among the excitonic states.**

Measuring Energy Transfer

MEASURING ENERGY TRANSFER

Transient Absorption Spectroscopy



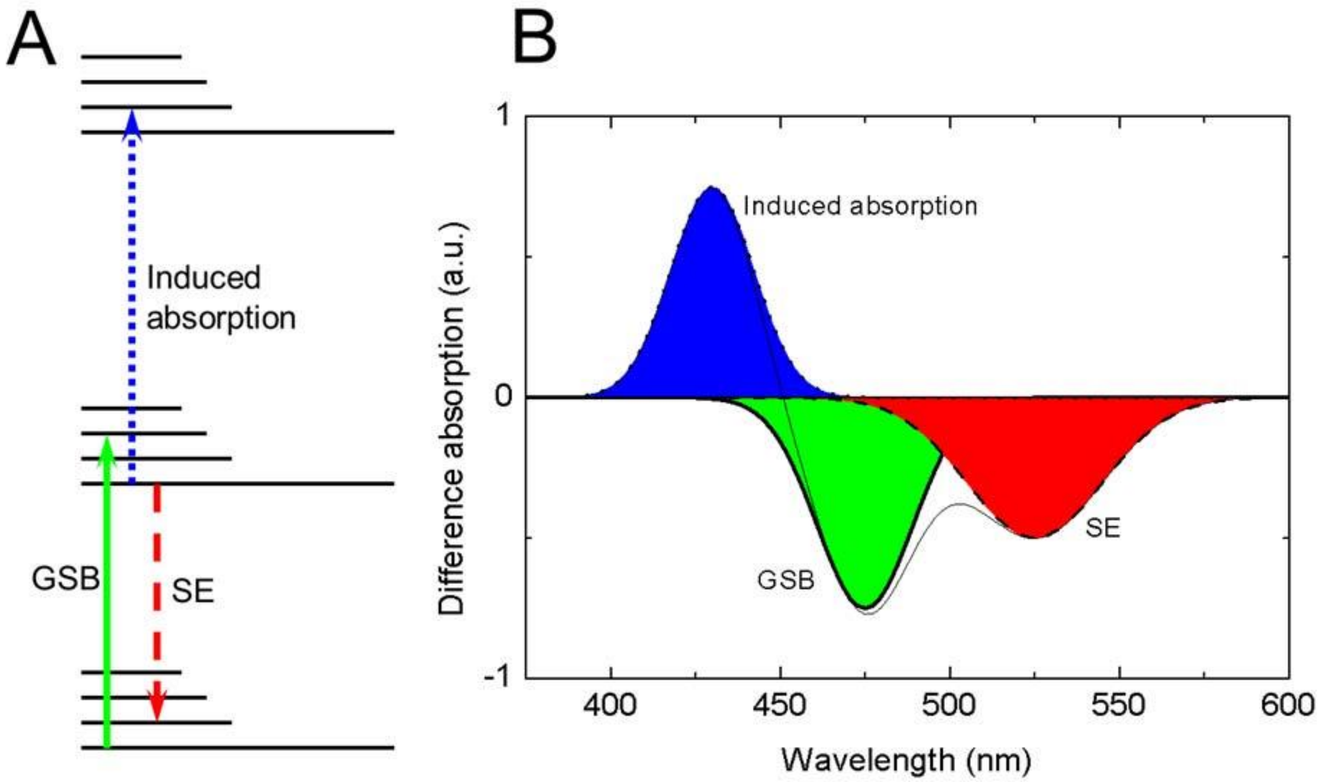
- 'Pump' pulse excites the system
- A subsequent 'probe' pulse measures the changes induced by the pump

$$\Delta A(\lambda, t) = A_{\text{with pump}} - A_{\text{without pump}}$$

- 3rd-order nonlinear response
- Sample interacts twice with the pump and once with the probe

$$\vec{k}_{sig} = \vec{k}_1 - \vec{k}_1 + \vec{k}_2 = \vec{k}_2$$

Transient absorption spectra



GSB - ground-state bleaching

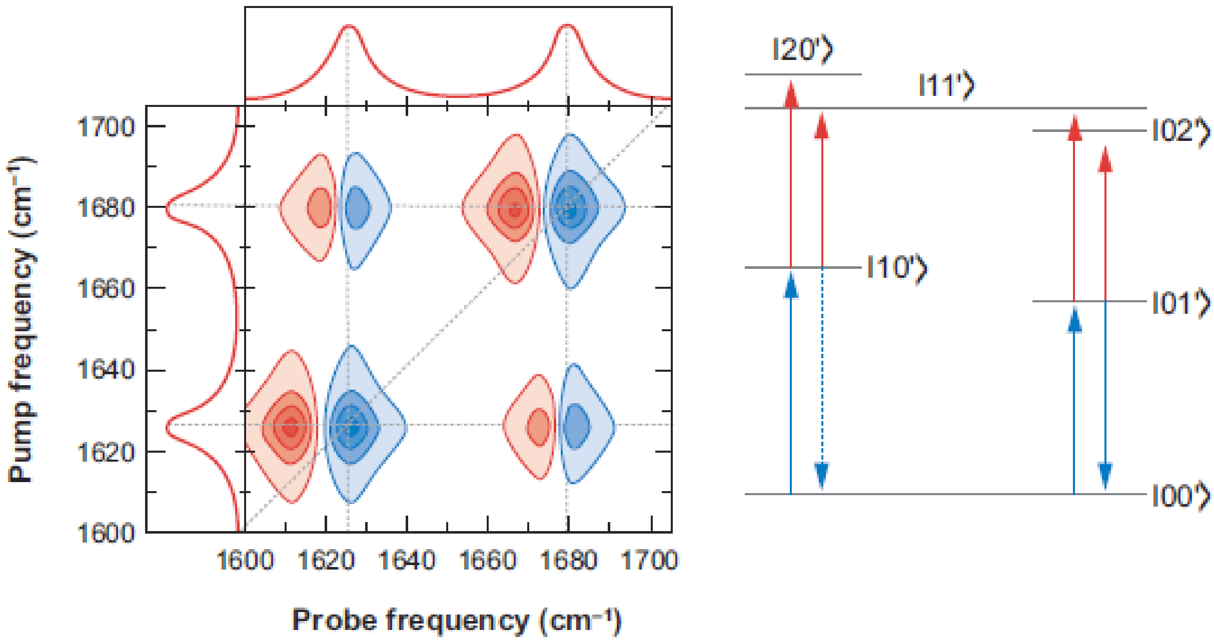
SE - stimulated emission

ESA, IA - excited-state absorption, induced absorption

M Vengris. Introduction to time-resolved spectroscopy

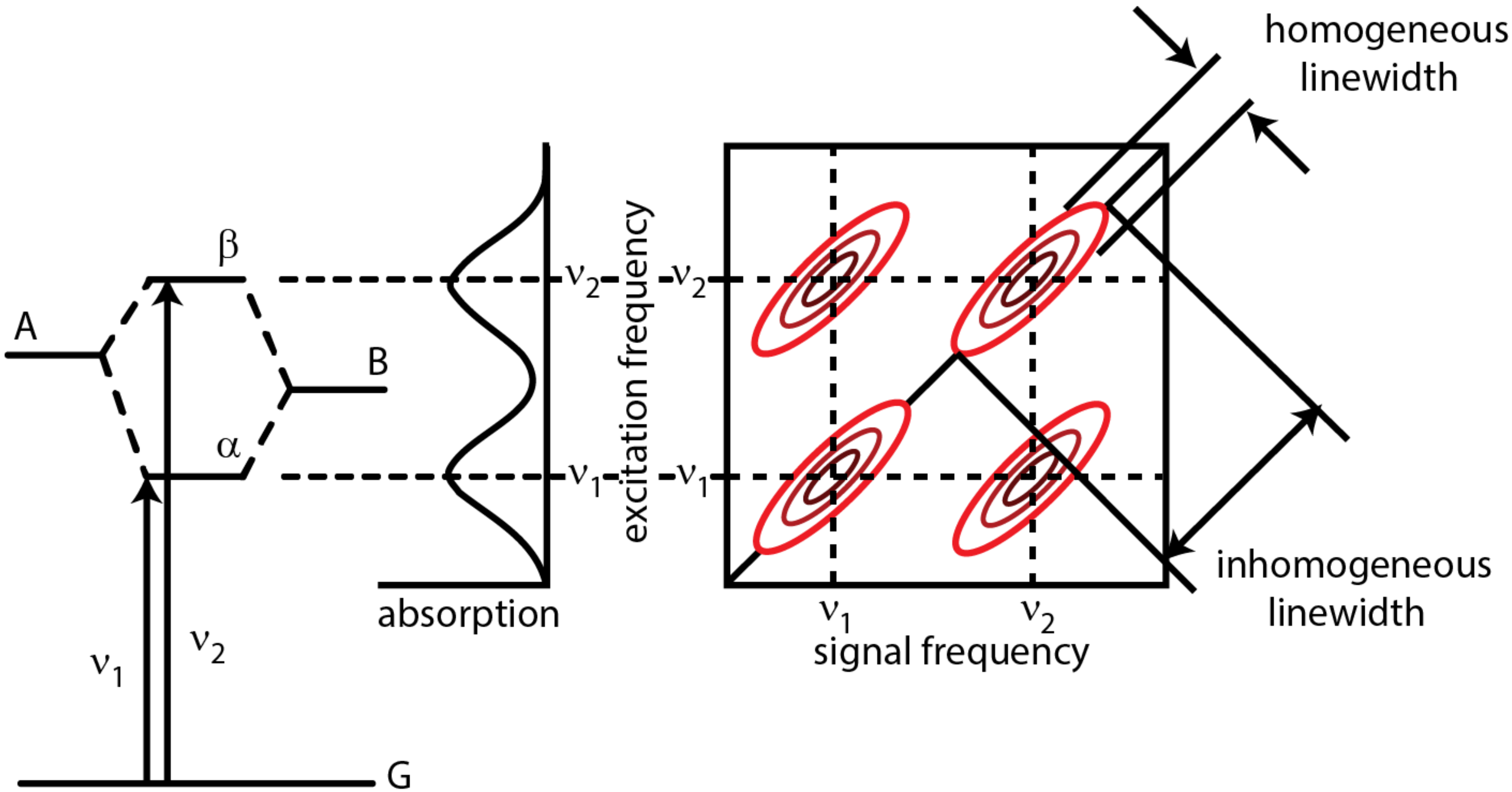
2D Coherent Optical Spectroscopy

Hamm *et al.* (2008) *Annu Rev Phys Chem*



- 2D optical spectroscopy is the optical analog of 2D NMR
- Two frequency axes (*pump* and *probe*)
- *Negative* signals (GS bleaching, emission) and *positive* signals (ES absorption)
- *Cross* peaks ($\omega_{\mathrm{pump}} \neq \omega_{\mathrm{probe}}$) reflect coupling between oscillators

2D Electronic Spectroscopy



Fassioli *et al.* (2014) *JRS Interface*

Quantum Coherence

Energy eigenstates:

- Solutions to the time-independent Schrödinger equation

$$\hat{H}\psi(\mathbf{r}) = E\psi(\mathbf{r})$$

- States of sharply defined energy:

$$\Delta E = \sqrt{\langle E^2 \rangle - \langle E \rangle^2} = 0$$

- Stationary states:

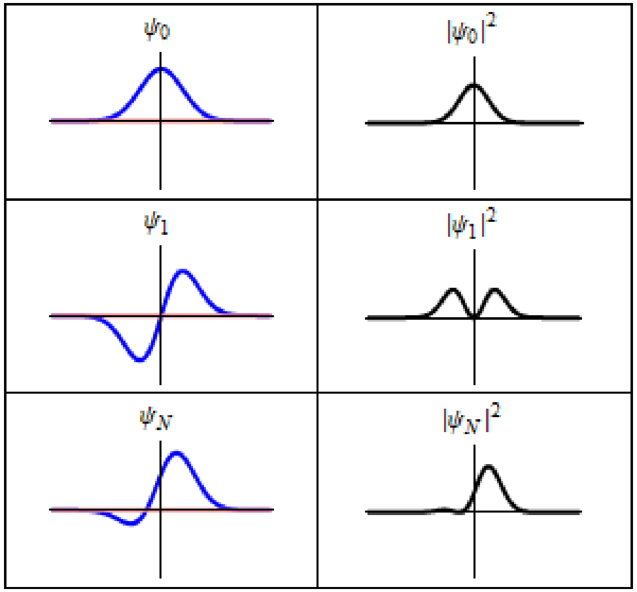
$$\Psi^*\Psi = \psi^* e^{+iEt/\hbar}\psi e^{-iEt/\hbar} = \psi^*\psi$$

Quantum superposition states:

$$\Psi_a = c_1\Psi_1 + c_2\Psi_2 =$$

$$= c_1\psi_1 e^{-iE_1 t/\hbar} + c_2\psi_2 e^{-iE_2 t/\hbar}$$

- Non-stationary
- Observable values oscillate in time
- Interference



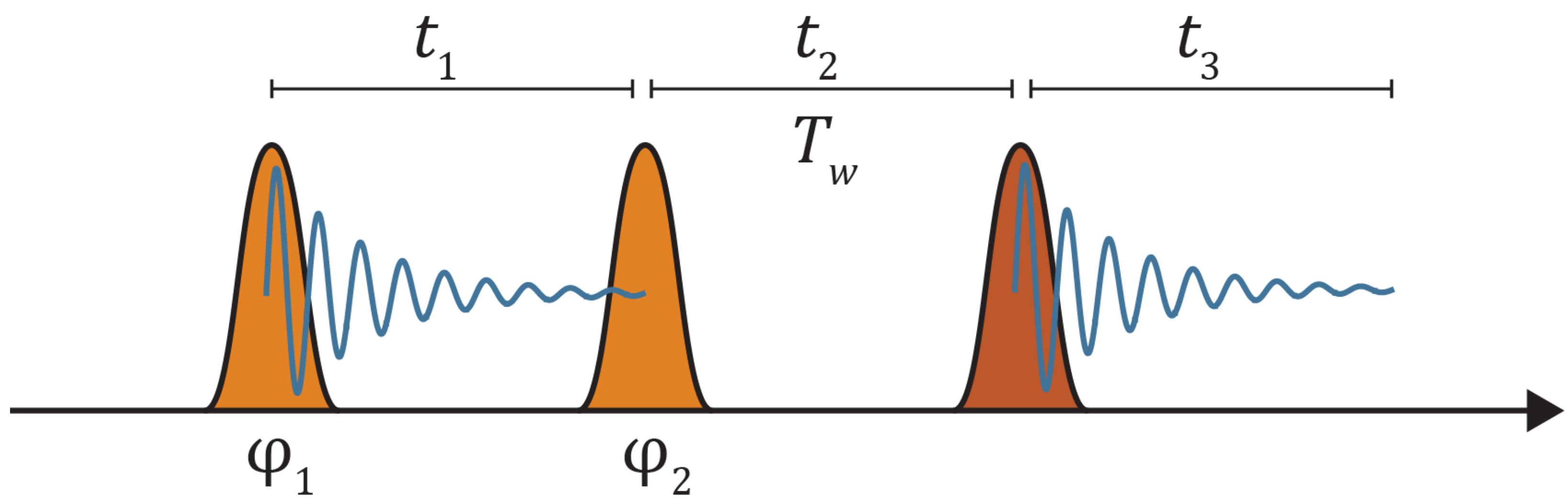
Wave functions of two eigenstates and one superposition state of a harmonic oscillator

Fourier Transforms

$$\hat{f}(\xi) = \int_{-\infty}^{\infty} f(x) e^{-2\pi i x \xi} dx$$

The frequency-domain function $\hat{f}(\xi)$ is the Fourier transform of the time-domain function $f(x)$

Pulse Sequence



The sample interacts with three pulses:

1. The 1st pulse creates coherence two states

   t_1 (*coherence time*): Oscillations with frequency ω_1

2. The 2nd pulse may create population, if in phase

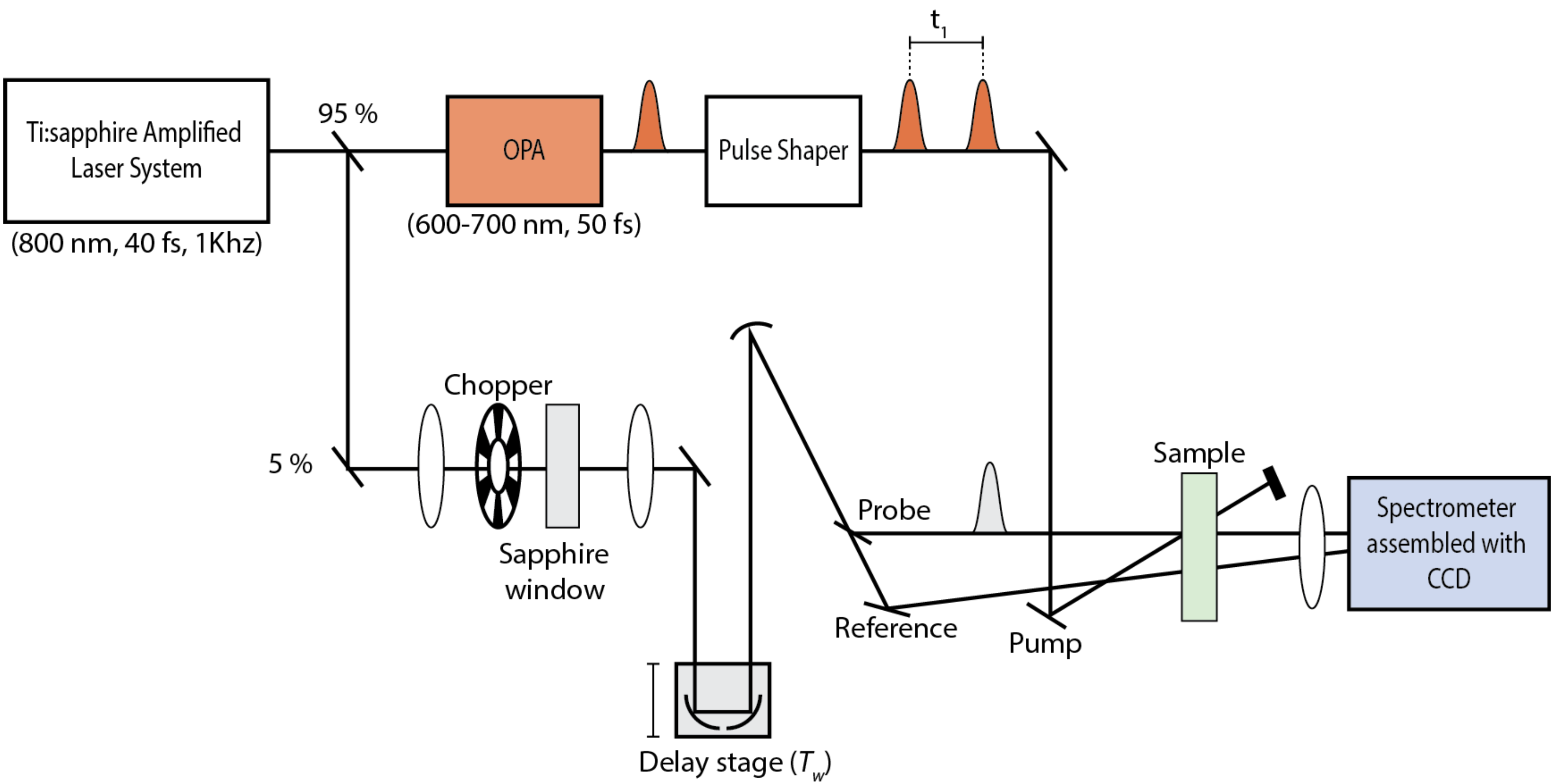
   t_2 (*population waiting time*, T_w): energy transfer, etc.

3. The 3rd pulse probes the system

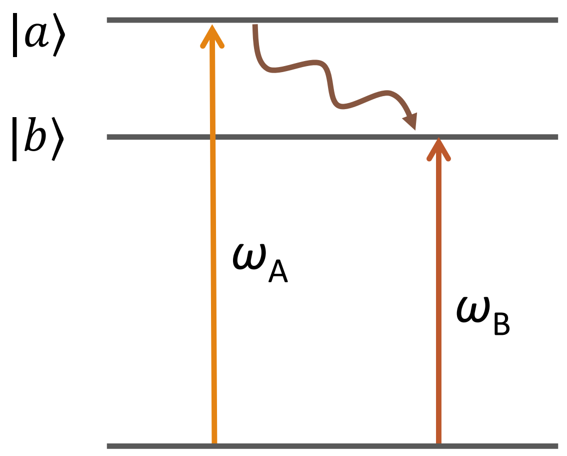
   t_3 (*detection time*): emitted photon echo signal oscillating with ω_3

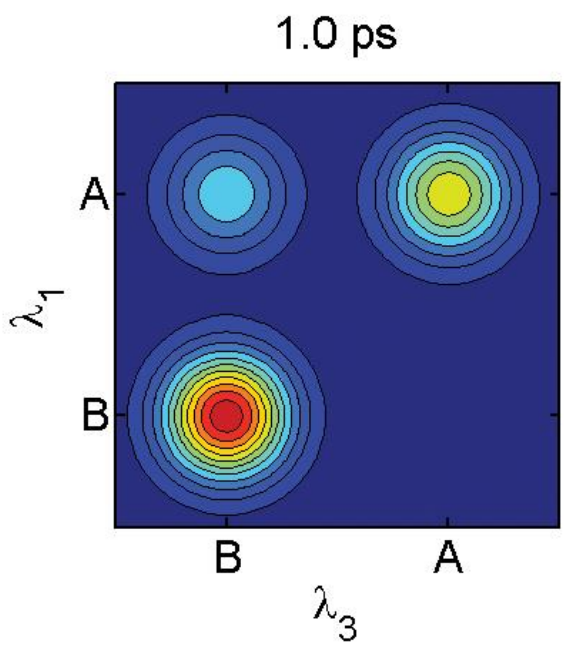
Stimulated echo signal is recorded as $S(\omega_1, t_2, \omega_3)$

Partially collinear "pump-probe" geometry 2DES



Transient 2D Electronic Spectroscopy





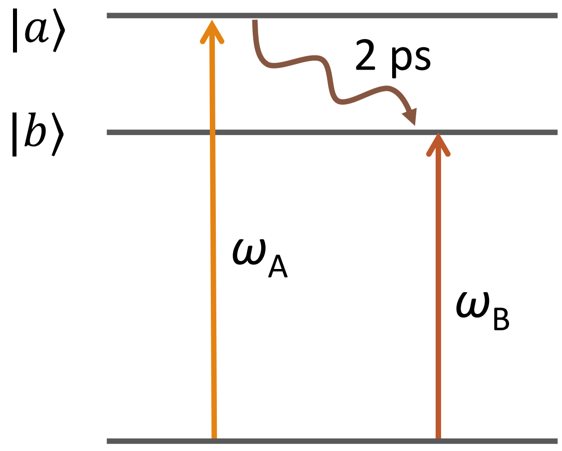
Diagonal peaks at the absorption wavelength of each state

Cross-peak at (λ_1 = A, λ_3 = B) reflects energy transfer from $|a\rangle$ to $|b\rangle$

λ_1 = A – donor's Abs wavelength

λ_3 = B – acceptor's Abs wavelength

Transient 2D Electronic Spectroscopy



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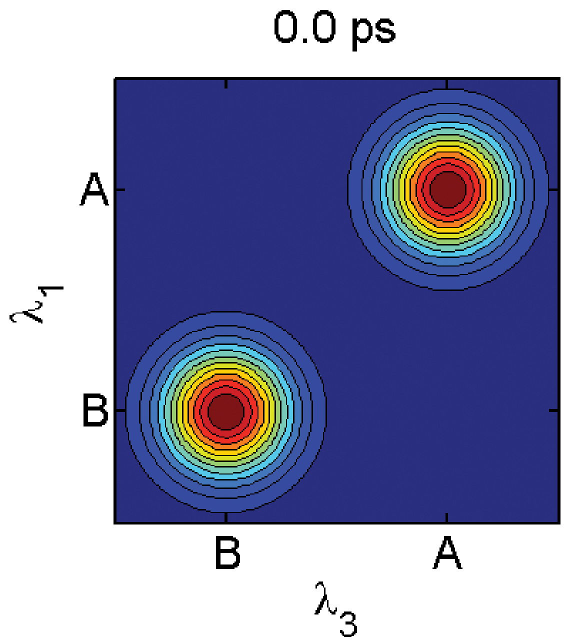
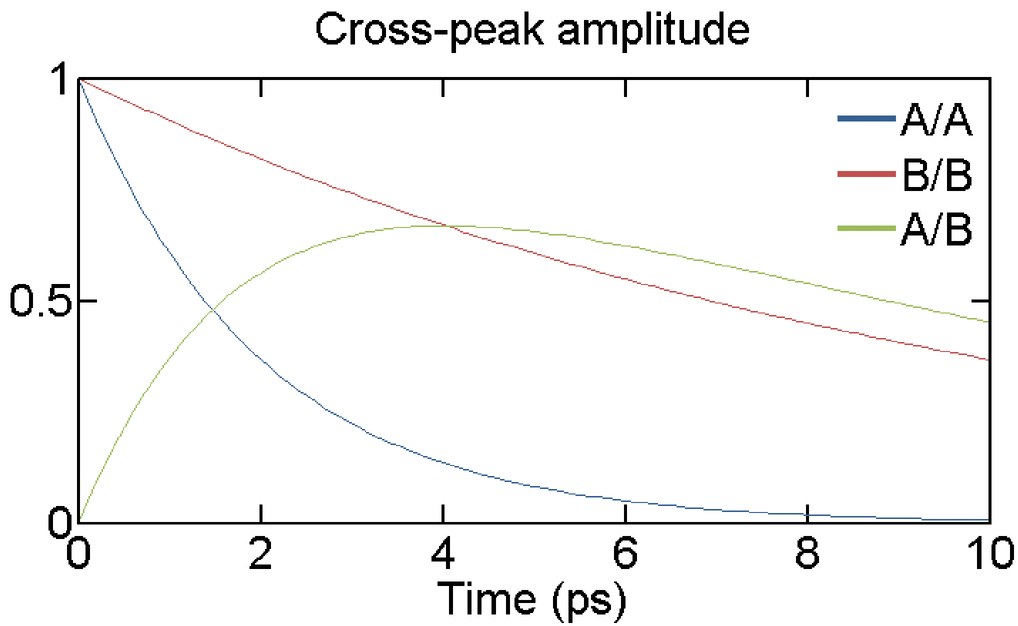
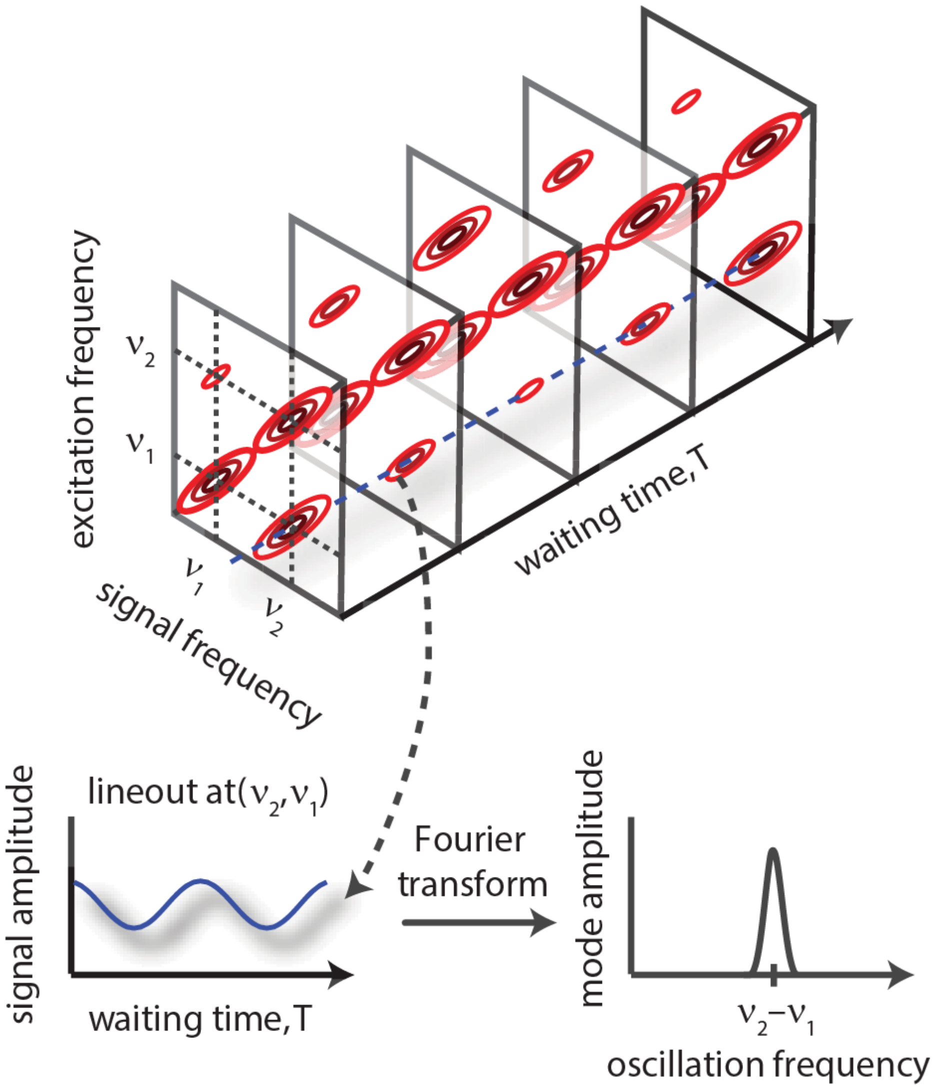


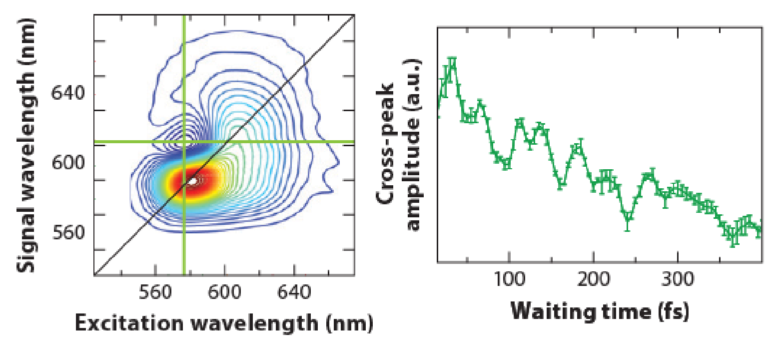


Coherent Dynamics

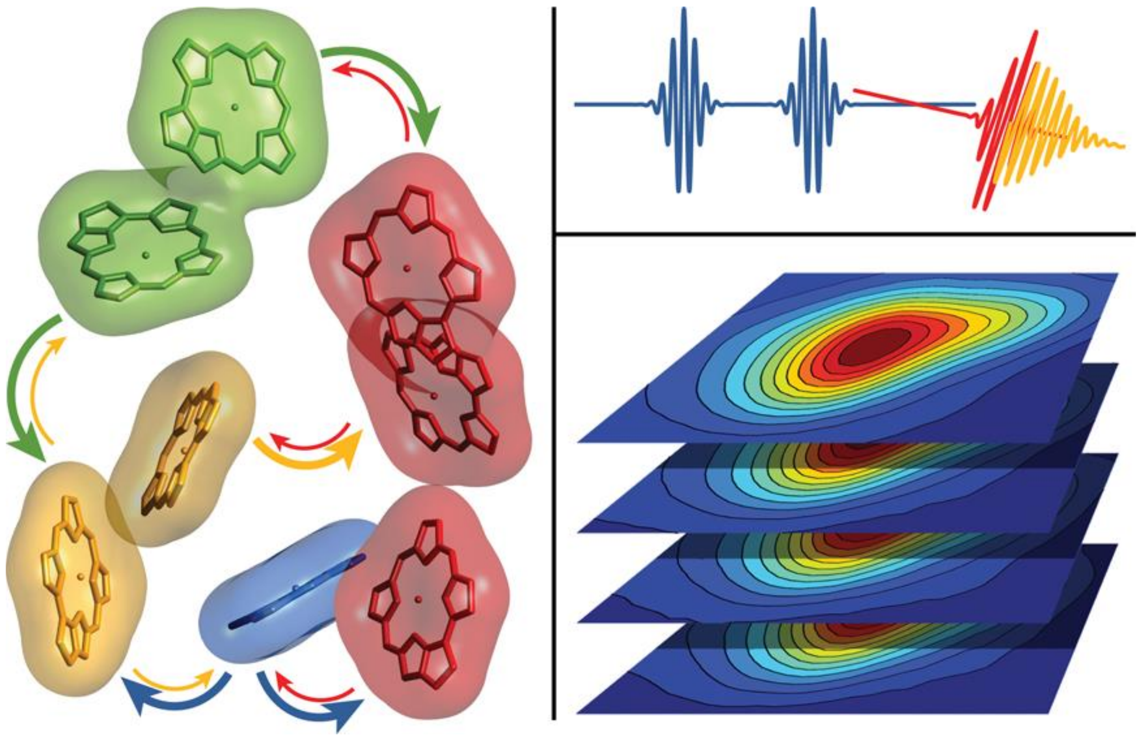


Exciton coherence:

- Coherent superposition of eigenstates exhibits oscillatory behavior
- Cross-peaks in the 2D spectrum oscillate with waiting time
- The oscillation frequency reflects the energy split between the eigenstates

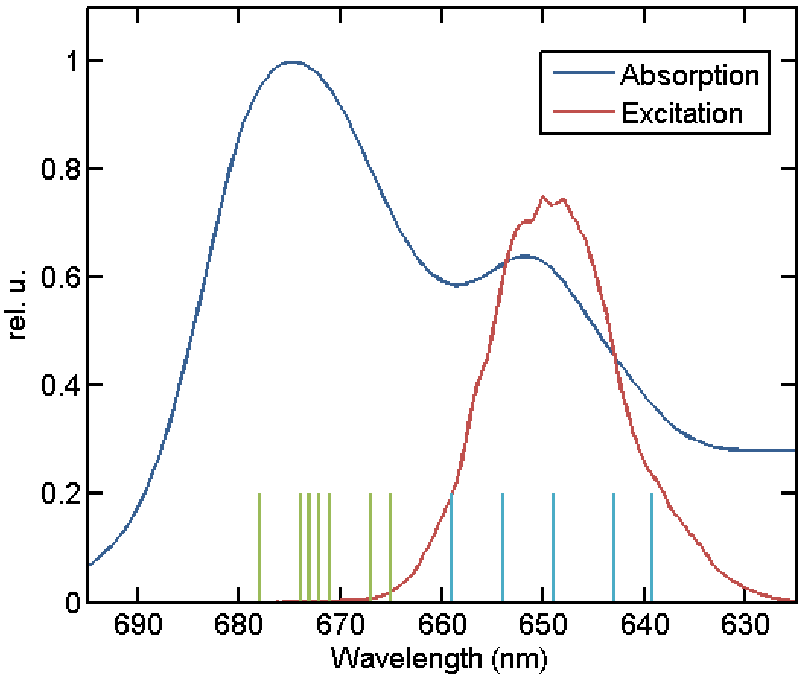


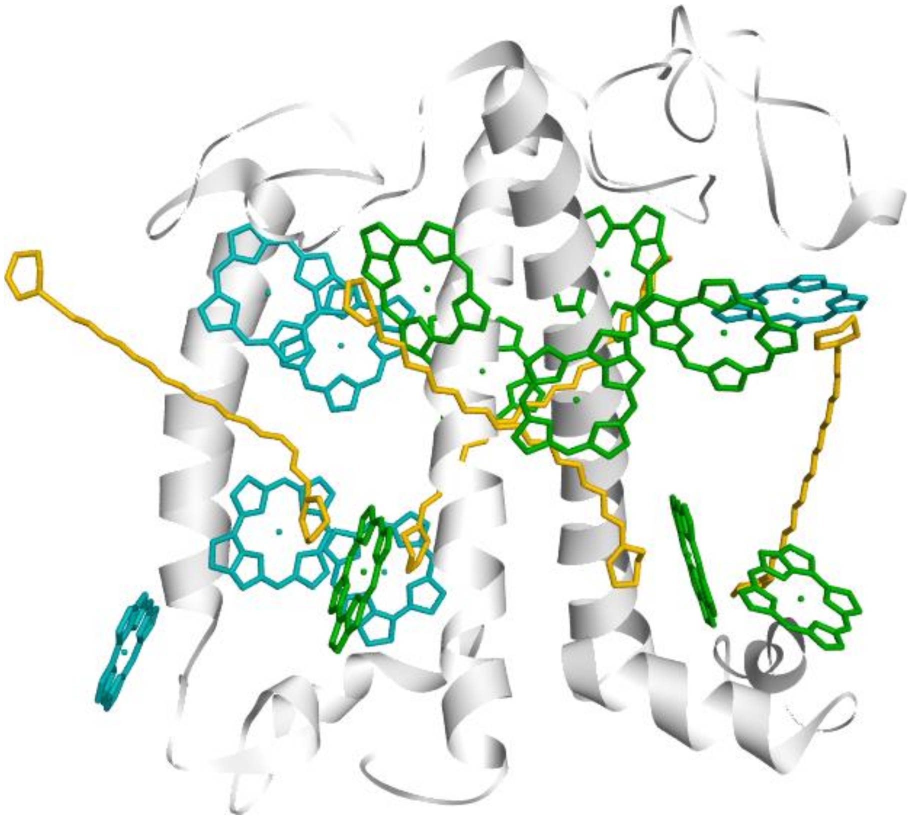
Chenu & Scholes 2015 *Annu Rev Phys Chem*



PROBING ENERGY TRANSFER IN LHCII BY 2DES

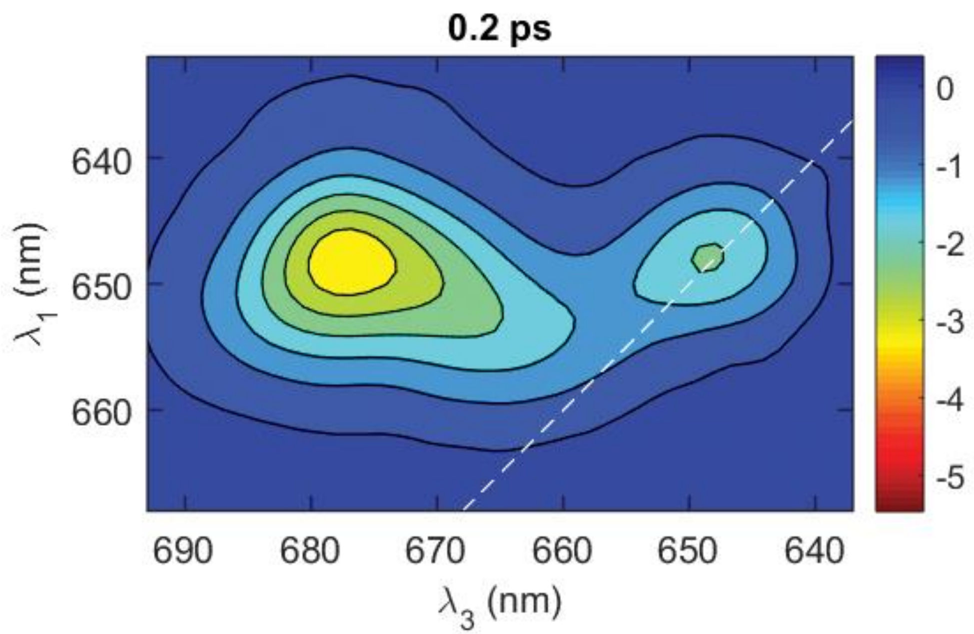
Chl *b* – Chl *a* Energy Transfe

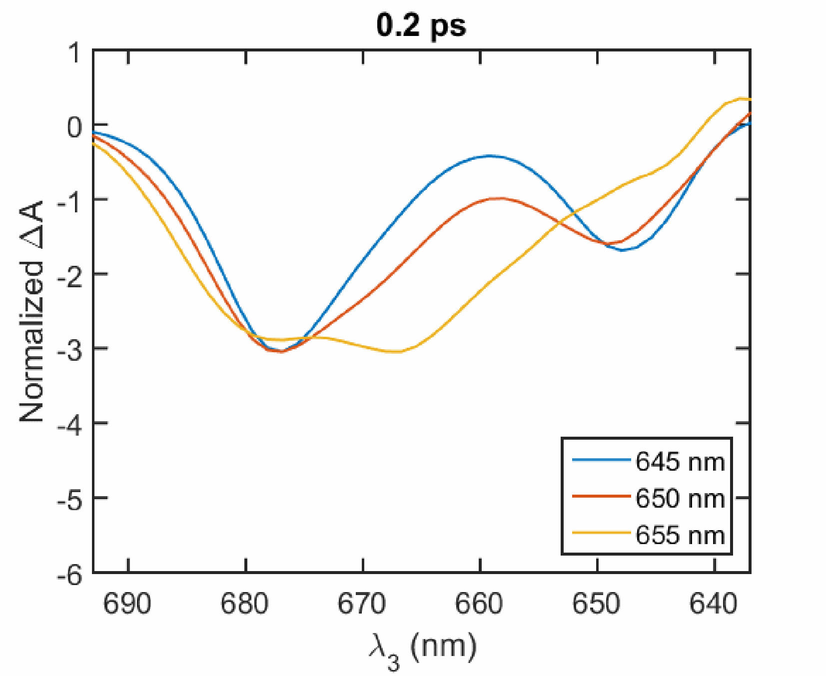




- Narrowband pump pulses centred at 650 nm excite Chl *b* states
- Broadband probe pulses cover all Chl Qy transitions

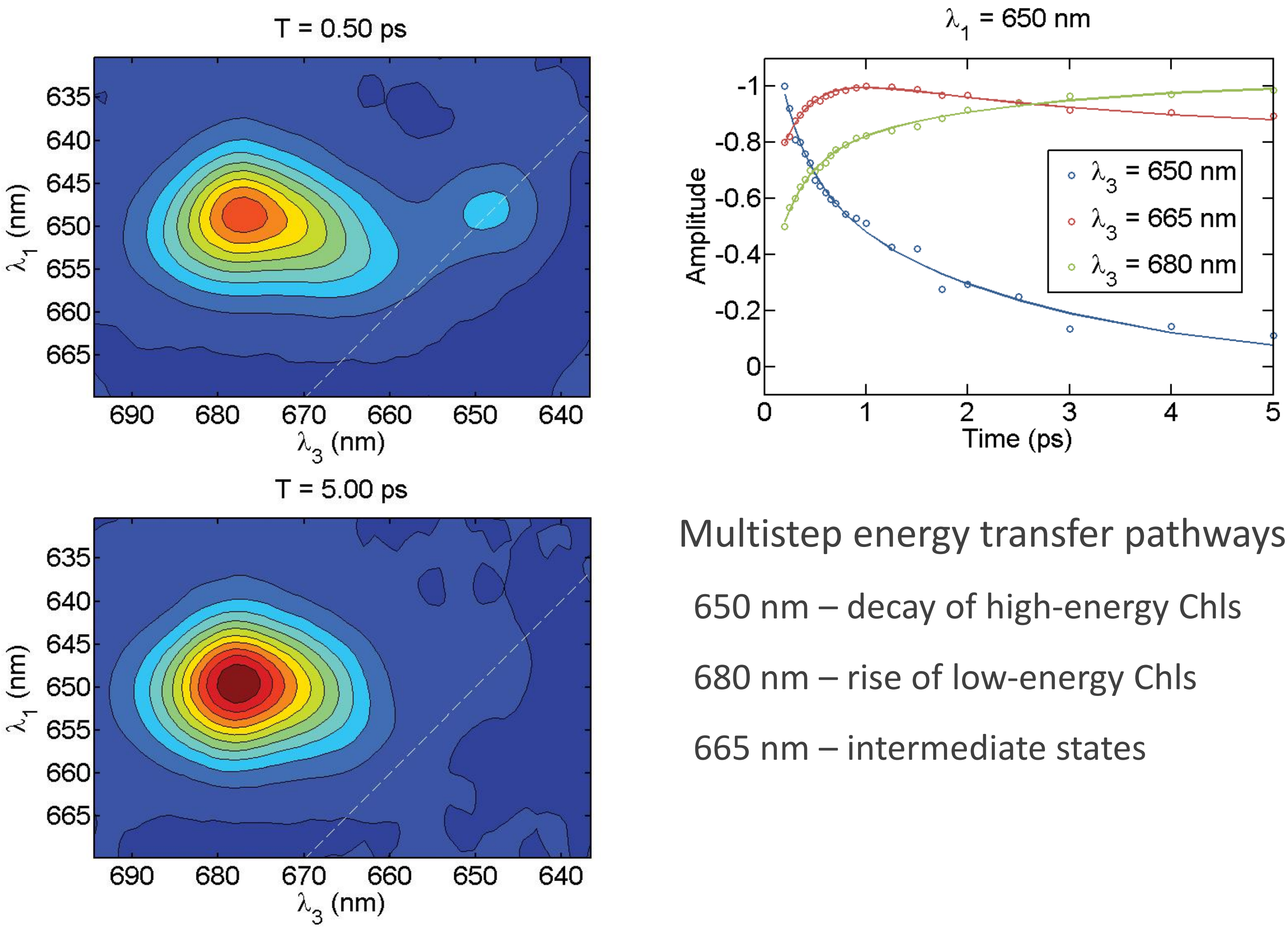
2D spectral evolution – Chl *b* excitation





Wells KL, Lambrev PH et al. (2014) *Phys Chem Chem Phys* 16:11640

2D Spectra of LHCII



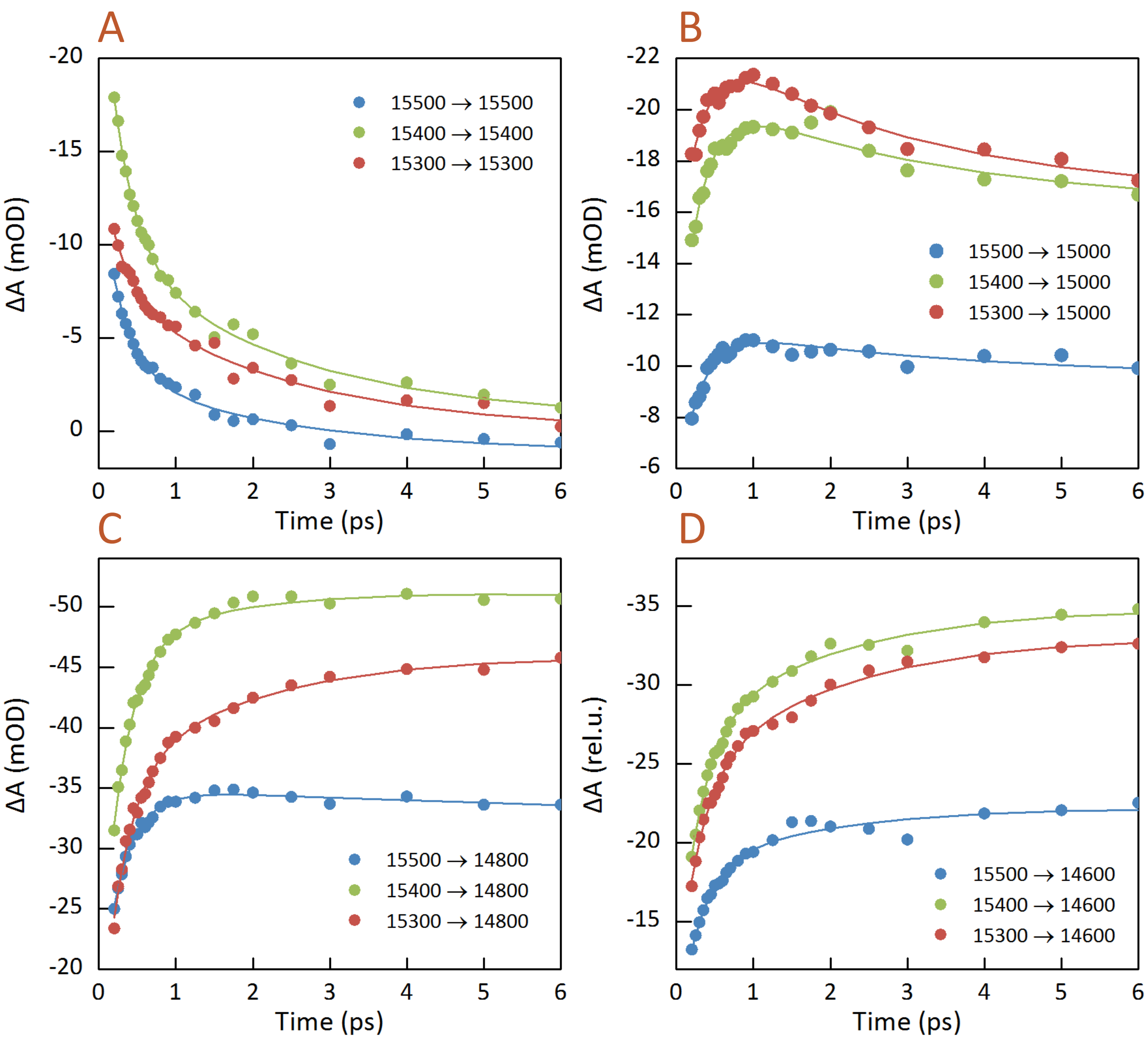
Multistep energy transfer pathways:

650 nm – decay of high-energy Chls

680 nm – rise of low-energy Chls

665 nm – intermediate states

2D Time Traces



Wells KL, Lambrev PH, Zhengyang Z *et al.* (2014) *Phys Chem Chem Phys*

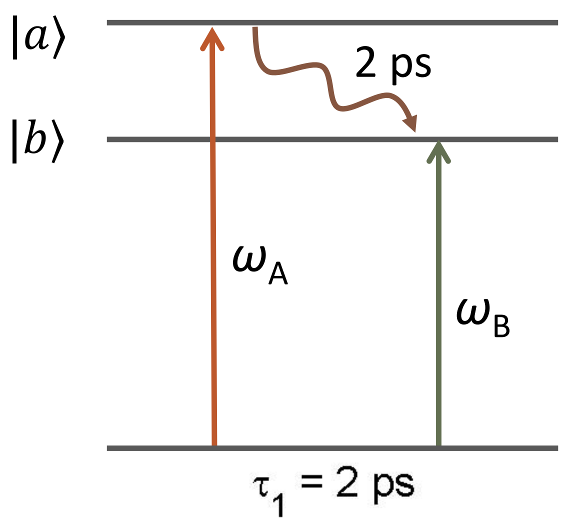
A. Diagonal traces (Chl *b*):
   - High-energy states decay faster
   - Low-energy states decay slower

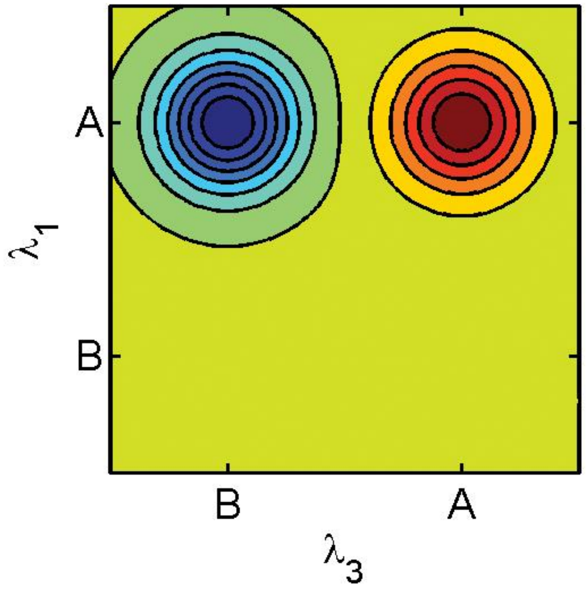
B. High-energy Chl *a*
   - Fast rise to a transient maximum
   - Slow (ps) decay
   - Strong coupling with 15300 cm^{-1}
   - Intermediate states

C. Mid-energy Chl *a*
   - Strong coupling with 15400 cm^{-1}
   - Strong dependence on ω_1

D. Low-energy Chl *a*
   - Multiphasic rise kinetics
   - Not dependent on ω_1
   - Final energy acceptor states

Global Analysis





$$S(\lambda_1, \lambda_3, t) = \sum_{i=1}^{n} A_i(\lambda_1, \lambda_3)\, e^{-\frac{t}{\tau}}$$

τ – lifetimes

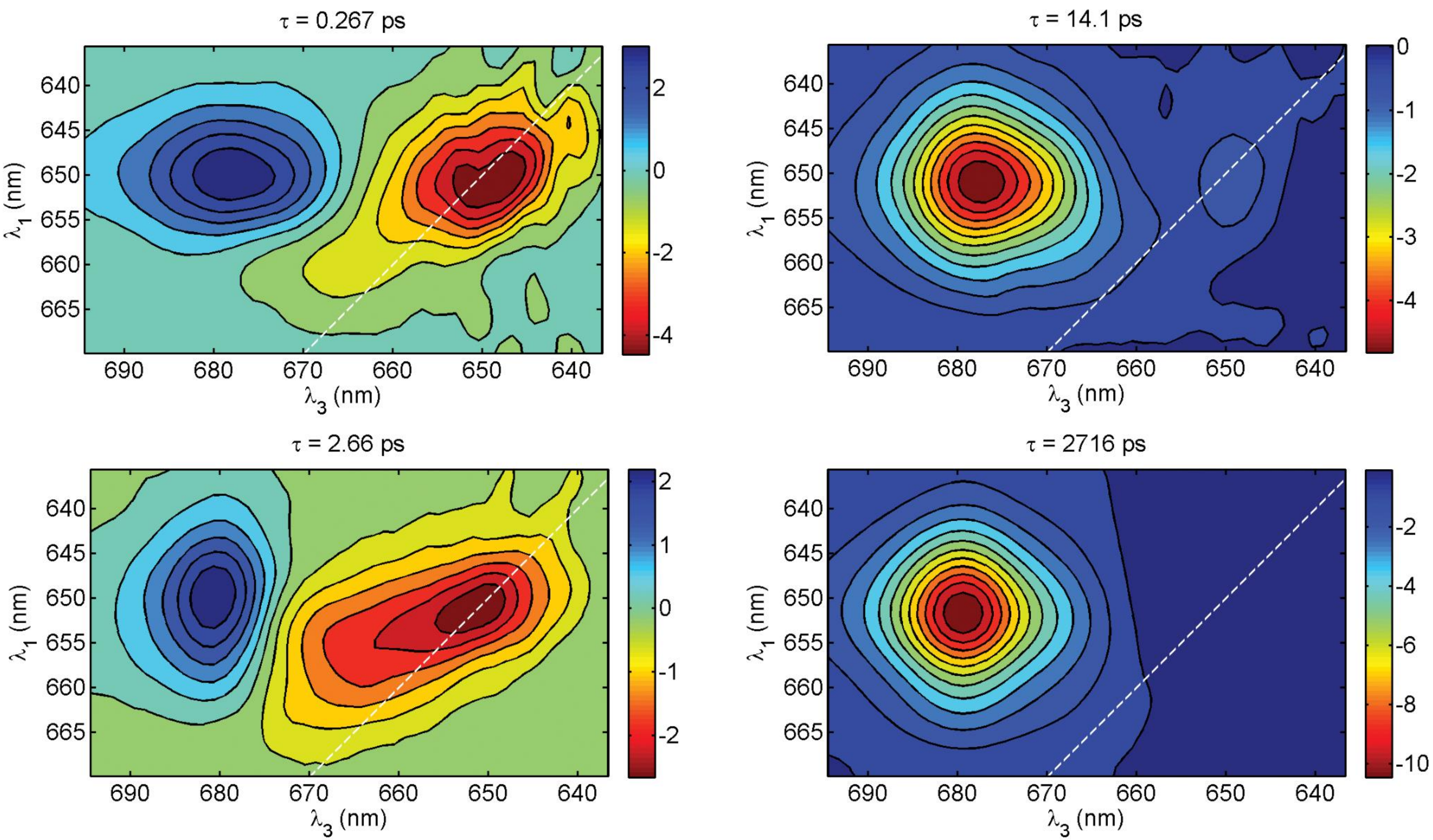
$A_i(\lambda_1, \lambda_3)$ – 2D DAS

negative peaks – population decay

positive peaks – population rise

Wells KL, Lambrev PH et al. (2014) *Phys Chem Chem Phys*

2D Decay-Associated Spectra



Enriquez MM, Akhtar P et al. (2015) *J Chem Phys*

Summary – Chl *b* excitation

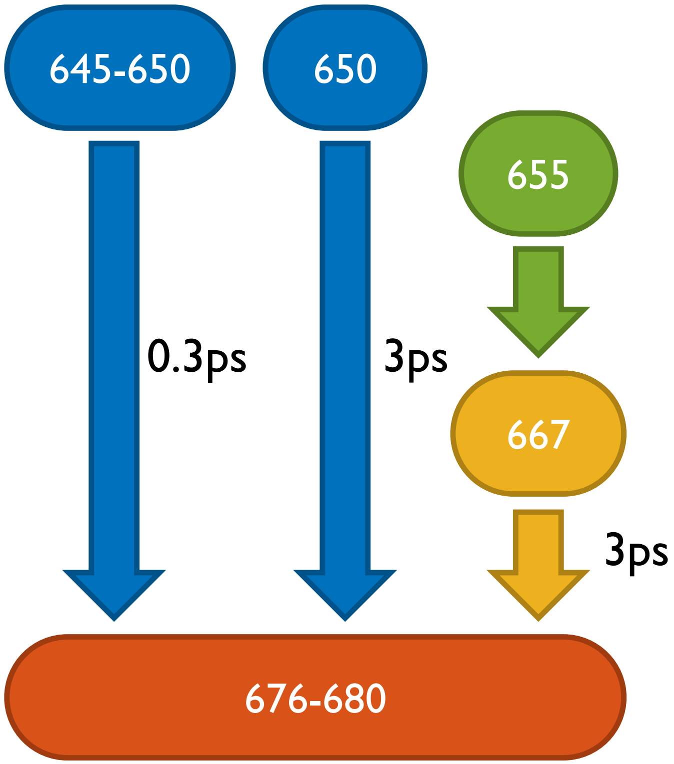
65% Chl *b* – Chl *a* in 0.2–0.3 ps

35% Chl *b* – Chl *a* in 2–3 ps

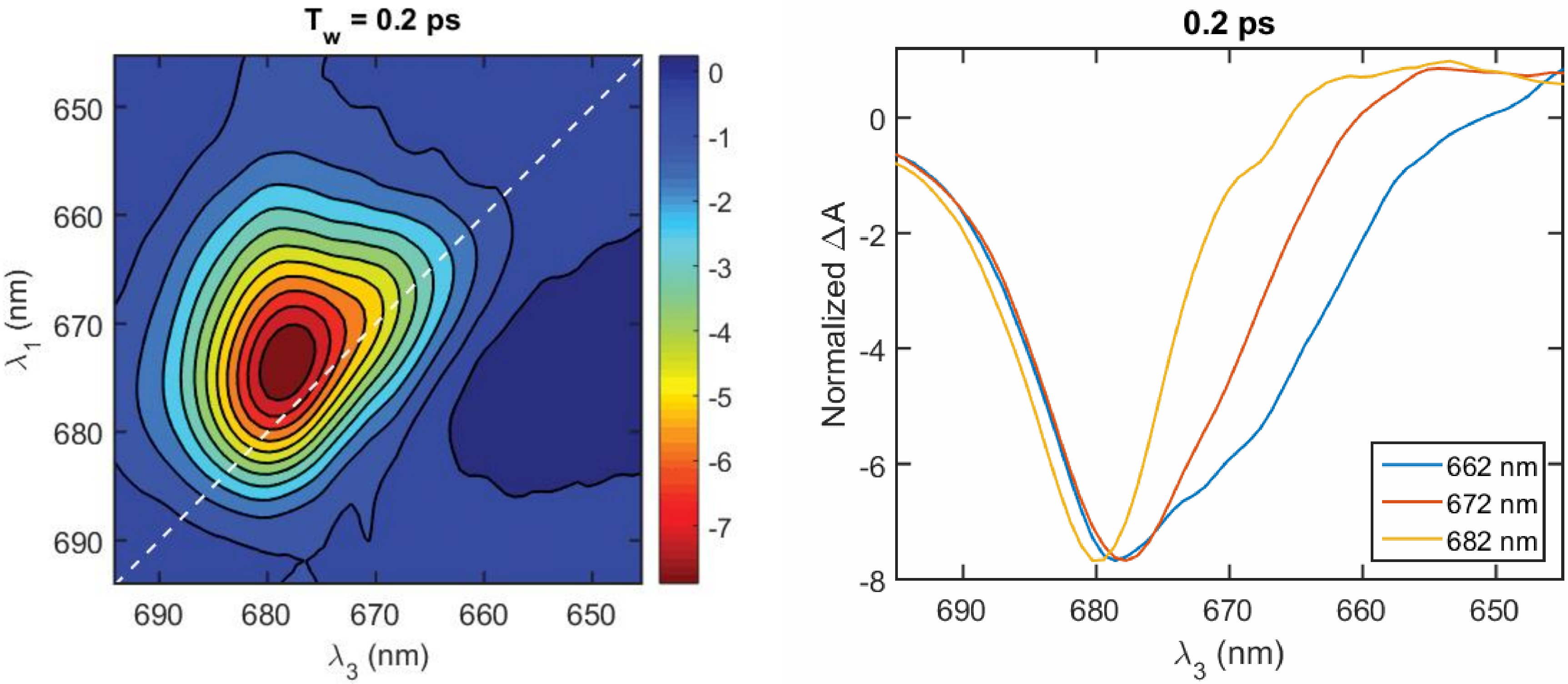
Intermediate Chl *a* states (665–670 nm) coupled to low-energy Chl *b*

Different Chl *a* – Chl *a* (670→680 nm) EET in trimers and aggregates

- > 3 ps in solubilized trimers
- 2–3 ps in LHCII aggregates



Exciton Relaxation in the Chl *a* Manifold

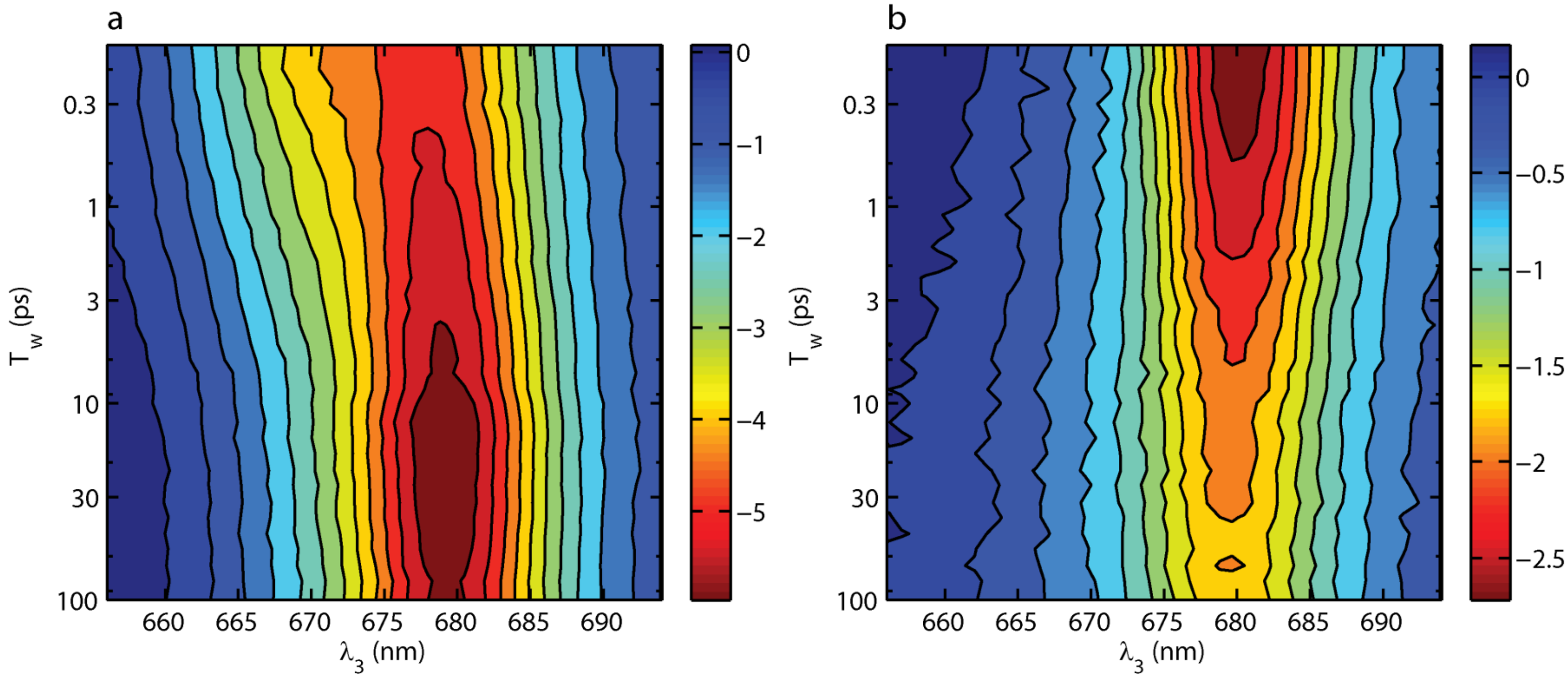


Akhtar P, Zhang C et al. (2017) *J Phys Chem Lett*

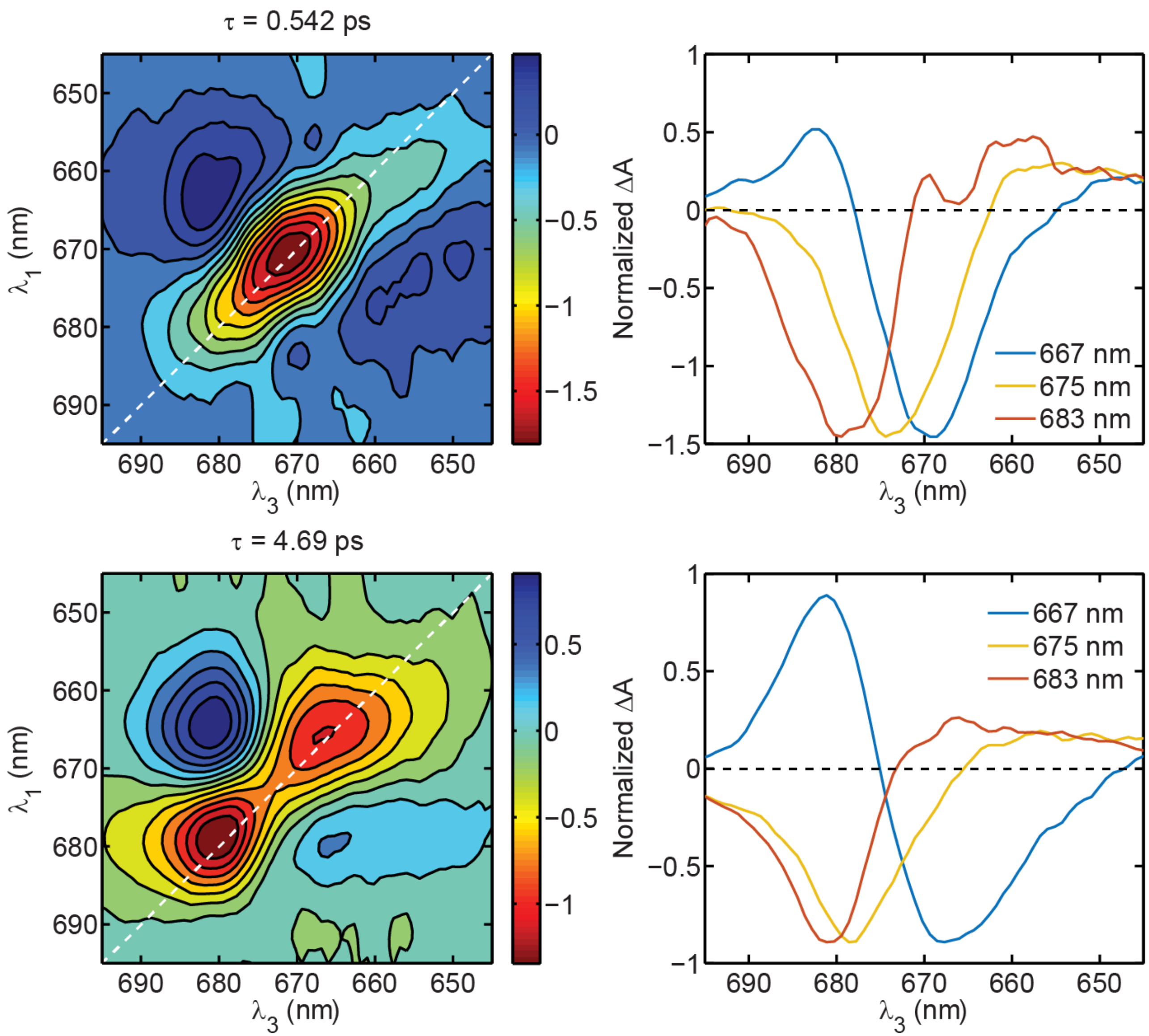
Spectral Evolution

λ_1 = 665 nm

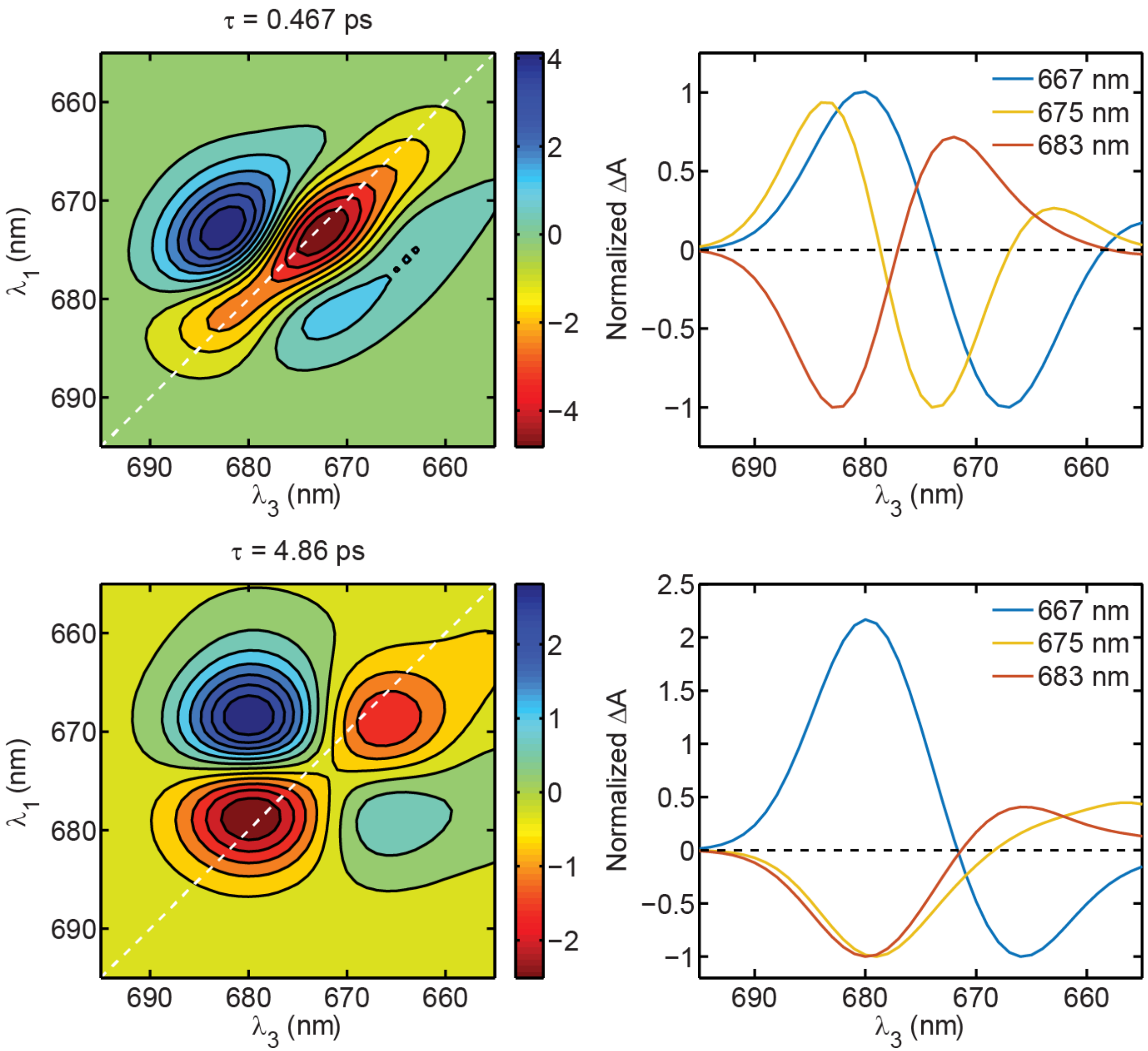
λ_1 = 685 nm



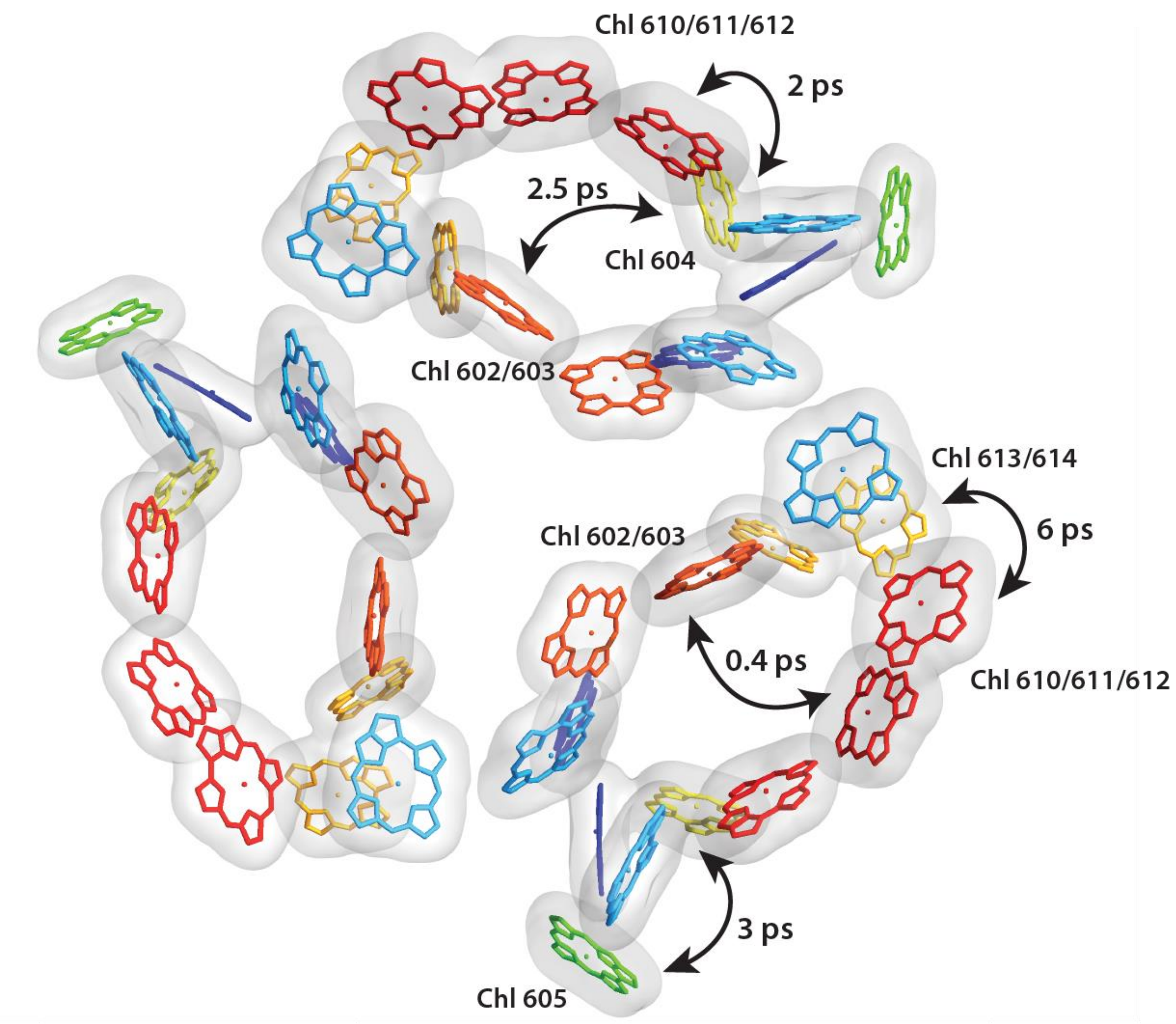
2D DAS of LHCII trimers



Simulated 2D Spectra



Energy Transfer between Chl *a* Domains



Thank you!