The architecture of native, reconstituted and artificial light-harvesting molecular (macro-)assemblies – as revelaed by polarization spectroscopic and microscopic techniques

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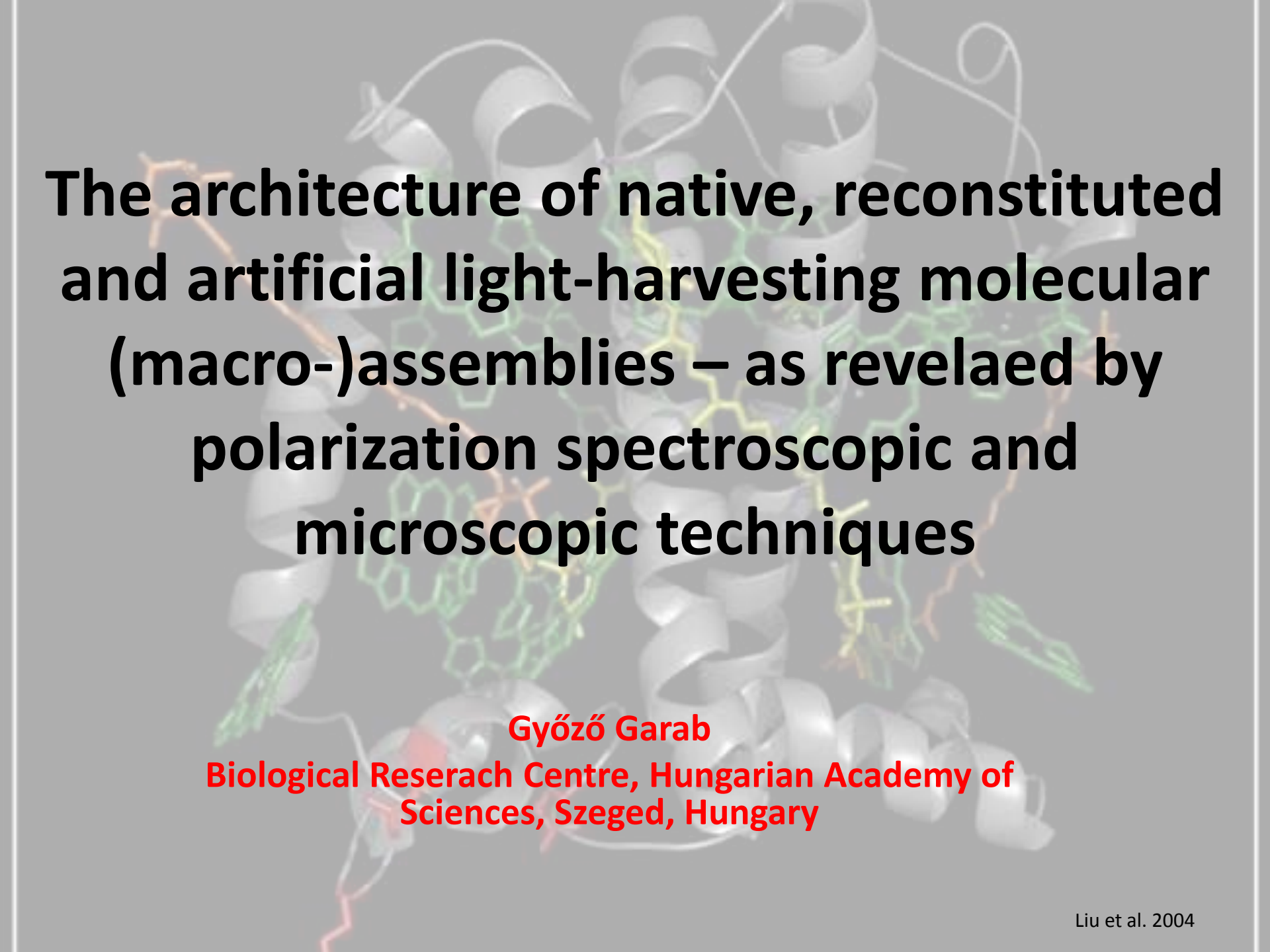

Liu et al. 2004

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Institute of Botany, CAS, Beijing

Chunhong Yang and coworkers

Aix-Marseille Univ., Marseille

Silviu T. Balaban and coworkers

Nanyang Techn. Univ., Singapore

Howe-Siang Tan and coworkers

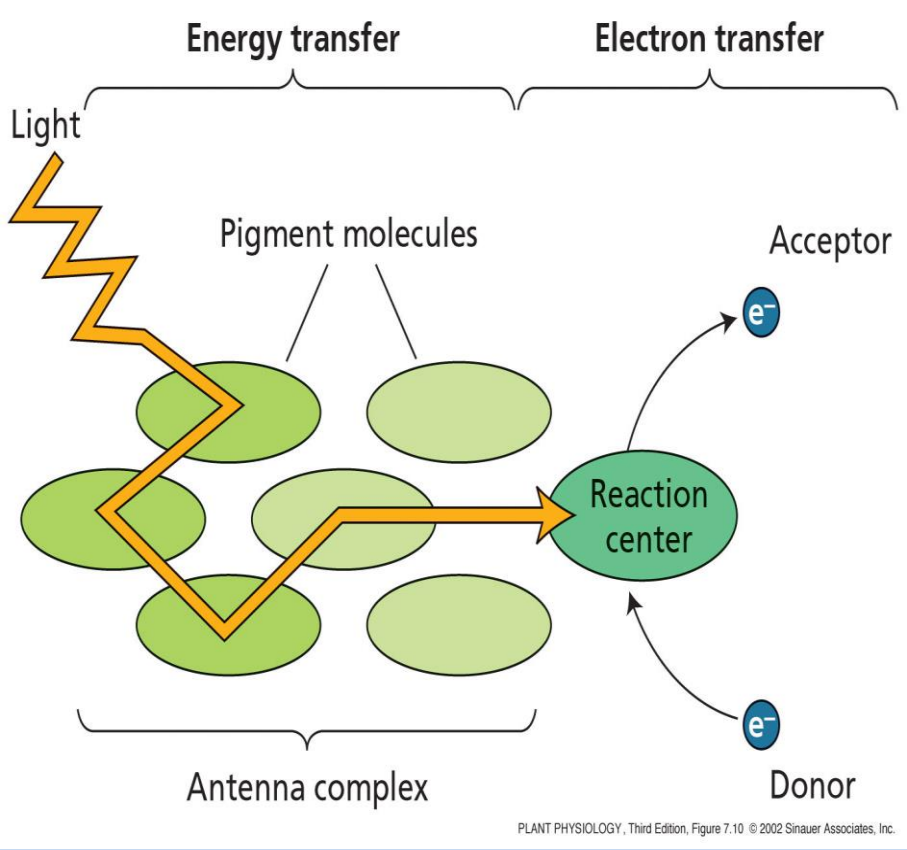
Technical University, Berlin

Gernot Renger and coworkers

In this talk:

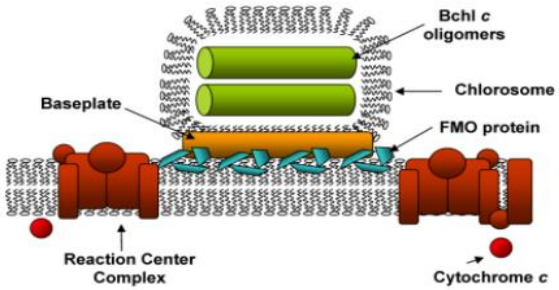
- Light-harvesting antennae in photosynthetic organisms
- Non-random orientation of the pigment molecules – linear dichroism spectroscopy
- Pigment-pigment (short-range) excitonic interactions and extended arrays of the chromophores with long-range order – circular dichroism spectroscopy techniques
- The migration of the excitation energy in LHCII - 2D spectroscopy, and in larger arrays – effective domain sizes
- ‚Artificial chlorosome'
- Microscopic order and micromanipulation of anisotropic particles

Light-harvesting antennae

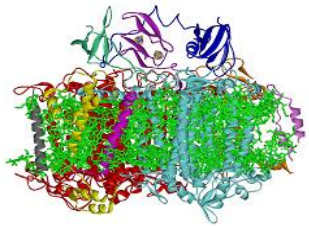


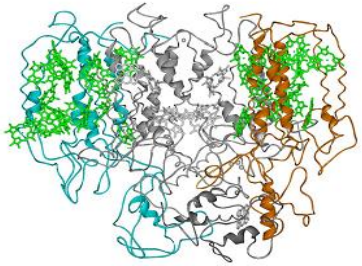
- ►Absorption of light and excitation energy transfer to the Reaction Center (RC)
- ►Increases the effective absorption cross-section.
- ►Important role in photoadaptation of photosynthetic organisms to the environment.

Antenna Complexes

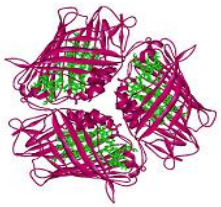


Chlorosomes

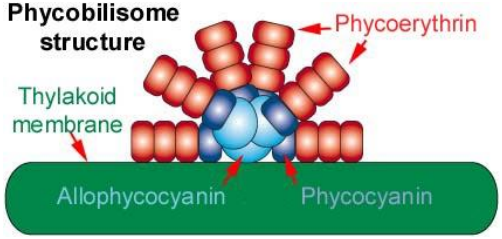


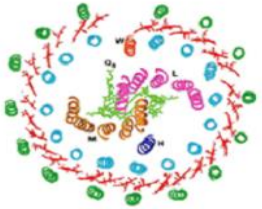


CP43 and CP47 from PSII

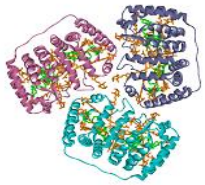


Fenna-Matthew-Olson Protein

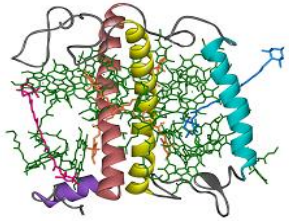




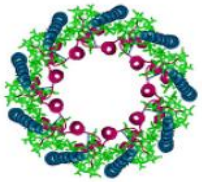
LH1 Core



Peridinin-Chl Complex



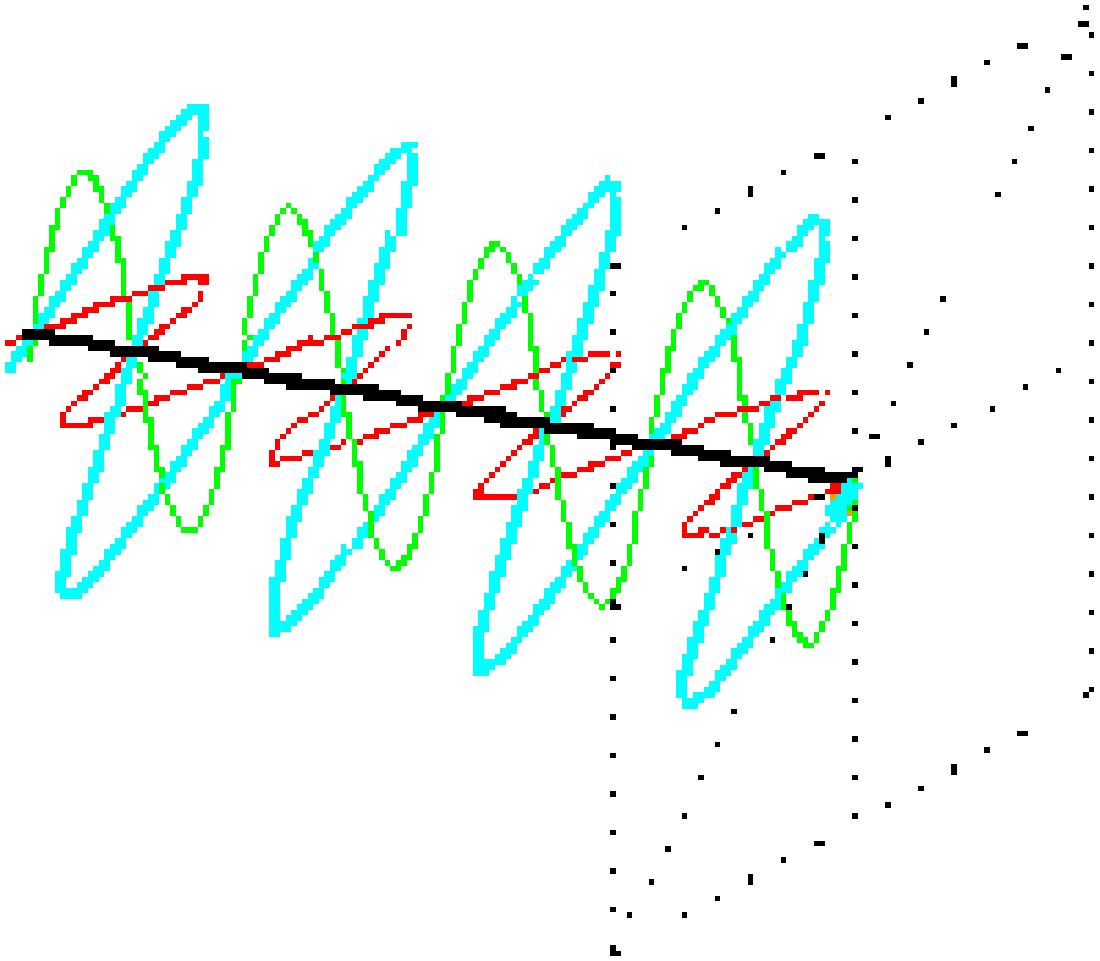
LHCII and LHCI



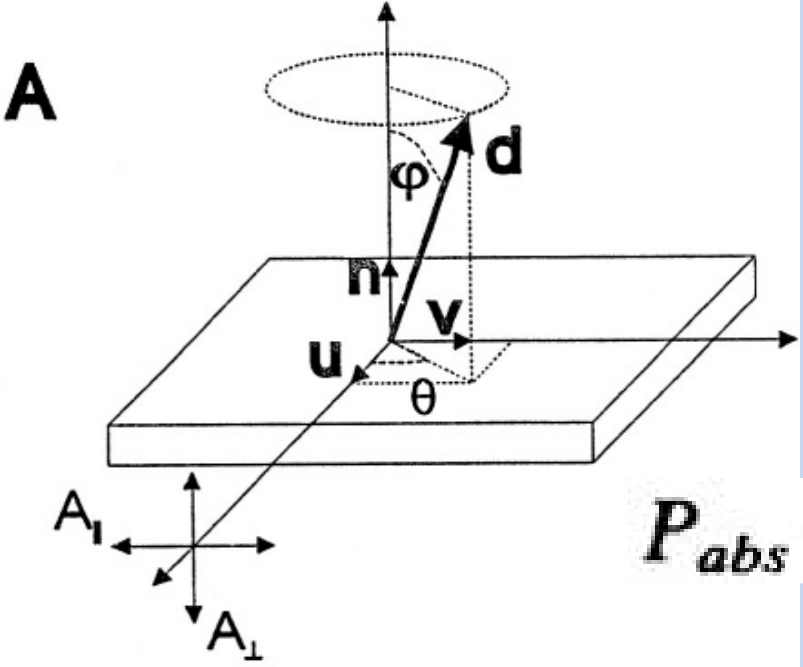
LH2

Extreme diversity of antenna systems strongly suggests multiple independent evolutionary origins

Linearly polarized light

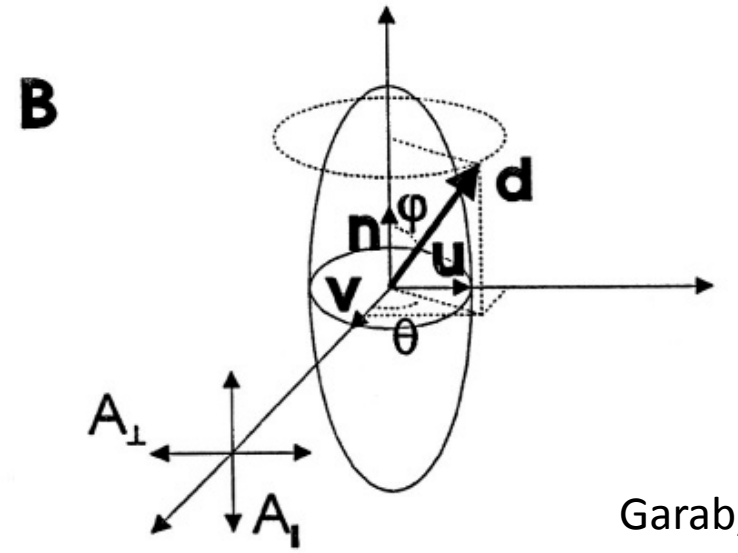


LD
basic principle and definition



$$P_{abs} \sim (E \cdot \mu)^2 = E^2 \mu^2 \cos^2 \alpha$$

$$LD \equiv A_{\parallel} - A_{\perp} = \frac{3}{2} A(1 - 3\cos^2 \varphi)$$

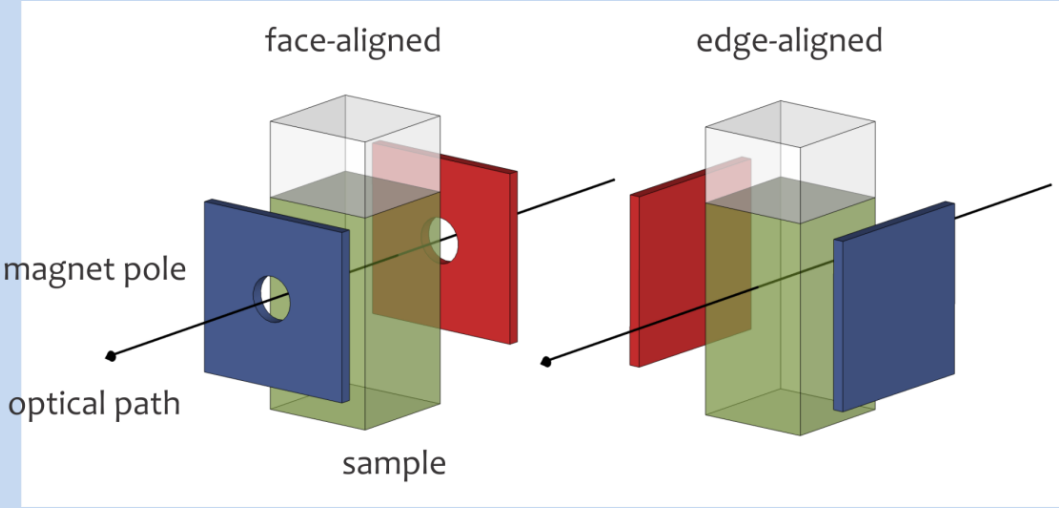


Orientation parameter:
S=LD/3A

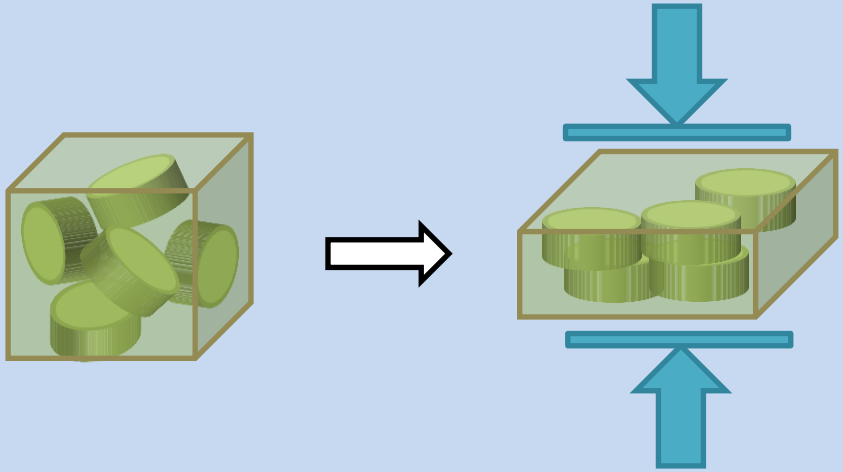
Also, polarized fluorescence emission

Garab, in Biophysical Techniques in Photosynthesis, Kluwer, 1996

Sample Orientation (Alignment)



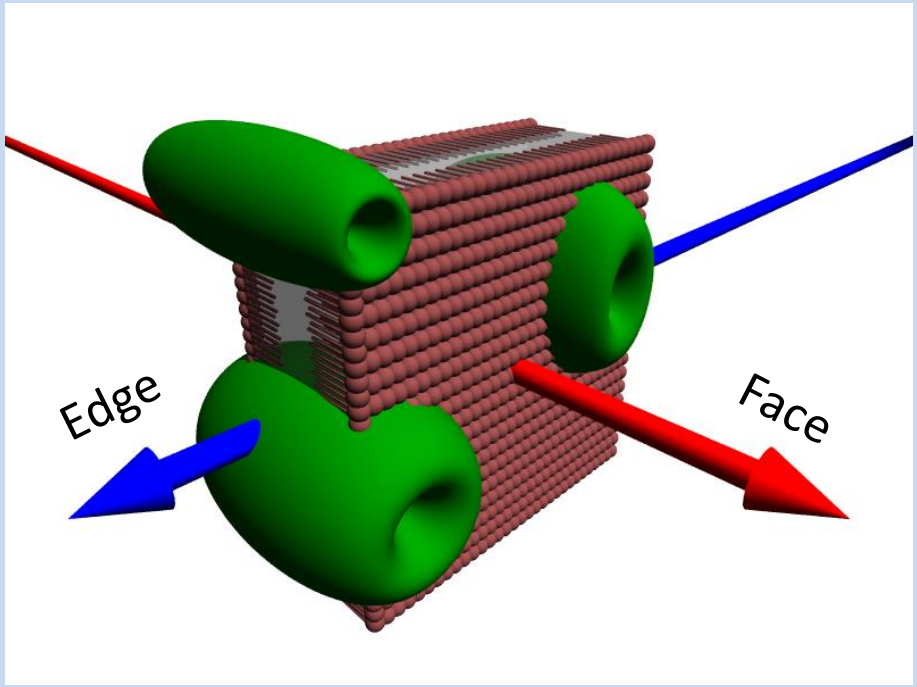
Magnetic orientation

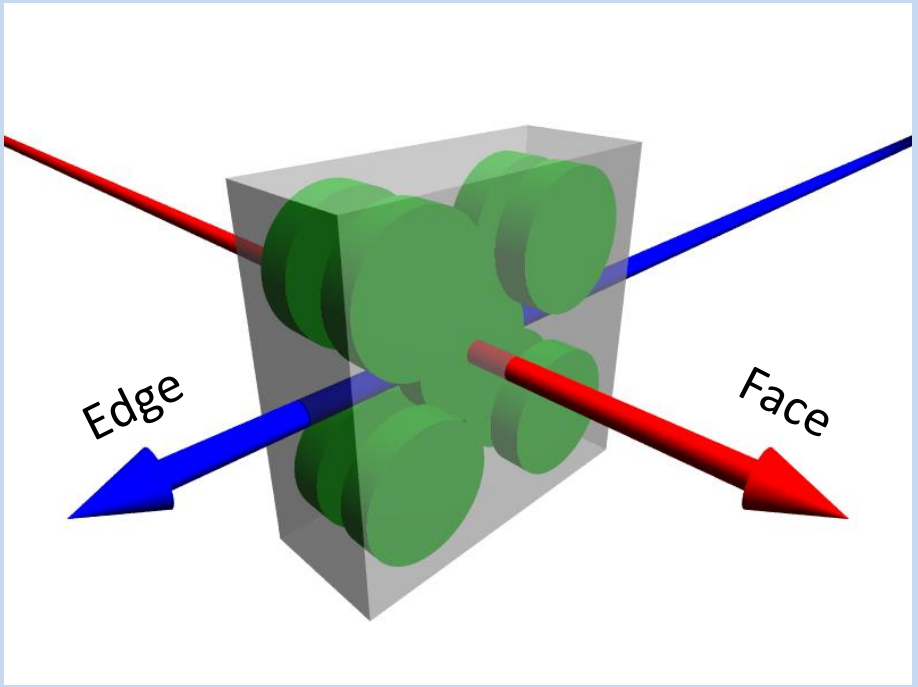


Orientation by gel squeezing

Miloslavina et al. 2011 Photosynth. Res.

Orientation of membranes

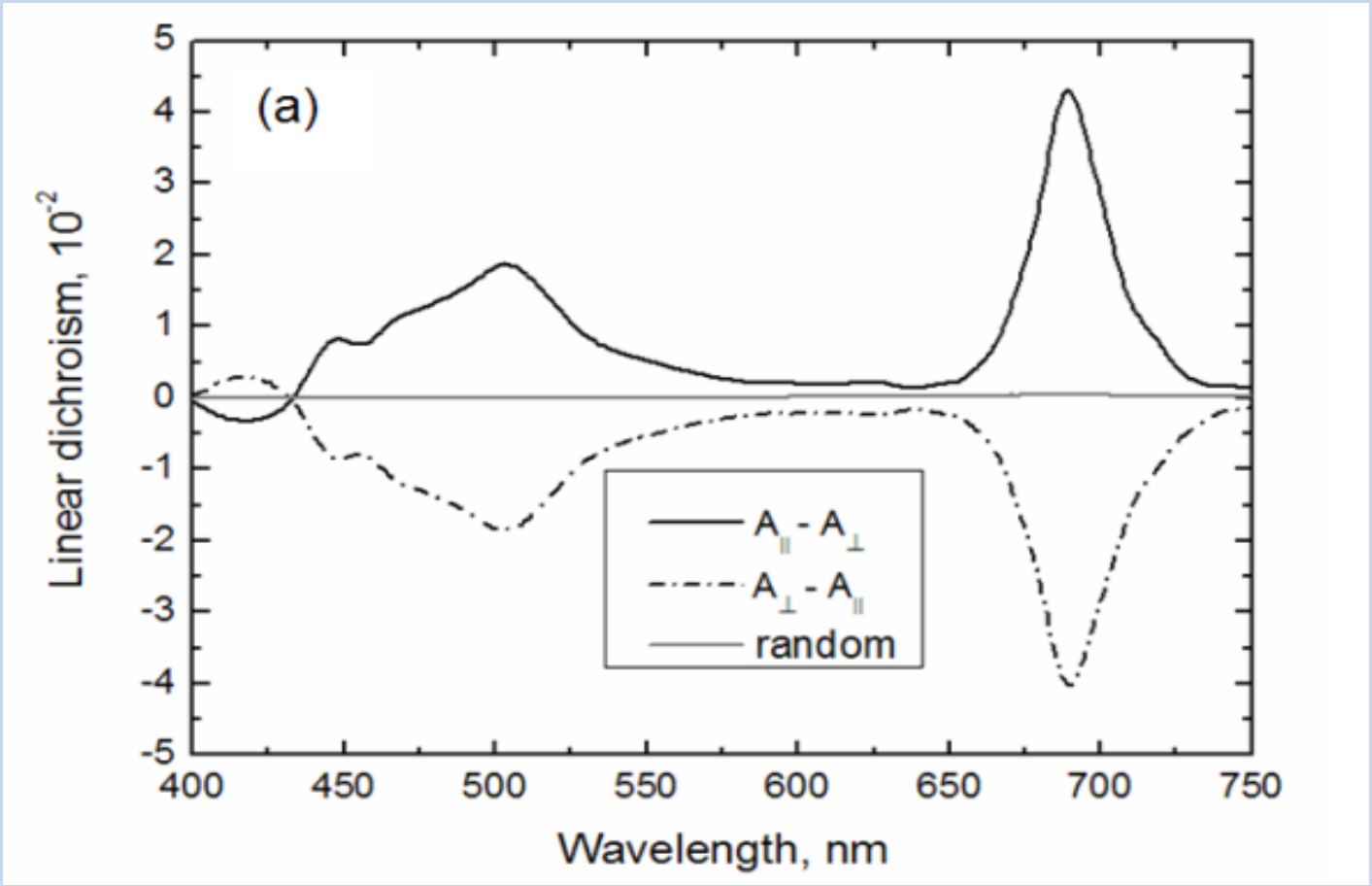


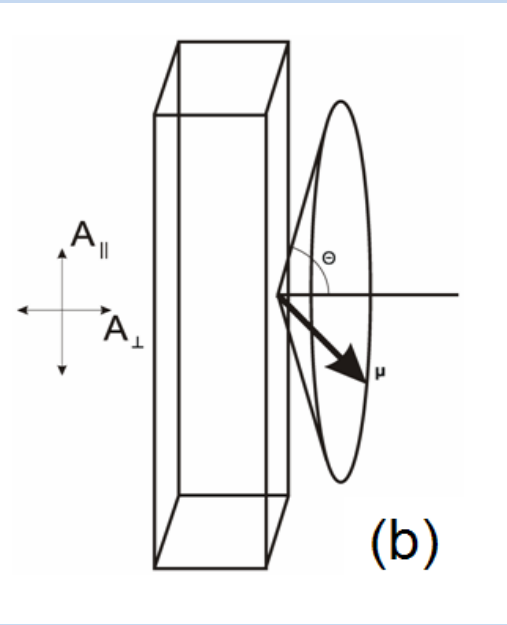


Face-aligned orientation should preferentially excite transitions in the membrane plane

Edge-aligned orientation shows transitions primarily perpendicular to the membrane.

LD of magnetically aligned thylakoid membranes, trapped in gel



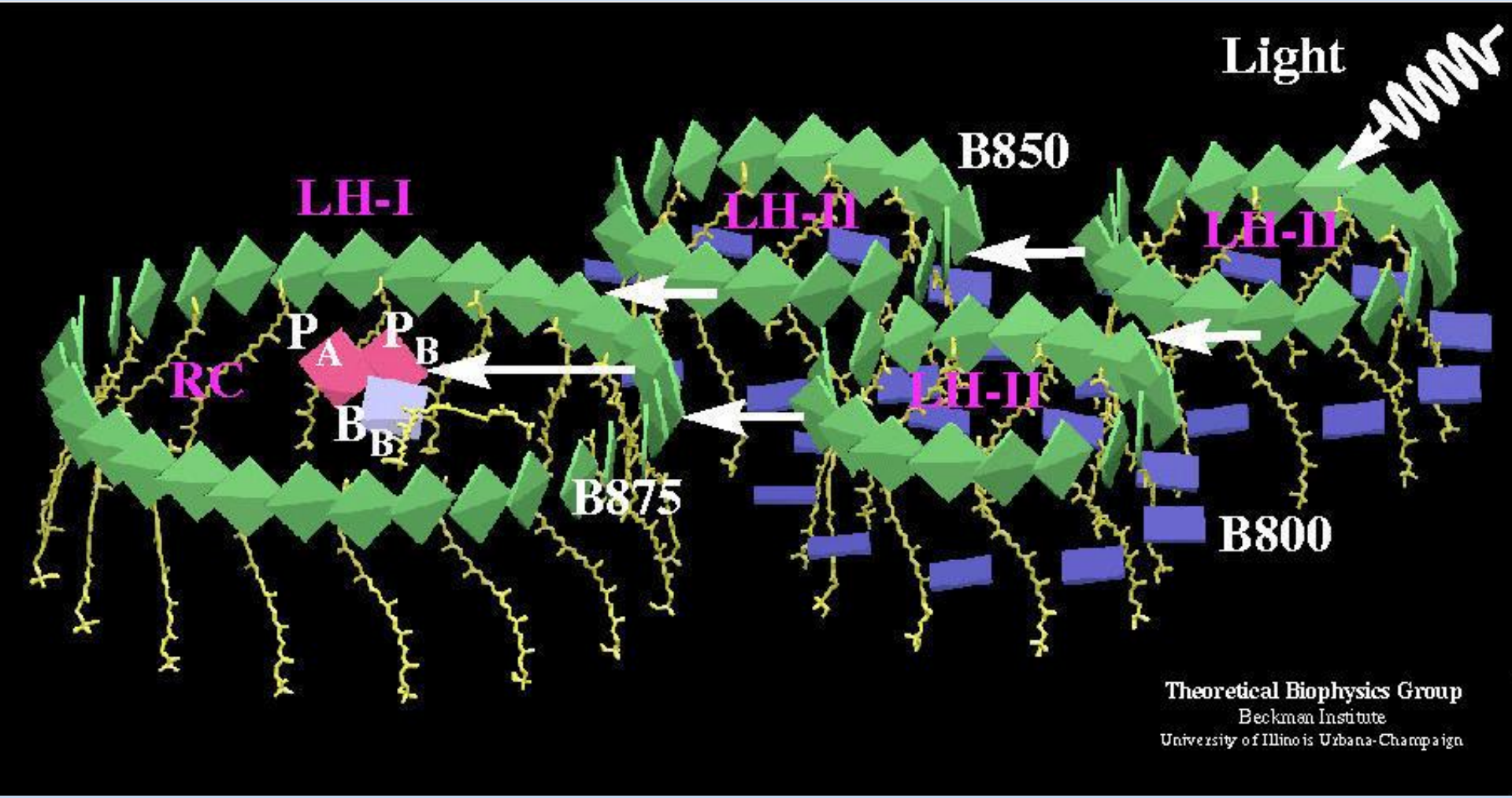


Garab and van Amerongen, 2009 Photosynth Res

Förster resonance interaction

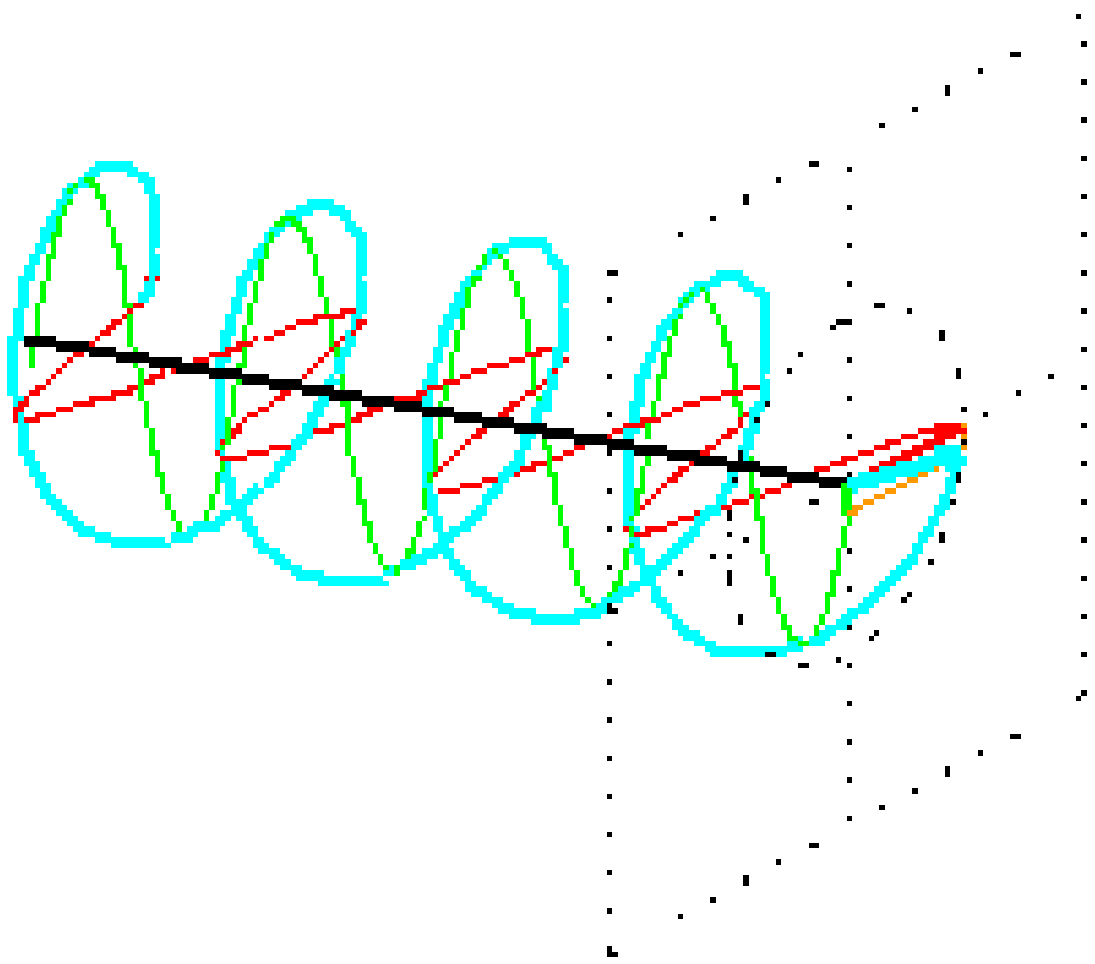
- Coulombic dipole-dipole interaction
- $V_{DA} = \frac{1}{4\pi\varepsilon_o n^2} \frac{\hat{\mu}_D \hat{\mu}_A}{R_{DA}^3} - 3 \frac{(\hat{\mu}_A \hat{R}_{DA})(\hat{\mu}_D \hat{R}_{DA})}{R_{DA}^5}$

- The rate of energy transfer depends on the mutual orientation of the donor (D) and acceptor molecules (dipoles)
- $k_{DA} = \frac{2\pi}{\hbar} |V_{DA}|^2 J_{DA}$
- $J_{DA} = \int_0^\infty f_D(\lambda) \varepsilon_A(\lambda) \lambda^4 \, d\lambda$


Light
B850
LH-I
LH-II
LH-II
RC
P_A
P_B
B_B
LH-II
B875
B800
Theoretical Biophysics Group
Beckman Institute
University of Illinois Urbana-Champaign

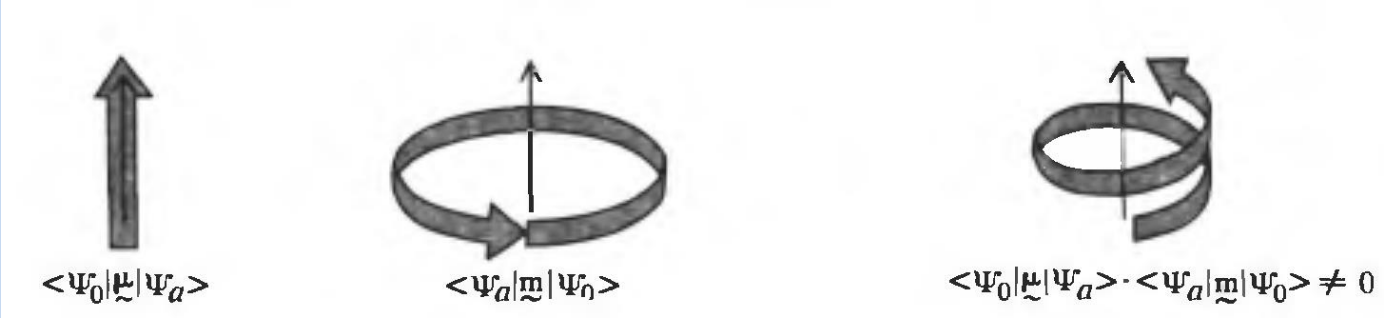
The non-random orientation of the chromophores / pigment molecules (dipoles) appears to be a universal property in photosynthetic system - it has a functional role in determining the excitation energy migration

Circularly polarized light



Physical origins of CD

Molecular charge motions induced by light



Interaction with the electric component

Interaction with the magnetic component

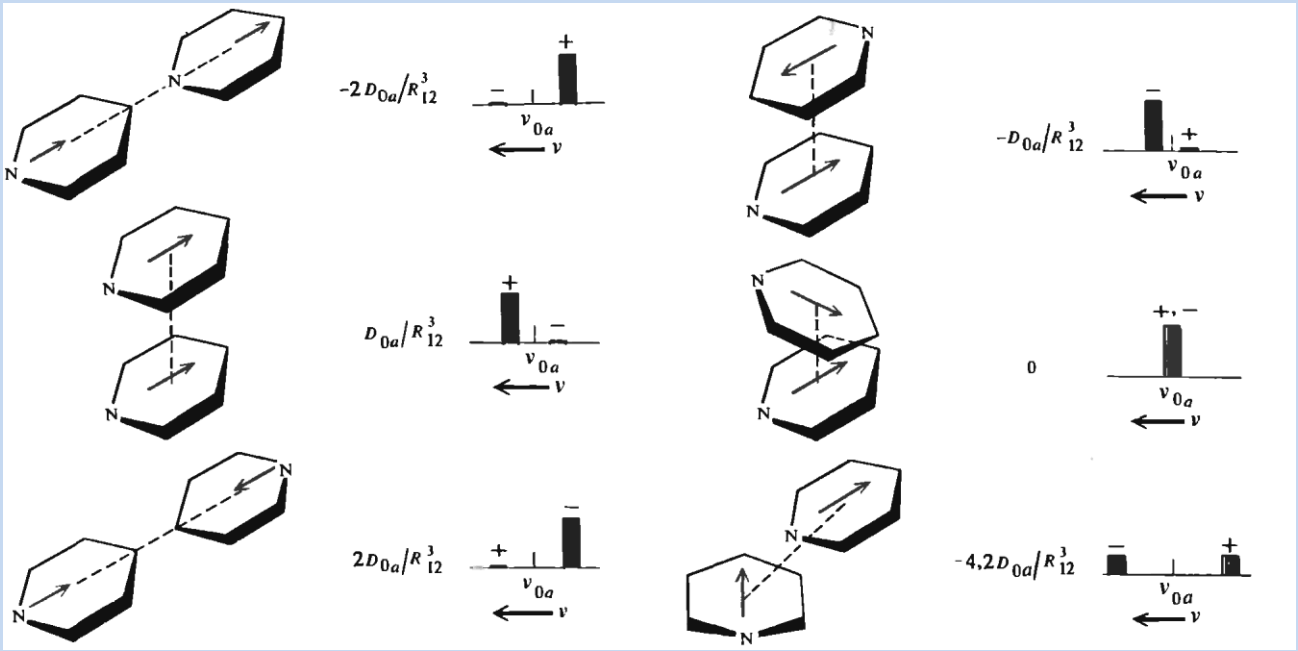
Optical activity

$R_{CD} = -Im(\boldsymbol{\mu}, \mathbf{m})$

Cantor C.R. & Schimmel P.R., Biophysical Chemistry, 1980, Freeman & Co.

Excitonic coupling

Dimer V_{12} Spectrum Dimer V_{12} Spectrum



Cantor C.R. & Schimmel P.R., Biophysical Chemistry, 1980, Freeman & Co.

$$\Re_{\pm CD} = \pm 1.7 \times 10^{-5} \nu_0 \boldsymbol{\mu}^2 R(\mathbf{r} \cdot \mathbf{d}_2 \times \mathbf{d}_1)$$

Short-range, dipole-dipole-interactions

see also Garab, 1996 in Biophysical Techniques ...

In large, densely packed ordered, so-called psi-type aggregates the electric field at any point is the superposition of the incident electric field and the sum of the fields produced by all oscillating dipoles - thus the CD is more complex, and is an attributum of the entire, highly organized macroaggregate (or the chiral macrodomains)

$$E_{dipole_i} = 4\pi k^2 \mathbf{\Gamma}(\mathbf{x}, \mathbf{x}') \cdot \boldsymbol{\mu}_i$$

$$E(x) = E_0(x) + \sum_i E_{dipole\ i}(x)$$

$$\mathbf{\Gamma}(x, x') = (3\hat{\mathbf{r}}\hat{\mathbf{r}} - 1)\frac{e^{ikr}}{4\pi k^2 r^3} - (3\hat{\mathbf{r}}\hat{\mathbf{r}} - 1)\frac{ie^{ikr}}{4\pi kr^2}$$

$$+ (1 - \hat{\mathbf{r}}\hat{\mathbf{r}})\frac{e^{ikr}}{4\pi r} - \frac{1}{3k^2}\delta^3(r)$$

$$\mathbf{\Gamma}(x, x') = (3\hat{\mathbf{r}}\hat{\mathbf{r}} - 1)\frac{e^{ikr}}{4\pi k^2 r^3} - (3\hat{\mathbf{r}}\hat{\mathbf{r}} - 1)\frac{ie^{ikr}}{4\pi kr^2} + (1 - \hat{\mathbf{r}}\hat{\mathbf{r}})\frac{e^{ikr}}{4\pi r} - \frac{1}{3k^2}\delta^3(r)$$

Keller and Bustamante, 1986, J Chem Phys

See also Garab 1996; Garab and van Amerongen, 2009 – and the references therein

Kim et al..
J. Chem. Phys.
Volume 84(6) March 1986 2981-2989

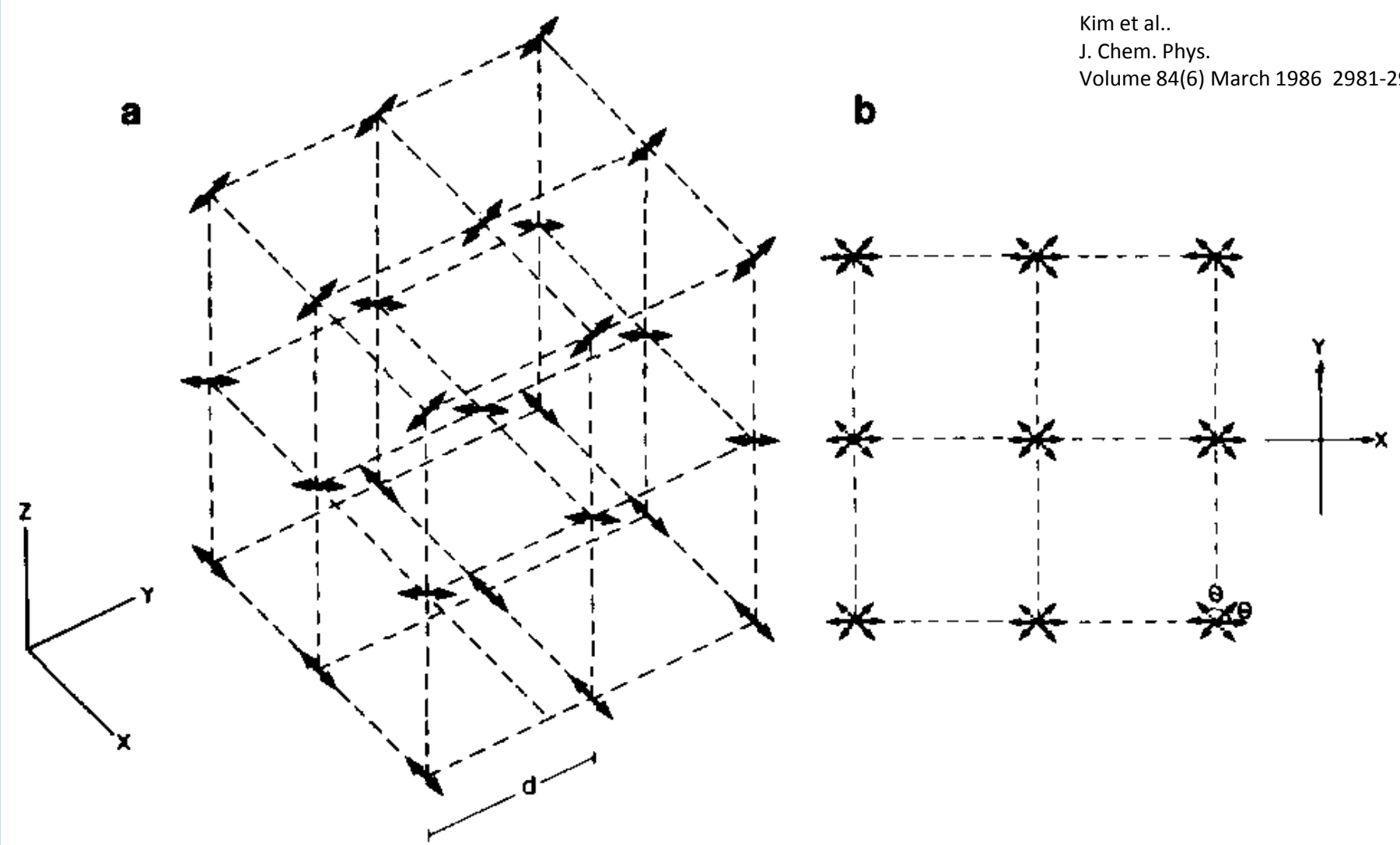


FIGURE 2 Geometry for the psi-type CD calculations: (a) 27 uniaxial polarizable axes with only *xy* components are situated on the cubic lattice points and each layer is twisted an angle, θ, which determines the pitch of the model system. *d* is the distance between the polarizable groups on each coordinate. Each polarizable axis represents the volume of d^3. Therefore, the volume which these 27 polarizable axes represent is $(3d)^3$; (b) top view of (a). The angle between the polarizable axes of adjacent layers is θ.

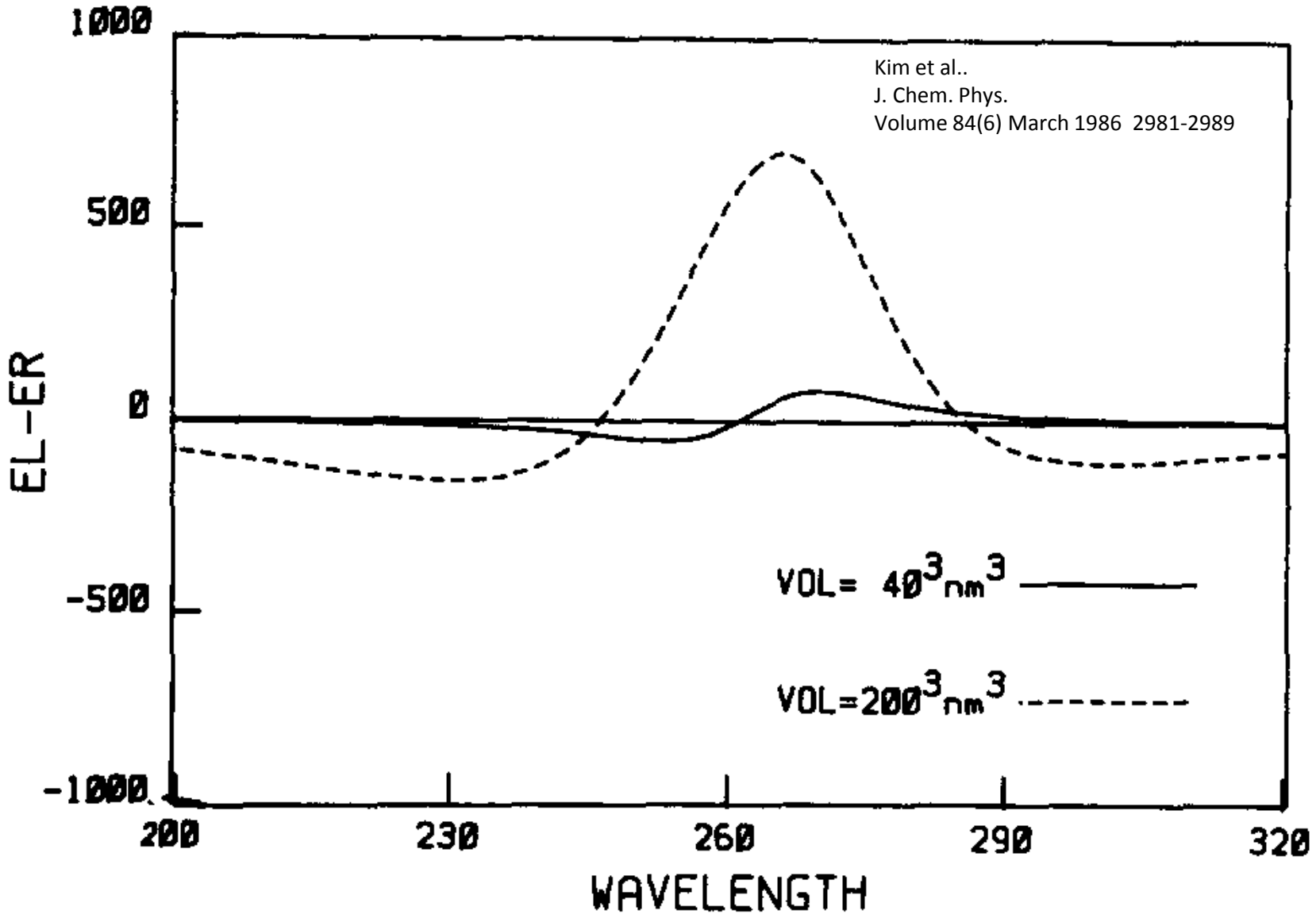
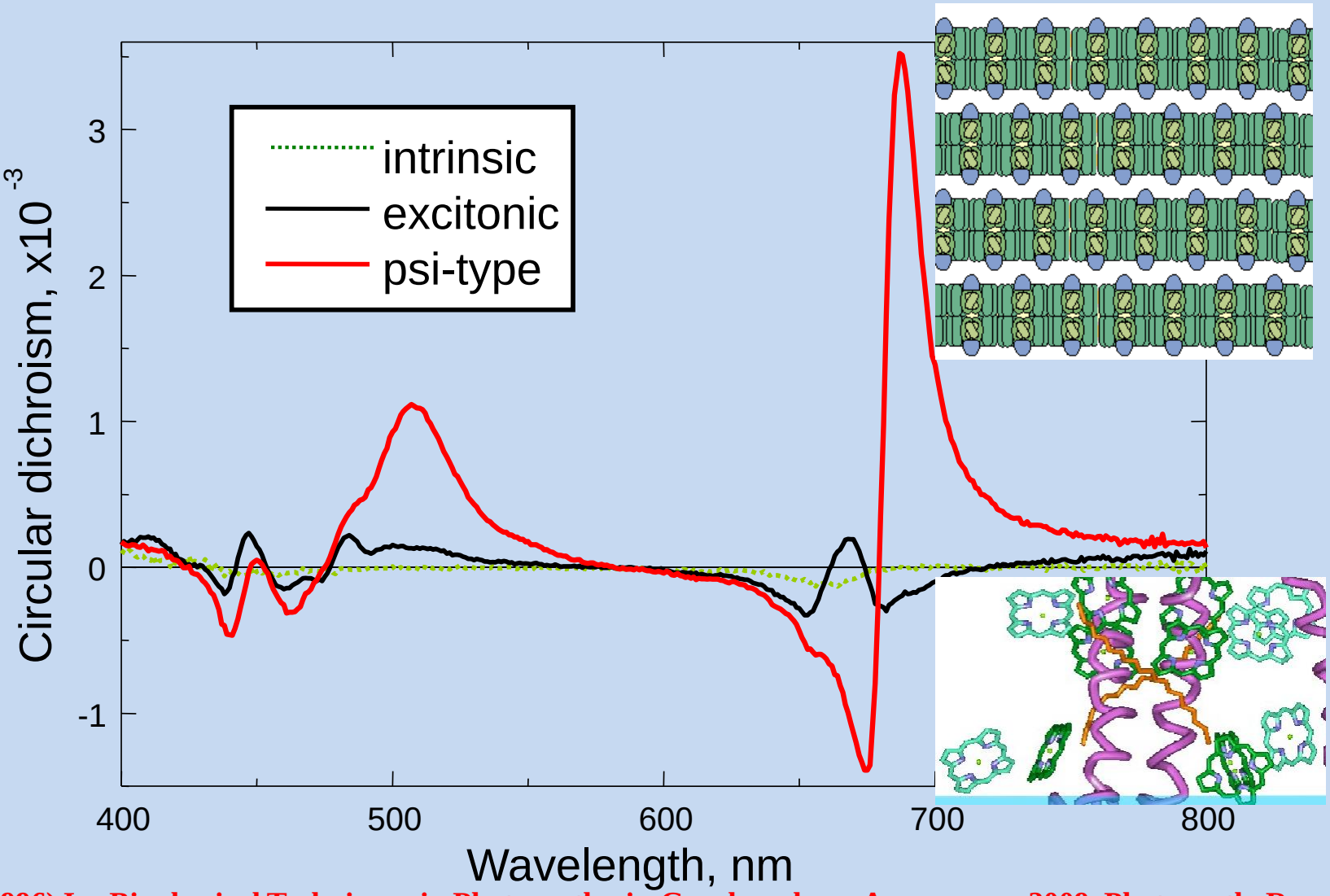
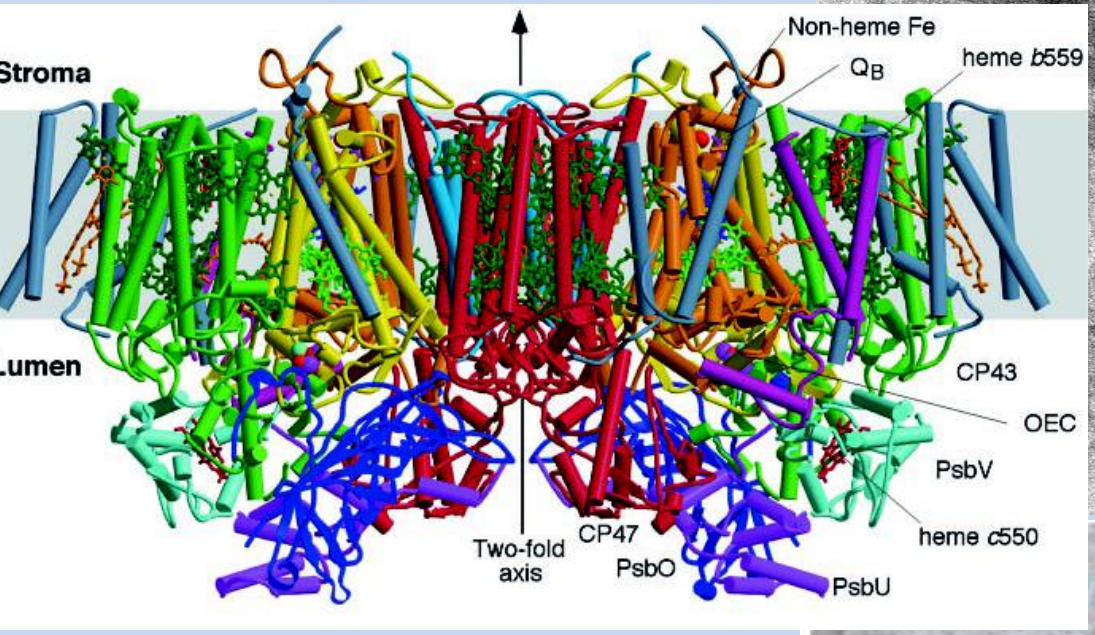


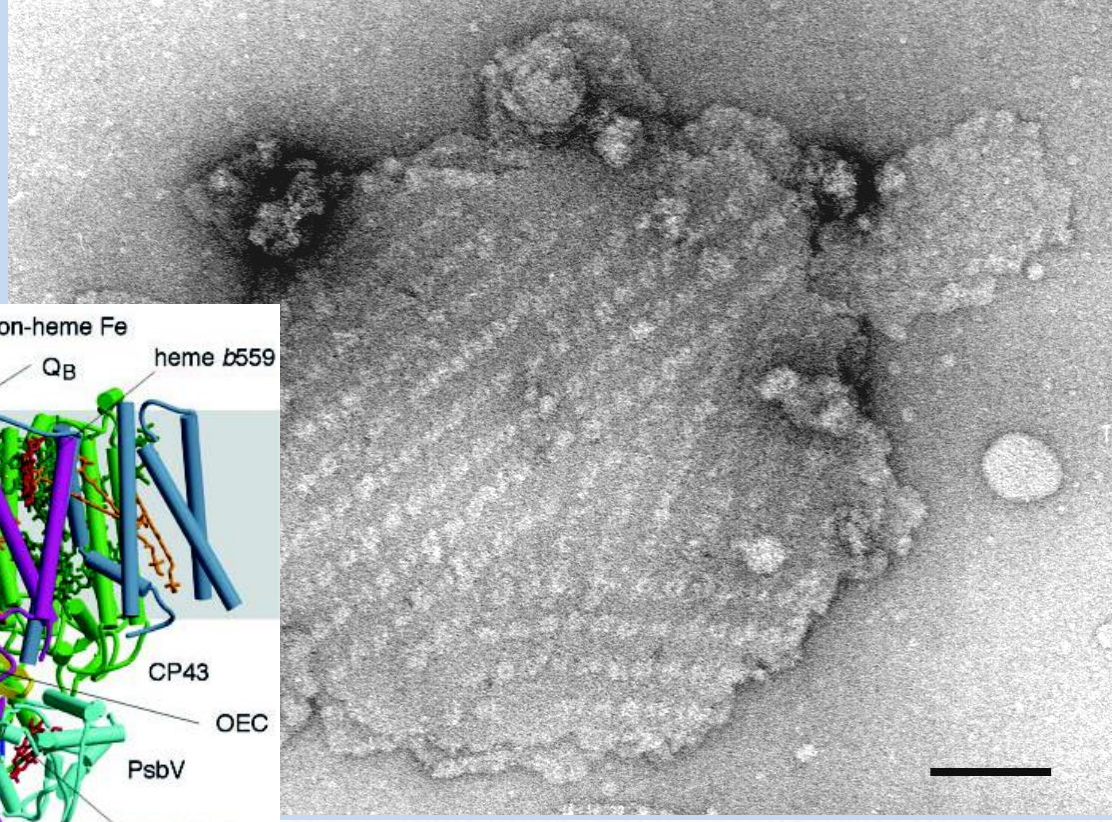
FIGURE 3 The CD spectra of aggregates with different sizes: The pitch and the chromophore density are the same for both aggregates, which are 300 nm and 2 chromophores/nm^3, respectively. (1) The solid line: volume = 40^3 nm^3; (2) the dashed line: volume = 200^3 nm^3.

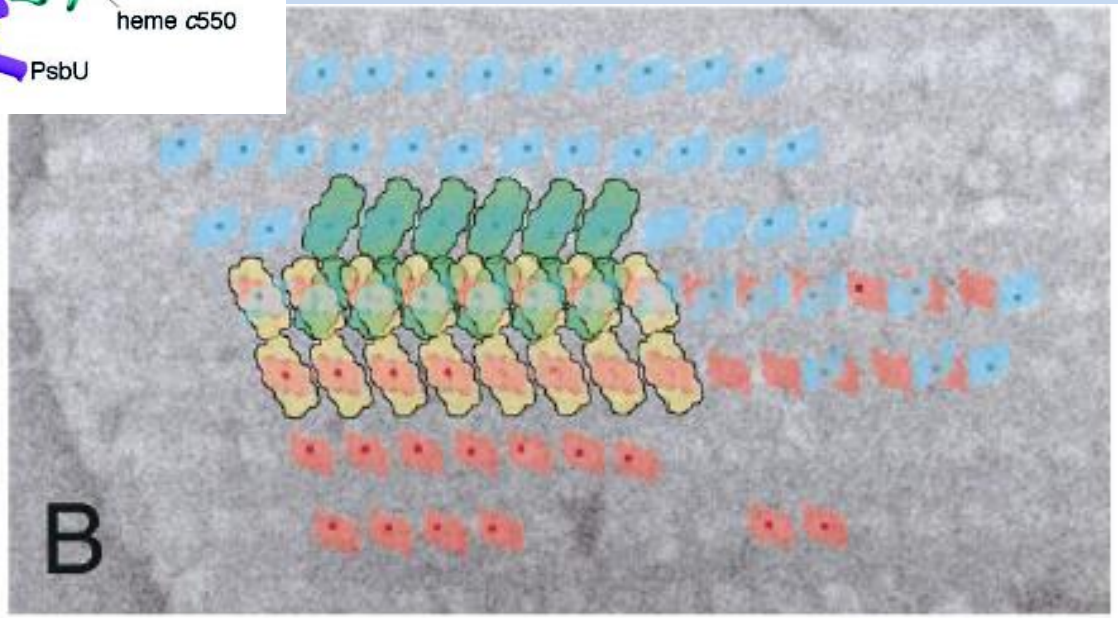
CD spectra of thylakoid membrane pigments at different levels of the structural hierarchy



Garab (1996) In: Biophysical Techniques in Photosynthesis, Garab and van Amerongen, 2009, Photosynth. Res.

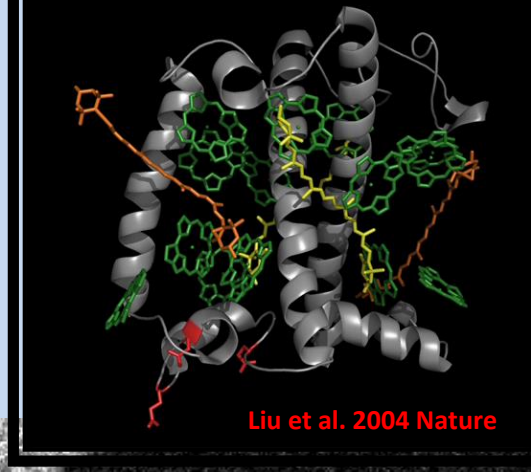


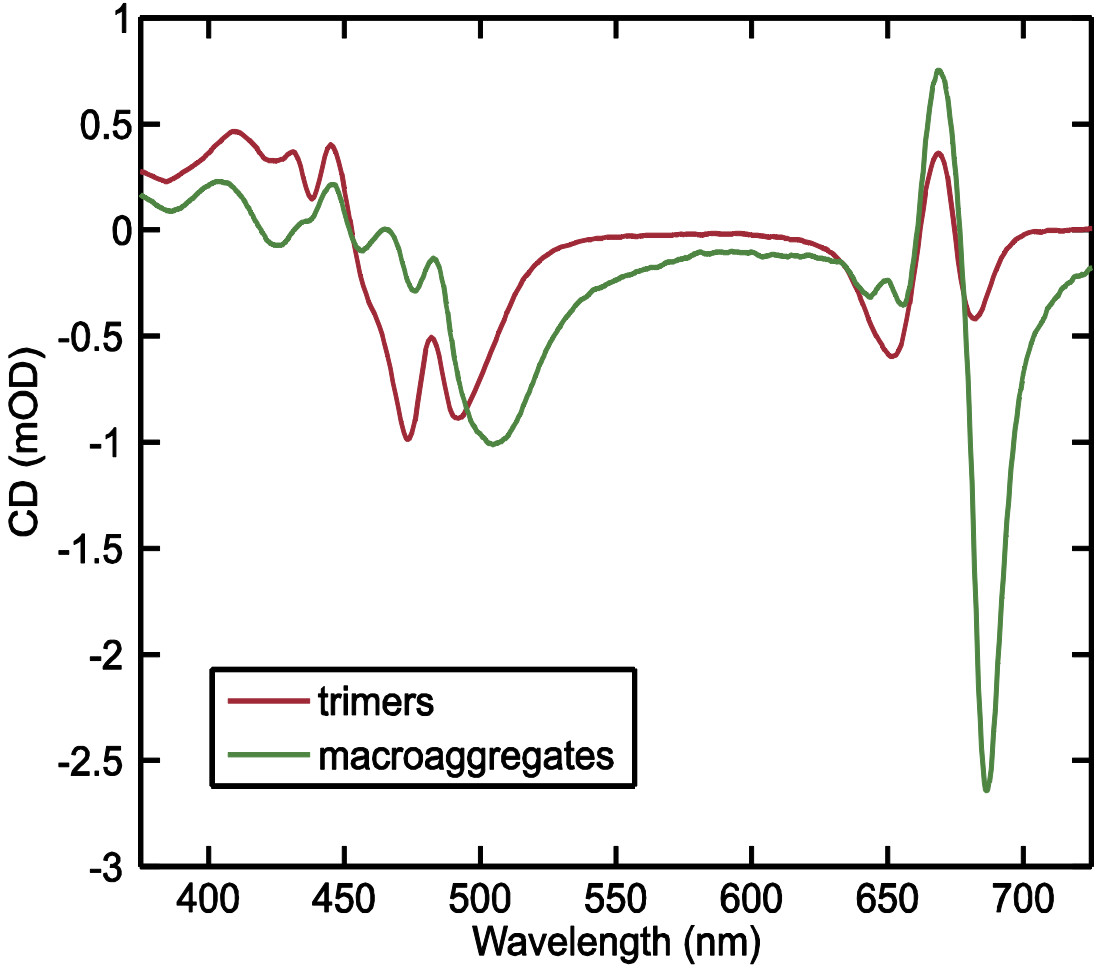


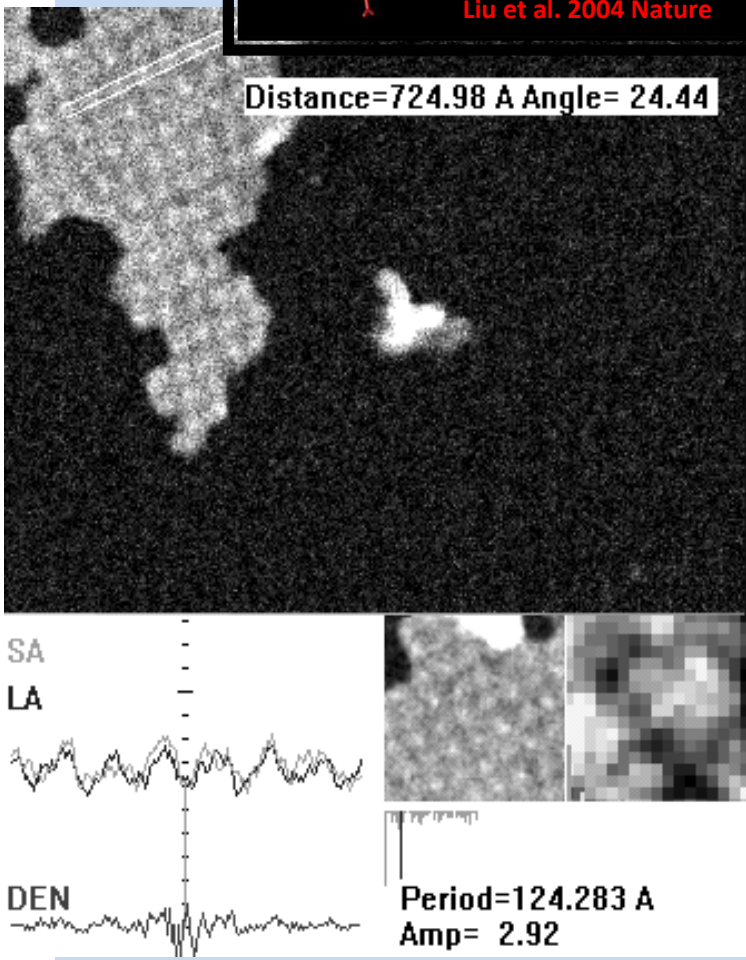


Boekema&Dekker; Barber

CD of LHCII samples





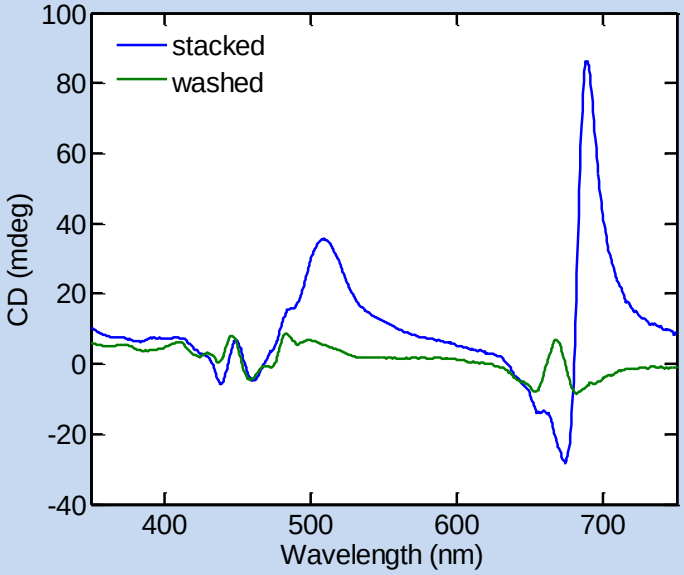


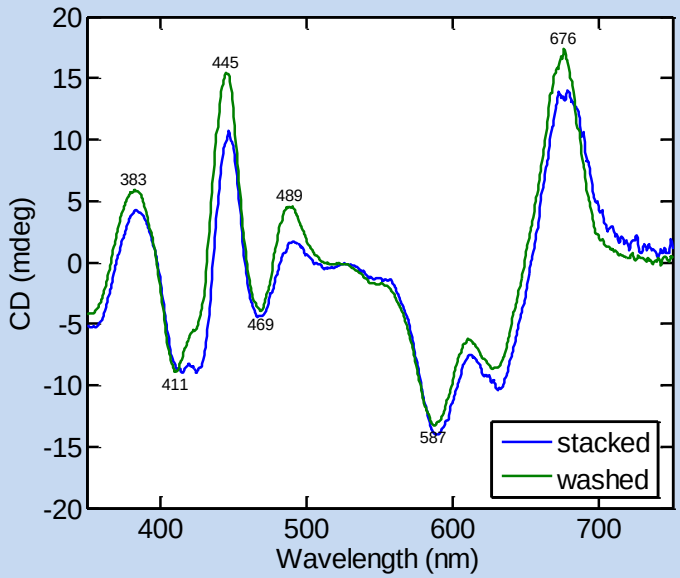
Miloslavina et al. 2011

CD and Magnetic CD

- MCD is the CD induced by magnetic field
- MCD is not affected by
  - supramolecular organization
  - orientation of the sample
  - differential scattering

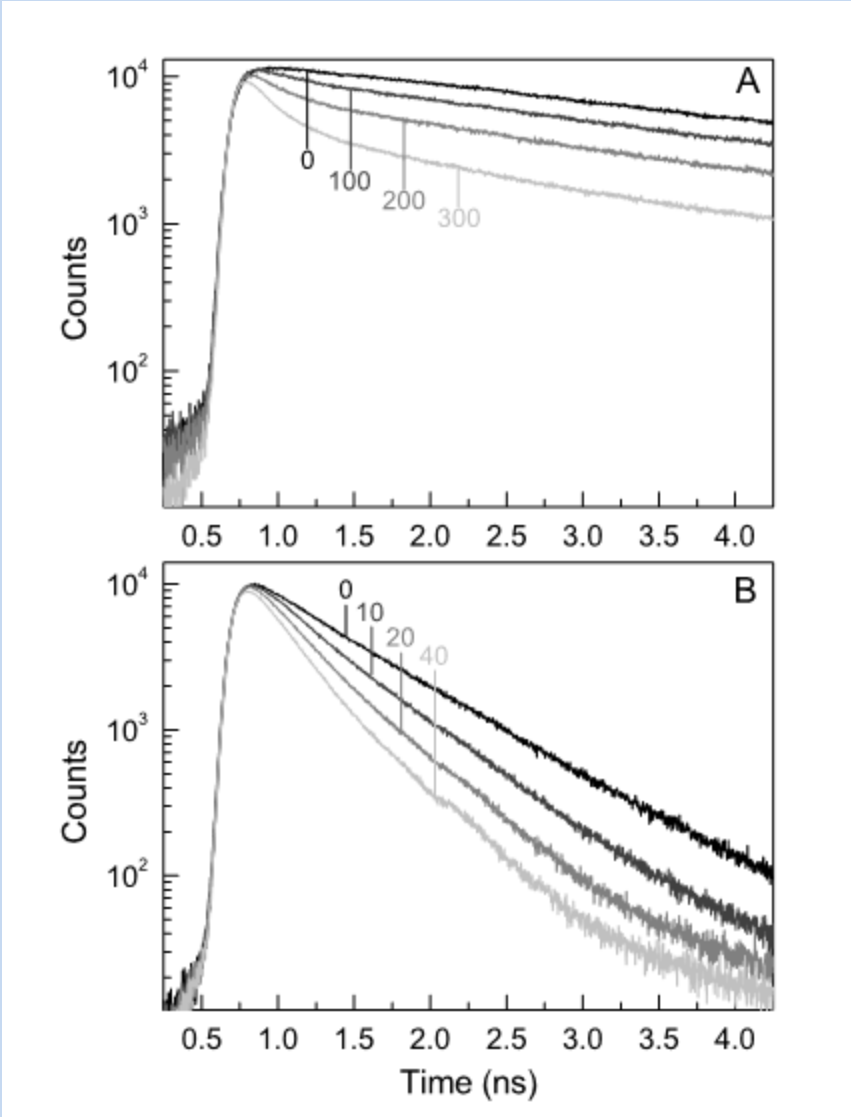
  Thus, an an ‚internal standard', shows that psi-type CD is not an optical artefact.





Miloslavina et al. 2011

The effective (functional) domain size



Fluorescence decay kinetics of solubilized LHCII trimers (exc. 632 nm, detected 682 nm)

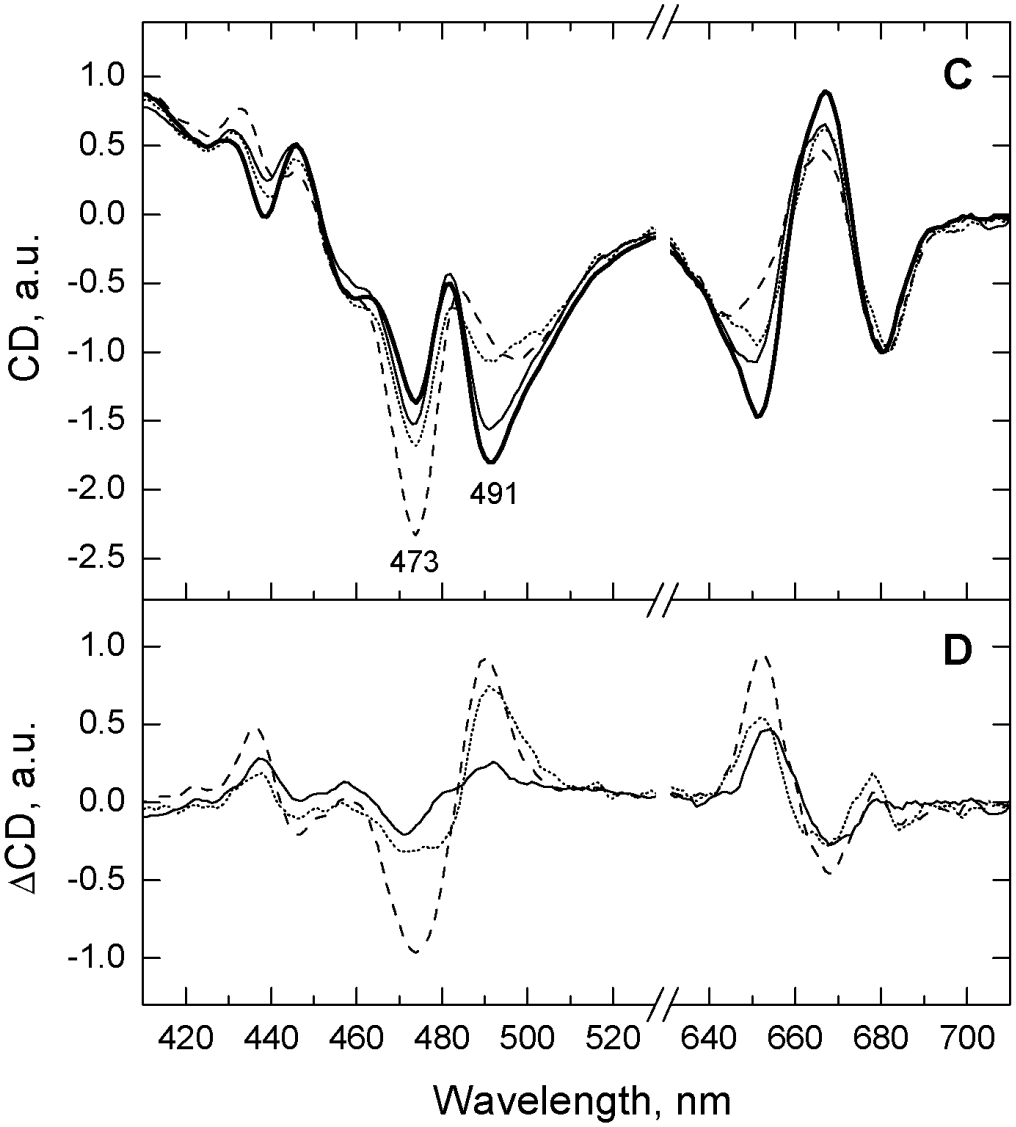
Fluorescence decay kinetics of LHCII macroaggregates in the presence of different concentrations of PPQ (phenyl-*p*-benzoquinone) (exc. 632 nm, detected 682 nm)

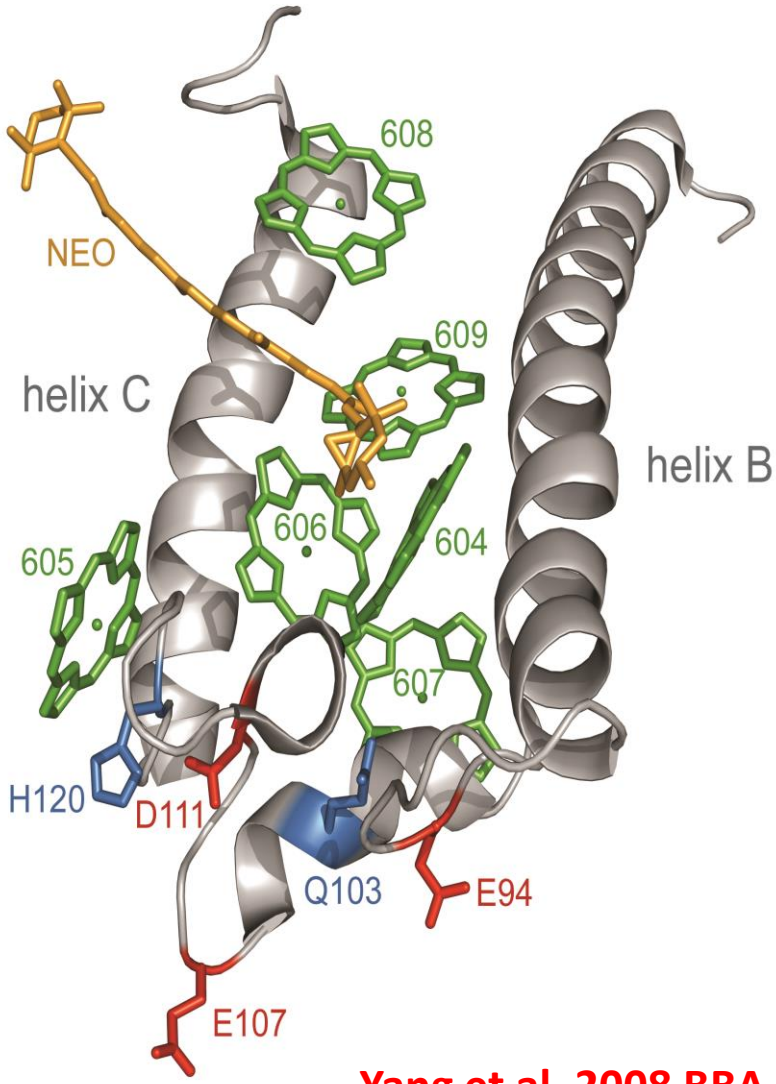
Lambrev et al. BBA 2011

Table 2. Apparent quenching rates K_Q and calculated domain sizes N in isolated LHCII.

Quencher	**LHCII type**	**K_Q (M^{-1} ps^{-1})**	***N***	**Unit**
PPQ	Aggregates (Bio-beads)	97.6	30	trimers
	Lamellar macroaggregates	83.0	25	trimers
	Aggregated LHCII monomers	47.9	31	monomers
	Trimers	3.3	3	monomers
	Monomers	1.5		
DNB	Aggregates (Bio-beads)	3.0	15	trimers
	Lamellar macroaggregates	4.9	25	trimers
	Trimers	0.2		

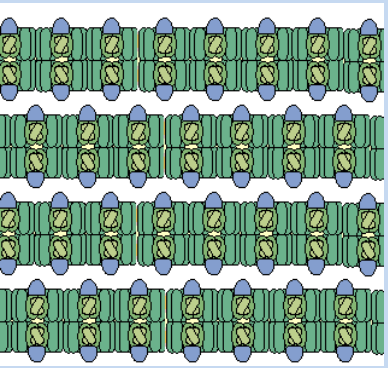
Sensitivity of excitonic interactions to minor changes in the protein scaffold

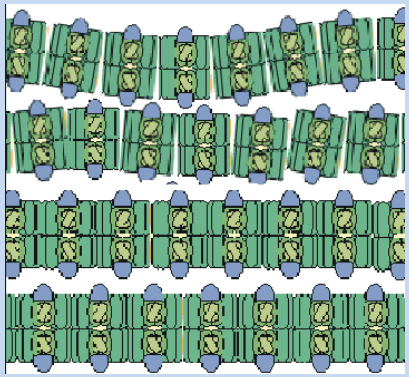


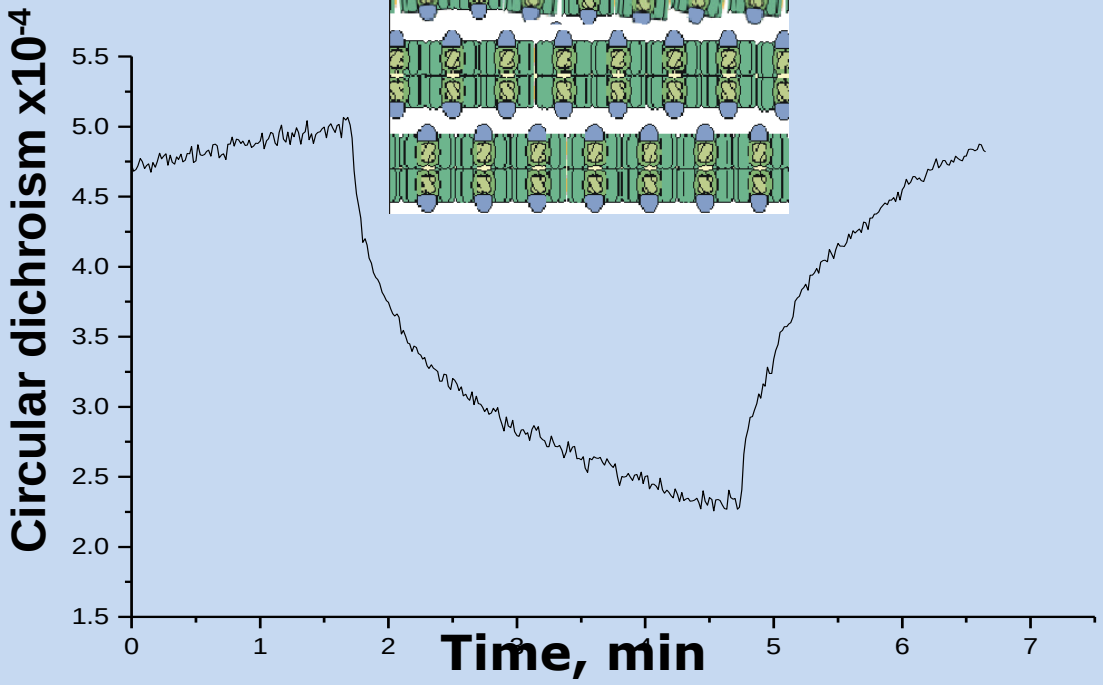


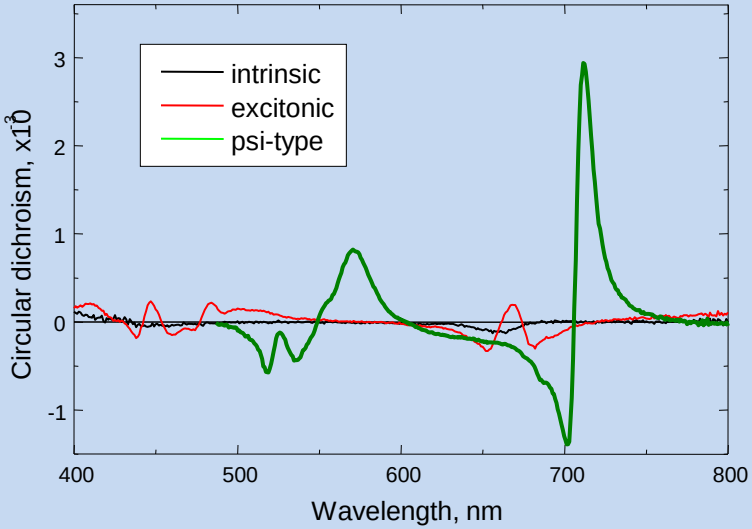
Yang et al. 2008 BBA

Light-induced reversible reorganizations in the chiral macrodomains of thylakoid membranes



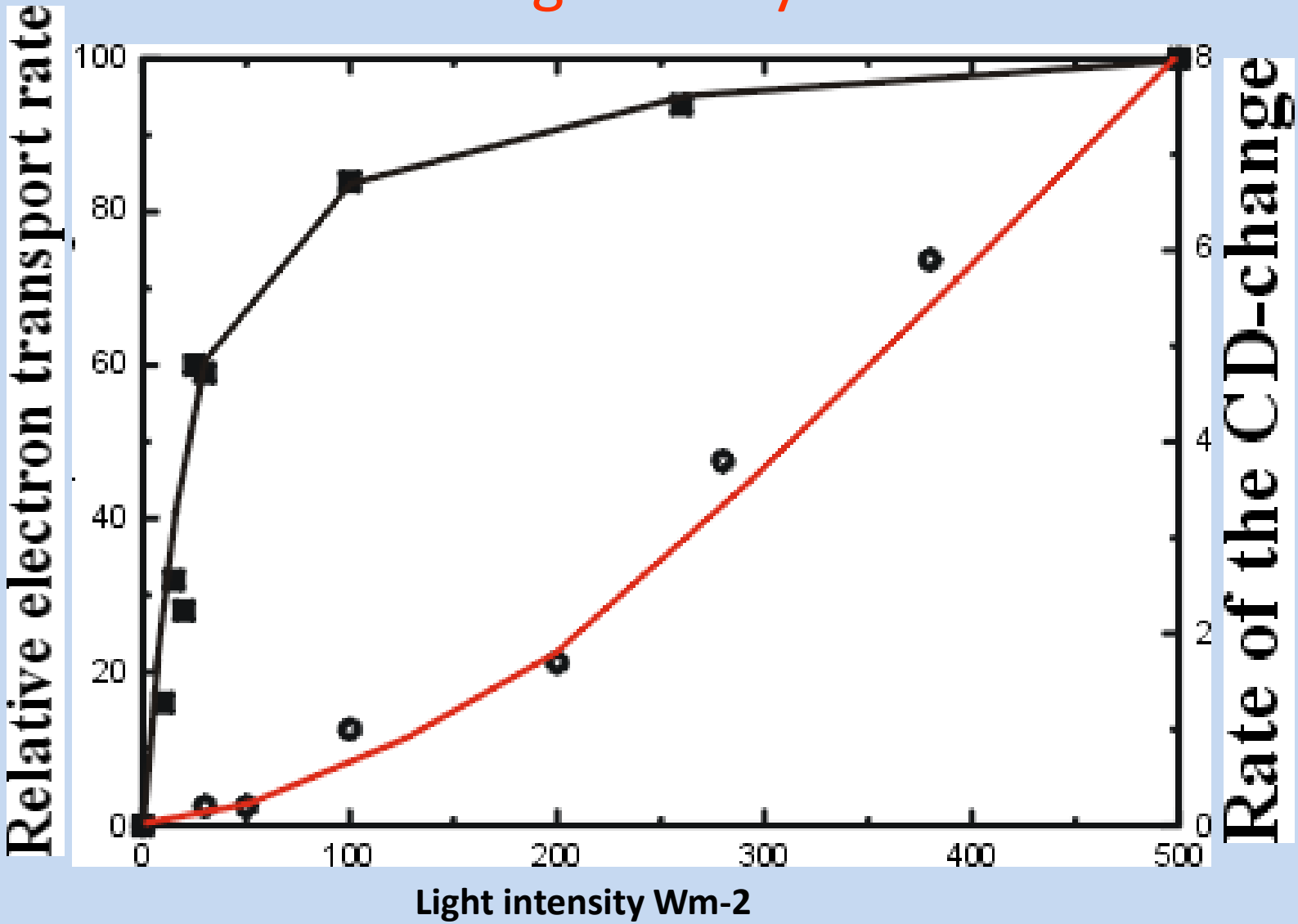






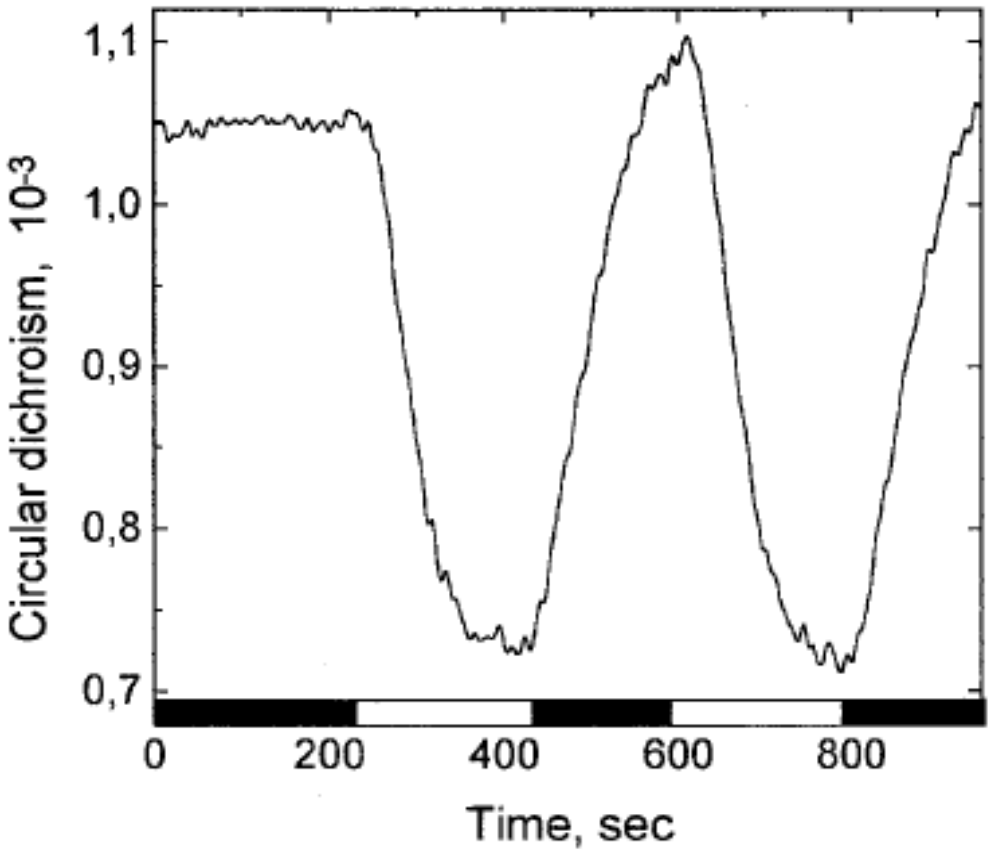
Garab et al. 1988, Biochemistry, Barzda et al. 1996 Biochemistry

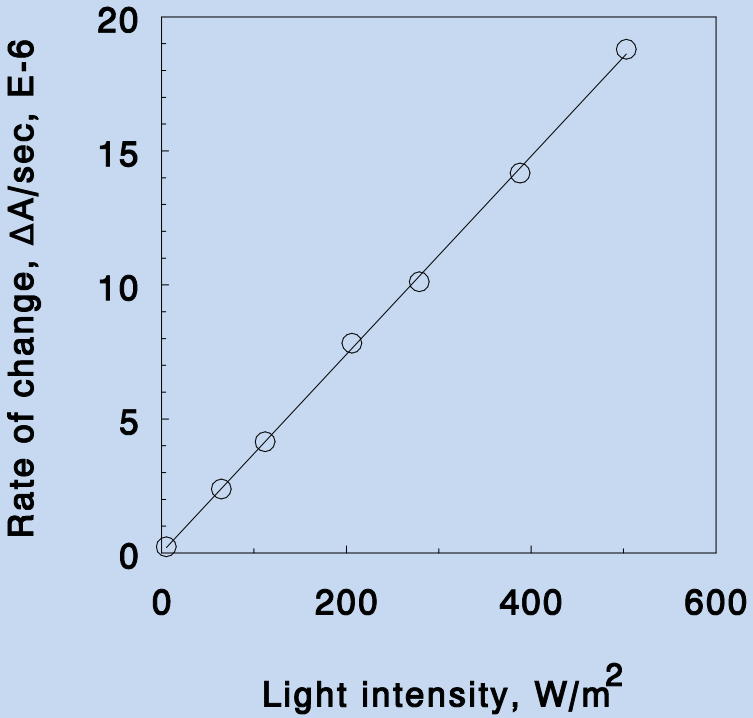
Quasi-linear light intensity dependence of the CD changes in thylakoids



Barzda et al. Biochemistry 1996

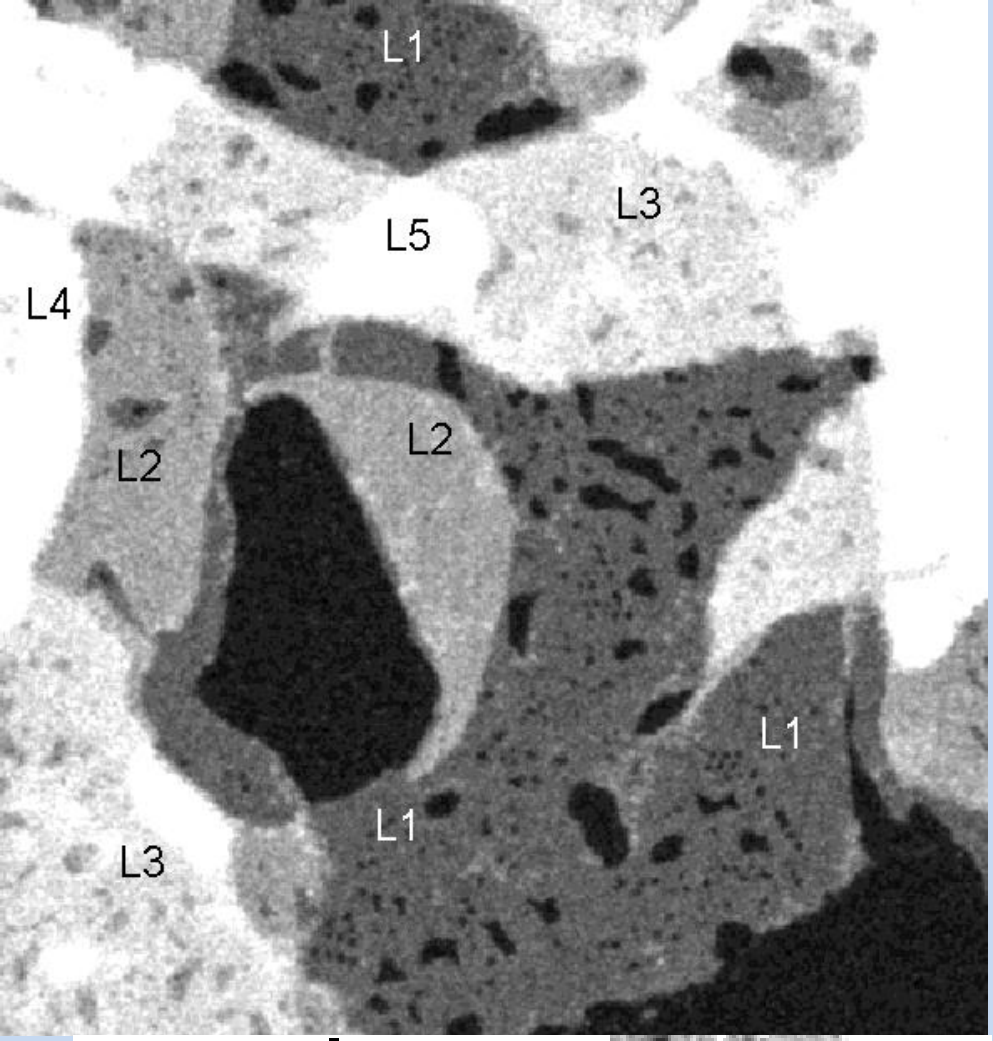
Thermo-optically induced reversible reorganizations in lamellar aggregates of LHCII

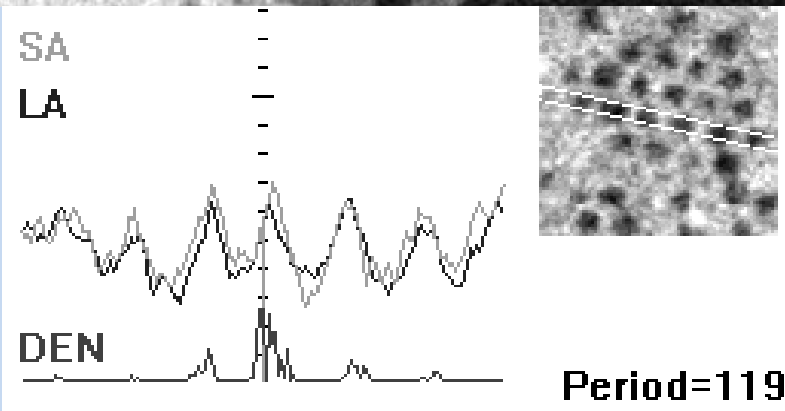


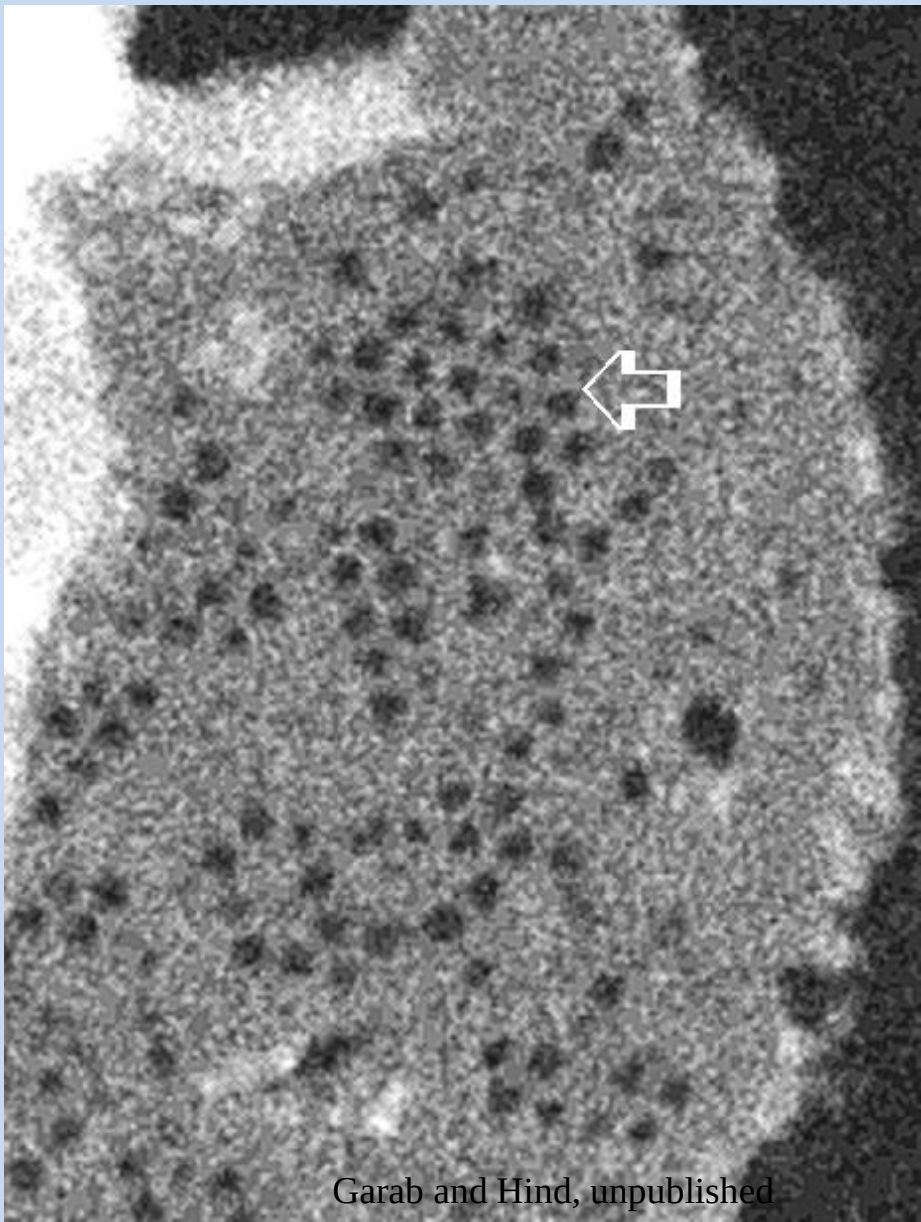


Simidjiev et al. Anal Biochem 1997, Cseh et al. 2005 Photosynth. Res.

STEM of LHCII in the dark

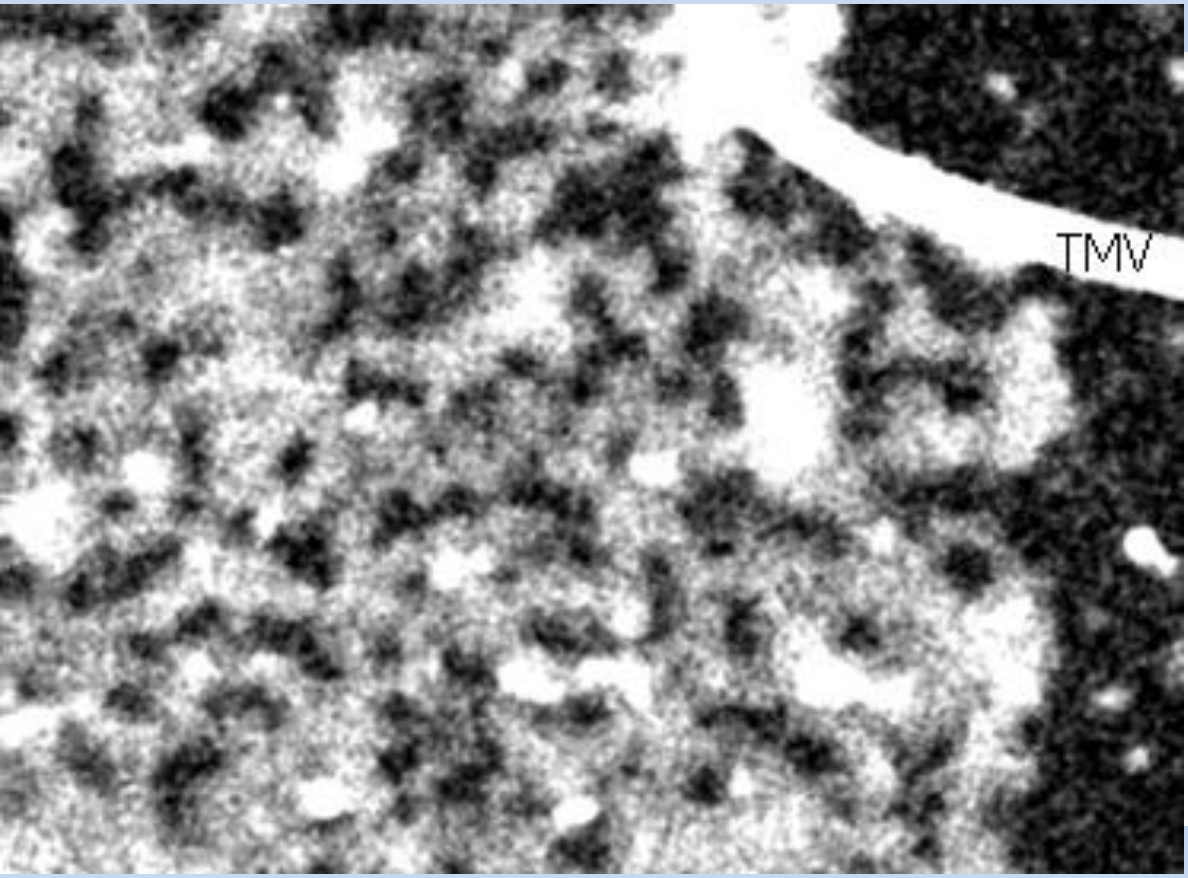


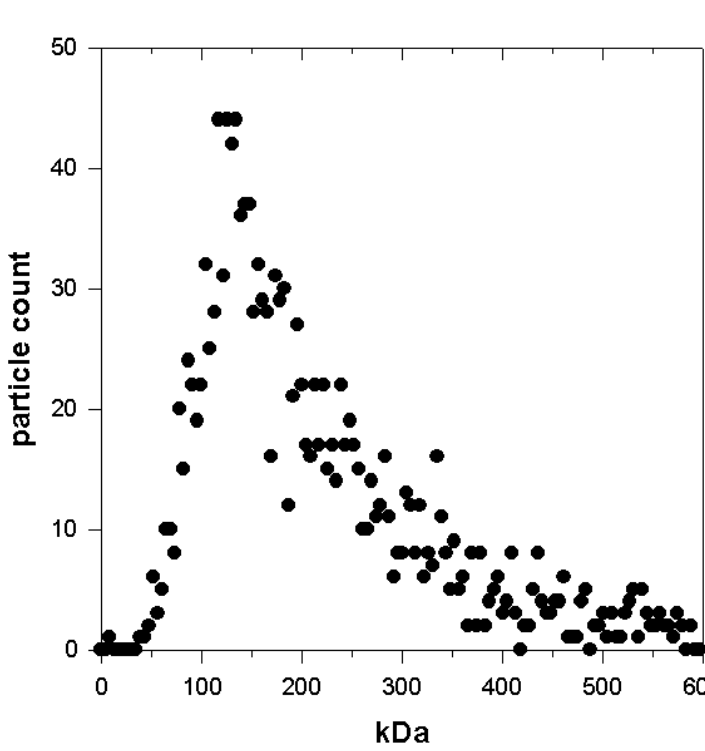




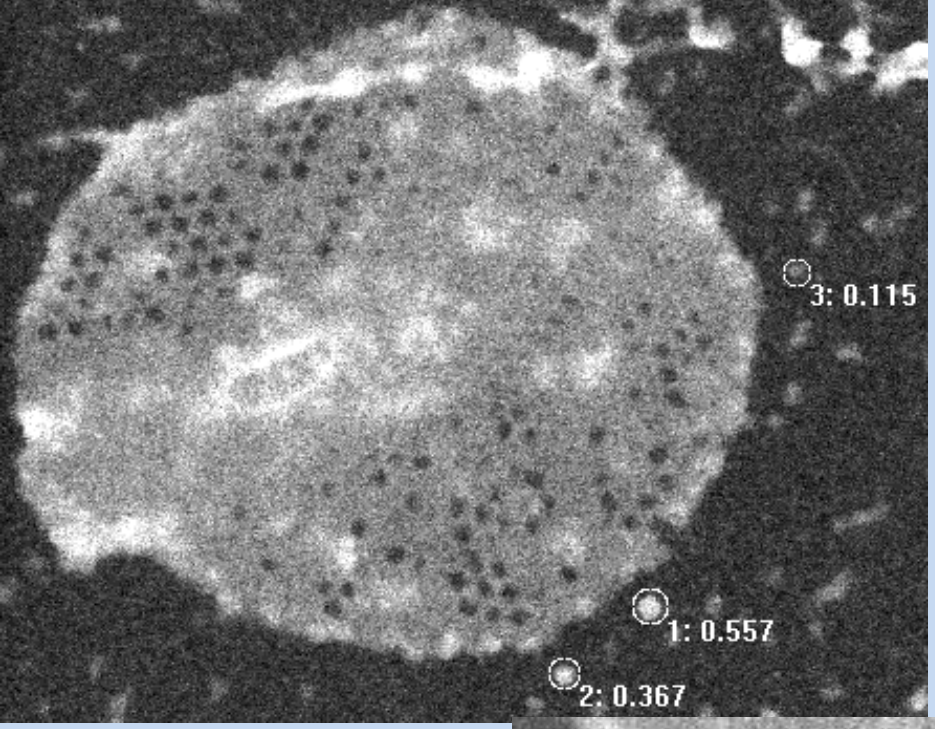
Garab and Hind, unpublished

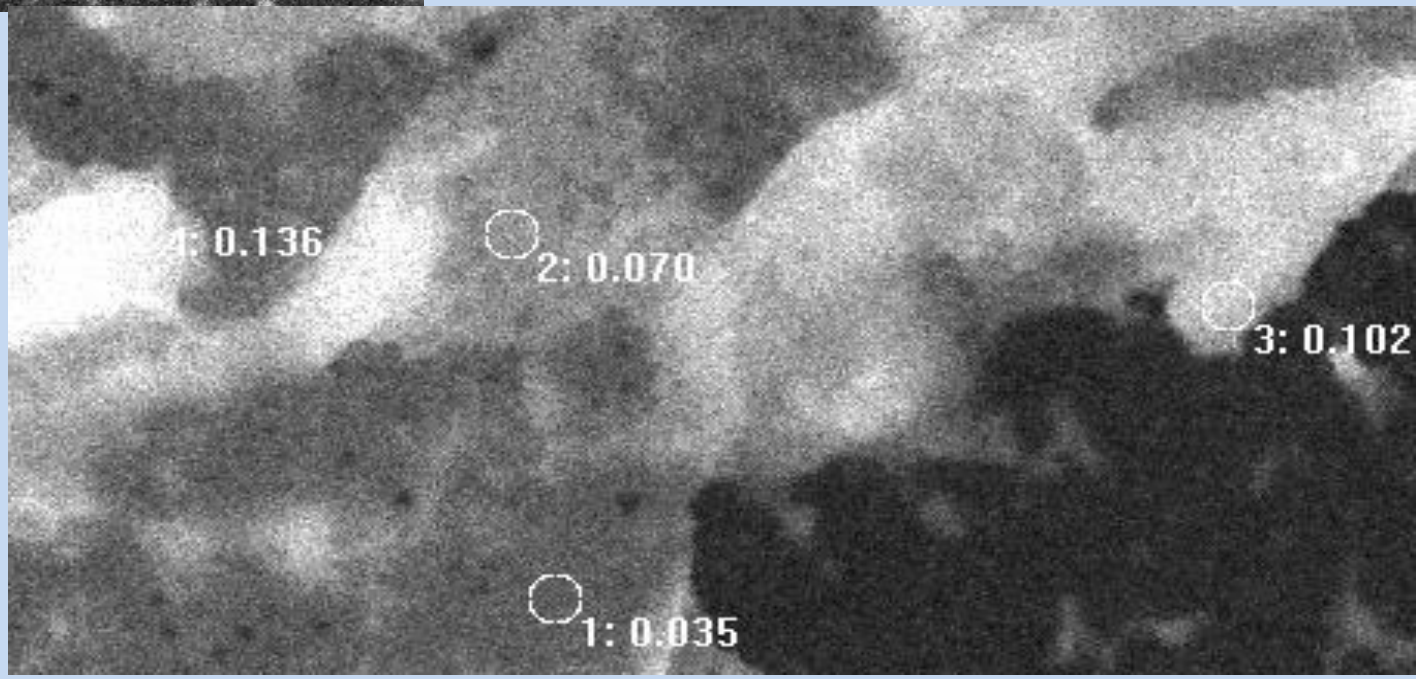
STEM of LHCII after preillumination (3 min 2000 µE)





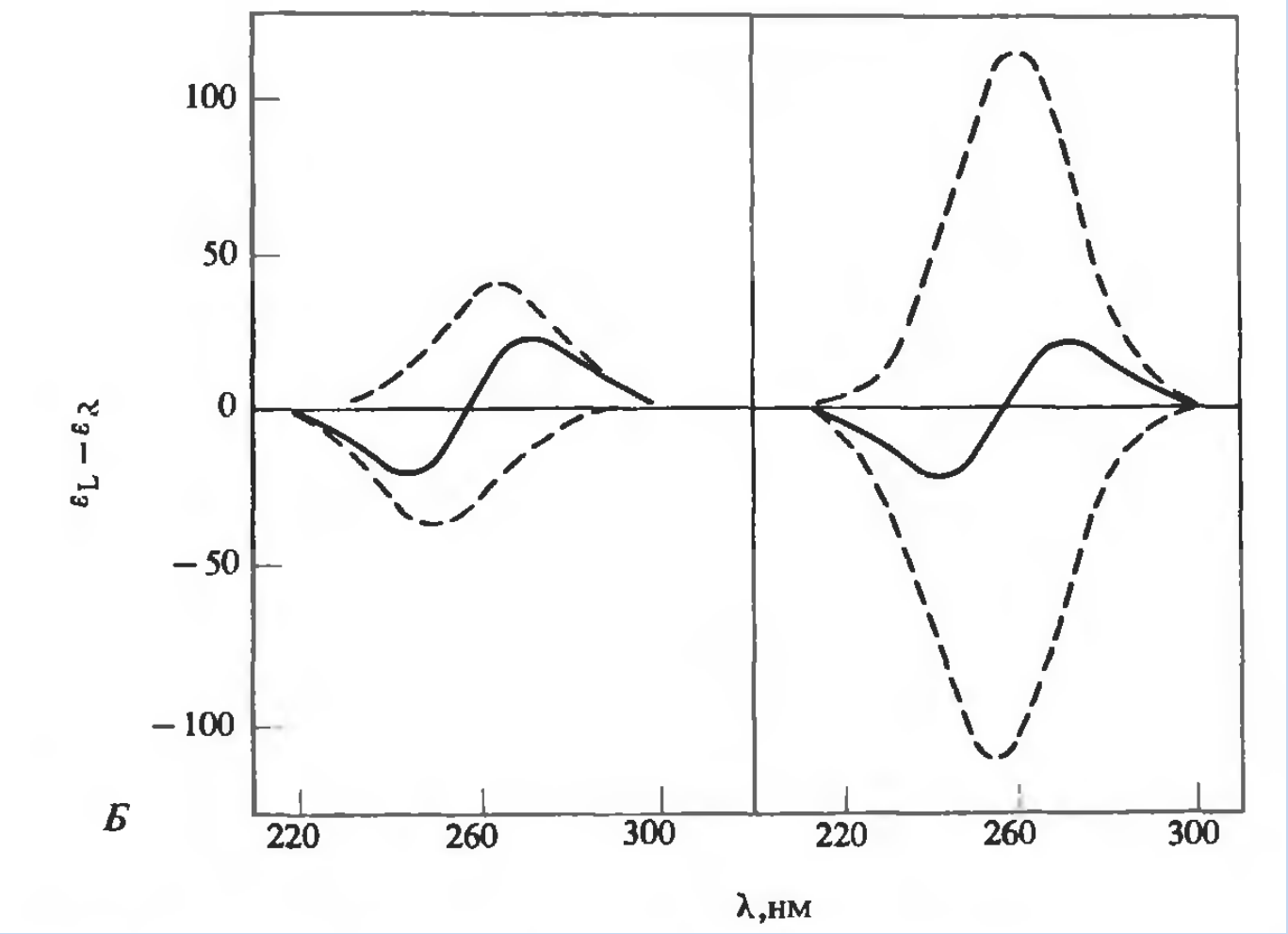
STEM of LHCII in the re-dark (10 min after preillumination)





G. Hind, J. Wall, Zs. Várkonyi, A. Istokovics, P. Lambrev, G. Garab – Plant&Cell Physiol 2014

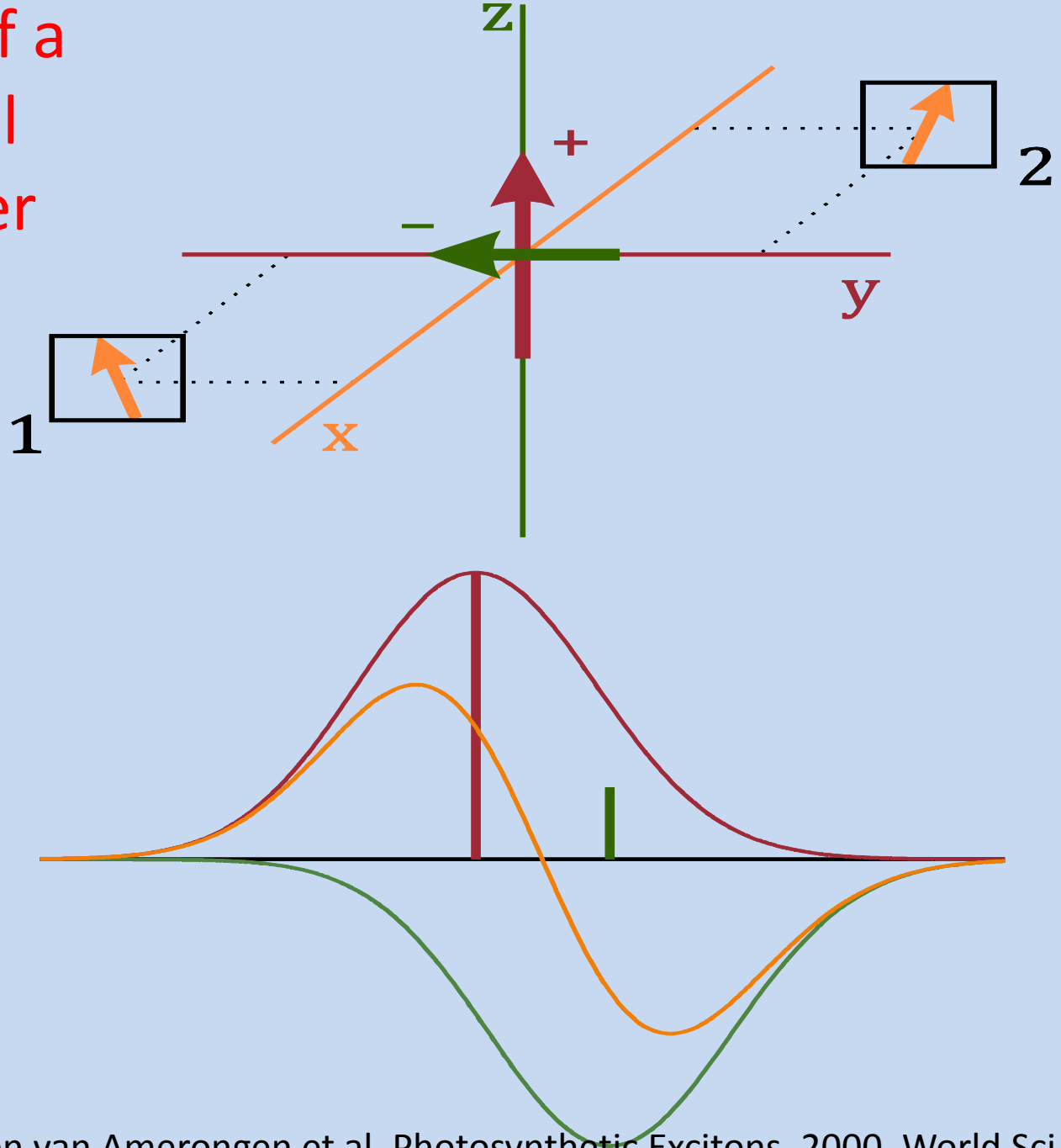
CD is a sensitive probe of the (dynamic) molecular architecture of complexes, and of their macroarrays



CD of excitonic dimers. Solid line – sum (isotropic) CD. Dashed lines – individual excitonic components. Left – monomers at 15°. Right – monomers at 75°.

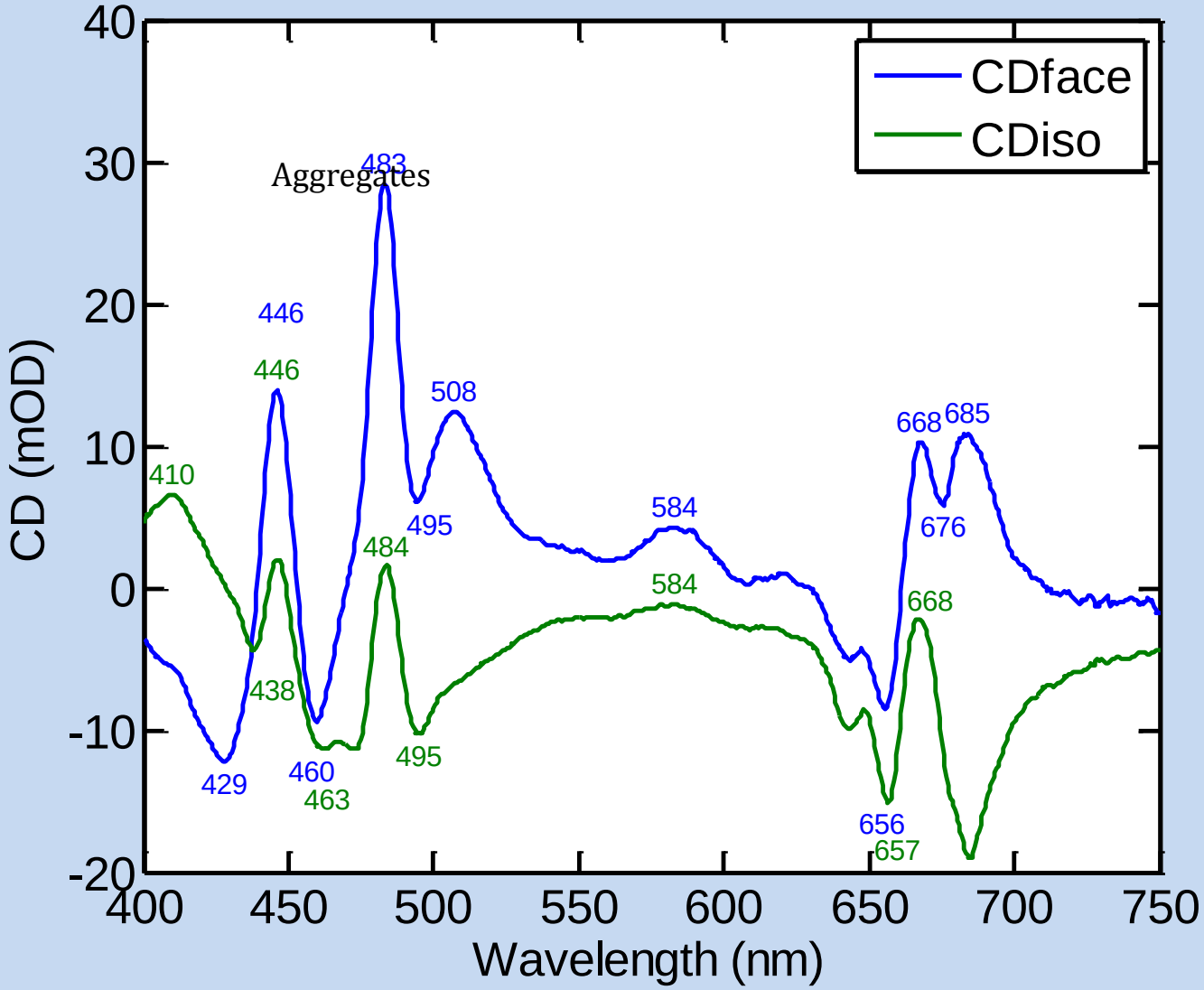
Cantor, C.R. & Schimmel 1980 Freeman & Co.

Abs and CD of a hypothetical exciton dimer

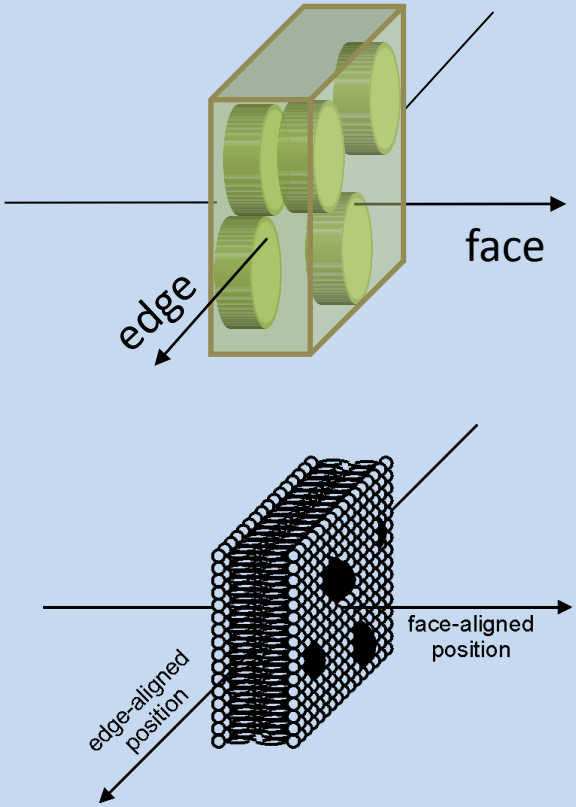


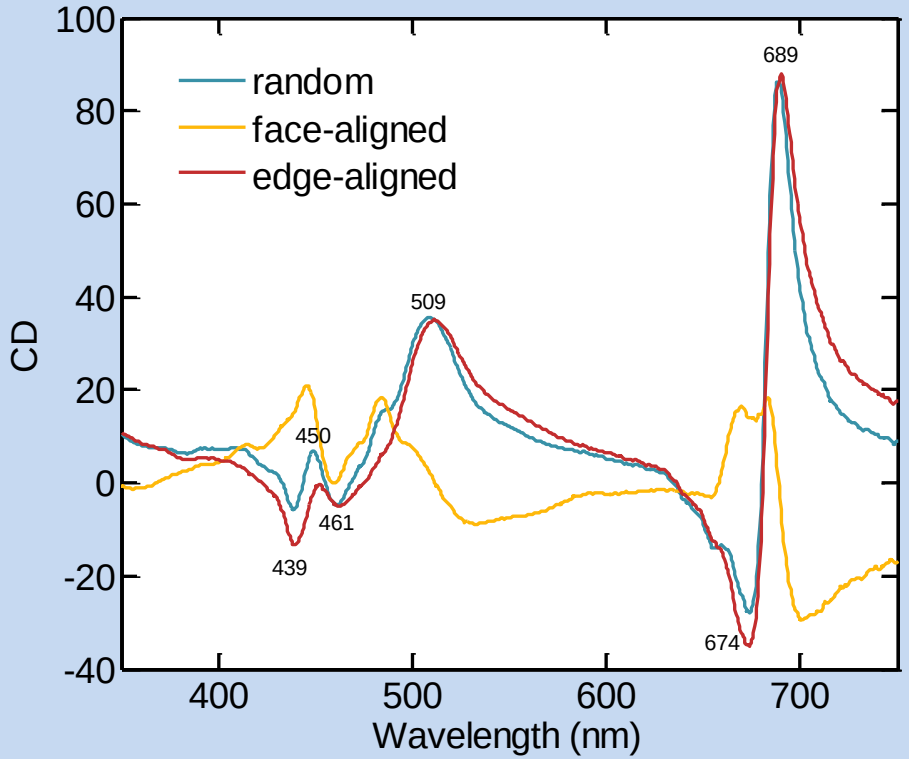
Lambrev and Garab– based on van Amerongen et al. Photosynthetic Excitons, 2000, World Sci

CD and ACD of LHCII aggregates



ACD of chloroplast membranes





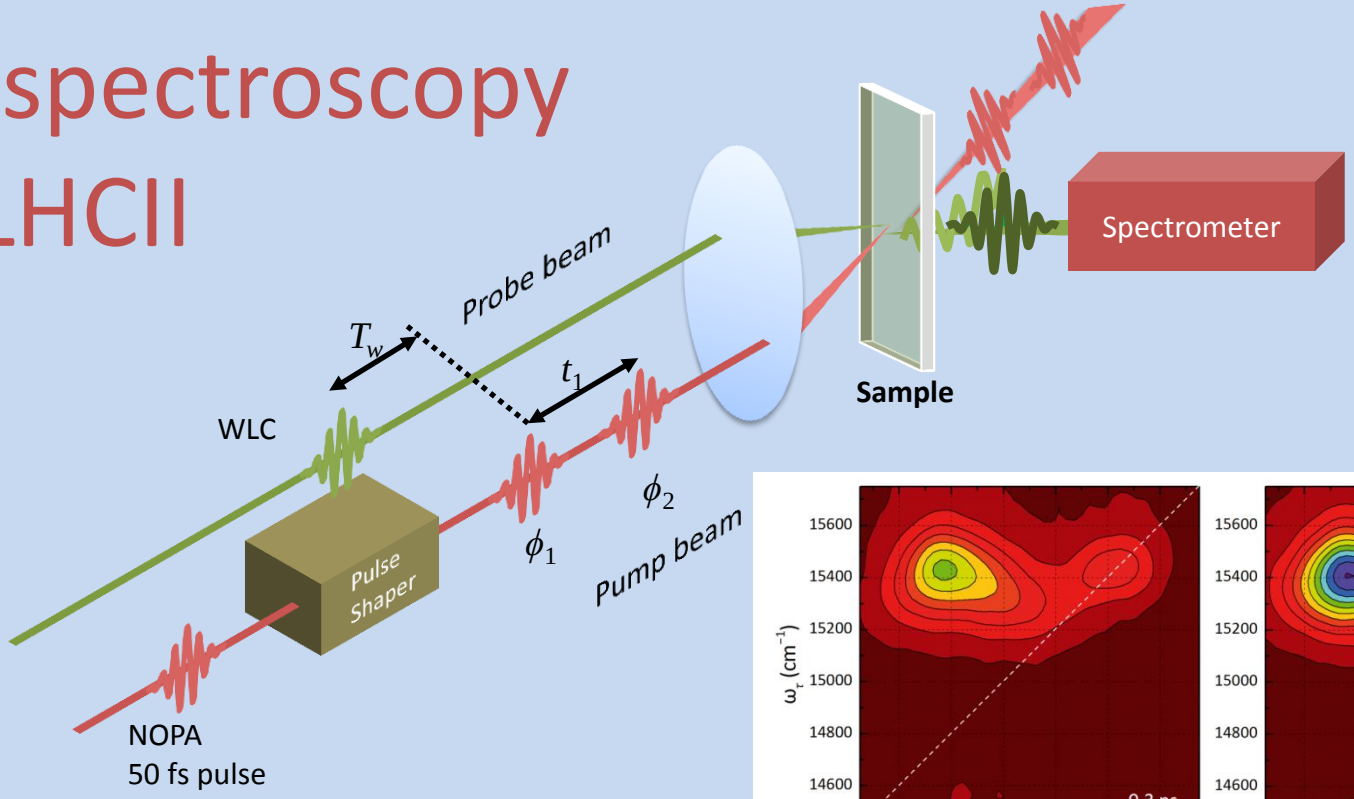
Miloslavina et al. 2011 Photosynth. Res. 111:29-39

ACD is a (novel) tool to reveal the origin of excitonic interactions in photosynthetic organisms

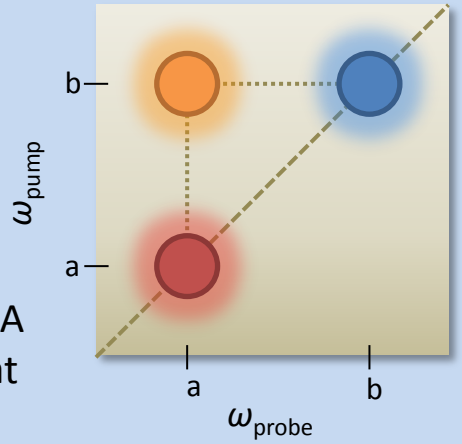
Light-Harvesting Complex II

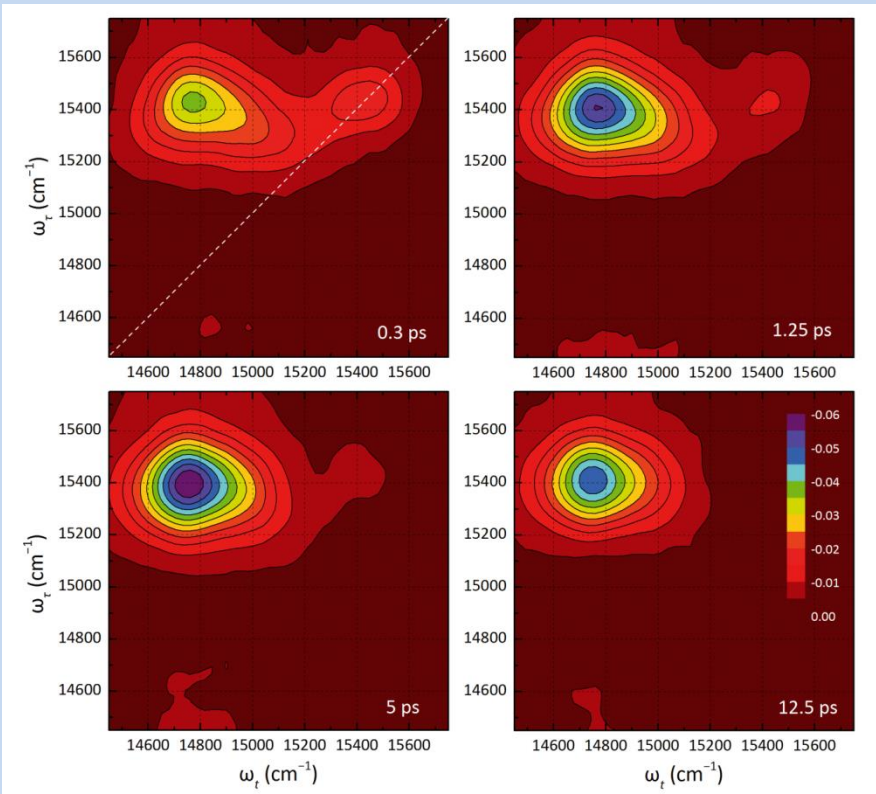
Liu et al. 2004 Nature

2D spectroscopy of LHCII



- Two frequency axes in 2D spectra:
  - 'pump' frequency reveals initially excited states
  - 'probe' frequency reveals coupled transitions
- Energy transfer from B to A manifested as a 2D peak at $\omega_{\mathrm{pump}} = \mathrm{b}, \omega_{\mathrm{probe}} = \mathrm{a}$



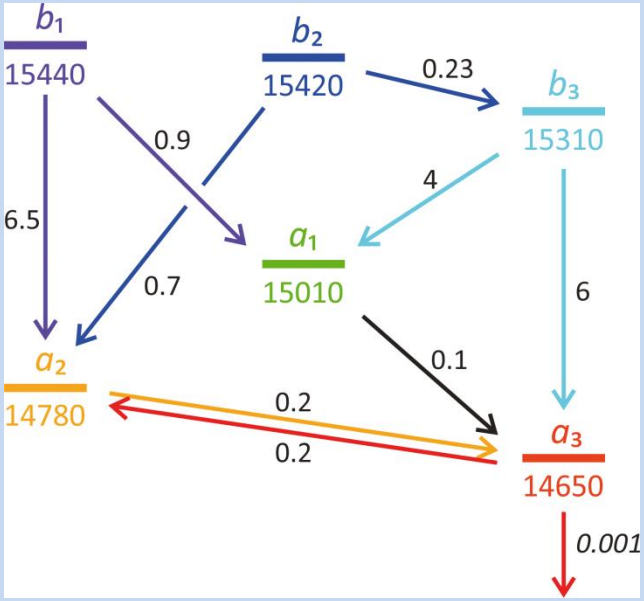


2D spectra of LHCII measured at different waiting times after Chl *b* excitation

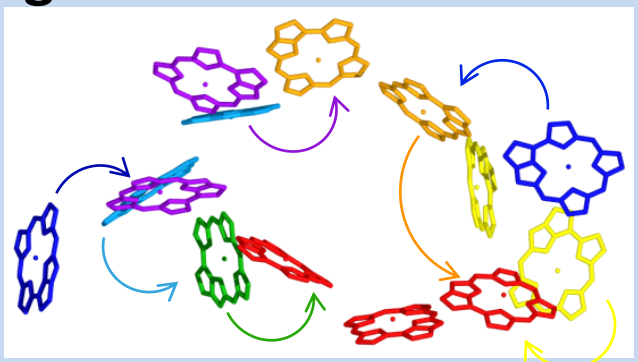
K Wells, Z Zhang, PH Lambrev, G Garab and H-S Tan Phys Chem Chem Phys 2014

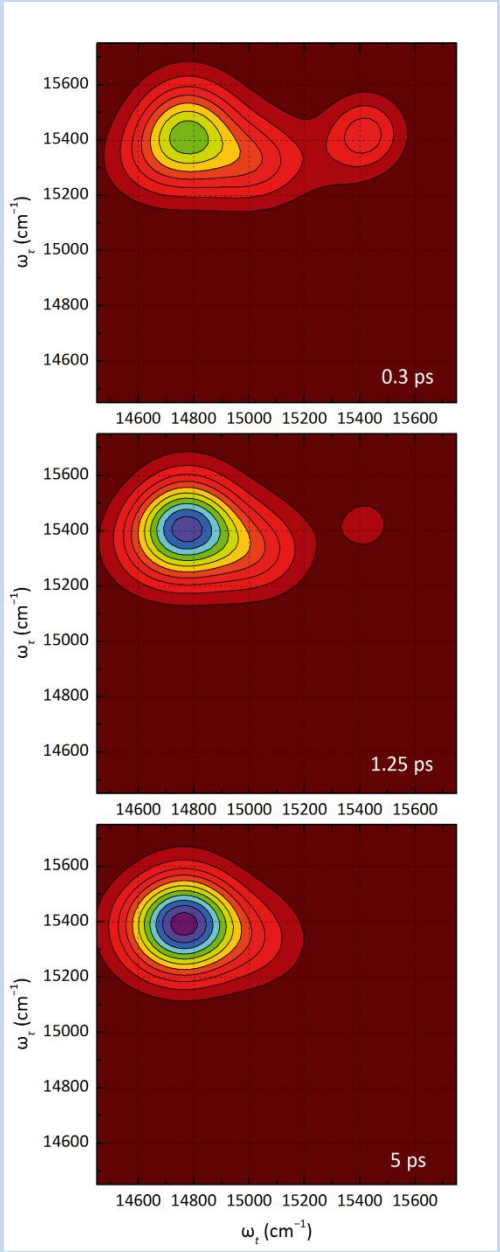
Modelling of the 2DES data

Model scheme



Pigment clusters in LHCII



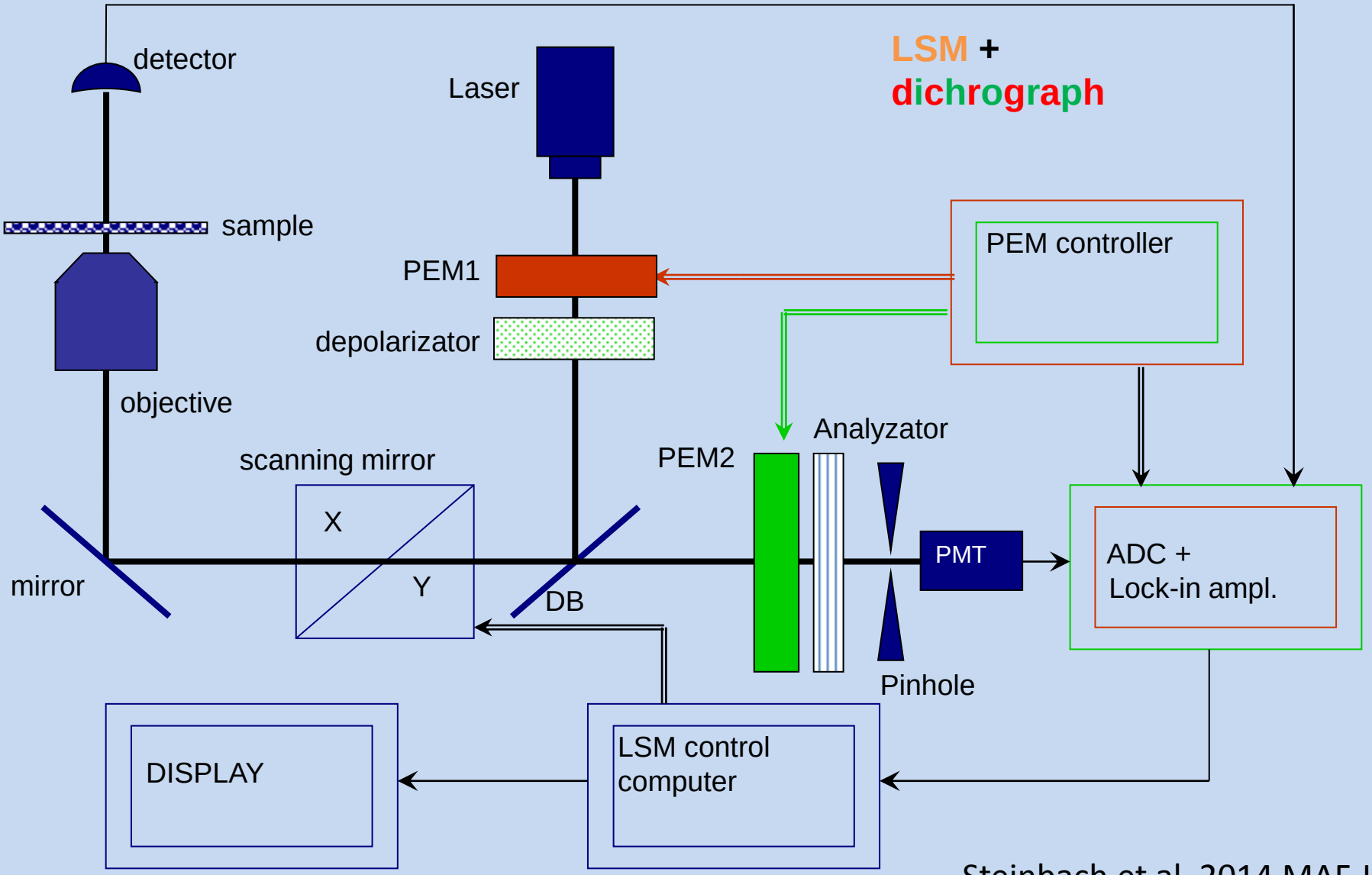


Simulated spectra

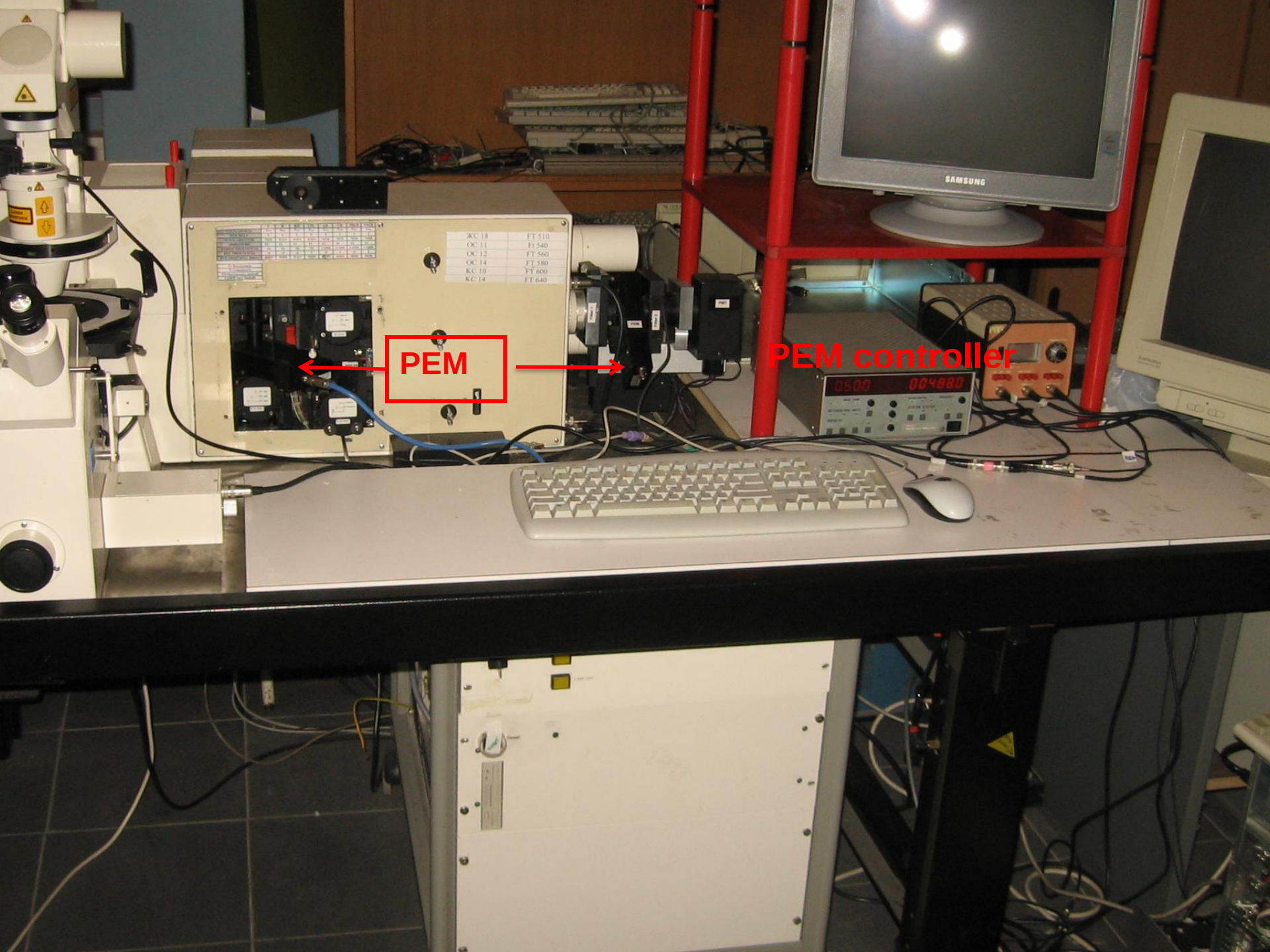
- The time-dependent 2D spectra are fitted with a spectro-temporal model
- The modelling results reveal exciton state energies and rate constants of energy transfer
- Simulated 2D spectra reproduce very well the measured ones at all T_w
- Exciton states can be assigned to actual pigments in the structure
- The first experimentally derived model detailed enough to disentangle the complex energy pathways in LHCII (between chl-b and chl-a)

2D spectroscopy is an efficient tool to map the energy migration pathways

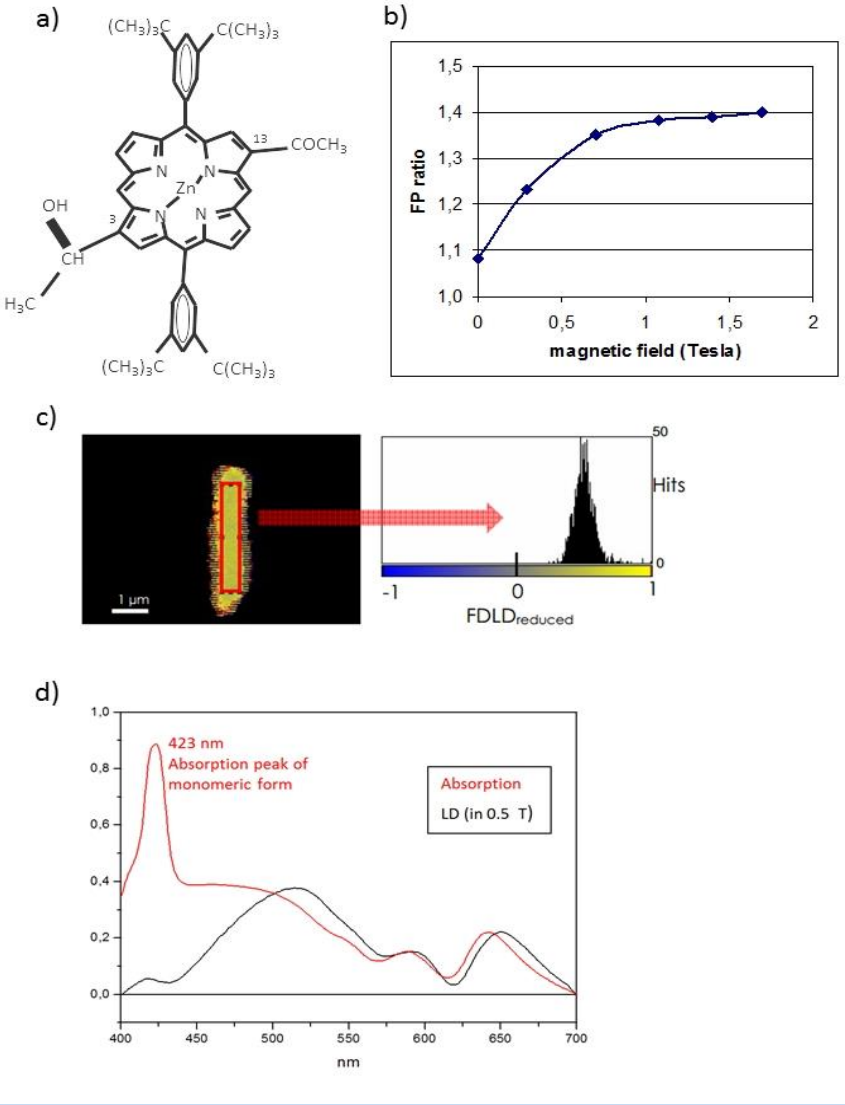
General Scheme of DP-LSM

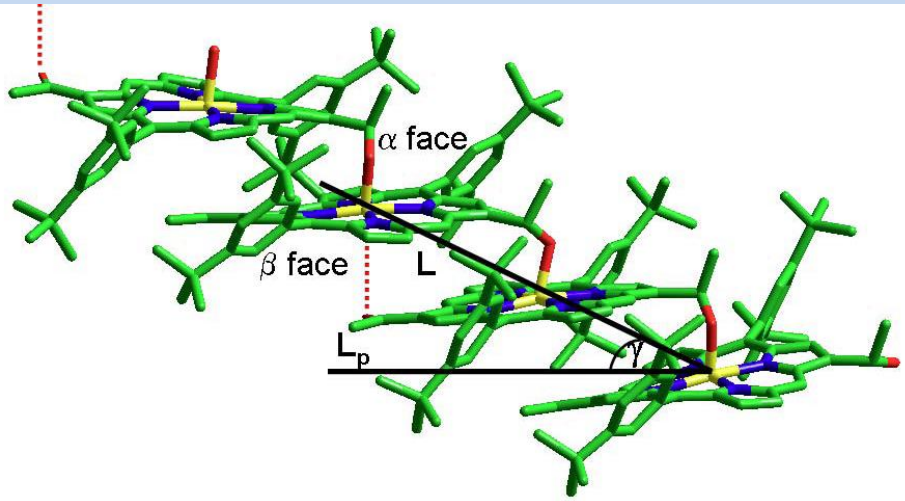


Steinbach et al. 2014 MAF J


PEM
PEM controller

Artificial chlorosome



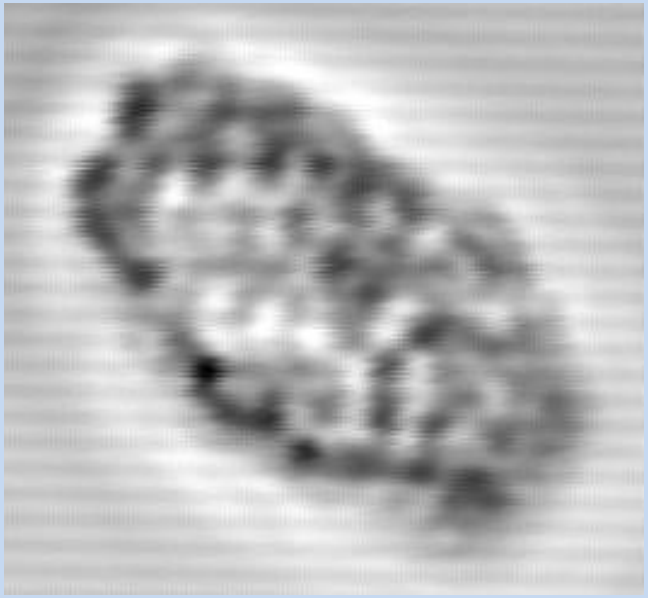


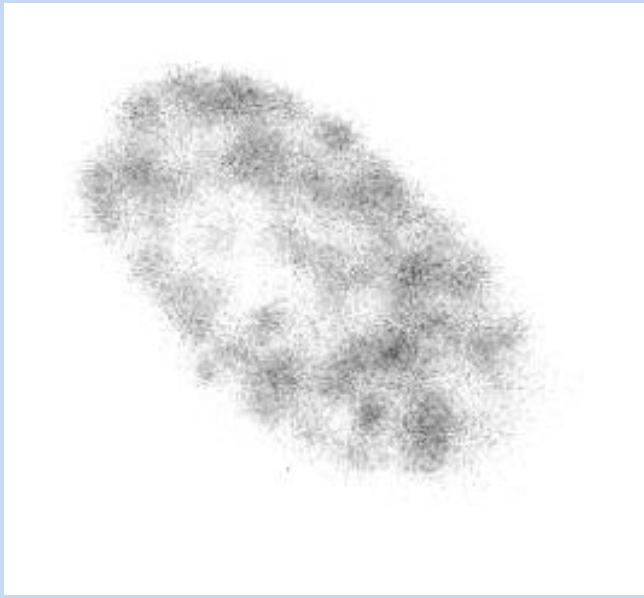
Orientation angles of the dipoles with respect to the long axes of the rods:

- Q_x 43°±8 (SE:5.6)
- Q_y 37°±7 (SE:0.42)
- B_x 39°±4 (SE:0.73)

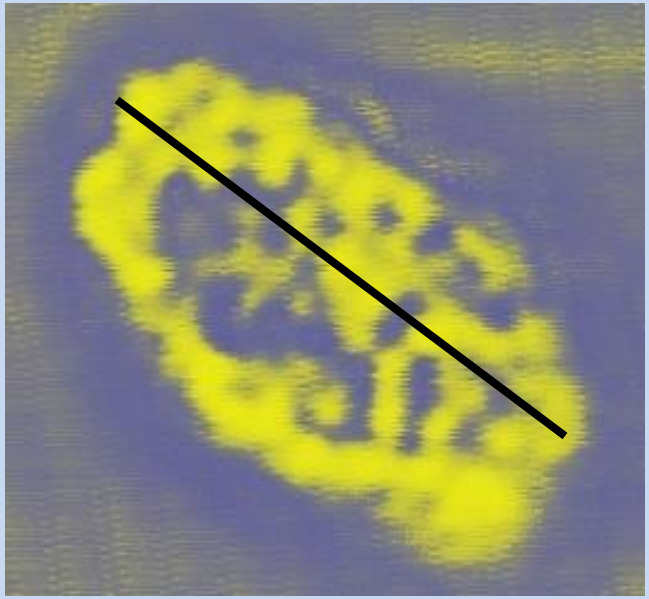
Chappaz-Gillot et al. 2011 JACS

Birefringence of chloroplasts





Transmission

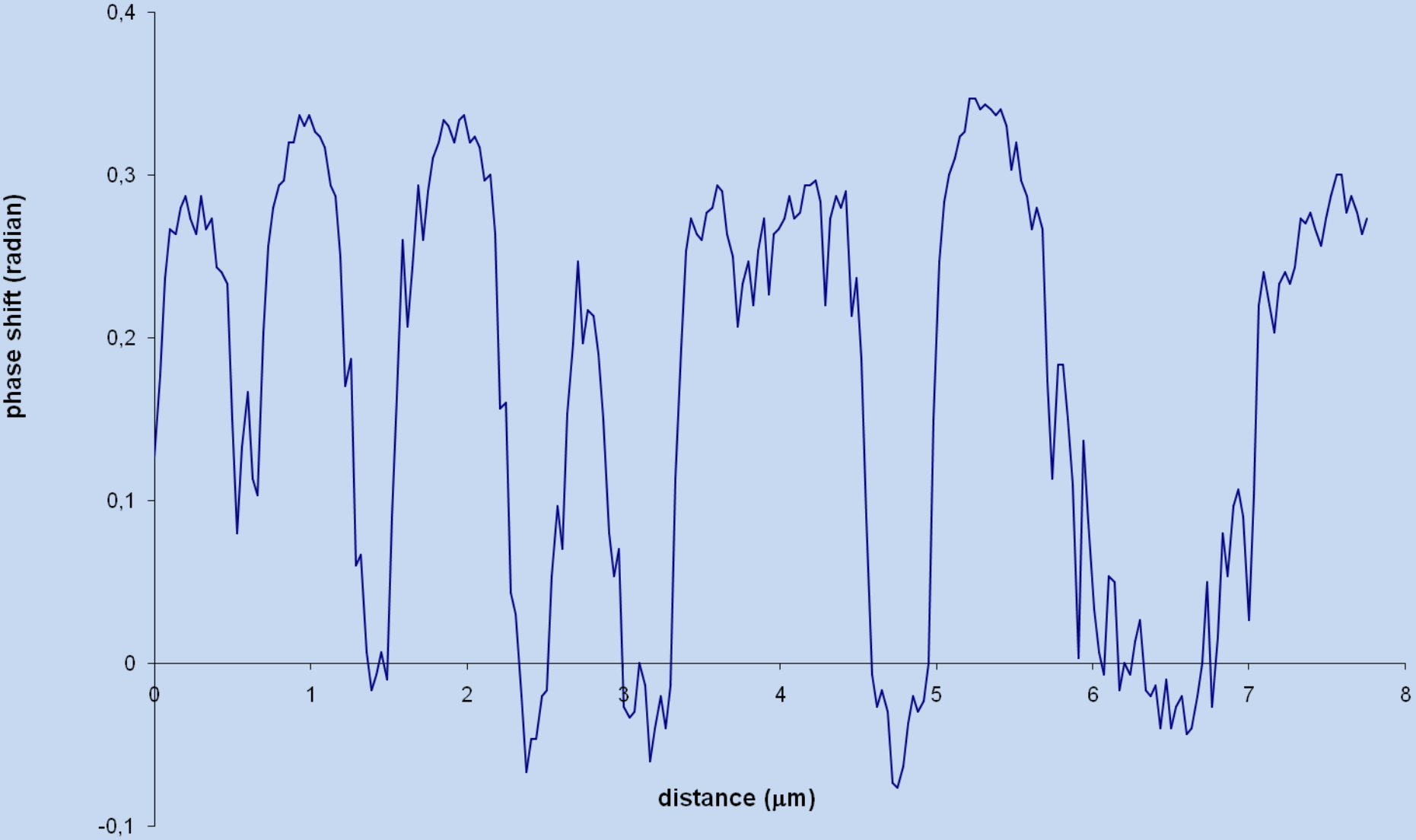


Fluorescence I

LB, linear birefringence

Garab et al. 2005, Eur. Biophys. J.; Steinbach et al SPIE Proc 2005

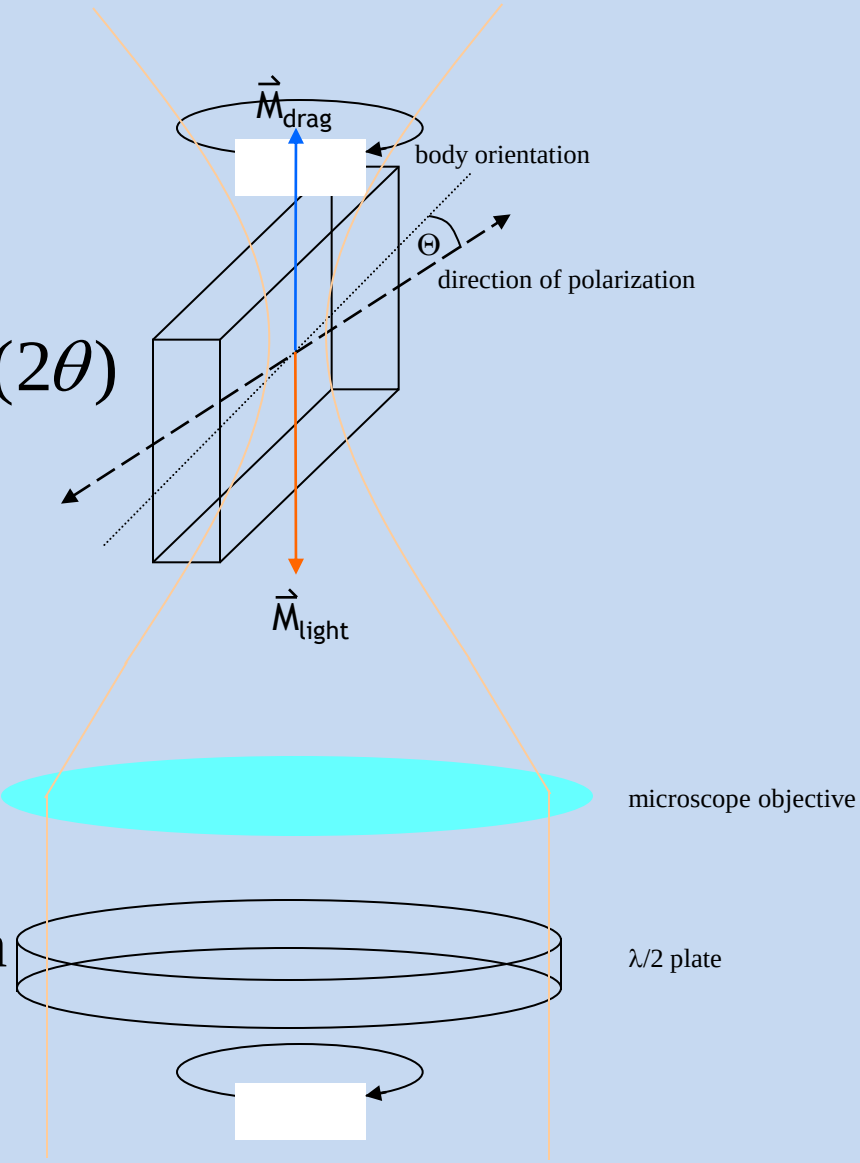
Values of LB (χ) along x



Garab et al. 2005, Eur. Biophys. J.

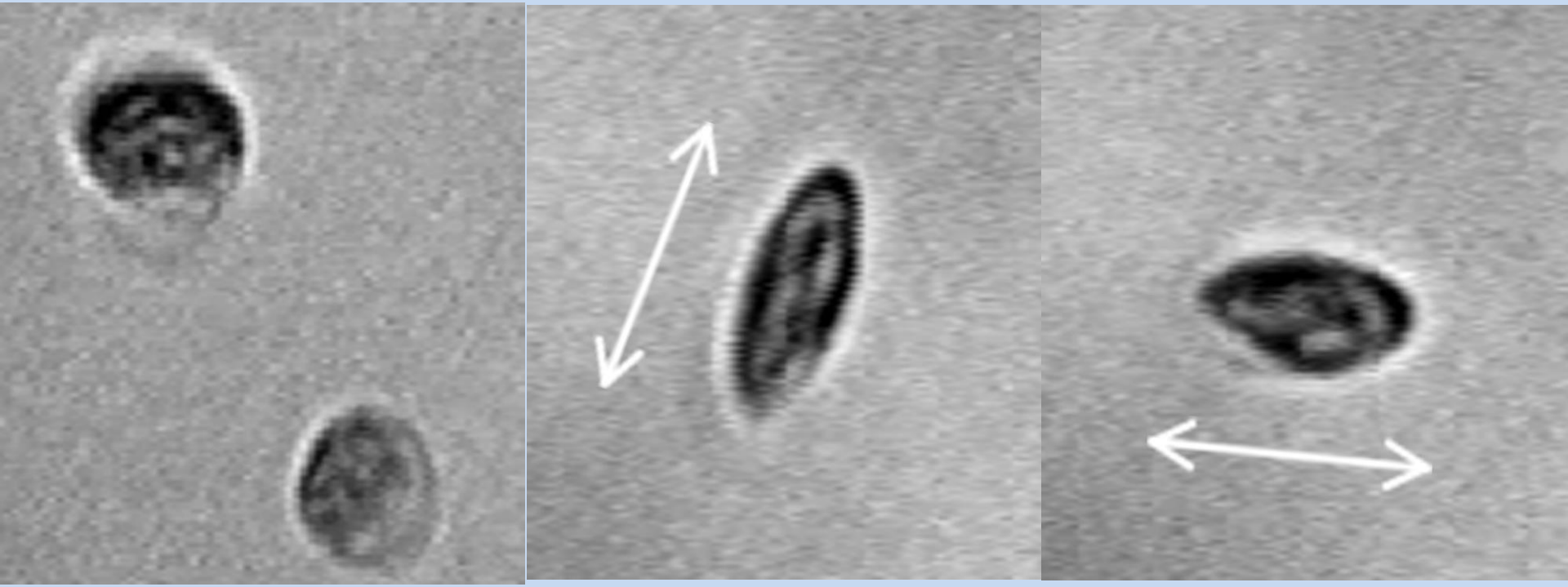
$$\tau_{lin} = -\frac{P}{\omega}\sin(2\pi\frac{R}{\lambda})\sin(2\theta)$$

$$R = \Delta n \times d$$



tau, torque
P, power of the laser beam
R, retardation
n, refractive index
d, thickness

Orientation (and rotation) of chloroplasts by (changing the plane of) linearly polarized beam of a laser tweezer



Garab G, Galajda P, Pomozi I, Finzi L, Praznovszky T, Ormos P, van Amerongen H. 2005 Eur Biopys J