BioSolar Cells

Global and target analysis of energy transfer and quenching in the thylakoid membrane

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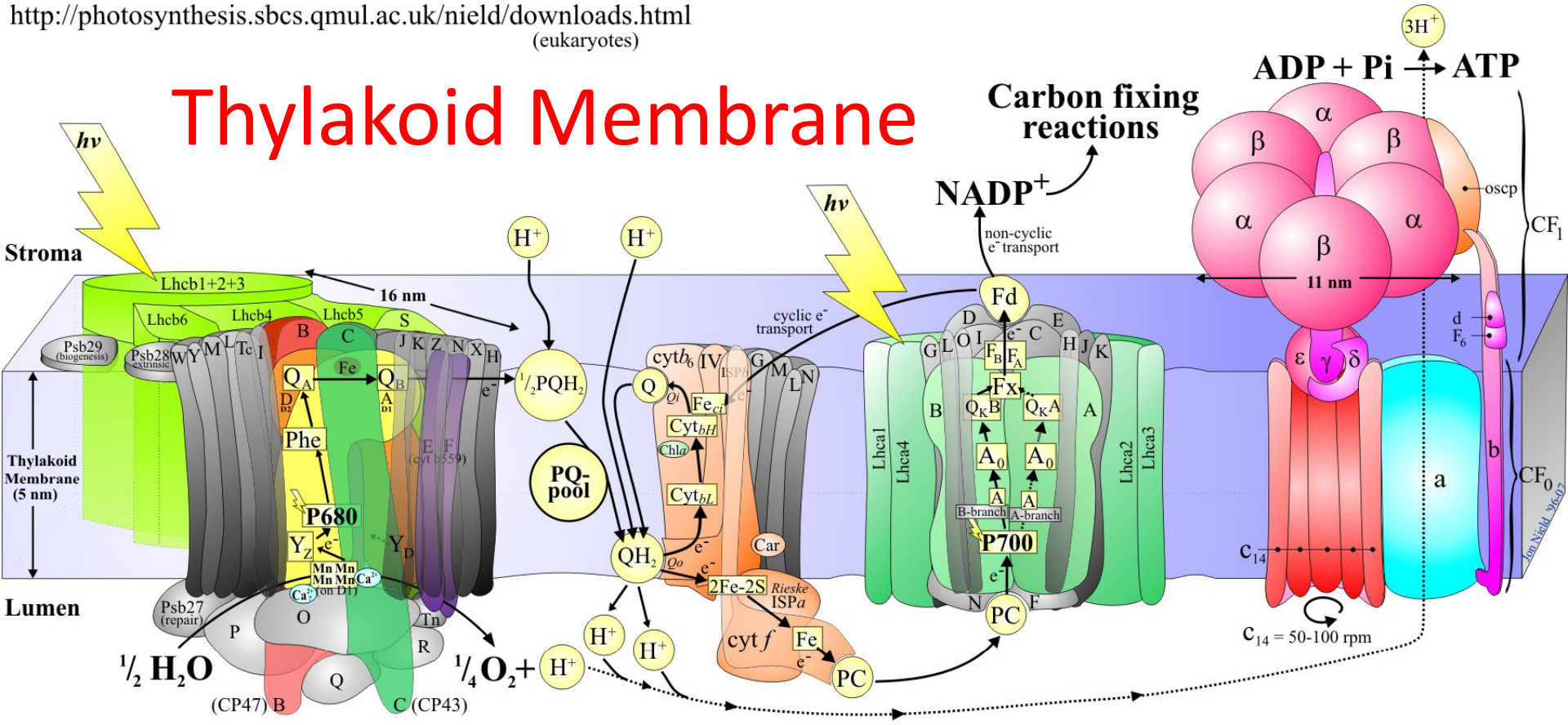
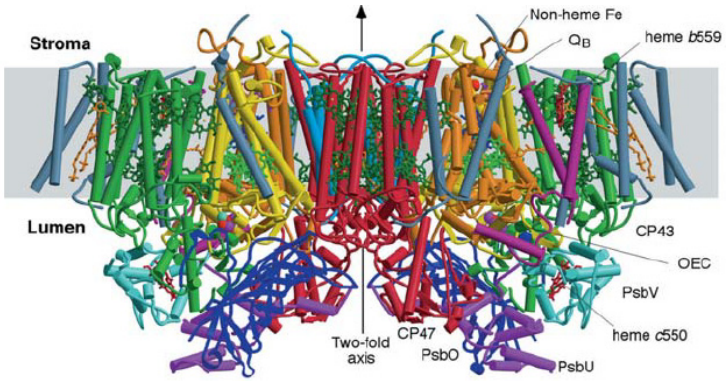
http://photosynthesis.sbcs.qmul.ac.uk/nield/downloads.html
(eukaryotes)

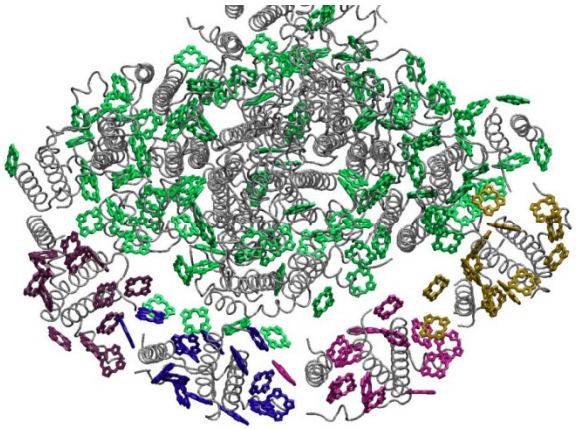
Thylakoid Membrane



PSII (*dimer) **Cyt b_6f** (*dimer) **PSI** **ATP synthase**



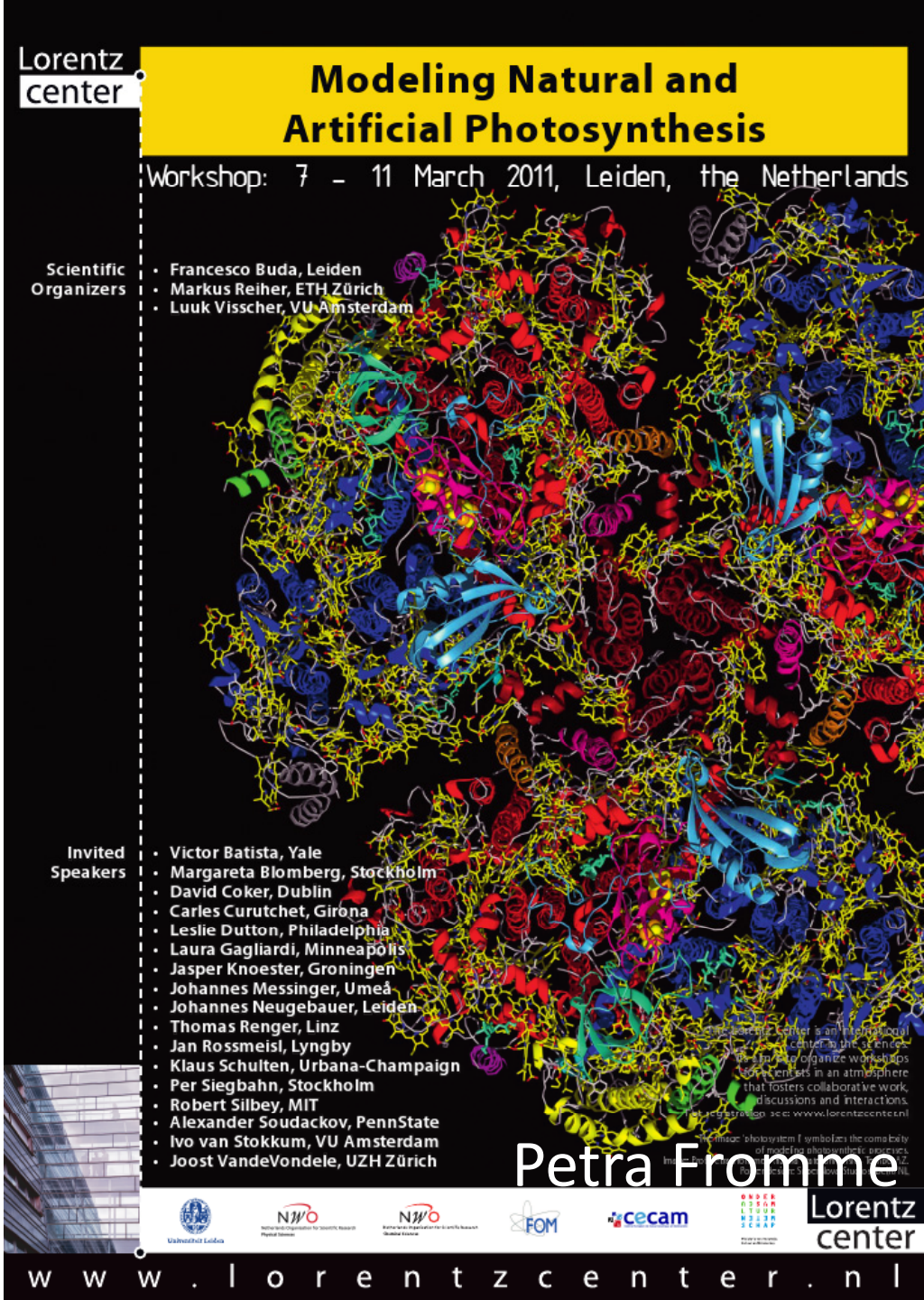
Each PS contains
~100 Chl
~50 Car



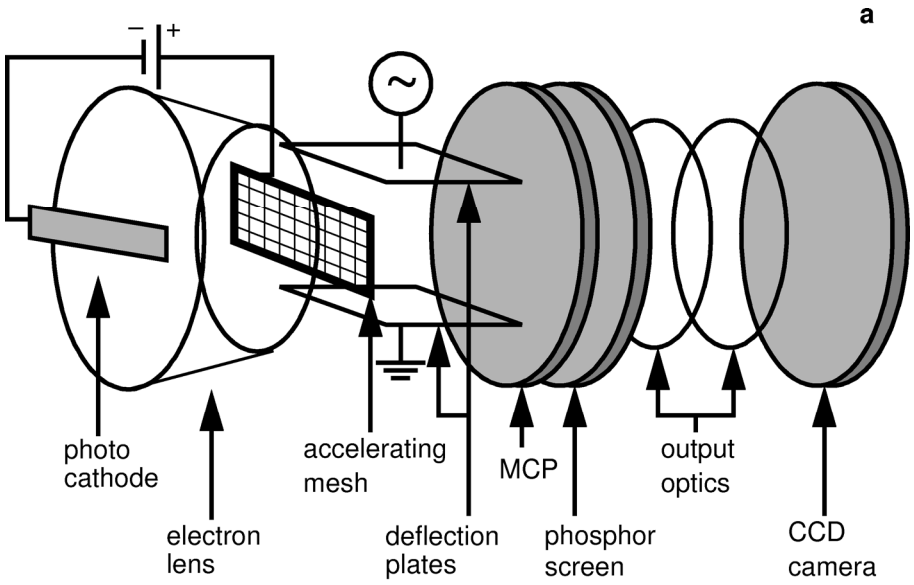
Q: how to study the function of PS I of Cyanobacteria ?

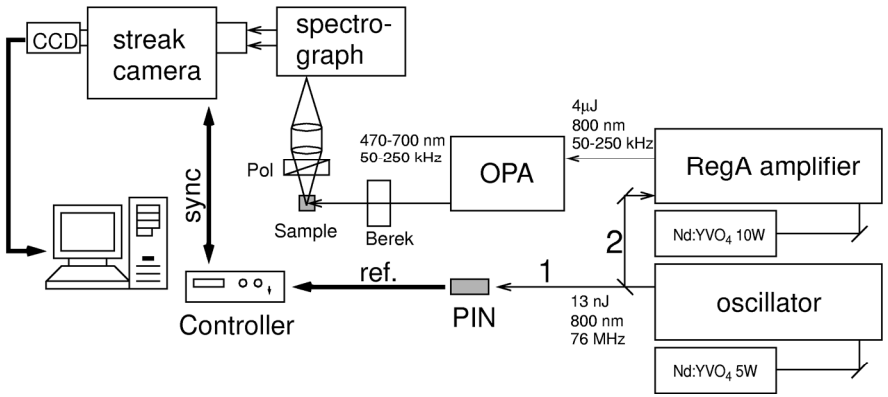
A: by measuring the time resolved emission spectrum with high time resolution using 400 nm (Soret) excitation with low power to avoid annihilation

And then try to model it ...



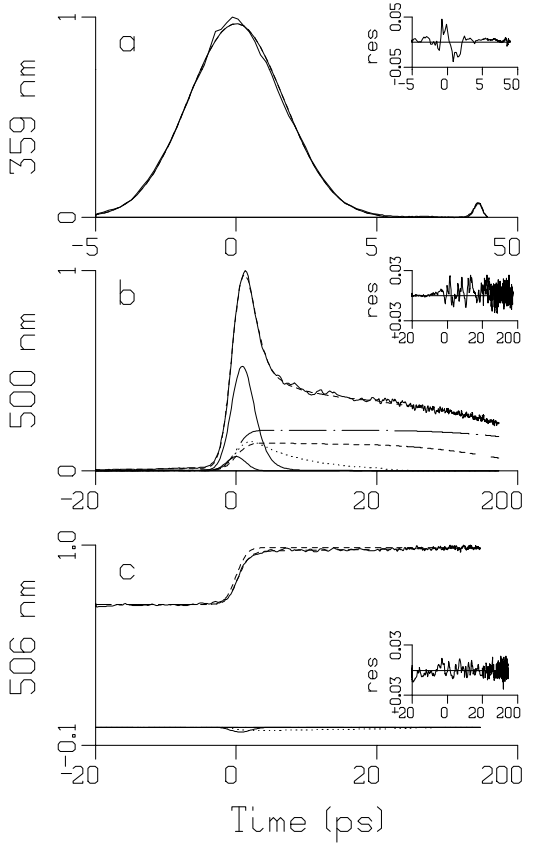
Time resolved emission





Synchroscan streak scope

Instrument Response Function at best ~4 ps FWHM



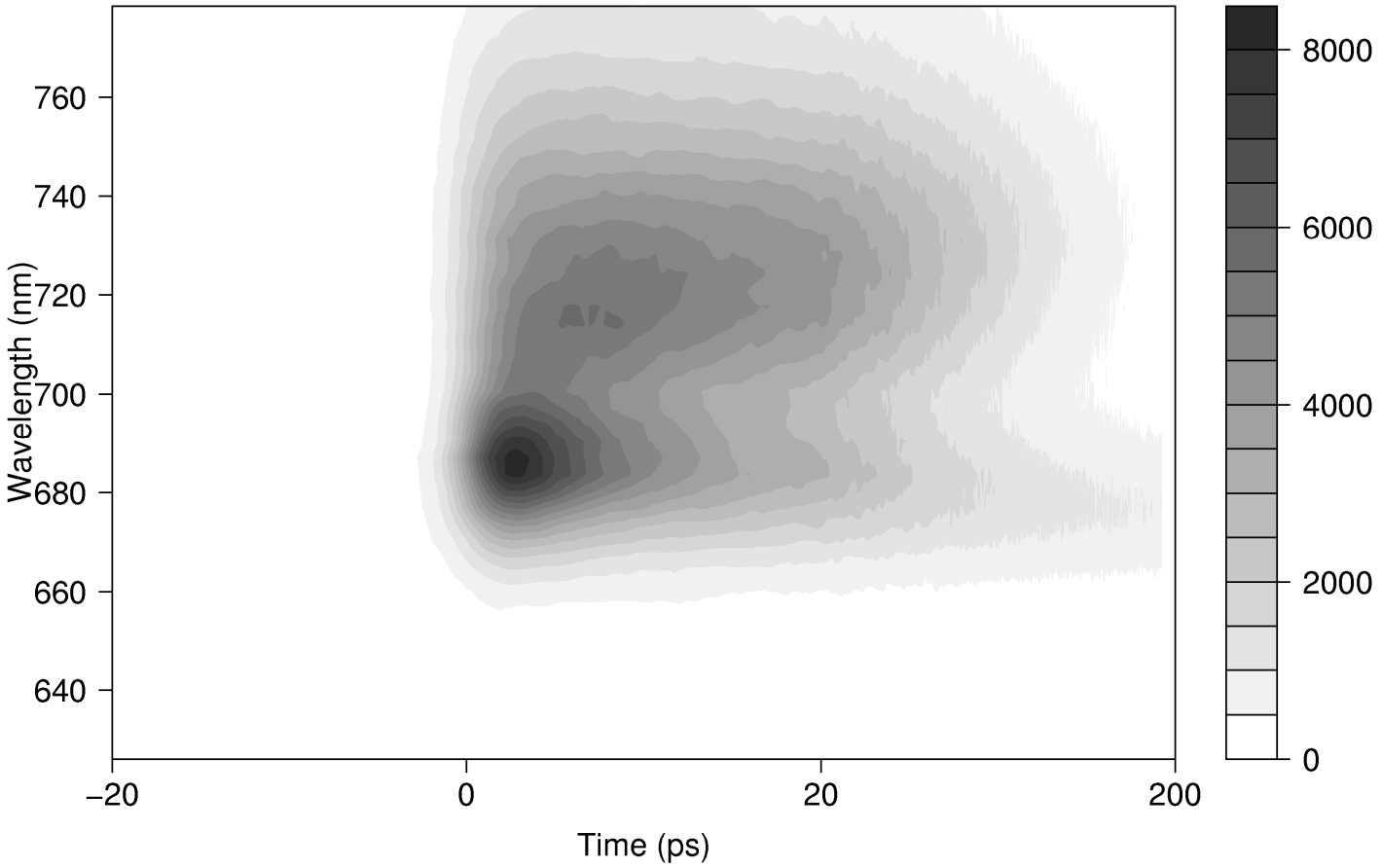
IRF

1.2 ps decay

17 ns decay

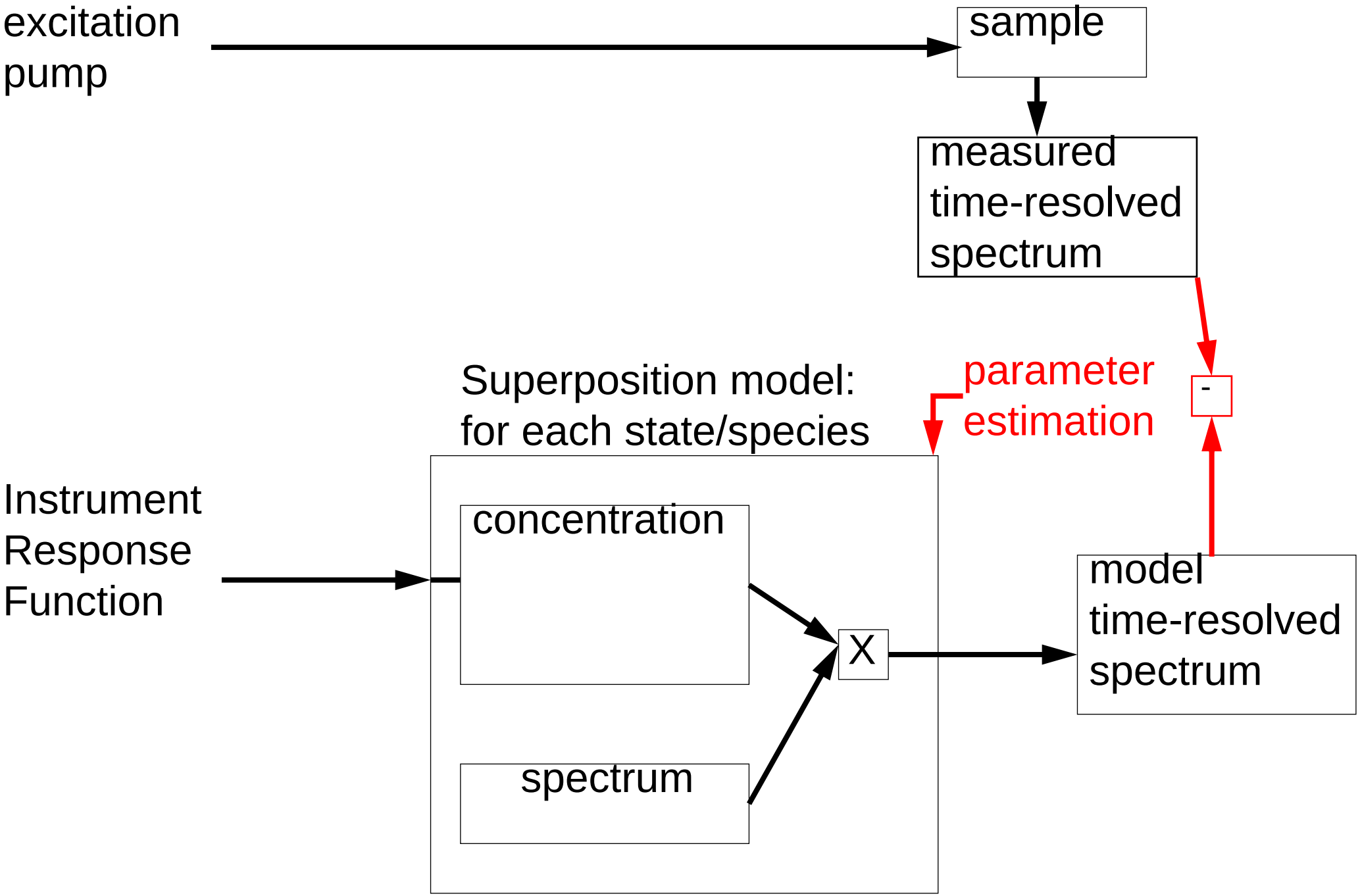
Gobets et al. (2001)

Measured PS I core emission $\Psi(\lambda, t)$



Q: How to analyse and model phenomenologically?

Modelling and parameter estimation scheme



Global and target analysis

Multiple experiments (detection wavelength, polarization angle, excitation wavelength, ...) at possibly multiple physiological conditions (temperature, pH, pD, open/closed RC, (un)quenched, ...) are simultaneously analysed.

Their information is integrated, and the parameters θ are estimated more precisely. When the residuals are satisfactory, the target model that is tested can be considered an adequate description of the data.

After fitting the data globally with a sufficient number of exponential decays (lifetimes) different compartmental schemes (target models) are tested.

Adequacy of a model can be judged from the plausibility of the estimated parameters, in particular the spectral shapes, and the microscopic rate constants. In case of equilibria also the free energy differences can be computed $\Delta G = k_B T \ln\left(\frac{k_{\text{forward}}}{k_{\text{backward}}}\right)$.

Superposition model for the observations

$$\psi_{qt_i\lambda_j} = \sum_{l=1}^{n_{\mathrm{comp},\lambda_j}} c_{q\lambda_j t_i l}(\Theta)\varepsilon_{\lambda_j l} = C_{q\lambda_j}(\Theta)\varepsilon_{\lambda_j} + \xi_{qt_i\lambda_j}$$

experiment q, $q = 1, \ldots, Q$,

time point t_i, $i = 1, \ldots, n_{t,\,q}$

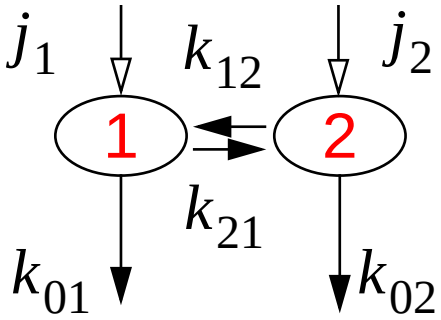
$n_{\mathrm{comp},\lambda_j}$ number of components that contribute at

wavelength λ_j, $j = 1, \ldots, n_{\lambda,\,q}$

additive normally distributed noise $\xi_{qt_i\lambda_j}$

nonlinear least squares model with intrinsically nonlinear parameters Θ and conditionally linear parameters $\varepsilon_{\lambda_j l}$, $(l = 1, \ldots, n_{\mathrm{comp},\lambda_j})$, $(j = 1, \ldots, n_{\lambda,\,\mathrm{tot}})$

Compartmental models

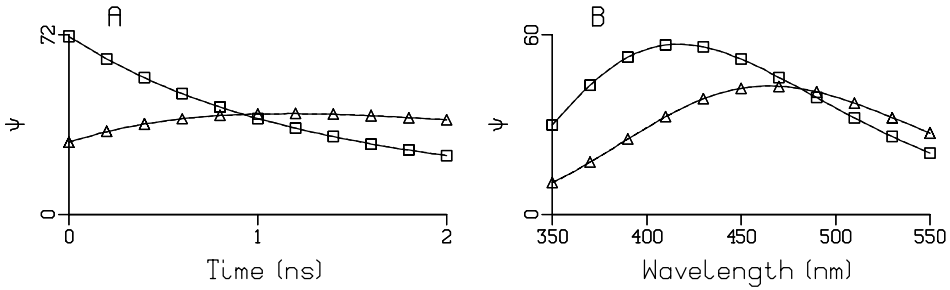


$$\frac{d}{dt}\begin{bmatrix} c_1(t) \\ c_2(t) \end{bmatrix} = \begin{bmatrix} -k_{01}-k_{21} & k_{12} \\ k_{21} & -k_{02}-k_{12} \end{bmatrix}\begin{bmatrix} c_1(t) \\ c_2(t) \end{bmatrix} + \begin{bmatrix} j_1 \\ j_2 \end{bmatrix} i(t)$$

$$\frac{dc}{dt} = Kc + j(t) \qquad c = \exp(Kt) \oplus j(t)$$

Solution: linear combination of exponential decays convolved with IRF $i(t)$

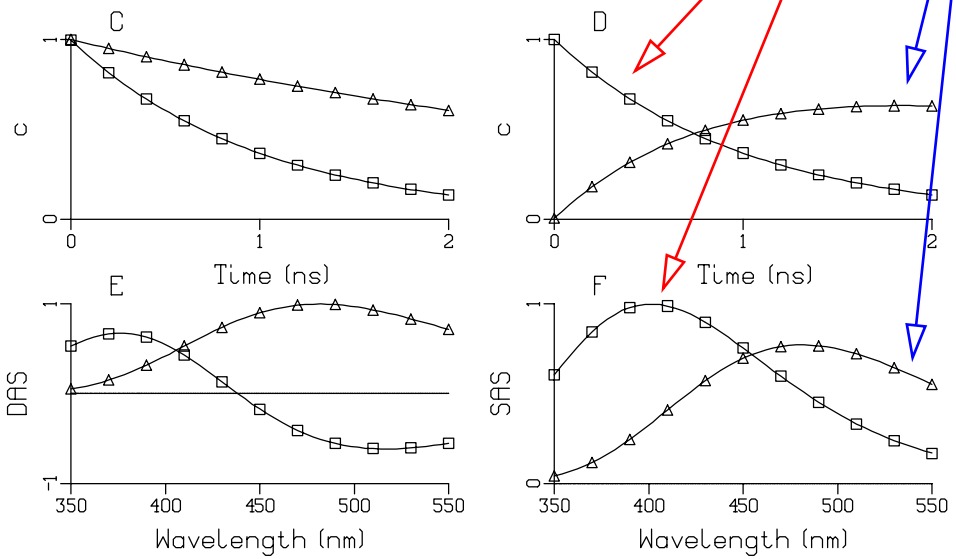
	K	j	c	$\Psi = CE^T$	spectral relation
parallel, decay associated	$\begin{bmatrix} -k_1 & 0 \\ 0 & -k_2 \end{bmatrix}$	$\begin{bmatrix} 1 \\ 1 \end{bmatrix}$	$\begin{bmatrix} \exp(-k_1 t) \\ \exp(-k_2 t) \end{bmatrix} \equiv C_I^T$	$C_I D A S^T$	
sequential, evolution associated, unbranched unidirectional	$\begin{bmatrix} -k_1 & 0 \\ k_1 & -k_2 \end{bmatrix}$	$\begin{bmatrix} 1 \\ 0 \end{bmatrix}$	$C_{II} = C_I R_{II}$	$C_{II} E A S^T$	$EAS\ R_{II}^T = DAS$
general, species associated			$C_{III} = C_I A_{III}$	$C_{III} S A S^T$	$SAS\ A_{III}^T = DAS$



❑ 400, ▲ 500 nm ❑ 0.4, ▲ 1.6 ns

Simulated data: sequential scheme $1 \rightarrow 2$, $\Psi = C_{II} EAS^{T}$

C_I



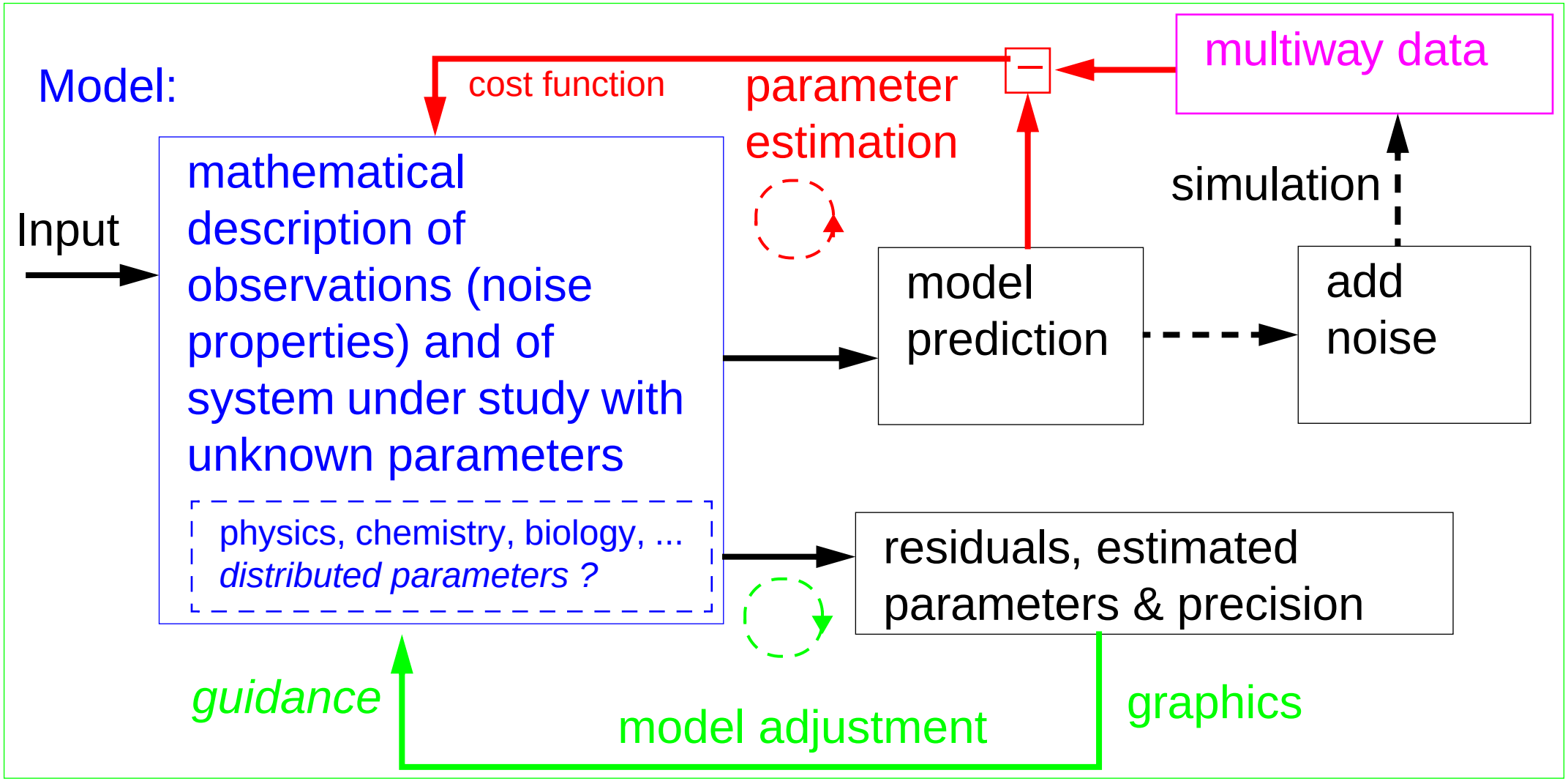
C_{II}

DAS

EAS or SAS

Analysis with wrong, parallel, kinetic scheme results in unrealistic DAS, which indicate rise of long lived component, and suggest $1 \rightarrow 2$

A Problem Solving Environment for interactive modelling of multiway data

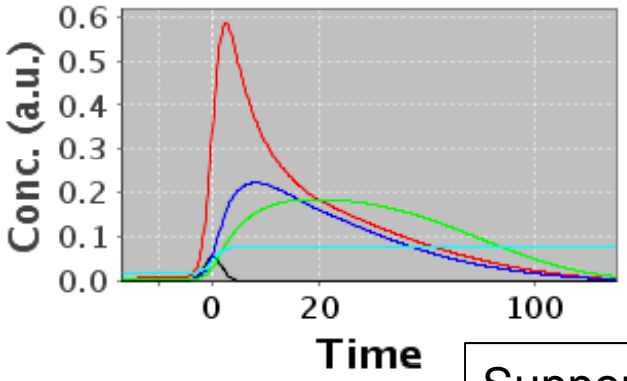


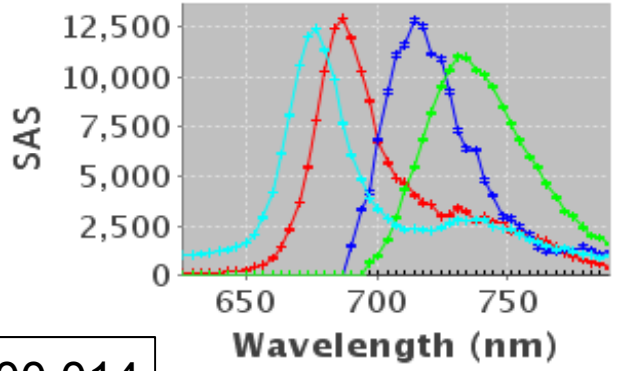
Glotaran

A tool for interactive global and target analysis of time-resolved spectroscopy and microscopy data

Joris Snellenburg, Sergey Laptenok, Katharine Mullen

- Open source, platform independent, developed on Java
- Uses the R-package TIMP as a computational back-end
- Interactive data-exploration and inspection of results
- Can be downloaded from glotaran.org



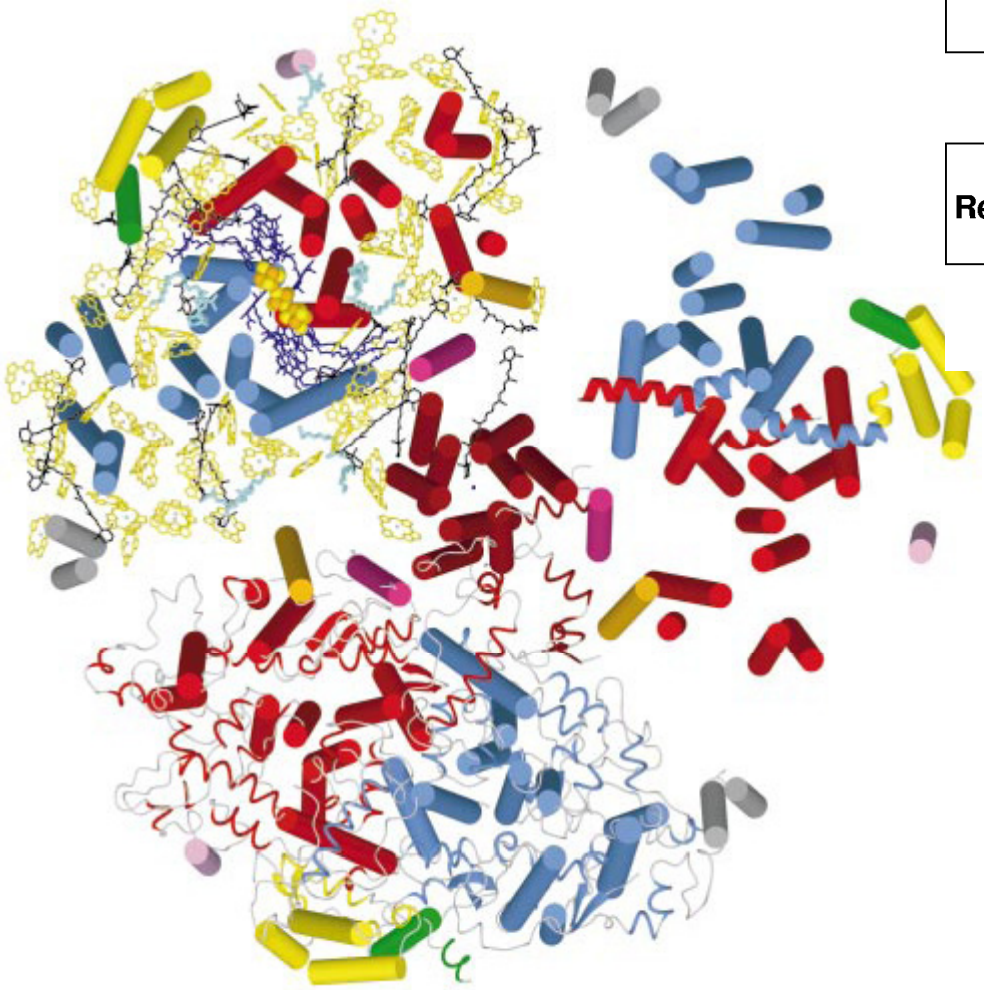


Supported by NWO CS grant 635.000.014

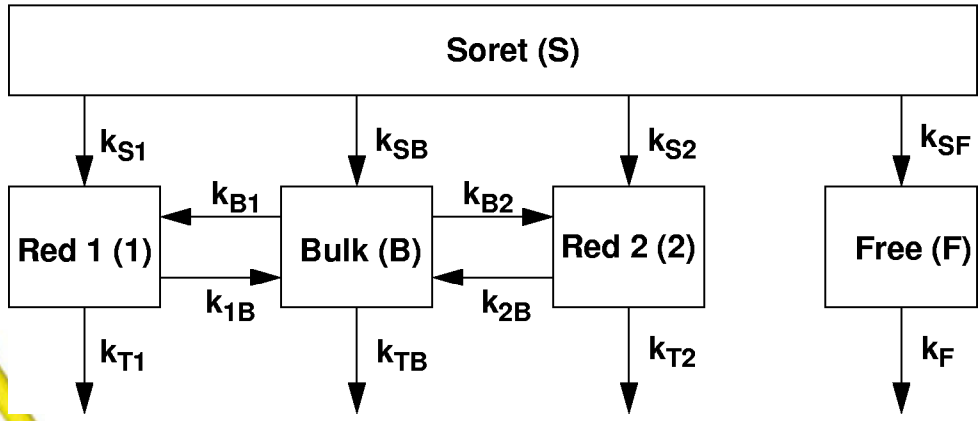
Netherlands Organisation for Scientific Research

vrije Universiteit amsterdam

Case study: cyanobacterial PS I core with two pools of red chlorophylls



Jordan et al. (2001)



Compartmental model with four types of Chls Bulk, Red 1 and 2, and a small fraction Free

Photochemical quenching with trapping rate $k_T \approx 1/(18\ ps)$

Gobets, B., van Stokkum, I.H.M., Rogner, M., Kruip, J., Schlodder, E., Karapetyan, N.V., Dekker, J.P., van Grondelle, R. 2001b. Time-resolved fluorescence emission measurements of photosystem I particles of various cyanobacteria: A unified compartmental model. Biophysical Journal 81, 407-424.

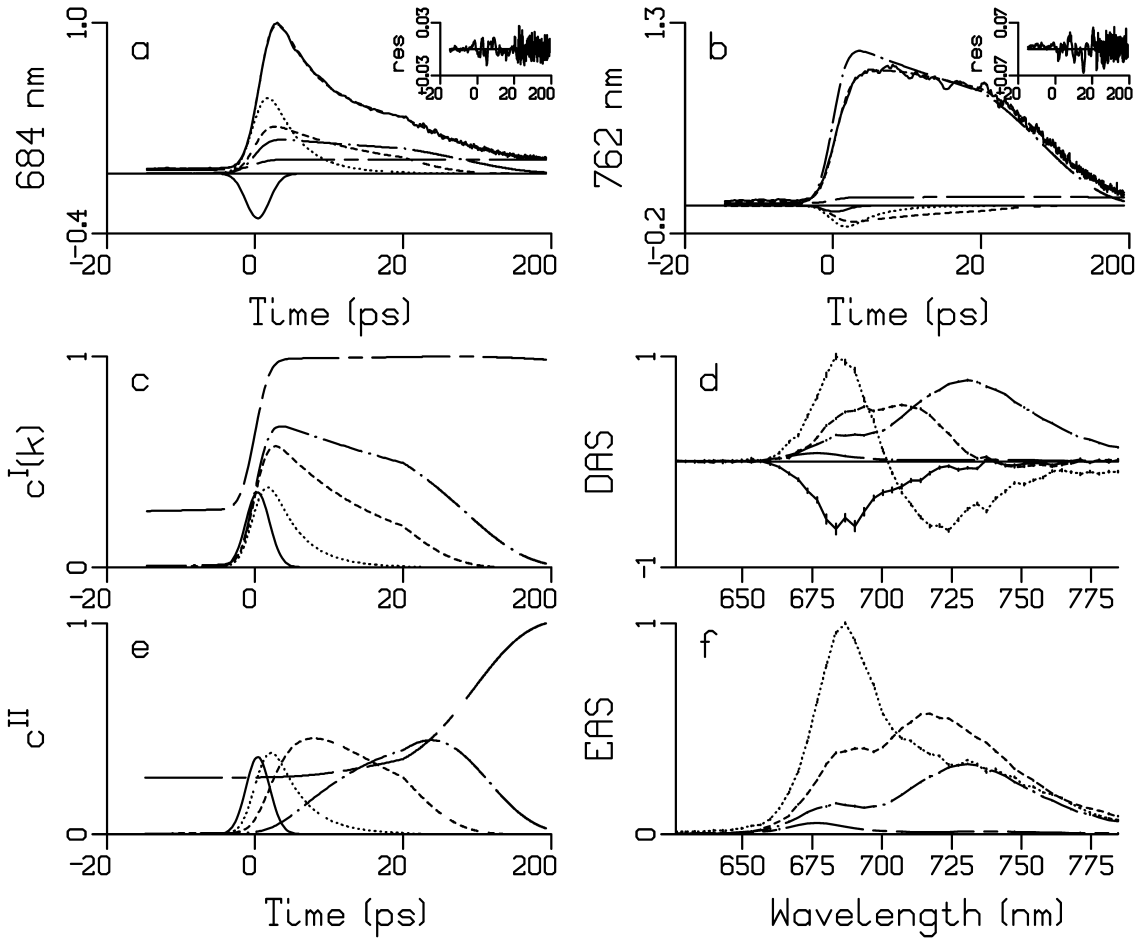


Fig. 4. Results from global analysis of PS I data depicted in Fig. 1. Note that in a–c and e the time axis is linear from –20 to +20 ps relative to the maximum of the IRF, and logarithmic thereafter. Insets in a, b show residuals. (a) Fit of bulk Chl *a* emission trace showing multiexponential decay. Contributions of the five exponential decays with different lifetimes (shown in c) are indicated by line type. (b) Fit of red Chl *a* emission trace showing multiexponential rise and decay. (c) Exponential decays $c^I(k_l)$. Estimated lifetimes: 0.4 ps (solid), 3.9 ps (dotted), 15 ps (dashed), 50 ps (dot dashed), and 4.9 ns (chain dashed). (d) Decay Associated Spectra (DAS), note that the first DAS which represents overall rise has been multiplied by 0.2. Vertical bars indicate estimated standard errors. (e) Evolutionary concentration profiles c_l^{II} (assuming a sequential kinetic scheme with increasing lifetimes). (f) Evolution Associated Spectra (EAS). Note that the first EAS is zero, since excitation was in the Soret band.

where each concentration is a linear combination of the exponential decays,

$$c_l^{II} = \sum_{j=1}^{l} b_{jl} c^I(k_l) \tag{13}$$

and the amplitudes b_{jl} are given by $b_{11} = 1$ and for $j \le l$:

$$b_{jl} = \frac{\prod_{m=1}^{l-1} k_m}{\prod_{n=1, n\neq j}^{l} \left(k_n - k_j\right)} \tag{14}$$

Examples of c_l^{II} are depicted in Fig. 4e, whereas the EAS estimated from the PS I trimer data are shown in Fig. 4f. With increasing lifetimes, and thus decreasing rates k_l, the first EAS (equal to the sum of DAS) corresponds to the spectrum at time zero with an ideal

infinitely small IRF, $i(t) = \Delta(t)$. In Fig. 4f, this first EAS is zero in the Q_y region. The second EAS (dotted), which is formed in 0.4 ps and decays in 3.9 ps, represents the sum of the spectra of all excitations that have arrived from the Soret region, and is dominated by bulk Chl *a*. The third EAS, which is formed in 3.9 ps and decays in 15 ps, is already dominated by red Chl *a* emission, which is even more the case with the fourth EAS (dot dashed, formed in 15 ps, decays in 50 ps). The final EAS (chain dashed, formed in 50 ps) is proportional to the final DAS, and represents the spectrum of the longest living component (4.9 ns). Clearly, these EAS do not represent pure species, except for the final EAS, and they are interpreted as a weighted sum (with only positive contributions) of true species spectra.

(3) When neither of these two simple models is applicable, a full kinetic scheme may be appropriate. The problem with such a scheme is that, while the kinetics are described by microscopic rate constants, the data only allows for the estimation of decay rates (or lifetimes). Thus additional information is required to estimate the microscopic rates, which can be spectral constraints (zero contribution of SAS at certain wavelengths) or spectral relations. This is explained in detail in van Stokkum et al. (2004).

Now the model reads:

$$\psi(t,\lambda) = \sum_{l=1}^{n_{comp}} c_l^{III} SAS_l(\lambda) \tag{15}$$

where the concentrations c_l^{III} are again linear combinations of the exponential decays, with coefficients that depend upon the microscopic rate constants that describe the transitions between all the compartments. Figure 5a depicts the kinetic scheme that was applied to the trimeric PS I data of Fig. 1. The concentrations of all compartments are collated in a vector $c(t) = [c_1(t)\ c_2(t)\ \ldots\ c_{n_{comp}}(t)]^T = [S(t)\ B(t)\ R_1(t)\ R_2(t)\ F(t)]^T$, which obeys the differential equation:

$$\frac{d}{dt}c(t) = Kc(t) + j(t) \tag{16}$$

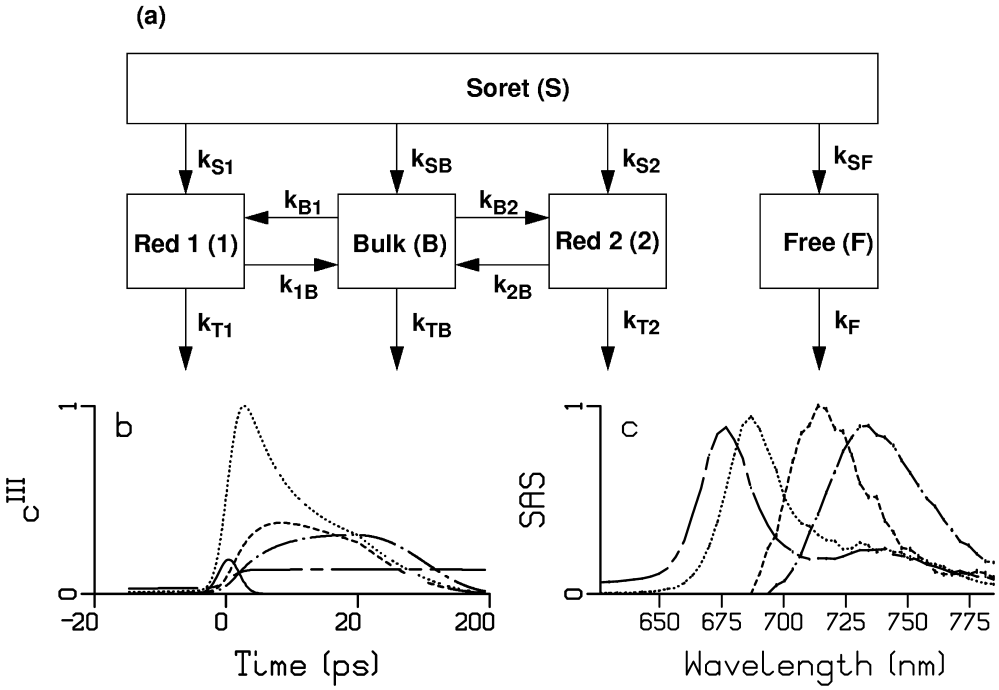


Fig. 5. (a) Kinetic scheme used for the target analysis of PS I data depicted in Fig. 1. After excitation in the Soret band four compartments are populated: bulk Chl *a* (B), two pools of red Chl *a* (1 and 2) and a small fraction of free Chl *a* (F). The first three compartments equilibrate, and excitations are trapped with different rates. (b) Concentration profiles c_l^{III}, note that the time axis is linear from –20 to +20 ps relative to the maximum of the IRF, and logarithmic thereafter. (c) Species Associated Spectra (SAS). Key in (b) and (c): bulk Chl *a* (dotted), red Chl *a* 1 (dashed), red Chl *a* 2 (dot dashed), free Chl *a* (chain dashed).