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

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Computational Biophysics
Thylakoid Membrane

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

Instrument Response Function at best $\sim 4$ ps FWHM

Synchroscan streak scope

IRF

1.2 ps decay

17 ns decay
Q: How to analyse and model phenomenologically?
Modelling and parameter estimation scheme

excitation pump → sample

measured time-resolved spectrum

Superposition model: for each state/species

Instrument Response Function

concentration

spectrum

X

parameter estimation

model time-resolved spectrum
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 $\theta$ 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

\[ \psi_{q t, \lambda_j} = \sum_{l=1}^{n_{\text{comp}, \lambda_j}} c_{q \lambda_j, t_i l}(\Theta) \varepsilon_{\lambda_j l} = C_{q \lambda_j}(\Theta) \varepsilon_{\lambda_j} + \xi_{q t, \lambda_j} \]

experiment \( q \), \( q = 1, \ldots, Q \),
time point \( t_i \), \( i = 1, \ldots, n_{t, q} \)

\( n_{\text{comp}, \lambda_j} \) number of components that contribute at

wavelength \( \lambda_j \), \( j = 1, \ldots, n_{\lambda, q} \)

additive normally distributed noise \( \xi_{q t, \lambda_j} \)

nonlinear least squares model with intrinsically nonlinear parameters \( \Theta \) and
conditionally linear parameters \( \varepsilon_{\lambda_j l}, (l = 1, \ldots, n_{\text{comp}, \lambda_j}), (j = 1, \ldots, n_{\lambda, \text{tot}}) \)
Compartmental models

\[
\begin{align*}
\frac{d}{dt} \begin{bmatrix} c_1(t) \\ c_2(t) \end{bmatrix} &= \begin{bmatrix} -k_{01} - k_{21} & k_{12} \\ k_{21} & -k_{02} - k_{12} \end{bmatrix} \begin{bmatrix} c_1(t) \\ c_2(t) \end{bmatrix} + \begin{bmatrix} j_1 \\ j_2 \end{bmatrix} i(t) \\
\frac{dc}{dt} &= Kc + j(t) \\
c &= \exp(Kt) \oplus j(t)
\end{align*}
\]

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

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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>spectral relation</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>( K )</td>
<td>( j )</td>
<td>( c )</td>
<td>( \Psi = CE^T )</td>
</tr>
</tbody>
</table>
| parallel, decay associated | \[
\begin{bmatrix}
-k_1 & 0 \\
0 & -k_2
\end{bmatrix}
\] | \[
\begin{bmatrix}
1 \\
1
\end{bmatrix}
\] | \[
\begin{bmatrix}
\exp(-k_1 t) \\
\exp(-k_2 t)
\end{bmatrix}
\] | \( C_IDAS^T \)       |
| sequential, evolution associated, unbranched unidirectional | \[
\begin{bmatrix}
-k_1 & 0 \\
k_1 & -k_2
\end{bmatrix}
\] | \[
\begin{bmatrix}
1 \\
0
\end{bmatrix}
\] | \( C_{II} = C_IR_{II} \) | \( C_{II}EAS^T \) | \( EAS R_{II}^T = DAS \)       |
| general, species associated |       |       |       | \( C_{III} = C_IA_{III} \) | \( C_{III}SAS^T \) | \( SAS A_{III}^T = DAS \)       |
Simulated data: sequential scheme \( 1 \rightarrow 2, \Psi = C_{II}EAS^T \)

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

Model:

- mathematical description of observations (noise properties) and of system under study with unknown parameters
- physics, chemistry, biology, ...
- distributed parameters?

Input

- cost function
- parameter estimation
- model prediction

Guidance

- model adjustment
- graphics

multiway data

- simulation
- add noise

residuals, estimated parameters & precision
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
Case study: cyanobacterial PS I core with two pools of red chlorophylls

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

Photochemical quenching with trapping rate \( k_T \approx 1/(18 \text{ ps}) \)

Q: what is the dynamics in PSII core with oxidized $Q_A$ (open RC)?
A: measure and model the emission after 400 nm (Soret) excitation with low power to avoid annihilation
Streak data and global analysis

DAS, 5 lifetimes:

<0.7 ps transfer from Soret
12, 49, 223 ps: energy transfer and trapping
1.4 ns: 1% closed RC
Kinetic model, SAS, and concentrations

Spectral assumptions needed to estimate equilibria, a priori knowledge used on rate constants

this model can be called *diffusion-to-the-trap-limited*
Miloslavina & Holzwarth: *trap-limited* kinetic model to fit the same data: SAS, and concentrations

* 0.73 $h$V
* 0.27 $h$V

same quality of fit with our data

large 111 ps component present in Miloslavina et al. (*Biochemistry* 2006, 45, 2436-2442) is not present in our data
Our model is in better agreement with the model proposed by Renger and Schlodder, ChemPhysChem 11 (2009) 1141

Plant photosynthesis is not efficient

Non-photochemical quenching (NPQ) in cyanobacteria is mediated by the orange carotenoid protein (OCP)

Non-photochemical quenching (NPQ) in intact cells of cyanobacterium *Synechocystis sp. PCC 6803*

Emission after antenna excitation

→wavelength

Unquenched  Quenched

→time

Molecular Model

Normal light  Strong BG light

OCP

PSII

RCP

Terminal emitter

PSI

Cyan pigments are quenched by RCP

Streak measurements

by Lijin Tian & Herbert van Amerongen Biophysics, Wageningen University WT and overexpressed OCP samples by Diana Kirilovsky (CNRS, Saclay, France)
Unquenched (black traces) and quenched (red traces) from 612-779 nm and till 700 ps with IRF width 8 ps

Quenching starts almost immediately, and is large above 650 nm
Target analysis

model: 

PBS

PSII

guidance

model adjustment

estimated parameters

parameter estimation

model prediction

fit

data

cost function

concentration

time (ps)

462 nm

647 nm

679 nm

Wavelength (nm)

SAS

Target analysis
Target analysis using a heterogeneous equilibrium model with fractions (non)quenched open/closed RC

PBS = PhycoBilisome antenna
(A)PC = (Allo) PhycoCyanin
S,L = Short,Long wavelength
Estimated concentrations and SAS
dashed = quenched concentration

\[ \theta^{16} \approx \frac{1}{33 \text{ ps}} \text{ when quenched} \]
## Target analysis of cyanobacteria emission data

### Hierarchical modelling of time-resolved emission spectra

<table>
<thead>
<tr>
<th>Level of modelling</th>
<th>Parametric description of linking of experiments</th>
<th>Relative scaling, linkage schemes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contribution of component</td>
<td>$l \ c_l(t) \varepsilon_l(\lambda)$</td>
<td>Spectrum of component $l$, $\varepsilon_l(\lambda)$</td>
</tr>
<tr>
<td>Convolution</td>
<td>$c_l^\delta(t) \otimes i(t)$</td>
<td>Instrument Response Function $i(t)$</td>
</tr>
<tr>
<td>MA concentration with $\delta$-input</td>
<td>$c_l^\delta(t)$</td>
<td>Compartmental scheme with microscopic rates</td>
</tr>
</tbody>
</table>

**Diagram:**

- **Superposition model:** for each state
- **Instrument Response Function**
- **Concentration**
- **Spectrum**
- **Model time-resolved spectrum**

Where $\Theta$ represents the model parameters.
Spectral assumptions, that $\varepsilon_l(\lambda) = 0$ for certain components $l$ in certain $\lambda$-ranges, or that some spectra are equal up to a scaling parameter $\Theta_\varepsilon$, are essential to estimate overlapping spectra, input fractions, and equilibria.

### Numbers and parameters with cyanobacteria emission data

<table>
<thead>
<tr>
<th></th>
<th>range</th>
<th>#</th>
<th>#parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>wavelength $\lambda$</td>
<td>612 – 787 nm</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>time $t$</td>
<td>800 ps</td>
<td>1000</td>
<td></td>
</tr>
<tr>
<td>unquenched &amp; quenched</td>
<td></td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>data points</td>
<td></td>
<td>100000</td>
<td></td>
</tr>
<tr>
<td>compartments</td>
<td></td>
<td>4*5=20</td>
<td></td>
</tr>
<tr>
<td>spectra</td>
<td></td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>microscopic rate constants (9) and input fractions (2)</td>
<td>11</td>
<td>11-1=10</td>
<td></td>
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<tr>
<td>instrument response location, width, dispersion, triplegaussian</td>
<td>9</td>
<td></td>
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<tr>
<td>scaling parameter</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>spectral parameters (VARPRO)</td>
<td>4#$\lambda$ – assumptions</td>
<td>200-7=193</td>
<td></td>
</tr>
<tr>
<td>spectral assumptions</td>
<td>blue SAS zero below 663 nm</td>
<td>1-1=0</td>
<td></td>
</tr>
</tbody>
</table>

Thus 20 truly nonlinear parameters and 193 conditionally linear parameters must be estimated.
But where is the emission from:
PS I bulk and red Chl
PS II CP43,CP47,RC
?
Hidden in the estimated “PBS” SAS, and more data and a more complicated model are needed (discussion !)
Target analysis

PBS

PSII

model:

cost function

parameter estimation

data

fit

model prediction

concentration

guidance

model adjustment

estimated parameters

612 nm

647 nm

662 nm

679 nm

Time (ps)

Time (ps)

Time (ps)

Time (ps)

610 630 650 670 700 730 750 770 790

Wavelength (nm)

SAS
Conclusions

• Target analysis of streak measurements with low power excitation contributes to functional models of energy transfer and quenching in the thylakoid membrane

• Energy transfer rate constants and free energies between compartments containing dozens of chromophores can be estimated

• Identity of quenched chromophores and dynamics of quenching can be estimated
Scaling and Integration of Kinetic Models of Photosynthesis: Towards Comprehensive E-Photosynthesis.

Plant Physiology 154, 410-422.
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Joris Snellenburg, Sergey Laptenok, Katharine Mullen, Bas Gobets, Chantal Van der Weij-de Wit, Jan Dekker, Rienk van Grondelle (Biophysics, VU University Amsterdam)
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