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Modeling the Excitation Energy Capture in Thylakoid Membranes

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Introduction

Organisms that carry out oxygenic photosynthesis have thylakoid membranes containing two photosystems (**PS I** and **PS II**) and a great variety of extrinsic and intrinsic peripheral light-harvesting complexes (**LHC**) that bind different classes of chromophores. Together, these perform an optimized conversion of light into chemical free energy. In the past decades detailed knowledge has accumulated on the structure and the arrangement of the different macromolecular supercomplexes, as well as on the spectral properties and the exciton dynamics in individual pigment-protein complexes. This knowledge can now be applied to computer-based modeling of light harvesting and the distribution of excitation energy to the photosystems in various kinds of thylakoid membranes.

The mathematical formalism needed requires extensive programming which may be prohibitive for many researchers and students. We are presently developing an interactive modeling program for the Internet which consists of several modules and allows the user to simulate numerical and graphical results for a large number of independent input parameters. Here we give a brief summary of the concepts and the mathematical formulation, that will be published elsewhere (Trissl, 2002).

Materials and Methods

Two levels of spectral simulation are considered. Absorption and fluorescence spectra of pigments or pigment-protein complexes are either calculated by pure Gauss functions (Modules I & II) or by sets of Gauss functions coupled to a master band (Q_y -band in the case of chlorophylls) to simulate real chromophore spectra. In either case a chromophore's absorption and fluorescence spectrum is defined by only two parameters, λ^{\max} and $\Delta\lambda$. For instance, a pigment absorption spectrum is computed according to:

$$A^p(\lambda, \lambda^{\max}, \Delta\lambda) = \sum_m \frac{a_m}{\sqrt{2\pi\Delta\lambda\beta_m}} e^{-\left(\frac{\lambda^{\max}\alpha_m - \lambda}{\sqrt{2\Delta\lambda\beta_m}}\right)^2}$$

The master Gaussian ($m=a_1=\alpha_1=\beta_1=1$) refers to the most intense bathochromic band. This definition implies that the oscillator strength is independent of the location of the maximum and spectral width. The parameters may be obtained from a fit of the pigment spectrum in organic solvent. In a further step the function is calibrated to the molar absorption coefficient and stored in a data base (Module III).

Such a spectrum can be assigned to a species (=species associated absorption spectrum, **SAAS**) being part (state) of a kinetic reaction scheme (consisting of s states):

$$SAAS_s(\lambda) = A^p(\lambda, \lambda_s^{\max}, \Delta\lambda_s)$$

Similarly, the fluorescence spectrum of a species $F(\lambda)$ can also be described by a set of Gauss functions coupled to a master band, which can be derived from that used for absorption by taking into account the Stokes shift Ξ . Thus, one can formulate the species associated emission spectra **SAES** _{s} by:

$$SAES_s(\lambda) = F^p(\lambda, \lambda_s^{\max} + \Xi, \Delta\lambda_s)$$

The Stokes shifts in turn can be calculated from the band widths applying known photo-physical rules. It is worth noting that the parameters $a_1=\alpha_1=\beta_1=1$ for Chl a are fine-tuned such that the absorption spectrum and the fluorescence spectrum fulfill the Kennard-Stepanov relation (Kennard, 1918; Stepanov, 1957) in the temperature range 240 to 300 K with good accuracy.

The kinetic simulation starts from a kinetic reaction scheme with s coupled states for which a square matrix (transfer matrix) T is formulated which can be solved by standard matrix operations for eigenvalues γ_i and the eigenvector matrix M . The apparent time constants are the reciprocal eigenvalues:

$$\tau_i = -\gamma_i^{-1}$$

In a next step the initial condition Z_s (**normalized fractional excitation** of the states, $\sum Z_s = 1$) or z_s (**not normalized fractional excitation** of the states, $z_s = SAES_s(\lambda)$) is taken into account by solving the linear system for the vector U_s :

$$M \cdot U_s = Z_s$$

The former initial condition is used for calculating quantum yields and the latter for calculating action or excitation spectra. Then a coefficient matrix $a_{i,s}$, required for calculating the transient kinetics of the states and the quantum yields, is obtained by:

$$a_{i,s} = U_s \cdot M^T$$

With the knowledge of the eigenvectors (γ_i) and the coefficient matrix ($a_{i,s}$) a variety of measurable quantities can be simulated, like kinetics, quantum yields and, in conjunction with the above spectra, **decay associated spectra (DAS)** and **time resolved emission spectra (TRES)**. To compute excitation spectra the initial condition has to refer to the **absolute fractional excitation** of the states, which has to be calculated from the intensity and spectral distribution of the incoming light and the molar absorption spectrum of the **photosynthetic entity (PSE, defined in Results)**.

Out of the variety of computable results, we quote exemplarily some selected equations for measurable quantities:

The time-courses of the states are given by:

$$L_s(t) = \sum_i a_{i,s} \cdot e^{\gamma_i t}$$

The total photochemical quantum yield - for the case that different states are able to form the product - are given by:

$$\Phi_{tot}^p = \sum_s \left[-kp_s \cdot \sum_i \frac{a_{i,s}}{\gamma_i} \right]$$

and the fluorescence quantum yields of the states by:

$$\Phi_s^f = -k_s^{rad} \sum_i \frac{a_{i,s}}{\gamma_i}$$

An action spectrum for photochemistry E^p is obtained by computing:

$$E^p(\lambda) = \Phi_{tot}^p(z_s(\lambda))$$

Finally, the stationary fluorescence spectrum due to the kinetic treatment is given by:

$$F^{kin}(\lambda) = \sum_s \Phi_s^f \cdot SAES_s(\lambda)$$

Results

I. Modeling of a green plant thylakoid membrane

To model the excitation energy capture in oxygenic thylakoid membranes and the distribution to the two photosystems in a quantitative way requires to take into account appropriately the various stoichiometries of the structural building blocks (**reaction center RC** plus LHCs). The scheme in Fig. 1 illustrates the definition of (i) a PSU, (ii) a stoichiometric **photosynthetic unit (sPSU)** and (iii) the stoichiometric ratio of the photosystems, all needed for quantification.

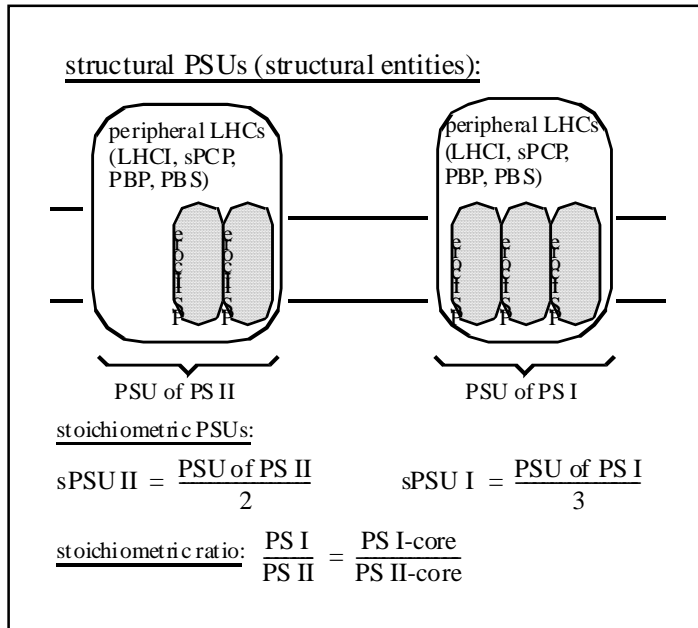


Fig. 1: Definition of photosynthetic unit (PSU), stoichiometric photosynthetic unit (sPSU) and the PS I/PS II-ratio.

With these definitions a **photosynthetic entity (PSE)** can be defined according to:

$$PSE = sPSU \text{ II} + \frac{PSI}{PSII} sPSU \text{ I}$$

Any thylakoid membrane possessing two photosystems is modeled in terms of the PSE!

As an example we want to show a computation of (i) the absorption spectra, (ii) an excitation spectrum and (iii) the kinetics of the closure of PS II-RC and PS I-RC as well as the sum of both, obtained from modeling a thylakoid membrane from chloroplasts of higher plants, assuming a 1:1 stoichiometry of the photosystems, grana-stoma differentiation (absence of spillover), and consensus kinetic data published in the current literature.

Specifically, we assume the following stoichiometries: 4 LHCII-trimers around PS II and 6 LHCI-dimers around PS I. Each LHC-monomer binds 12 Chl *a/b* molecules. The core complex of PS II binds 50 Chl *a* and the core complex of PS I binds 96 Chl *a* (Jordan et al., 2001). All complexes contain carotenoids according to published stoichiometries. The peripheral LHCs and the PS I-core are modeled with different states that account for red form chlorophylls. Altogether the PSE is modeled by 15 states. It is worth mentioning that only the combination of both photosystems in one reaction scheme allows to study the effect of exciton spill-over between them.

Fig. 2 shows the chosen absorption spectra of sPS I, sPS II and the PSE in units of molar absorption coefficients. The peaks at 440 and 680 nm belong to Chl *a*. Chl *b* causes a broadening of the 440 nm band a satellite band at 655 nm. The satellite band around 480 nm belongs to carotenoids. A red-shift of PS I compared to PS II in the Q_y -region may be noticed.

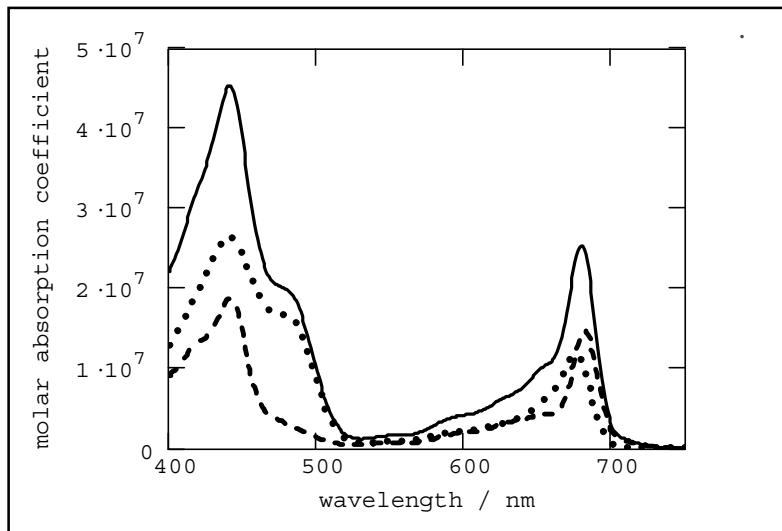


Fig. 2: Absorption spectra of sPS I (dotted line), sPS II (dashed line) and the PSE (solid line).

Fig. 3 illustrates on the sub-nanosecond scale (i) the faster closure of PS I-RCs compared to the PS II-RCs, (ii) the turn-over rates of the two photosystems under light-limiting conditions, and (iii) the total photochemical quantum yield of the PSE, both given by the limiting values at infinite times. Excitation of the PSE was at 660 nm (Gaussian width $\Delta\lambda = 4$ nm).

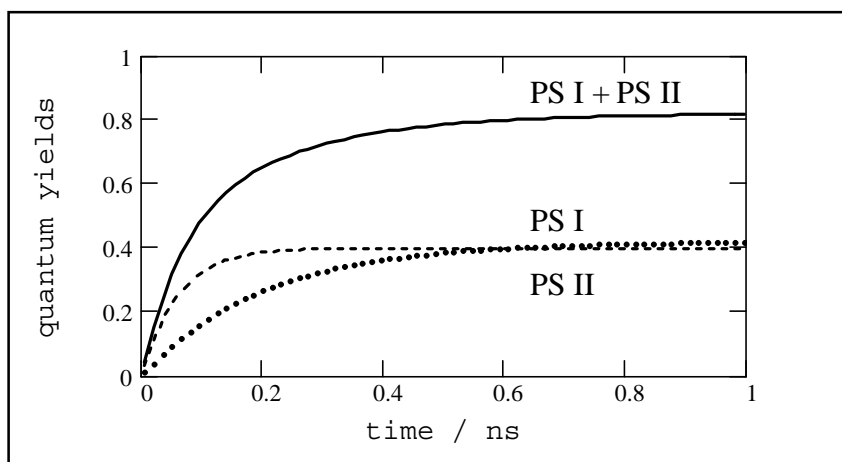


Fig. 3: Computed kinetics of trap closure in the two photosystems and in the PSE. The limiting values at infinite times are a measure for the turn-over rates of the two photosystems under light-limiting conditions.

Finally, Fig. 4 shows a computed action spectrum for the photochemistry in PS II (dashed line) in comparison with its absorption spectrum (dotted line). The lower amplitude of the action spectrum with respect to the PS II absorption spectrum correlates quantitatively with the low quantum yield of 0.75 of this photosystem. For comparison the absorption spectrum of the PSE is also shown (solid line).

II. Internet modeling modules

The modules on the Internet allow also the computation of the fluorescence excitation spectrum. Furthermore, the appliance of long- and band-pass filters can be simulated.

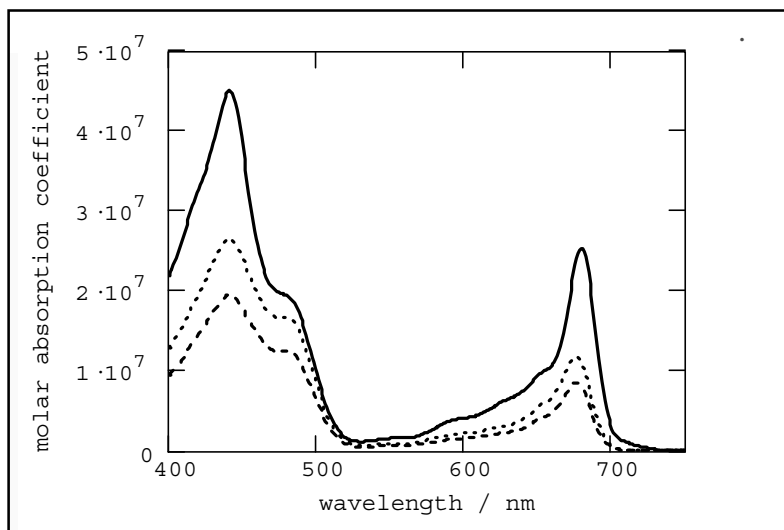


Fig. 4: Excitation spectrum of PS II-photochemistry (dashed line) and absorption spectrum of sPS II (dotted line). Absorption spectrum the PSE (solid line).

The following modules based on the above-mentioned concepts:

I. Three-state System	II. Four-state System
III. Spectral (De-)Composition	IV. Exciton Radical-pair Equilibrium Model

are presently (Aug. 2001) available under the Internet address:

<http://www.biologie.uni-osnabrueck.de/biophysik/trissl/modeling.htm>.

Further planned modules comprise: V. PS I, VI. PS II, VII. the PSE of green plants and algae and VIII. the PSE of phycobilisome containing organisms. All modules allow the export of the simulated data and the import of own experimental data, both in the ASCII format.

The computed results comprise the following items:

1. Numerical results (apparent time constants, yields, Boltzmann distribution, etc.)
2. Absorption spectra
3. Fluorescence spectra
4. Excitation spectra
5. Time dependence of states
6. Decay-associated spectra (DAS)
7. Decay-associated fluorescence yield spectra (DAQYS)

8. Transfer equilibria (TE)
9. 3D Time-resolved emission spectra (TRES)
10. 2D Time-resolved emission spectra (TRES)
11. Temperature dependence of linewidths
12. Temperature dependence of fluorescence
13. Fluorescence decay (TRES areas)
14. Fluorescence decays at selected wavelengths

Discussion

Model calculations serve several fruitful purposes: (i) Different models can be tested for compatibility with experimental data. (ii) The parameters of a model may be determined directly by global fitting of experimental data (= target analysis). (iii) Experimentally accessible as well as inaccessible quantities are predicted by the simulations. (iv) Model calculations can be used to test in advance whether planned experiments can potentially distinguish between alternative hypotheses.

Acknowledgements

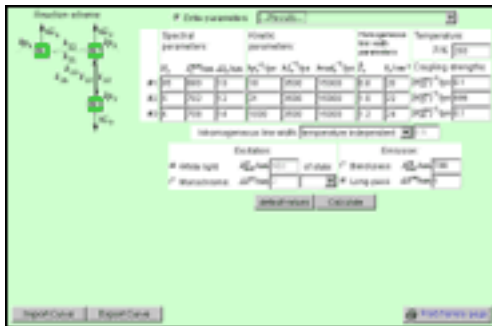
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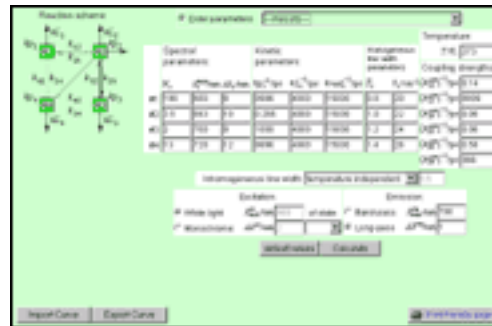
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Modeling Modules

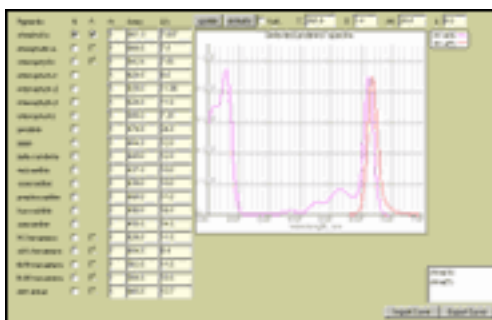
Three-state System



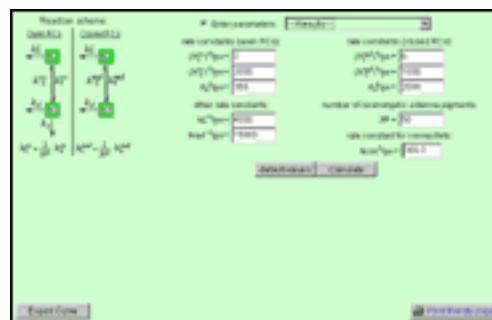
Four-state System



Spectral (De-)Composition



Exciton Radical-pair Equilibrium Model



Last modification: July 2001