

Chapter 4

Photophysics of Photosynthetic Pigment-Protein Complexes

Evgeny E. Ostroumov and Yaser R. Khan
*Department of Chemistry, University of Toronto,
80 St. George Street, Toronto, ON M5S 3H6, Canada*

Gregory D. Scholes*
*Department of Chemistry, Princeton University,
Washington Rd, Princeton, NJ 08544, USA*

and

Govindjee
*Department of Plant Biology,
Department of Biochemistry and Center of Biophysics and Quantitative Biology,
University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA*

Summary	98
I. Introduction.....	99
II. Chromophores in Photosynthesis and Their Electronic Properties	100
A. Chlorophylls.....	100
B. Carotenoids.....	102
III. Radiative Transitions.....	104
IV. Nonradiative Transitions.....	106
V. Radiative Versus Nonradiative Processes in Chlorophyll	107
VI. Excitation Energy Transfer, Förster Theory.....	109
VII. Considerations Beyond Förster Theory	113
VIII. Delocalization of Excitation, Molecular Excitons	114
IX. Excited State Complexes.....	117
X. Basic Photophysics of Non-Photochemical Quenching of Chlorophyll Fluorescence	118
XI. Concluding Remarks	120
Acknowledgments	120
References	120

*Author for correspondence, e-mail: gscholes@princeton.edu

Dedication This chapter is dedicated to the late Professor Robert M. Clegg (July 18, 1945 — October 15, 2012) of the University of Illinois at Urbana-Champaign (UIUC). He was a pioneer of the physics of living cells, and a world leader in fluorescence lifetime-resolved imaging microscopy (FLIM) of biological systems. He was an authority on fluorescence spectroscopy and rapid kinetic methods as applied to biological systems. Bob was a great human being, an intellectual, a “living library” to his students and his colleagues, a “connoisseur” of creative literature (not only in English, but in French and German as well), a historian of science, a great teacher, a poet, a storyteller, a great chef of diverse cuisine for friends and family, and a friend to all with whom he associated. One of us (Govindjee) was fortunate to have worked with him since he came to UIUC; they used FLIM in studying photoprotection against excess light in the green alga *Chlamydomonas reinhardtii*, and in avocado, a higher plant.

Summary

Photosynthesis converts solar energy into energy of chemical bonds. This process is initiated when a photon of sunlight is absorbed by a photosynthetic pigment molecule, followed by a highly efficient transfer of the excitation energy, excitation trapping, and charge separation at the reaction center. The excited state dynamics initiated by light absorption are central to the primary reactions of photosynthesis. Unless successfully transferred away from the excited chromophore within the excitation lifetime, the excitation energy relaxes back to the electronic ground state, either via emission of a photon (radiative decay) or through various nonradiative processes. The photosynthetic machinery can control the nonradiative relaxation rate: it can increase it under stress conditions (e.g., high light) by adjusting electronic properties of chromophores as well as their interaction, or decrease it under optimal conditions reaching >90 % efficiency of energy transfer. Some background in photophysics is, therefore, needed to understand the mechanistic aspects of the initial events following photoexcitation of photosynthetic complexes. The goal of this chapter is to describe the excited states involved in photoreactions and to outline the physical basis of photophysical processes involved in photosynthesis. We introduce the principles of light absorption and the nature of electronic excited states and light-initiated dynamics in photosynthetic complexes. De-excitation pathways, rate constants, quantum yields and lifetimes of fluorescence, excitation energy transfer and related photophysics are discussed. In the concluding section, we present an overview of the mechanisms of non-photochemical quenching (NPQ) of chlorophyll fluorescence in terms of photophysics of the excited states of photosynthetic pigments.

Abbreviations A_g, B_u, E_u – Excited state symmetry; a_w, b_g, e_g – Molecular orbital symmetry; B, Q_x, Q_y – Chlorophyll singlet states; C_w, S_w, σ_h, I, e – Molecular symmetry operators; Chl – Chlorophyll; EET – Excitation energy transfer; EM – Electromagnetic; FRET – Förster resonance energy transfer; HOMO – Highest occupied molecular orbital; IC – Internal conversion; ISC – Intersystem crossing; LUMO – Lowest unoccupied molecular orbital; LH2 – Light-harvesting complex 2 of purple bacteria; LHC – Light-harvesting complex; LHCII – Light-harvesting complex II, a major antenna of PS II in plants and green algae;

NPQ – Non-photochemical quenching of chlorophyll fluorescence; PS I, PS II – Photosystem I, photosystem II; PSU – Photosynthetic unit; RC – Reaction center; $S_0, S_1,$ and S_2 – Ground, first excited and second excited singlet states of carotenoids, not to be confused with similar names for the states of the oxygen evolving complex, the 4Mn-Ca (water) complex; TDC – Transition density cube; $\Phi_f, \Phi_{ET}, \Phi_{tr}, \Phi_{PQ}, \Phi_{NPQ}$ – Quantum efficiencies of fluorescence (f), energy transfer (ET), trapping (tr), photochemical (PQ) and non-photochemical quenching (NPQ) respectively; note that Φ_{PQ} is equivalent to Φ_p used by other authors

I Introduction

Photosynthesis starts with absorption of light by a pigment molecule that, in most photosynthetic organisms, is embedded in pigment-protein complexes. The processes following light absorption occur on remarkably fast timescales by vastly distinctive mechanisms. The aim of this chapter is to present an overview of photophysical background relevant for the light reactions of photosynthesis. For background on physical aspects of the interaction of light with living matter, see Clayton (1970); for basic background knowledge on photosynthesis, see Rabinowitch and Govindjee (1969) and Blankenship (2014); for details on the entire process of photosynthesis, see Eaton-Rye et al. (2012).

An array of different pigments is used for the harvesting of light. Among them are members of the porphyrin family – chlorophylls or bacteriochlorophylls – found in all photosynthetic membranes (see Grimm et al. 2006). Light absorption promotes the light-absorbing molecule (chromophore) from the ground state to an excited electronic state, thereby storing much of the energy of the photon in the molecule. This energy, however, is only stored transiently because excited electronic states decay back to the ground state by de-excitation processes involving either emission of light (fluorescence or phosphorescence) or “rapid cooling” by nonradiative processes, e.g., heat emission. Isolated chlorophylls, for example, have an excited-state lifetime on the order of 5 ns (Brody and Rabinowitch 1957; Kaplanová and Parma 1984). For fluorescence properties of photosynthetic pigments in vitro, see Seely and Connolly (1986). When chlorophyll *a* molecules are embedded in a protein, nonradiative quenching of their excited states increases due to interactions between the pigments and their environment, decreasing their lifetime to ~4 ns (Mullineaux et al. 1993; Connelly et al. 1997). The deactivation of excited states is determined by the intrinsic properties of the chromophore and how it interacts with the environment. It is through such interactions that the protein can tune, sometimes quite remarkably, the properties of the chromophores in light-harvesting complexes, either directly or by arrangement

of several chromophores into aggregates with a strong inter-chromophore interaction.

The primary event in photosynthesis, after light is absorbed by chromophores in a light-harvesting complex, is transfer of that excitation energy to reaction centers (RC) where the energy is stored by charge separation. This *excitation energy transfer* (EET) process must be faster than the excited-state lifetime of chlorophyll in order for the excitation energy to reach the RC rather than be lost via fluorescence or nonradiative decay. We know that nature does this successfully because the efficiency of energy transfer from arrays of light-harvesting complexes to reaction centers is very high (typically >90 %, see e.g., Krause and Weis 1991; Wientjes et al. 2013). This efficiency, which in some cases requires rapid jumps (~300 fs on average) of excitation from molecule to molecule, is facilitated by chlorophyll molecules being present in thylakoid membranes at a typical concentration of >0.2 M. In solution, isolated chlorophylls are efficiently quenched by concentration quenching at concentrations as low as ~0.1 M (Beddard and Porter 1976). The remarkably high efficiency of energy transfer achieved in natural photosynthetic complexes is due partly to the arrangement of chlorophyll and other chromophores in a way that prevents concentration quenching. Other physical principles, such as a dense, ordered arrangement of bacteriochlorophylls in a ring of the light-harvesting complexes, LH2, of purple photosynthetic bacteria, allow the chromophores to work cooperatively. States termed *exciton states* are formed (Monshouwer and van Grondelle 1996; van Amerongen et al. 2000a) leading to a shift of the absorption spectrum, delocalization of excitation energy, and efficient intra- and inter-protein energy transfer (Sundström et al. 1999; Scholes and Fleming 2000; Robert 2008). Another example is the exceptionally rapid energy transfer from carotenoids to (bacterio-)chlorophylls in light-harvesting complexes (LHCs) despite the ultrashort lifetimes of carotenoid excited states (Gradinaru et al. 2000; Croce et al. 2003; Frank and Polívka 2008). For overviews on the photochemistry of carotenoids, see Frank et al. (1999).

Efficient harvesting of solar energy is achieved by using more than one type of chromophore in antenna complexes. For a discussion of various light-harvesting antenna systems of plants, see chapters in Green and Parson (2003). Accessory pigments used by plants and photosynthetic microbes include, e.g., carotenoids that absorb blue-to-green light and phycobilins that absorb green-to-orange light. These pigments transfer absorbed energy to chlorophyll *a* with high efficiency (see, e.g., Duysens 1952; Govindjee 1999; Clegg et al. 2010). Despite their simple molecular structure, carotenoids have unusual electronic properties, e.g., an optically forbidden lowest singlet state with an ultrashort (~ 10 ps) lifetime (Christensen 2004). Because of their short excited-state lifetime, carotenoids are efficient quenchers of both singlet and triplet excited states (Telfer et al. 2008). Moreover, carotenoids can form charge-transfer complexes with chlorophylls, creating efficient traps for excitation energy (Gradinaru et al. 2000; Hsu et al. 2001).

A significant feature of LHCs is their ability to respond to ambient light conditions, and modify chromophores and/or interactions among chromophores such that excess electronic excitation is dissipated by heat if the amount of the absorbed light exceeds the capacity of the photochemical machinery (i.e., charge separation in RCs). This process, accessed via non-photochemical quenching (NPQ) of chlorophyll fluorescence, can decrease the efficiency of energy transfer by a factor of 2–10 (as judged by fluorescence lifetime) on a time scale of several seconds to minutes (see, e.g., Weis and Berry 1987; Krause and Weis 1991; Horton et al. 1996; Gilmore 1997; Gilmore and Govindjee 1999). The mechanisms of photoreactions in photosynthesis remain incompletely understood due to the complexity of the electronic structure of chromophores (chlorophylls and carotenoids in algae and higher plants), the sophisticated schemes of chromophore interaction (excitonic coupling, charge transfer states, forbidden transitions), and the significant role

of the protein environment in tuning the properties of embedded chromophores (heterogeneity of electronic properties, polar environment, alteration of chromophore geometry). In this chapter, we focus on electronic properties of chlorophylls and carotenoids, the interaction of their excited states, and excitation energy pathways relevant to NPQ of fluorescence of chlorophyll *a* molecules.

II Chromophores in Photosynthesis and Their Electronic Properties

A Chlorophylls

Chlorophyll molecules serve as the primary photoreceptors in most photosynthetic organisms (Scheer 2006). The latter molecules belong to the cyclic tetrapyrrole family (porphyrins) with four pyrrole residues in the macrocycle (Fig. 4.1). In different chlorophylls, the peripheral pyrrole carbons bear various side chains. The nitrogen atoms of the porphyrin ring bind magnesium ion (Mg^{2+}).

The porphyrin ring is near-planar, and the electron density of its π -electrons is strongly delocalized over the entire ring. In accordance with its square symmetry, porphyrin belongs to the D_{4h} point group (Rubio et al. 1999; Liao and Scheiner 2002). Defining symmetry of a molecule provides a convenient way to label molecular orbitals and thus electronic transitions. Well established rules then allow prediction of the spectroscopic properties of the transitions, for example, whether they are allowed (a bright absorption band) or forbidden (a dark state – not seen in the absorption spectrum, but nevertheless present in the ladder of electronic states). In this context, symmetry indicates operations on the molecule leading to a state indistinguishable from the original state (see Fig. 4.2), e.g., rotation of the molecule by $180^\circ/n$ (C_n), reflection in a given plane (σ_h), identity operation (e), center of inversion (I), and rotary reflection S_n (Willock 2009). The symmetry operations and the corresponding irreducible representations (a symmetry

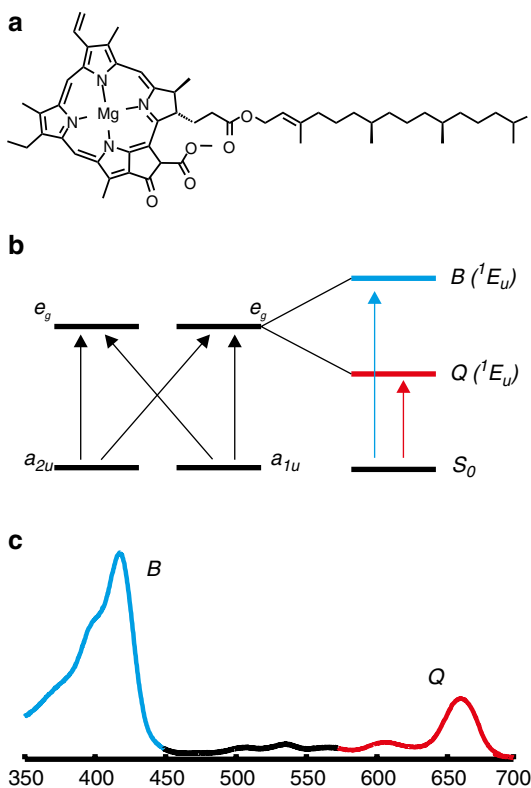


Fig. 4.1. Chlorophyll *a* molecule. (a) Molecular structure, (b) molecular orbitals and electronic level structure, (c) absorption spectrum. The *capital letters* indicate states, *lower case letters* indicate orbitals.

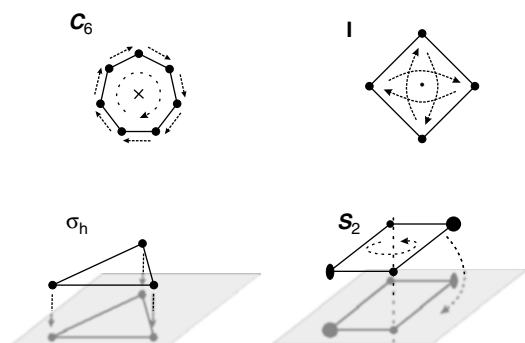


Fig. 4.2. Molecular symmetry operations. C_6 – rotation by $360^\circ/6=60^\circ$, i – center of inversion, σ_h – plane reflection, S_2 – rotary reflection by $360^\circ/2=180^\circ$.

property of an excited state) for D_{4h} point group are shown in Table 4.1. Here, A and B designate singly degenerate symmetric and anti-symmetric representations with respect

to rotation (C_n), E designates the doubly degenerate representation with respect to rotation (C_n), the g and u subscripts designate symmetric (gerade: even) and anti-symmetric (ungerade: odd) representation with respect to the center of inversion (I), and ' and '' designate symmetric and anti-symmetric representation, respectively, with respect to plane reflection (σ_h).

In chlorophylls, the lowest-energy optical absorption is due to two electronic π - π^* transitions between the two a_{1u} and a_{2u} highest occupied molecular orbitals (HOMO) and two e_g lowest unoccupied molecular orbitals (LUMO) (Rubio et al. 1999). The relative energies corresponding to these two transitions depend on the central metal ion and the ring substituents. These two configurations mix quantum-mechanically to yield electronic excited states that can be measured by spectroscopy. Owing to that mixing, the electronic states in the absorption spectrum are split into two excited states of 1E_u symmetry. The higher energy state is known as the Soret band (B band) and the lower energy state is known as the Q band (Gouterman 1961; Nemykin and Hadt 2010). Each band is further decomposed into two sub-bands corresponding to x -polarized and y -polarized transitions relative to the square symmetry of the macrocycle (e.g., Q_x and Q_y ; Gouterman 1961; Weiss 1978; Shipman 1982; Scheer 2006). The intense Soret band with an absorption maximum at ~ 400 nm is associated with the symmetric nitrogen atoms of the macrocycle (Britton 1983). The weaker Q band is more strongly perturbed by the peripheral groups of the macrocycle. For instance, the elongation of the π -system in bacteriochlorophylls causes a shift of the Q_y band to 770 nm (in solution). Notably, the Q_x state (Q band is polarized along the x -axis of the macrocycle) is weaker and less sensitive to the peripheral groups, showing no red-shift. Distortions of the chlorophyll ring from ideal planar geometry serve as an additional perturbation factor (Zucchelli et al. 2007).

When bound to protein, chlorophyll's electronic properties change and both the total absorption spectrum and the excitation

Table 4.1. Symmetry characters and irreducible representations of porphyrins (D_{4h} point group). Numbers designate the level of degeneracy (1 or 2), the sign designates symmetric/antisymmetric representation (+/-)

D_{4h}	E	$2C_4$	C_2	$2C_2'$	$2C_2''$	I	$2S_4$	σ_h	$2\sigma_v'$	$2\sigma_d''$
a_{1g}	1	1	1	1	1	1	1	1	1	1
a_{2g}	1	1	1	-1	-1	1	1	1	-1	-1
b_{1g}	1	-1	1	1	-1	1	-1	1	1	-1
b_{2g}	1	-1	1	-1	1	1	-1	1	-1	1
e_g	2	0	-2	0	0	2	0	-2	0	0
a_{1u}	1	1	1	1	1	-1	-1	-1	-1	1
a_{2u}	1	1	1	-1	-1	-1	-1	-1	1	1
b_{1u}	1	-1	1	1	-1	-1	1	-1	-1	1
b_{2u}	1	-1	1	-1	1	-1	1	-1	1	-1
e_u	2	0	-2	0	0	-2	0	2	0	0

lifetime are affected. For a discussion of the lifetime of fluorescence in vivo, see Moya et al. (1986), and for basics on lifetimes measured by fluorescence, see Noomnarm and Clegg (2009). There are two common kinds of excited states of molecules, designated as singlet or triplet states, that differ in the arrangement of electron spins among the orbitals involved in the transition. Further details can be found elsewhere (see, for example, Zahlan 1967; Angerhofer 1991). The ground electronic state of photosynthetic pigments is singlet, such that the transitions seen as absorption bands (and those that transfer energy for light harvesting) are the singlet states. Singlet-to-triplet transitions are forbidden at first order of theory, but can occur in relaxation pathways, as we describe later in this chapter. The triplet states of chlorophyll can activate a highly reactive and potentially destructive excited state of oxygen called *singlet oxygen*. Triplet states of carotenoid molecules play an important role by quenching singlet oxygen (Ke 2001a; Telfer et al. 2008).

Chlorophylls not only absorb light, but also function as efficient electronic energy donors and acceptors, mediating ultrafast energy transfer across the photosynthetic unit. For a discussion of which properties allow Chl a to have different functions, see, e.g., Björn et al. (2009). After light absorption, the excitation often has to travel up to

tens of nanometers in order to reach the RC. A large number of chlorophyll-chlorophyll energy-transfer events are required in sequence and in competition with the excited-state lifetime. Many details of this process are optimized, as summarized by Scholes et al. (2011). One interesting example of optimization involves the central element of the chlorophyll molecule, Mg^{2+} , that maximizes the lifetime of the excited states, lengthening the time for the excitation to reach the reaction center (Kobayashi et al. 2006).

B Carotenoids

As mentioned above, photosynthetic cells also contain carotenoids that serve as accessory pigments (Govindjee 1999; Ke 2001b; Telfer et al. 2008). Carotenoids consist of long conjugated alternating single and double carbon bonds, and hydrocarbon side chains. The molecular structure of one of the most ubiquitous carotenoids, β -carotene, is shown in Fig. 4.3a. Owing to their special electronic structure, carotenoids have remarkable electronic and spectroscopic properties (Christensen 2004).

The electronic properties of carotenoids, as polyenes, are dictated mostly by the π -electronic structure of conjugated double carbon bonds. Polyenes have thus been extensively used as a model system of carotenoids both experimentally and in theoretical

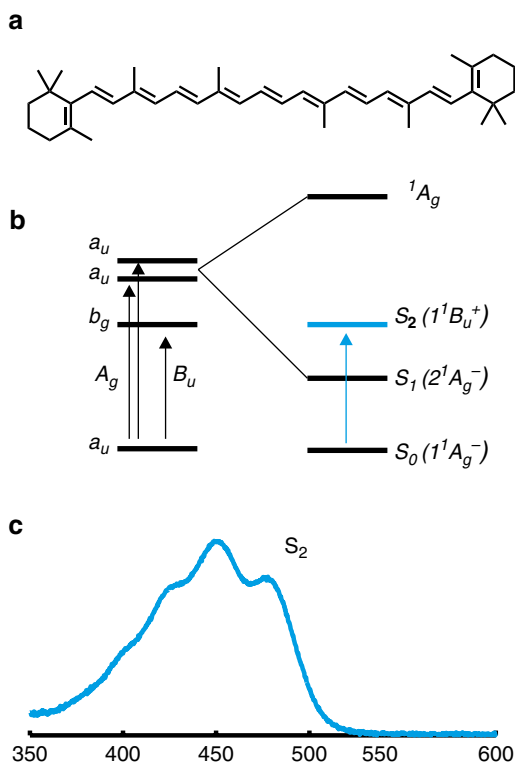


Fig. 4.3. β -carotene molecule. (a) Molecular structure, (b) molecular orbitals and electronic level structure, (c) absorption spectrum. The capital letters indicate states, lower case letters indicate orbitals.

Table 4.2. Symmetry table of linear polyenes (C_{2h} point group). All orbitals have single degeneracy level; the sign designates symmetric/antisymmetric representation (+/-)

C_{2h}	E	C_2	I	σ_h
a_g	1	1	1	1
b_g	1	-1	1	-1
a_u	1	1	-1	-1
b_u	1	-1	-1	1

calculations. Polyenes belong to the C_{2h} point group. The symmetry operations and irreducible representations for this point group are summarized in Table 4.2. The excited states of polyenes are of A_g and B_u symmetries only (see Fig. 4.3b) because all π -orbitals (a_u and b_g) are anti-symmetric with respect to reflec-

tion operation (σ_h in Table 4.2). According to the simple free-electron model, where no correlation effects are taken into account, the lowest excited state is predicted to have B_u symmetry. However, numerous experiments have shown that the first excited state is a different state and has A_g symmetry. The latter low-energy $2A_g^-$ (S_1) state is, similarly to porphyrin Q and B states, due to configuration interaction. It results from mixing of two different transition configurations involving the a_u orbitals as shown in Fig. 4.3b.

The S_1 excited state has the same symmetry as the ground state S_0 ($1A_g^-$), and the corresponding transition is thus forbidden by selection rules. The S_1 state is often referred to as the “dark” state. However in some cases, e.g., when the conjugated chain of the molecule is short, the S_1 state couples to the higher lying S_2 ($1B_u^+$) state and gains weak transition dipole moment. Several studies have shown that in these cases weak fluorescence is emitted from the S_1 state of carotenoids (Mimuro et al. 1992; DeCoster et al. 1992). An exceptional feature of the S_1 state of carotenoids, as compared to that of other pigment molecules, is an ultrafast relaxation time on the order of ~ 10 ps (Polívka and Sundström 2004). This efficient thermal relaxation to the ground state makes carotenoids efficient quenchers of excited electronic states and points to their possible role in NPQ (Frank et al. 1994; see also, e.g., Polívka and Frank, Chap. 8; Walla et al., Chap. 9; van Amerongen, Chap. 15). Carotenoids are well known for their photoprotective functions, including quenching of chlorophyll triplet states as well as singlet oxygen via triplet-triplet excitation energy transfer (Truscott and Edge 2004; Telfer et al. 2008). Quenching of excess chlorophyll excitation via singlet-singlet EET to the carotenoid S_1 state would augment carotenoid photoprotective function by preventing formation of singlet oxygen and chlorophyll triplet states. For a more complete picture of the photochemistry of carotenoids, see chapters in Frank et al. (1999).

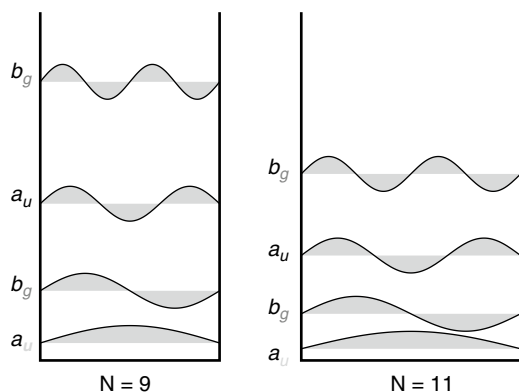


Fig. 4.4. Free electron (particle-in-a-box) model calculation showing the effect of an increase in conjugation length, N , from nine to eleven double carbon bonds on the lowest orbital energies. The orbitals are labeled by their respective symmetry (see Table 4.2).

The transition from the ground state to the S_2 (IB_u^+) state is strongly allowed and is responsible for the absorption band with notable vibrational structure in the 400–500 nm spectral range (Fig. 4.3c). The S_2 state has a very short lifetime (100–200 fs) owing to fast relaxation to the lower S_1 state by internal conversion. Despite such ultrafast internal conversion, the S_2 state contributes significantly to carotenoid-to-chlorophyll energy transfer in several light-harvesting complexes, and especially in LH2 complexes of purple bacteria (MacPherson et al. 2001; Cong et al. 2008).

The properties of the electronic states depend strongly on the structure of the carotenoid molecule, and in particular on the length of the conjugation chain. The $S_0 \rightarrow S_1$ and $S_0 \rightarrow S_2$ transition energies are decreased as conjugation length of the molecule increases. The qualitative trend in transition energies can be predicted by a calculation using the free-electron model (“particle-in-a-box”; Christensen 2004; Bittner 2009; Scherer and Fischer 2010), as shown in Fig. 4.4. The dependence of the excited-state relaxation rate on the conjugation length corresponds to the changes of the transition energies and can usually be described by the energy-gap law (Englman and Jortner 1970). The energy-gap law allows satisfactory

prediction of the energies of the S_1 state (Andersson et al. 1995; Chynwat and Frank 1995), whereas substantial deviation from the law is found for the S_2 state (Frank et al. 1997; Kosumi et al. 2006). One of the explanations of this disagreement with the energy-gap law is the presence of an intermediate “dark” state below the S_2 state. The early theoretical work by Tavan and Schulten (1986) predicted that two dark states, of A_g^- and B_u^- symmetry, should be found below the S_2 state for carotenoids with a conjugation system longer than 10 (see also Schmidt and Tavan 2012). However, experimental observations of these states have been controversial, and the existence of such states has been hotly debated during the last decade (Wohlleben et al. 2004; Yoshizawa et al. 2006; Polívka and Sundström 2009; Christensson et al. 2010).

III Radiative Transitions

Optically allowed electronic states of the photosynthetic pigments may be excited and de-excited radiatively, i.e., by absorption and emission of a photon (Fig. 4.5a). With the notable exception of the dark S_1 state of the carotenoids, the lowest excited singlet states of other photosynthetic pigments (e.g., chlorophylls) absorb light and subsequently emit fluorescence.

Light absorption can be modeled by describing a pigment molecule as a classical oscillator with a natural frequency and oscillation direction. A harmonic electromagnetic (EM) field can transfer energy to such a molecule only if the electric field (i) has a non-zero component in the direction of the pigment’s natural oscillation (Fig. 4.5b), and (ii) drives the pigment’s electrons at a frequency close to resonance with the pigment’s natural frequency (in other words, if the energy of a photon, $h\nu$, is close to the transition energy $\varepsilon(S_1) - \varepsilon(S_0)$ of the pigment molecule, Fig. 4.5a). These principles can be described quantitatively in a quantum-mechanical context. Interaction between the radiation field (E) and the pigment is

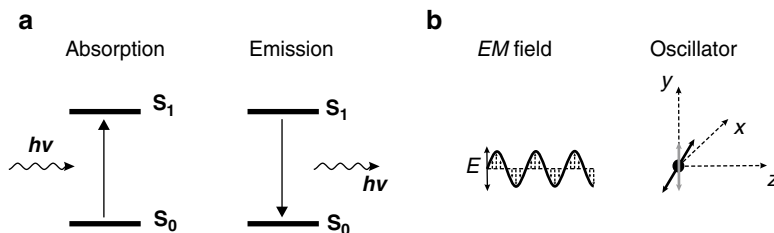


Fig. 4.5. (a) Radiative transitions and (b) interaction of y -polarized electromagnetic (EM) wave with an oscillator. Projection of the oscillator polarization vector on the y -axis is shown in grey.

described in terms of a coupling (V_{nm}), which mediates an electronic transition from state $|m\rangle$ to state $|n\rangle$, and is given by

$$V_{nm} = -\langle n | \hat{E} \cdot \hat{\mu} | m \rangle = -E \cdot \langle n | \hat{\mu} | m \rangle = -E \cdot \mu_{nm} \quad (4.1)$$

where $\hat{\mu}$ is a transition dipole moment vector – the operator behind the optical transition between all of the electronic states of the molecule, and μ_{nm} is the transition dipole moment between states $|m\rangle$ and $|n\rangle$.

Equation 4.1 is a simplification of a more general expression, where we have assumed that the electric field strength $E(r)$, as a function of coordinate “ r ”, is constant over the spatial extent of the pigment. For radiation in the infrared, visible and UV regions of the spectrum, this simplification is an excellent approximation because the wavelength of the light (>100 nm) is two orders of magnitude larger than the size of a pigment molecule ($r \sim 1$ nm). Thus, the phase of the EM wave and, therefore, the EM-field amplitude do not vary appreciably over the molecule. The result of such an analysis is that the electronic transitions of a molecule can be described as a transition-point dipole. The point-dipole approximation does not account for spatial inhomogeneity of electronic features of the molecule, i.e., the extent of delocalization or shape of the transition density; this approximation retains only a specific magnitude and direction in analogy to the classical oscillator model discussed above (van Amerongen et al. 2000a; Renger and

Holzwarth 2008). Despite its simple form, the point-dipole approximation is often sufficient to describe a field-matter interaction with good precision.

For the discussion of dark states, the model of purely electronic transition dipoles needs modification. Formally, this involves making corrections beyond the Born-Oppenheimer approximation that considers changes in electronic and nuclear properties induced by excitation of a molecule to be independent. The corrections to the Born-Oppenheimer approximation open up mechanisms whereby dark states can “borrow” transition strength from a bright state if vibrations appropriately distort the molecule during the transition. A good example is the carotenoid S_1 state. Although in linear polyenes, the optical transition to this state is strictly forbidden by selection rules and the coupling element V_{nm} ($\sim \mu_{nm}$) = 0, the S_1 state of carotenoids can gain weak transition dipole moment μ_{nm} by various mechanisms, e.g., due to deviation of carotenoid geometry from linear polyene (side groups, protein environment) or by coupling to an optically allowed state. The latter mechanism is known as Herzberg-Teller vibronic coupling, where a dark excited state gains weak transition dipole moment μ_{nm} by coupling to an optically allowed excited state lying in the vicinity of the dark state (Herzberg and Teller 1933; Zgierski 1974). In accordance with this mechanism in short-chain carotenoids, where S_2 and S_1 states are close in energy, the dark S_1 state shows fluorescence due to vibronic coupling to S_2 state (Mimuro et al. 1992; DeCoster et al. 1992). However,

the yield of this fluorescence is very low due to extremely fast (~ 10 ps) internal conversion to the ground state.

IV Nonradiative Transitions

After light absorption, the energy stored in the excited state of a chromophore can decay by competing pathways that are summarized by the Jabłoński diagram, shown in Fig. 4.6 (Lakowicz 1999). These pathways can be separated into radiative transitions, such as fluorescence and phosphorescence, and non-radiative transitions, such as internal conversion and intersystem crossing. According to the Kasha rule (Kasha 1950), vibrational relaxation and radiationless processes from higher excited states are much faster than fluorescence. Therefore, steady-state radiative emission (e.g., fluorescence) is observed predominantly from the lowest vibrational level of the lowest excited electronic state of a certain spin multiplicity (Lakowicz 1999). In isolated molecules, the three main processes competing with each other during singlet electronic state de-excitation are fluorescence, internal conversion (IC), and intersystem crossing (ISC). Fluorescence is a convenient probe of the efficiencies of de-excitation processes, because any increase in efficiency (rate) of IC or ISC will immediately suppress fluorescence yield and decrease overall excited-state lifetime, while the rate corresponding to the fluorescence

(also known as radiative) pathway will remain unchanged.

The two main non-radiative transitions in photosynthetic pigments, IC and ISC, differ from each other by the spin multiplicity of the acceptor state. IC refers to the redistribution of electronic excitation energy into vibrational motions of a lower-lying electronic state of the same spin multiplicity as the donor state (see Fig. 4.6), and is highly efficient when a dense manifold of vibronic states of the acceptor state is resonant with the vibrationally relaxed level of the donor state. Typically, the higher-energy excited states have very high IC rates and energy relaxes to the lowest excited state within <1 picosecond (ps). This is the case for both carotenoid and chlorophyll molecules. In contrast, the lowest excited state usually shows slower relaxation, on a timescale of several nanoseconds (ns). The Q_y state of chlorophylls is a good example of that rule, showing a lifetime of 5 ns (Brody and Rabinowitch 1957; Livingston 1960; Kaplanová and Parma 1984). IC does not contribute to the relaxation of the Q_y state of chlorophyll in solution (Livingston 1960; Bowers and Porter 1967), and it only slightly increases the overall relaxation rate in chlorophylls embedded in proteins (Mullineaux et al. 1993; Connelly et al. 1997). The lowest excited state of the carotenoid, the S_1 state, is a notable exception from the latter rule. The relaxation of the carotenoid S_1 state is very fast, due to highly efficient IC, and has a typical lifetime of several ps (Polívka and Sundström 2004). As a result of high IC rates, very little, or no fluorescence is observed from carotenoids.

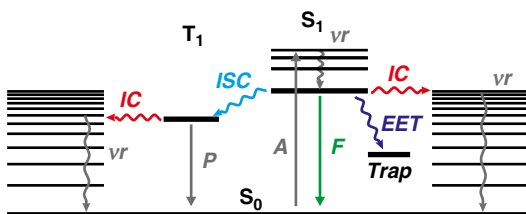


Fig. 4.6. Simplified Jabłoński diagram showing energy pathways in a molecule. S_0 : ground state; S_i : excited singlet state; T_i : excited triplet state; A: absorption of a photon; F: fluorescence; P: phosphorescence; IC: internal conversion; ISC: intersystem crossing; EET: excitation energy transfer; vr: vibrational relaxation.

Intersystem crossing is a non-radiative transition between two electronic states with different spin multiplicity. Formally, transitions with changing multiplicity are spin forbidden, and can only take place when additional processes relax the selection rules. Most of the observed singlet-triplet transitions are due to spin-orbit coupling. Spin-orbit coupling is a relativistic effect and is especially pronounced when heavy atoms are involved in the excitation process.

Coupling strength can also be influenced by a small energy gap, a change in the orbital type, strong displacement of the potential energy surfaces, and vibronic interaction of the singlet and triplet states (Marian 2012).

In chlorophyll molecules, ISC is facilitated by spin-orbit and spin-vibronic coupling between $\pi\pi^*$ triplet state and $n\pi^*$ singlet state (change of orbital type). This coupling is mostly due to nitrogen and oxygen atoms of the porphyrin ring, whereas the central Mg^{2+} is not significantly involved in ISC (Clarke et al. 1976). ISC has a substantial yield in chlorophylls (>60 % in isolated chlorophylls; Bowers and Porter 1967) and the generated chlorophyll triplet states ($^3Chl^*$) can be easily quenched by oxygen, forming reactive oxygen species. Because reactive oxygen species can potentially significantly damage photosynthetic apparatus of the organism, ISC in chlorophylls is a critical process.

In carotenoids, ISC is very inefficient owing to ultrafast IC rates (<1 ps). However carotenoid triplet states play a crucial role in quenching $^3Chl^*$ and in deactivating reactive oxygen species, and therefore they can protect the organism against photodamage (Truscott and Edge 2004; Telfer et al. 2008). This function of carotenoids is especially important in the RCs of photosystems I and II (PS I and PS II), where an additional pathway of $^3Chl^*$ formation is activated as a result of the charge recombination (Ke 2001b).

V Radiative Versus Nonradiative Processes in Chlorophyll

In isolated chlorophyll molecules, fluorescence (radiative transition, with a rate constant k_{rad}) from the Q_y excited electronic state back to the ground state competes with other de-excitation processes, such as ISC and IC (with rate constants k_{ISC} and k_{IC}). The total lifetime $\tau=5$ ns of the excited state is the inverse of the total k (total transitions per unit time) of excitation quenching pathways:

$$\tau = \frac{1}{k} = \frac{1}{k_{rad} + k_{IC} + k_{ISC}} \quad (4.2)$$

The quantum yield of each quenching process can be calculated as a ratio of the rate of the process of interest to the total rate of excitation quenching (inverse of lifetime). The quantum yield of fluorescence, for instance, can be calculated as follows:

$$\Phi_{fl} = \frac{k_{rad}}{k_{rad} + k_{IC} + k_{ISC}} \quad (4.3)$$

For chlorophyll in solution, fluorescence competes mostly with ISC, while k_{IC} is negligible (Fig. 4.7a; Bowers and Porter 1967). However, chlorophylls embedded in isolated proteins show a decrease of the fluorescence lifetime to ~4 ns by a small amount of thermal dissipation k_{IC} (Mullineaux et al. 1993; Connelly et al. 1997).

In vivo, two more processes contribute to chlorophyll de-excitation – photochemical quenching k_{PQ} and thermal de-excitation reflected in non-photochemical quenching k_{NPQ}

$$\tau = \frac{1}{k_{rad} + k_{IC} + k_{ISC} + k_{PQ} + k_{NPQ}} \quad (4.4)$$

In photosynthesis, photochemical quenching of chlorophyll fluorescence refers to processes of de-excitation of chlorophyll excited states that lead to photochemical charge separation in the reaction center. It is important to note that in Fig. 4.7 and Table 4.3, k_{PQ} is rate constant of the first reaction of charge separation. In closed RCs, the electron transfer chain is blocked after the second charge separation step, while the first charge separation reaction is still active. Therefore, the efficiency diagrams for open and closed RCs, as shown in Fig. 4.7, do not differ significantly. The **rate** of photochemical quenching k_{PQ} is very high and, as a result, the **yield** of chlorophyll fluorescence is reduced by a factor of >10 (see Fig. 4.7b;

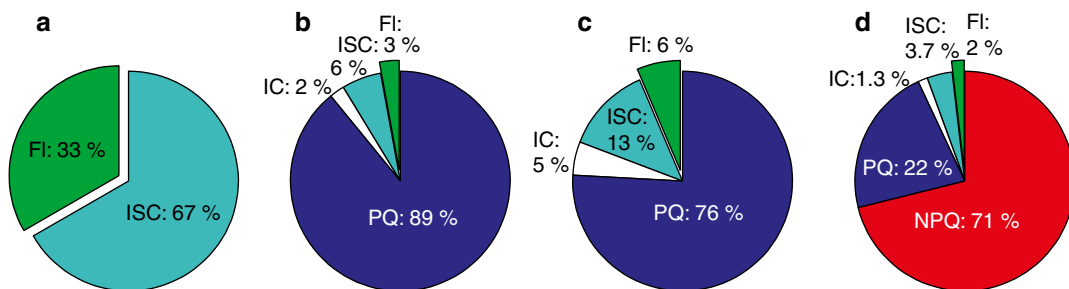


Fig. 4.7. Yields of various de-excitation mechanisms of chlorophyll *a* in (a) solution, (b) PS II with open reaction centers, (c) PS II with closed reaction centers, (d) PS II with closed reaction centers and under NPQ conditions. *Fl* fluorescence (green), *IC* internal conversion (white), *ISC* intersystem crossing (cyan), *PQ* photochemical quenching (trapping by RC, dark blue), *NPQ* non-photochemical quenching (red). The yields were calculated (see Eqs. 4.3 and 4.5) from kinetic rates shown in Table 4.3 and should not be confused with yields based on fluorescence intensity (Brody and Rabinowitch 1957; Barber et al. 1989).

Table 4.3. Rate constants involved in de-excitation of chlorophyll *a*.

	FI	IC	ISC	PQ (open RC)	PQ (closed RC)	NPQ
k, s^{-1}	$6.5 \cdot 10^7$	$5 \cdot 10^7$	$1.3 \cdot 10^8$	$2.0 \cdot 10^9$	$7.7 \cdot 10^8$	$2.5 \cdot 10^9$
Refs.	1,2,3	4,5	1,2,3	6,7	6,7,8	9,10

References: 1 (Shipman 1980); 2 (Renger 1999); 3 (Peterman et al. 1995); 4 (Mullineaux et al. 1993); 5 (Connelly et al. 1997); 6 (Schatz et al. 1988); 7 (Hansson and Wydrzynski 1990); 8 (Brody and Rabinowitch 1957); 9 (Miloslavina et al. 2008); 10 (Gilmore et al. 1995)

Barber et al. 1989; Krause and Weiss 1991). Non-photochemical quenching of chlorophyll fluorescence (the topic of this book) is used as a measure of all other processes of chlorophyll de-excitation that do not result in charge separation. When activated, some of these processes create additional pathways for chlorophyll de-excitation. The rate of de-excitation measured as NPQ varies depending on plant species and conditions, with $k_{NPQ} = 2.5 \cdot 10^9 \text{ s}^{-1}$ reported for higher plants (Gilmore et al. 1995; Miloslavina et al. 2008; see Demmig-Adams et al., Chap. 24, for pronounced differences in NPQ among plants). This means that fluorescence yield is strongly diminished when the organism is exposed to high-light conditions (Fig. 4.7c). The values of the rate constants of the above-described processes are summarized in Table 4.3.

Chlorophyll fluorescence is a sensitive probe of the energy conversion processes in photosynthesis and it is broadly used for monitoring the “metabolic status” of cyanobacteria, algae and plants, as well as

for studying the mechanisms of responses to the environment in vivo (Govindjee et al. 1986; Papageorgiou and Govindjee 2004; Papageorgiou 2012). A number of techniques have been developed for fast and efficient measurements of fluorescence yield of both terrestrial plants (from leaves) and algae (in the liquid phase). Responses to the environment and/or metabolic status are readily assessed by measuring fluorescence upon exposure to high light intensity, as what is termed the Kautsky effect (Govindjee 1995, 2004; Kolber et al. 1998; Schreiber 2004; Strasser et al. 2004). Moreover, photosynthetic activity can be monitored remotely using laser-based instruments, LIDARs – light detection and ranging (Gorbunov et al. 2000; Burikov et al. 2001; Ananyev et al. 2005). Typically, the chlorophyll fluorescence spectrometry techniques provide the following quantities: minimal fluorescence yield of dark- and light-exposed organism (F_0 and F_0'), maximal fluorescence yield of dark and light-exposed organism (F_m and F_m'), fluorescence yield under ambient conditions

(F) and their products. The quantum yields (i.e., efficiencies) of photochemical (Φ_{PQ}) and non-photochemical quenching/thermal de-excitation (Φ_{NPQ}) can be calculated using both rate constants, measured by time-resolved spectroscopies, and fluorescence yields (F ; F_m and F_m'), measured by chlorophyll fluorescence techniques, as follows:

$$\begin{aligned}\Phi_{PQ} &= \frac{F}{F_m'} - \frac{F}{F_m} \\ &= \frac{k_{PQ}}{k_{rad} + k_{IC} + k_{ISC} + k_{PQ} + k_{NPQ}}\end{aligned}\quad (4.5a)$$

$$\begin{aligned}\Phi_{NPQ} &= \frac{F_m' - F}{F_m'} \\ &= \frac{k_{NPQ}}{k_{rad} + k_{IC} + k_{ISC} + k_{PQ} + k_{NPQ}}\end{aligned}\quad (4.5b)$$

Detailed information on calculation of quantum yields using chlorophyll fluorescence parameters and rates can be found in Kasajima et al. (2009; see also, e.g., Logan et al., Chap. 7).

VI Excitation Energy Transfer, Förster Theory

Light-harvesting proteins are organized into photosynthetic units (PSU), where a large number of pigments (~300) harvest light and funnel excitation energy to the RC. For the concept of the PSU, i.e., of an antenna and a reaction center and experiments supporting this concept, see Clegg et al. (2010) and Govindjee and Björn (2012). For an overview of the primary processes of photosynthesis, see Renger (2008a). While various pigments absorb light and convert it to molecular excitation, it is the chlorophyll-type molecules that are predominantly responsible for migration of the excitation in PSU – the process of

accepting excitation energy from neighboring excited chromophores and forwarding it to other chlorophylls, – eventually leading to trapping of excitation by the RC. The accessory pigments (e.g., carotenoids) only transfer energy that they acquire by absorbing light themselves to chlorophyll a molecules. An exception to this rule are bilin-containing antenna complexes, where no chlorophyll is present and the excitation migration is facilitated by the phycobilin chromophores, such as phycocyanins and phycoerythrins. EET can be considered macroscopically, when the excitation dynamics in PSU are treated kinetically, and microscopically, by calculating actual energy transfer rates based on the electronic properties of interacting chlorophylls.

On a macroscopic scale, electronic properties of chlorophyll molecules are approximated by a simple two-level system (van Amerongen et al. 2000b). This approximation is qualitatively reasonable because the actual migration of excitation takes place only between the Q_y states of chlorophylls, i.e., between the thermally equilibrated lowest excited chlorophyll states. To a first degree of accuracy, excitation energy migration in the antenna complex can be described by the “random walk” model with hops (single EET event) only between nearest-neighbor pigments organized into a square lattice (Pearlstein 2005). In that case, average migration time $\tau_{mig} = 1/k_{mig}$ of the excitation to the RC can be calculated as follows:

$$\tau_{mig} = \frac{1}{\pi} N \cdot \log(N) \cdot \tau_{hop} \quad (4.6)$$

where, N is the number of chlorophyll molecules per RC (the size of the PSU) and τ_{hop} is the average hopping time of excitation between neighboring chlorophyll molecules (Montroll 1969). The size of the PSU varies among organisms, with an average of ~300 chlorophyll molecules per PSU (Malkin et al. 1981; Clegg et al. 2010; Govindjee and Björn 2012). Hopping time τ_{hop} can be estimated from Eq. 4.6 if migration time is known, e.g., from the expression for quantum efficiency of excitation trapping:

$$\Phi_{tr} = \frac{k_{tr}}{k_{loss} + k_{tr}} \quad (4.7)$$

where,

$k_{loss} = k_{rad} + k_{IC} + k_{ISC}$, $k_{tr} = 1 / (t_{mig} + t_{cs})$ is total rate constant of excitation trapping by RC (Pearlstein 1982; van Grondelle and Gobets 2004; Broess et al. 2006), τ_{mig} is the effective time constant of excitation migration to RC, τ_{cs} is the effective time constant of charge separation in RC, and Φ_{tr} is the quantum efficiency of excitation trapping.

However, the approximation of the antenna by a regular two-dimensional square lattice of pigments in Eq. 4.7 is a significant simplification. Natural PSUs typically consist of several light-harvesting antenna proteins, where each pigment experiences a different environment, and intra-pigment EET rates are, therefore, different for each step. In addition, intra-protein EET rates can be substantially different from rates of inter-protein EET. Therefore, τ_{mig} contains several components describing inter-protein and intra-protein migration rates. Several experimental studies have addressed the problem of migration and equilibration dynamics of excitation energy in natural PSUs, and three models have emerged: (i) trap-limited, (ii) diffusion-limited and (iii) diffusion-to-trap limited (for reviews, see Barter et al. 2005; Croce and van Amerongen 2011). In each model, one of the three kinetic rates, indicated in Fig. 4.8, limits energy equilibration. In the trap-limited model, migration occurs on an ultrafast timescale, followed by slower trapping in the RC, k_{CS} . The PSUs with a small antenna (e.g., core complexes of PS II) can be described by the latter model (Miloslavina et al. 2006). The most broadly used trap-limited model is the exciton/radical pair equilibrium (ERPE) model (van Grondelle 1985; Nuijs et al. 1986; Schatz et al. 1988; Miloslavina et al. 2006), in which the primary and secondary charge separation steps are included to describe the temporal response of the system. In the ERPE model, both forward and back electron transfer pro-

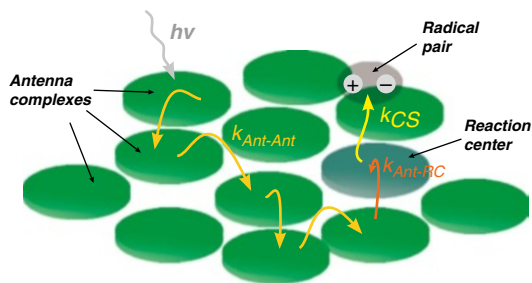


Fig. 4.8. Migration of excitons in PSU after absorption of light photon by antenna chlorophyll molecules. Three kinetic rates are shown: between neighboring antenna complexes ($k_{Ant-Ant}$), from antenna complex to reaction center (k_{Ant-RC}), and trapping of exciton in reaction center by charge separation (k_{CS}).

cesses are included; therefore, the total charge separation τ_{cs} time constant is the product of all of these processes. In the diffusion-limited model, it is migration of the excitation ($k_{Ant-Ant}$) that slows down equilibration of the excitation energy, while charge separation is relatively fast (Melkozernov et al. 2004; Miloslavina et al. 2006; Broess et al. 2008; Van Oort et al. 2010). This model was reported to be appropriate for systems with a large antenna (Caffarri et al. 2011). Furthermore, some experiments have indicated that energy equilibration can occur via an intermediate mechanism, a diffusion-to-trap limited mechanism, where the limiting step is the transfer of the excitation energy from the antenna complex to the RC, k_{Ant-RC} (Visscher et al. 1989; Valkunas et al. 1995; Dekker and van Grondelle 2000; Vasil'ev et al. 2001; Raszewski and Renger 2008).

The rate constants of excitation migration and charge separation, reported in numerous experimental and theoretical studies, have shown a large range of variation. The primary charge separation time constant has been reported to be between 300 fs and 8 ps (Wasielewski et al. 1989; Greenfield et al. 1997; van Amerongen et al. 2000c; Dekker and van Grondelle 2000; Holzwarth et al. 2006; Raszewski and Renger 2008), while the total charge separation time, τ_{cs} , has been estimated to be in the range of 60–180 ps (Miloslavina et al. 2006; Broess et al. 2006). Values for excitation migration to RC have

been reported between 9 and 150 ps (Miloslavina et al. 2006; Van Oort et al. 2010), while hopping time is between <1 and >10 ps (Barzda et al. 2001; Broess et al. 2006; Caffarri et al. 2011). For detailed reviews on migration and trapping of excitation in PS II-containing systems, see Croce and van Amerongen 2011 and van Amerongen and Croce 2013; for a discussion of basics of excitation-energy transfer, see Clegg 2004).

It is important to emphasize that in Eq. 4.7, the quantum efficiency does not account for the energy of excitation quanta and only reports the ratio of the number of excitation quanta that have reached the RC (and resulted in charge separation) to the number of photons absorbed in the antenna. The quantum efficiency of excitation trapping in systems without regulated thermal de-excitation (NPQ) and open RCs typically ranges between 85 % and 95 % (Wraight and Clayton 1974; Clayton and Yamamoto 1976; Rijgersberg et al. 1980; Vredenberg 2004; Wientjes et al. 2013). It is important to distinguish *quantum efficiency* from the absolute efficiency of EET that is often referred to as *storage efficiency* (Ross and Calvin 1967; Jursinic and Govindjee 1977; Dau and Zaharieva 2009; Shevela et al. 2013). Storage of the absorbed energy in a primary charge-separated state is calculated in terms of *free energy* (see Boeker and van Grondelle 2011). In these calculations, the decrease of energy of an excitation quantum during its migration to RC, followed by charge separation, is accounted for. The resulting values of storage efficiency have been estimated to be 68–73 % (Duysens 1958; Ross and Calvin 1967; Jursinic and Govindjee 1977). If efficiency of light absorption over the entire solar spectrum is included in the calculation (i.e., the relative area of solar spectrum covered by absorption bands of photosynthetic pigments), the efficiency drops to <34 % (Dau and Zaharieva 2009).

In the excitation-energy transfer (EET) studies on a microscopic scale, not only the electronic properties of chlorophylls are taken into account, but also their arrangement in the protein complex. Several mechanisms of EET in LHCs are found depending

on the interaction of chlorophylls with each other and with their protein environment. For a review of the mechanism of excitation energy transfer, see Renger (2008b).

It is often assumed that the electronic interaction between two chromophores is weak in comparison to chromophore interaction with the environment. In that case, the key parameter of the EET is the Coulombic interaction (coupling V_{DA}) between donor D and acceptor A molecules. When the distance between chromophores is large (3–10 nm), coupling can often be approximated by a dipole-dipole interaction:

$$V_{DA} = \frac{1}{4\pi\epsilon_0 n^2} \frac{\check{\mu}_D \check{\mu}_A}{R_{DA}^3} - 3 \frac{(\check{\mu}_A \check{R}_{DA})(\check{\mu}_D \check{R}_{DA})}{R_{DA}^5} \quad (4.8)$$

where, $\check{\mu}_A$ and $\check{\mu}_D$ are unit vectors of the acceptor and donor transition dipole moments, respectively, and \check{R}_{DA} is unit vector pointing from the center of the donor molecule to the center of the acceptor. This is how T. Förster (Förster 1946; for English translation, see Förster 2012) introduced the theory of energy transfer known as Förster Resonance Energy Transfer (FRET; Knox 2012). For a history of FRET, see Clegg (2006). The Förster coupling regime is often called the “weak coupling” limit and the rate of resonance energy transfer from donor D to acceptor A , k_{DA} , can be calculated using Fermi’s Golden Rule (Dirac 1927):

$$k_{DA} = \frac{2\pi}{\hbar} |V_{DA}|^2 J_{DA} \quad (4.9)$$

where $\hbar = h/2\pi$ is reduced Planck’s constant, V_{DA} is electronic coupling of D and A excited states from Eq. 4.8, and J_{DA} is the spectral overlap integral between the area-normalized donor fluorescence spectrum (f_D) and the area-normalized acceptor absorption spectrum (ϵ_A). If the spectra are plotted on a wavelength (λ) scale, the expression is (Scholes 2003):

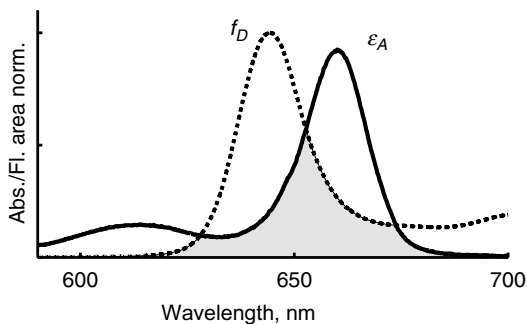


Fig. 4.9. Overlap region of area normalized chlorophyll *b* fluorescence spectrum f_D (····) and chlorophyll *a* absorption spectrum ϵ_A (—).

$$J_{DA} = \int_0^{\infty} f_D(\lambda) \epsilon_A(\lambda) \lambda^4 d\lambda \quad (4.10)$$

In simple terms, the rate of EET from the excited state of donor D^* to the ground state of acceptor A depends upon the coupling strength between the transitions $D^* \rightarrow D$ and $A \rightarrow A^*$, and their spectral properties. The example of spectral overlap between chlorophyll *b* and chlorophyll *a* molecules in solution is shown in Fig. 4.9. For basic principles of calculation of spectral overlap see Lakowicz 1999.

In experimental studies of energy transfer, the characteristic distance at which the efficiency of the energy transfer is 50 % is of special importance. Because the main competing de-excitation process is donor fluorescence, the rate of Förster energy transfer (k_{FRET}) can be rewritten as follows (Förster 1946, 1948; Braslavsky et al. 2008):

$$k_{FRET} = \frac{1}{\tau_d} \left(\frac{R_0}{R_{DA}} \right)^6 \quad (4.11)$$

where, $1/\tau_d$ is the fluorescence rate of the donor in the absence of acceptor, and R_0 is the Förster radius (Förster 1965, 1967):

$$R_0^6 = \frac{9(\ln 10) \kappa^2 \Phi_D I}{128 \pi^5 N_A n^4} \quad (4.12a)$$

$$\kappa = \frac{\bar{\mu}_D \cdot \bar{\mu}_A - 3(\bar{\mu}_D \cdot \bar{r})(\bar{\mu}_A \cdot \bar{r})}{\mu_D \mu_A} \quad (4.12b)$$

Here, κ is an orientation factor associated with donor-acceptor spatial geometry, $\bar{\mu}_D$ and $\bar{\mu}_A$ are donor and acceptor transition dipole moment vectors, r is the unit vector in the direction of R_{DA} , n is the effective refractive index of the medium surrounding donor and acceptor, N_A is Avogadro's number, and Φ_D is the fluorescence quantum yield of the donor in the absence of the acceptor, I is Förster spectral overlap integral defined like J_{DA} in Eq. 4.10 except that the acceptor absorption spectrum is not normalized, but is plotted as extinction coefficient vs wavelength λ (Braslavsky et al. 2008). The Förster radius for a pair of photosynthetic chromophores is estimated to be approximately 60–100 Å (Colbow 1973; van Grondelle 1985; Dekker et al. 1998; Berghuis et al. 2010).

Another important quantity is FRET transfer efficiency (Φ_{ET}), defined as the probability of the excitation being transferred away from the chromophore rather than being quenched by another process (e.g., IC, ISC). Transfer efficiency, expressed as a function of donor-acceptor separation, is

$$\Phi_{ET} = \frac{1}{1 + (R/R_0)^6} \quad (4.13)$$

The EET efficiency is highly sensitive to donor-acceptor separation and decreases quickly at distances greater than the Förster radius R_0 of a given donor-acceptor pair (Fig. 4.10). The significance of Förster's theory is that all quantities appearing in the FRET expressions, Eqs. 4.11, 4.12a, 4.12b and 4.13, can be obtained from experimental data (van Zandvoort et al. 1995).

Owing to the strong coupling of pigments to their dissipative environment (coupling to the bath), energy transfer processes in the weak chromophore-coupling limit (when inter-chromophore coupling is weaker than chromophore-to-bath coupling) are

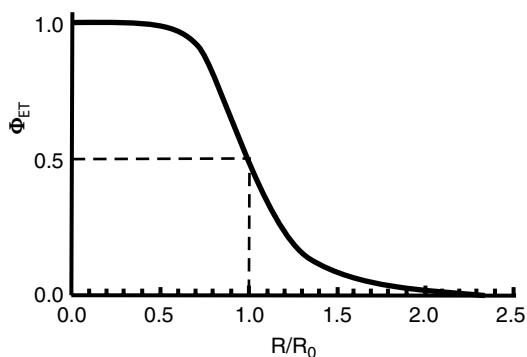


Fig. 4.10. The transfer efficiency (Φ_{ET}) as a function of the donor-acceptor distance (R).

characterized by transfer from a thermally equilibrated (thermalized) donor. After the event of EET, excitation is, once more, thermalized on the excited electronic state of the acceptor on a time scale that is fast compared to energy migration rate. Rapid thermalization of excitation causes what is termed “loss-of-memory” of the excitation trajectory, meaning that each new EET process is independent of the history of the migration of the excitation energy before reaching the last chromophore. Therefore, FRET represents a diffusive hopping of energy from chlorophyll to chlorophyll through the PSU (Fig. 4.8; Jean et al. 1989; Pullerits and Freiberg 1992; Pullerits et al. 1994; Somsen et al. 1994).

VII Considerations Beyond Förster Theory

While Förster theory has proven to be extremely successful and predictive, it does have shortcomings, which can pose a challenge when chromophores are packed close together – like in LHCs. Over the past years, studies of LHCs have helped reveal and clarify processes of energy transfer by mechanisms not explained by Förster theory (Scholes and Fleming 2005; van Grondelle and Novoderezhkin 2006; Beljonne et al. 2009; Novoderezhkin and van Grondelle 2010; Scholes et al. 2011). The main problem with Förster theory is that it works well only when the distance between chromophores is large compared to the dimensions of

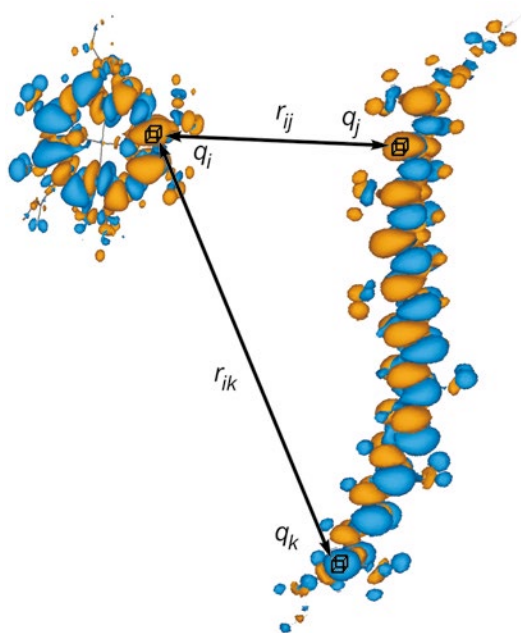


Fig. 4.11. Coulombic interaction between the transition density cubes of a bacteriochlorophyll (*left*) and a carotenoid molecule (*right*). The diagram shows a transition density cube q_i centered on the bacteriochlorophyll interacting with two cubes, q_j and q_k , centered on the carotenoid and their corresponding separation r_{ij} . The sum over all such interactions gives the total Coulombic coupling (Scholes and Fleming 2005).

the chromophores, r_D and r_A , for instance when donor and acceptor chromophores are located on different protein complexes. However, certain ingredients within the theory need to be modified when inter-chromophore distance R_{DA} is comparable or smaller than r_D or r_A , or when chromophore-chromophore interaction competes with chromophore-environment interaction; in the latter events, dipole-dipole electronic coupling approximation fails, and, in some cases, orbital overlap effects start to contribute to the EET.

A good example is the energy transfer between the carotenoid and the bacteriochlorophyll in LH2 complexes of purple bacteria, where the distance between the chromophores is much smaller than their dimensions (see Fig. 4.11). The dipolar approximation forbids energy transfer to and from optically dark electronic states since these states have negligible transition dipole

moments and V_{DA} in Eq. 4.9 therefore equals zero. This prediction is contradicted by the observation of energy transfer from the optically dark carotenoid S_1 state to the chlorophyll Q_y state in LH2 complexes (Gradinaru et al. 2000; Hsu et al. 2001; Croce et al. 2003). The breakdown of the dipole approximation occurs because transition dipoles do not account for the shape or extent of the transition densities (Scholes 2003) that are highly elongated in carotenoid molecules. In such cases, size and shape of molecular transition densities should be taken into account when calculating V^{Coul} , which is done by summation of Coulombic interactions between monopoles distributed at points r_i around the chromophores, i.e., using the transition density cube (TDC) method (Krueger et al. 1998):

$$V_{DA}^{Coul} = \frac{1}{4\pi\epsilon_0 n^2} \sum_{i,j} \frac{q_i q_j}{r_{ij}} \quad (4.14)$$

where, the index i (j) runs over the grid discretizing the donor (acceptor) transition density, q_i (q_j) are the discrete charges associated with the donor (acceptor) transition density at position r_i (r_j), and $r_{ij} = |r_i - r_j|$ is the distance between the points r_i and r_j (Fig. 4.11).

The accuracy of the TDC method can be improved by taking successively smaller volume elements (cubes) until, for infinitely small cubes, the calculated coupling becomes exact and limited only by the accuracy of the quantum-chemical determination of the wavefunction. In this implementation limit of the TDC method, the coupling term is calculated using the following expression:

$$V_{DA}^{Coul} = \frac{1}{4\pi\epsilon_0 n^2} \int dr_D \int dr_A \frac{\rho_D(r_D) \rho_A(r_A)}{r_{DA}} \quad (4.15)$$

Here, $\rho_D(r_D)$ and $\rho_A(r_A)$ denote the transition density associated with the donor/acceptor at position r_A and r_D , and the integration

coordinates are varied over the entire three dimensional coordinate space.

VIII Delocalization of Excitation, Molecular Excitons

Insight into thermal de-excitation mechanisms (assessed via measurements of NPQ) requires an understanding of the principles of excitation-energy migration through the antenna. Excitation energy migration is, in turn, determined by interaction of the chromophores with each other as well as with their environment, the pigment-binding protein. In a number of photosynthetic complexes, the coupling between chlorophylls is substantially stronger than the coupling to the “bath” (pigment-binding protein), which determines the homogeneous broadening. In other words, electronic coupling energy is larger than the width of spectral absorption bands. Under these conditions (with what is termed a strong coupling limit), Förster’s theory fails to describe EET as diffusive hopping of excitation among molecules. In particular, when the coupling between pigments, V_{DA} , is greater than the transition energy difference between pigment excited states ΔE as well as the coupling to the environment of each individual pigment V_{bath} , excitation becomes coherently delocalized over the system of interacting pigments, and it is no longer possible to identify a donor and acceptor molecule. The latter, newly formed delocalized excited state belongs to all of the coupled pigments and is called an exciton state $|M\rangle$, as compared to localized states of individual pigments $|M\rangle$ (van Grondelle and Novoderezhkin 2006; Scholes and Rumbles 2006). Each exciton state, also simply termed exciton, is given as a linear combination of the localized states:

$$|M\rangle = \sum_n c_n^{(M)} |n\rangle \quad (4.16)$$

where, $c_n^{(M)}$ are the elements of the eigenvector matrix of the system Hamiltonian H_S ,

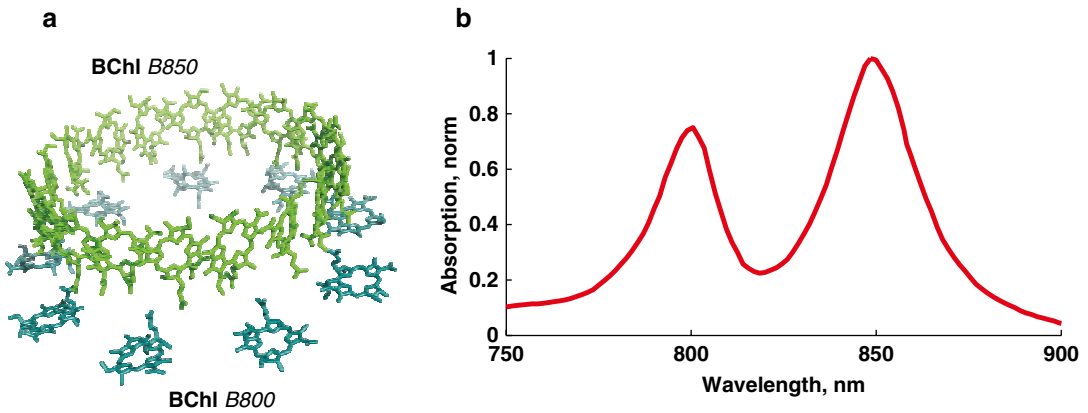


Fig. 4.12. (a) Molecular structure of LH2 complex from *Rhodospirillum rubrum* (*Rba.*) *sphaeroides*, showing only bacteriochlorophyll (BChl) molecules. Strongly coupled BChls (B850 ring) are shown in green, weakly coupled BChls (B800 ring) are shown in blue. (b) Absorption spectrum of LH2 complex from *Rba. sphaeroides*.

consisting of localized state energies E_m and couplings V_{mn} :

$$H_S = \sum_m E_m |m\rangle\langle m| + \sum_{mn} V_{mn} |m\rangle\langle n| \quad (4.17)$$

The work of Fidler et al. (1991) provides a clear and practical example for how exciton states can be calculated. The latter approach has found numerous applications in the interpretation of excited states and ultrafast dynamics in LHCs. Delocalization of the excitation energy results in a collective, or coherent, behavior of the excited electronic states, and, as a consequence, the properties of excitons can be markedly different from those of the excited states of individual pigments. A characteristic example of exciton delocalization in photosynthetic complexes is that of bacteriochlorophyll (BChl) light-harvesting LH2 proteins of purple bacteria shown in Fig. 4.12a (Novoderezhkin et al. 1999a, b). In these complexes, 18 of the 27 BChls are arranged in a ring with an ~ 0.9 nm center-to-center inter-chromophore distance (McDermott et al. 1995). Because of strong electronic coupling between two nearest neighbour BChls in this B850 ring (~ 300 cm $^{-1}$), 18 exciton states are formed. The red-shift of the bright (optically allowed) exciton transitions, in combina-

tion with pigment-protein interactions, shifts the absorption spectrum from 800 to 850 nm, as shown in Fig. 4.12b (Jimenez et al. 1996; Hu and Schulten 1997; Chachisvilis et al. 1997; Kennis et al. 1997; Monshouwer et al. 1997).

Deviation of a chlorophyll aggregate's exciton properties from those of its constituent individual chlorophyll molecules can be illustrated by considering a simple dimer system like that shown in Fig. 4.13, which represents what is termed the “J” and “H” aggregates (Kirstein et al. 2000). In the absence of interaction between the individual pigments of the dimer, it has a doubly-degenerate excited state (two states of the same energy) arising from the excitation of each pigment (Fig. 4.13a). The coupling between pigment molecules results in exciton splitting (formation of two exciton states of different energy) because the light-absorbing quantum mechanical states are a linear combination of indistinguishable excitations localized on each chromophore (Scholes and Rumbles 2006). When pigments are arranged in “side-to-side” geometry (*H*-aggregate), the exciton state with antiparallel (\ominus) transition dipole moments is optically forbidden (“dark”) and has lower energy, while the exciton state with parallel (\oplus) transition dipole moments is higher in energy and optically accessible (Fig. 4.13b).

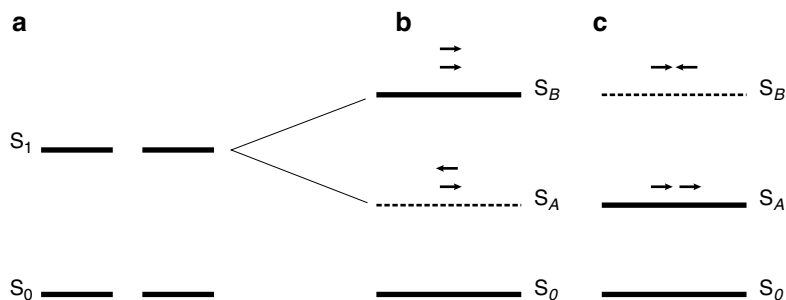


Fig. 4.13. Effect of coupling on excited states. The direction of the transition dipoles are shown by *small arrows* above the corresponding exciton states. (a) Non-interacting molecules; (b) dimer with ‘sandwich’ transition dipole arrangement (*H*-aggregate); (c) dimer with ‘head-to-tail’ transition dipole arrangement (*J*-aggregate). Optically accessible exciton states are shown in *solid line*, dark states are shown in *dotted line*.

When pigments are arranged with their transition dipoles in a co-linear, “end-to-end” geometry, parallel arrangement ($\rightarrow\rightarrow$) minimizes the interaction energy and is optically accessible, while antiparallel arrangement ($\rightarrow\leftarrow$) maximizes the energy and is optically forbidden (Fig. 4.13c). For the ring geometry of BChls in the LH2 complex, the lowest exciton state is formally forbidden (Hu and Schulten 1997).

The effect of delocalization and exciton formation can, however, be diminished by disorder in excited-state energies of the BChls contributing to the exciton. In most photosynthetic complexes, each pigment experiences a different protein environment, which causes a shift of its absorption band. As a result, the total absorption spectrum is defined not only by the homogeneous broadening of the absorption band, which is identical for each pigment, but also by inhomogeneous broadening – distribution of the band positions and widths among different pigments of the complex. Inhomogeneous broadening leads to decreased delocalization. The effect of disorder in photosynthetic proteins has been extensively studied (for a review, see van Grondelle and Novoderezhkin 2006; see also, e.g., Krüger et al., Chap. 6). A good example of the role of inhomogeneous broadening is observation of the formally forbidden lowest exciton state of LH2 complexes (Kennis et al. 1997; Monshouwer et al. 1997).

Excitation in the B850 band (of an LH2 complex) does not migrate as diffusive hopping, but is instead often described as a

wave-like movement (Chachisvilis et al. 1997). Theoretical techniques other than FRET have to be used to describe this EET. One of the approaches, the Redfield theory, is based on the density-matrix formalism and is described in detail in May and Kühn (2011). In the Redfield theory, chromophore-bath interaction is considered to be small and is treated as a perturbation of inter-chromophore interaction. This perturbative approach is known as the Born approximation, and is an opposite limiting case to FRET, where chromophore-bath coupling is assumed to be much stronger compared to inter-chromophore coupling. Within the second approximation of the Redfield theory, Markov approximation, the time evolution of the system does not depend on the past history (previous EET steps) and no memory effects are taken into account.

As a result of interaction among different LHCs, the EET in photosynthetic organisms often has to be described by a combination of Redfield theory, accounting for strongly interacting intra-aggregate chlorophylls (here aggregate is an assembly of chlorophylls bound together by strong interaction), and Förster theory, accounting for weak inter-aggregate interaction. This combined approach leads to the *generalized* Förster theory (Mukai et al. 1999; Sumi 1999; Scholes and Fleming 2000; Yang et al. 2003; van Grondelle and Novoderezhkin 2006; Novoderezhkin and van Grondelle 2010), which allows a better microscopic description of EET in PSU.

Over the last decade, a number of researchers have proposed quantum-coherence effects to be involved in excitation-energy migration in LHCs (Engel et al. 2007; Collini et al. 2010; Calhoun and Fleming 2011; Ishizaki and Fleming 2012). The mechanism behind quantum-coherent energy transfer can be thought of as the interference of all possible EET pathways. These pathways contribute either constructively (add up) or destructively (annihilate), and result in the most efficient transfer of energy to RC. This is a formal statement and the precise physical meaning depends on the dynamics model and the basis in which the system is described (e.g., exciton *vs* localized). Coherent EET can more appropriately be thought of as transfer occurring with changes in delocalization. Coherent EET is discussed from our point of view in Fassioli et al. 2014.

IX Excited State Complexes

After photoexcitation, excited molecules or excitons can sometimes interact with, or become coupled to, neighboring non-excited molecules. The complex formed by such interaction is called an exciplex (“excited complex”; Birks 1970; Förster 1975). Because the exciplex is formed after excitation, and the pigments interact with the radiation field independently, the absorption spectrum of the system remains unchanged (McGlynn et al. 1965). The excitation is subsequently delocalized over the coupled molecules, forming the exciplex, which leads to altered fluorescence properties in the form of a red-shifted emission wavelength. Formation of an exciplex is sometimes coupled to a geometric reorganization of the pigments and their solvent environment (Scholes et al. 1991). The process of exciplex formation can be demonstrated for the example of two identical anthracene molecules, one in the excited and the other in the ground state, as shown in Fig. 4.14. When two identical molecules interact, the exciplex is often called an excimer (“excited dimer”).

Interaction of individual pigments within an exciplex can be studied by either assuming

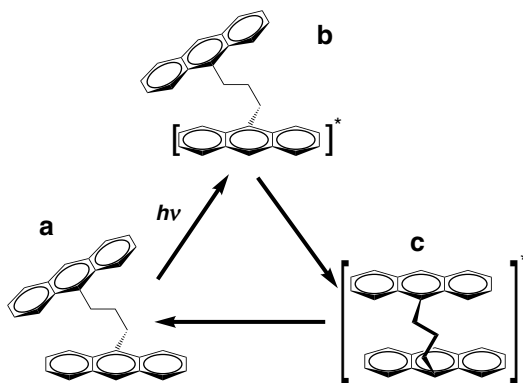


Fig. 4.14. The dynamics of an excimer formation from two anthracene molecules, 9-(9-anthryl) anthracene. A photon is absorbed by an individual chromophore (a), resulting in a localized excited state (b). The excitation is subsequently delocalized over interacting pigments to form an excimer (c).

(i) that molecules comprising the exciplex are bound together by molecular exciton interactions (Förster 1963), or (ii) that the complex is formed by the coulombic attraction between an electron donor and acceptor pair (Ferguson 1958). In both limits, formation of the exciplex leads to lowering of excitation transition energy. Exciplexes can be accurately described by a superposition of exciton and charge transfer character (Murrell and Tanaka 1964).

In photosynthesis, exciplexes can be formed between chlorophyll and carotenoid molecules via a charge-transfer mechanism. Such exciplexes have been observed in minor light-harvesting complexes of PS II (Holt et al. 2005; Avenson et al. 2008), and are associated with one of the components of regulated thermal de-excitation (reflected in NPQ) activated under excess light conditions (see, e.g., Walla et al., Chap. 9; Polivka and Frank, Chap. 8). Here, excitation energy localized on a chlorophyll molecule that is placed very close to a zeaxanthin molecule is quenched by transfer of an electron from the highest occupied molecular orbital (HOMO) localized on zeaxanthin to the lowest unoccupied molecular orbital (LUMO) localized on the chlorophyll molecule. This process is followed by rapid charge recombination and relaxation to the ground state (Holt et al.

2005; Dreuw et al. 2005; Avenson et al. 2008).

An excimer can be present in photosynthetic complexes even without involvement of carotenoids, and can be formed by interaction of two chlorophyll molecules. If chlorophylls are less than 10 Å apart, they can form an excimer trap, and excitation energy absorbed by any of them will be quenched by the trap. This effect has been observed in light-harvesting proteins of PS I (Romero et al. 2009) and has been suggested to occur in LHCII complexes as well (Miloslavina et al. 2008; Müller et al. 2010; Wahadoszamen et al. 2012; see also, Holzwarth and Jahns, Chap. 5). The protein scaffold that dictates the spacing and relative orientation of pigments therefore plays an essential role in regulating the efficiency of energy transfer and photochemical charge separation (see Horton, Chap. 3; Pascal et al., Chap 10; Büchel, Chap. 11; Morosinotto and Bassi, Chap. 14; van Amerongen, Chap. 15; Garab, Chap. 16; Ruban and Mullineaux, Chap. 17).

X Basic Photophysics of Non-Photochemical Quenching of Chlorophyll Fluorescence

Intense light can create excitations in photosynthetic systems at a rate exceeding the capacity for efficient photochemical charge separation (Demmig-Adams and Adams 1992; Horton et al. 1996). The resulting increase in excited-state lifetime of chlorophylls, caused by closing of RCs, makes the photosynthetic apparatus susceptible to oxidative damage from singlet oxygen produced via the chlorophyll excited triplet state (Ke 2001b). In plants and algae, thermal energy dissipation (for which NPQ is an indicator) is the regulatory process that involves nonradiative dissipation, as heat, of singlet excitation energy (for general introduction into NPQ, see, e.g., Papageorgiou and Govindjee, Chap. 1; Horton, Chap. 3; Holzwarth and Jahns, Chap. 5; Logan et al. Chap. 7; Morosinotto and Bassi, Chap. 14; Garab, Chap. 16; Ruban and Mullineaux, Chap. 17; Murchie and Harbinson, Chap. 25). Thermal

dissipation protects plants and algae from photodamage occurring under excess light absorption (see Adams and Demmig-Adams, Chap. 2; Lavaud and Goss, Chap. 20; Demmig-Adams et al., Chap. 24). While the mechanisms of NPQ can vary among pigment-protein complexes and different organisms, there are two main strategies: (i) energy transfer to an individual pigment molecule that dissipates excitation energy on an ultra-short timescale (see section IV of this Chapter) and (ii) thermal dissipation in an excitation trap, formed by interaction of several pigment molecules (see section IX of this Chapter).

Numerous experimental studies have shown that carotenoids are involved in NPQ (see, e.g., Adams and Demmig-Adams, Chap. 2; Polivka and Frank, Chap 8; Walla et al., Chap. 9; Esteban and Garcia-Plazaola, Chap. 12; van Amerongen, Chap. 15). Currently, two general NPQ mechanisms have been formulated. In the first group, carotenoids are presumably directly involved in quenching of the excessive excitation energy, whereas in the second group, carotenoids presumably create conditions for the quenching and are not involved in direct de-excitation processes.

In algae and plants, NPQ is associated with one of three different xanthophyll cycles, in each of which a polar carotenoid is chemically transformed to a less polar carotenoid under excess light conditions (see Papageorgiou and Govindjee, Chap. 1; Adams and Demmig-Adams, Chap. 2; Morosinotto and Bassi, Chap. 14), with (i) the major conversion in all plants and green algae, involving violaxanthin and zeaxanthin (see, e.g., Demmig-Adams et al., Chap 24; Finazzi and Minagawa, Chap. 21), (ii) an additional, more minor conversion in specific plant families, involving lutein epoxide and lutein (Esteban and Garcia-Plazaola, Chap. 12), and (iii) conversion of diadinoxanthin to diatoxanthin in several algal groups (Büchel, Chap. 11; Lavaud and Goss, Chap. 20). The proponents of an involvement of zeaxanthin as a quencher have long proposed that zeaxanthin is rapidly “engaged” and “disengaged” by a

mechanism dependent on the trans-thylakoid pH gradient in rapidly growing plants, and that zeaxanthin is continuously engaged in quenching by a pH-independent mechanism in evergreens under growth-arresting conditions (reviewed by Adams and Demmig-Adams, Chap. 2 and Demmig-Adams et al., Chap. 24). Frank et al. (1994; see also Owens 1994) had calculated energies of violaxanthin and zeaxanthin in accordance with the energy-gap law, and had suggested that zeaxanthin can quench chlorophyll excitation by direct energy transfer from Q_y excited state to the carotenoid S_1 state. Indeed, the increase of conjugation chain length from 9 (in violaxanthin) to 11 (in zeaxanthin) leads to lowering of the singlet-transition energies in zeaxanthin (see its justification by reference to the simple particle-in-a-box model, Fig. 4.4). However, these energies have since been shown to be even lower, such that both violaxanthin and zeaxanthin fall below the lowest singlet-excited state of chlorophyll (Polivka et al. 1999; Polivka and Frank, Chap. 8). It is therefore clear that conversion of violaxanthin to zeaxanthin alone cannot account for NPQ. Another possible direct mechanism was reported to take place in minor light-harvesting complexes of higher plants (Holt et al. 2005; Dreuwe et al. 2005; Avenson et al. 2008; see also Walla et al., Chap. 9). After conversion of violaxanthin to zeaxanthin, zeaxanthin binds to a light-harvesting protein, where a neighboring excited chlorophyll molecule may form an excited state charge-transfer complex (charge transfer exciplex) with the newly bound zeaxanthin molecule via transfer of an electron from zeaxanthin to chlorophyll. A chlorophyll-zeaxanthin charge transfer state would be stabilized with respect to the localized excitation on the chlorophyll by the coulombic attraction between the resulting electron and hole. The exciplex energy is thermalized along with rapid charge recombination to the ground state (see section IX of this Chapter).

Formation of carotenoid-chlorophyll exciton states was proposed to be the mechanism of NPQ in LHCII complexes (Razi

Naqvi 1998; van Amerongen and van Grondelle 2001), where the S_1 state of a xanthophyll carotenoid molecule is coupled to the Q_y excited state of chlorophyll (see, e.g., Walla et al., Chap. 9; van Amerongen, Chap. 15). Recently, an experimental confirmation of this process in LHCII has been reported using two-photon excitation (Liao et al. 2010a, b). However, an independent study, using LH2 complexes, showed that the results of two-photon excitation experiments on chlorophyll-containing proteins can be misinterpreted (Krikunova et al. 2002). Therefore, a confirmation of that mechanism by alternative techniques is necessary.

Zeaxanthin has also been proposed to play an indirect role in NPQ by changing the hydrophobicity of light-harvesting proteins (see, e.g., Horton, Chap 3). Allosteric regulation, triggered by binding of the hydrophobic zeaxanthin to the surface of the LHCII, can change the conformation of the light-harvesting complex, thus switching it to a quenched state (Horton et al. 2000; Pascal et al. 2005). Alternatively, zeaxanthin can act as a stabilizer of the quenched conformation, which is an intrinsic feature of the LHCII complex (Krüger et al. 2010, 2012; see also Krüger et al., Chap. 6). In this new, quenched conformation, direct energy transfer between excited chlorophyll and a carotenoid (suggested to be neoxanthin), located within the protein, was proposed to be activated (Ruban et al. 2007; see also, e.g., Pascal et al., Chap. 10; van Amerongen, Chap. 15). While neoxanthin has been used as an indicator for a conformational change of LHCII during the onset of NPQ (Ruban et al. 2011; Illoiaia et al. 2011; Zubik et al. 2011), Dall'Osto et al. (2007) showed that neoxanthin is not involved in NPQ.

Finally, the conformational change of LHCII has also been suggested to lead to formation of charge-transfer states between two (or more) chlorophylls without direct participation of a carotenoid molecule (Miloslavina et al. 2008; Müller et al. 2010; see also Holzwarth and Jahns, Chap. 5). The resulting chlorophyll exciplex traps may be dissipating excitation energy by nonradiative internal conversion (heat).

XI Concluding Remarks

In this chapter, we have focused primarily on electronic properties of two classes of photosynthetic chromophores, chlorophylls and carotenoids, and on investigations into their role(s) in primary photoprocesses, such as: light absorption and emission, excitation-energy transfer, thermal dissipation, and trapping in RCs. As numerous examples from different pigment-protein complexes have shown, interactions between chromophores are of critical importance in the overall light-harvesting process (Scholes et al. 2011). Assembly of chromophores in a protein scaffold allows fine-tuning of their spectral properties leading to broadening and distribution of absorption bands over a wide spectral range, thereby improving total absorption cross-section. By orienting chromophores at specific distances and angles within the protein, remarkably high EET efficiencies are achieved, e.g., up to 99 % for single chromophore-chromophore EET (Duysens 1952; Song et al. 1976; Frank and Cogdell 1996; van Amerongen and van Grondelle 2001) and >90 % for total EET within PSU (Wraight and Clayton 1974; van Grondelle et al. 1994; Vredenberg 2004). Depending on the intra-chromophore distances, orientations and couplings different mechanisms dominate the EET, such as FRET between weakly coupled chromophores in biliproteins of cyanobacteria (Sharkov et al. 1992), Redfield energy transfer in LH2 antenna of purple bacteria (Novoderezhkin and van Grondelle 2010) or recently proposed coherent energy transfer in Fenna-Matthews-Olson complex of green sulfur bacteria (Ishizaki and Fleming 2009).

Of particular interest for the current book are changes of photophysical properties of chromophores induced by variation in chromophore environment, because these changes are the foundation of physiological acclimation (and genetic adaptation) of photosynthetic organisms to their natural environments. Different mechanisms of thermal dissipation of excess excitation energy can, and may, contribute to NPQ. The basis of all of these mechanisms is, as discussed

above, strong interaction among chromophores. This interaction, measured in terms of coupling of excited states of the interacting molecules, is able to produce drastic increases of local internal conversion rates and formation of excitation-energy traps. Once it hops onto such a chromophore-trap, excitation is removed from the antenna and efficiently dissipated as heat. Thermal dissipation processes eliminate excess excitation energy from antenna, avoid damage to the photosynthetic apparatus, and protect the organism's ability to quickly return to a conformation with highly efficient light harvesting and photochemistry under constantly fluctuating natural environmental conditions.

Acknowledgments

The authors thank R. van Grondelle and H. van Amerongen for their suggestions and critical comments. Authors also acknowledge the Natural Sciences and Engineering Research Council of Canada (NSERC) and the Defense Advanced Research Projects Agency (DARPA, QuBE) for financial support. Govindjee acknowledges the support and friendship of the photosynthesis community at large.

References

- Ananyev G, Kolber ZS, Klimov D, Falkowski PG, Berry JA, Rascher U, Martin R, Osmond B (2005) Remote sensing of heterogeneity in photosynthetic efficiency, electron transport and dissipation of excess light in *Populus deltoides* stands under ambient and elevated CO₂ concentrations, and in a tropical forest canopy, using a new laser-induced fluorescence transient device. *Glob Chang Biol* 11:1195–1206
- Andersson PO, Sergei T, Chen MBR, Gillbro T (1995) Solvent and temperature effects on dual fluorescence in a series of carotenes. Energy gap dependence of the internal conversion rate. *J Phys Chem* 5639:16199–16209
- Angerhofer A (1991) Chlorophyll triplets and radical pairs. In: Scheer H (ed) *Chlorophylls*. CRC Press, London, pp 945–991

- Avenson TJ, Ahn TK, Zigmantas D, Niyogi KK, Li Z, Ballottari M, Bassi R, Fleming GR (2008) Zeaxanthin radical cation formation in minor light-harvesting complexes of higher plant antenna. *J Biol Chem* 283:3550–3558
- Barber J, Malkin S, Telfer A, Schreiber U (1989) The origin of chlorophyll fluorescence in vivo and its quenching by the photosystem II reaction centre. *Philos Trans R Soc Lond B Biol Sci* 323:227–239
- Barter LMC, Klug DR, van Grondelle R (2005) Energy trapping and equilibration: a balance of regulation and efficiency. In: Wydrzynski TJ, Satoh K, Freeman JA (eds) *Photosystem II*. Springer, Dordrecht, pp 491–514
- Barzda V, Gulbinas V, Kananavicius R, Cervinskis V, van Amerongen H, van Grondelle R, Valkunas L (2001) Singlet-singlet annihilation kinetics in aggregates and trimers of LHCII. *Biophys J* 80:2409–2421
- Beddard GS, Porter G (1976) Concentration quenching in chlorophyll. *Nature* 260:366–367
- Beljonne D, Curutchet C, Scholes GD, Silbey RJ (2009) Beyond Förster resonance energy transfer in biological and nanoscale systems. *J Phys Chem B* 113:6583–6599
- Berghuis BA, Spruijt RB, Koehorst RBM, van Hoek A, Laptinok SP, Van Oort B, van Amerongen H (2010) Exploring the structure of the N-terminal domain of CP29 with ultrafast fluorescence spectroscopy. *Eur Biophys J* 39:631–638
- Birks JB (1970) *Photophysics of Aromatic Molecules*. Wiley, New York
- Bittner ER (2009) *Quantum Dynamics: Applications in Biological and Materials Systems*. CRC Press, New York
- Björn LO, Govindjee (2012) Dissecting oxygenic photosynthesis: the evolution of the “Z”-scheme for thylakoid reactions. In: Itoh S, Mohanty P, Guruprasad KN (eds) *Photosynthesis: Overviews on Recent Progress and Future Perspective*. I.K. International Publishers, New Delhi, pp 1–27
- Björn LO, Papageorgiou GC, Blankenship RE, Govindjee (2009) A viewpoint: Why chlorophyll *a*? *Photosynth Res* 99:85–98
- Blankenship RE (2014) *Molecular Mechanisms of Photosynthesis*, 2nd edn. Wiley-Blackwell, Oxford
- Boeker E, van Grondelle R (2011) *Environmental Physics: Sustainable Energy and Climate Change*, 3rd edn. Wiley, Chichester
- Bowers PG, Porter G (1967) Quantum yields of triplet formation in solutions of chlorophyll. *Proc R Soc Lond A* 296:435–441
- Braslavsky SE, Fron E, Rodriguez HB, Roman ES, Scholes GD, Schweitzer G, Valeur B, Wirz J (2008) Pitfalls and limitations in the practical use of Förster’s theory of resonance energy transfer. *Photochem Photobiol Sci* 7:1444–1448
- Britton G (1983) Tetrapyrroles. In: Britton G (ed) *The Biochemistry of Natural Pigments*. Cambridge University Press, Cambridge, pp 156–222
- Brody SS, Rabinowitch E (1957) Excitation lifetime of photosynthetic pigments in vitro and in vivo. *Science* 125:555
- Broess K, Trinkunas G, van der Weij-de Wit CD, Dekker JP, van Hoek A, van Amerongen H (2006) Excitation energy transfer and charge separation in photosystem II membranes revisited. *Biophys J* 91:3776–3786
- Broess K, Trinkunas G, van Hoek A, Croce R, van Amerongen H (2008) Determination of the excitation migration time in photosystem II: consequences for the membrane organization and charge separation parameters. *Biochim Biophys Acta* 1777:404–409
- Burikov SA, Klimov DV, Litvinov PN, Maslov DV, Fadeev VV (2001) Shore-based LIDAR for monitoring coastal sea water areas. *Quantum Electron* 31:745–750
- Caffarri S, Broess K, Croce R, van Amerongen H (2011) Excitation energy transfer and trapping in higher plant photosystem II complexes with different antenna sizes. *Biophys J* 100:2094–2103
- Calhoun TR, Fleming GR (2011) Quantum coherence in photosynthetic complexes. *Phys Status Solidi (B)* 248:833–838
- Chachisvilis M, Kühn O, Pullerits T, Sundström V (1997) Excitons in photosynthetic purple bacteria: wavelike motion or incoherent hopping? *J Phys Chem B* 101:7275–7283
- Christensen R (2004) The electronic states of carotenoids. In: Frank HA, Young AJ, Britton G, Cogdell RJ (eds) *The Photochemistry of Carotenoids. Advances in Photosynthesis and Respiration, Volume 8*. Springer, Dordrecht, pp 137–159
- Christensson N, Milota F, Nemeth A, Pugliesi I, Riedle E, Sperling J, Pullerits T, Kauffmann HF, Hauer J (2010) Electronic double-quantum coherences and their impact on ultrafast spectroscopy: the example of β -carotene. *J Phys Chem Lett* 1:3366–3370
- Chynwat V, Frank HA (1995) The application of the energy gap law to the S_1 energies and dynamics of carotenoids. *Chem Phys* 194:237–244
- Clarke RH, Connors RE, Schaafsma TJ, Kleibeuker JF, Platenkamp RJ (1976) The triplet state of chlorophylls. *J Am Chem Soc* 98:3674–3677
- Clayton RK (1970) *Light and Living Matter. Physical Part, Volume 1*. McGraw-Hill, New York
- Clayton RK, Yamamoto T (1976) Photochemical quantum efficiency and absorption spectra of reaction centers from *Rhodospseudomonas sphaeroides* at low temperature. *Photochem Photobiol* 24:67–70
- Clegg RM (2004) Nuts and bolts of excitation energy migration and energy transfer. In: Papageorgiou GC, Govindjee (eds) *Chlorophyll *a* Fluorescence: A Signature of Photosynthesis. Advances in*

- Photosynthesis and Respiration, Volume 19. Springer, Dordrecht, pp 83–105
- Clegg RM (2006) The history of FRET: from conception through the labors of birth. In: Geddes CD, Lakowicz JR (eds) *Reviews in Fluorescence*. Springer, New York, pp 1–45
- Clegg RM, Sener M, Govindjee (2010) From Förster resonance energy transfer (FRET) to coherent resonance energy transfer (CRET) and back. A wheel o' mickles mak's a muckle. In: Alfano RR (ed) *SPIE Proceedings in Optical Biopsy VII*, Volume 7561. SPIE, Bellingham, pp 1–21
- Colbow K (1973) Energy transfer in photosynthesis. *Biochim Biophys Acta* 314:320–327
- Collini E, Wong CY, Wilk KE, Curmi PMG, Brumer P, Scholes GD (2010) Coherently wired light-harvesting in photosynthetic marine algae at ambient temperature. *Nature* 463:644–647
- Cong H, Niedzwiedzki DM, Gibson GN, LaFountain AM, Kelsch RM, Gardiner AT, Cogdell RJ, Frank HA (2008) Ultrafast time-resolved carotenoid to bacteriochlorophyll energy transfer in LH2 complexes from photosynthetic bacteria. *J Phys Chem B* 112:10689–10703
- Connelly JP, Müller MG, Hucke M, Gatzen G, Mullineaux CW, Ruban AV, Horton P, Holzwarth AR (1997) Ultrafast spectroscopy of trimeric light-harvesting complex II from higher plants. *J Phys Chem B* 101:1902–1909
- Croce R, van Amerongen H (2011) Light-harvesting and structural organization of photosystem II: from individual complexes to thylakoid membrane. *J Photochem Photobiol* 104:142–153
- Croce R, Müller MG, Caffarri S, Bassi R, Holzwarth AR (2003) Energy transfer pathways in the minor antenna complex CP29 of photosystem II: a femtosecond study of carotenoid to chlorophyll transfer on mutant and WT complexes. *Biophys J* 84:2517–2532
- Dall'Osto L, Cazzaniga S, North H, Marion-Poll A, Bassi R (2007) The Arabidopsis *aba4-1* mutant reveals a specific function for neoxanthin in protection against photooxidative stress. *Plant Cell* 19:1048–1064
- Dau H, Zaharieva I (2009) Principles, efficiency, and blueprint character of solar-energy conversion in photosynthetic water oxidation. *Acc Chem Res* 42:1861–1870
- DeCoster B, Christensen RL, Gebhard R, Lugtenburg J, Farhoosh R, Frank HA (1992) Low-lying electronic states of carotenoids. *Biochim Biophys Acta* 1102:107–114
- Dekker JP, van Grondelle R (2000) Primary charge separation in photosystem II. *Photosynth Res* 63:195–208
- Dekker JP, van Grondelle R, Völker S (1998) Spectral distributions of “trap” pigments in the RC, CP47, and CP47–RC complexes of photosystem II at low temperature: a fluorescence line-narrowing and hole-burning study. *J Phys Chem B* 102:11007–11016
- Demmig-Adams B, Adams WW (1992) Photoprotection and other responses of plants to high light stress. *Annu Rev Plant Physiol Plant Mol Biol* 43:599–626
- Dirac PAM (1927) The quantum theory of the emission and absorption of radiation. *Proc R Soc Lond A* 114:243–265
- Dreuw A, Fleming GR, Head-Gordon M (2005) Role of electron-transfer quenching of chlorophyll fluorescence by carotenoids in non-photochemical quenching of green plants. *Biochem Soc Trans* 33:858–862
- Duysens L (1952) *Transfer of Excitation Energy in Photosynthesis*, Doctoral Thesis, State University Utrecht, Netherlands, p 96
- Duysens LNM (1958) The path of light energy in photosynthesis. *Brookhaven Symp Biol* 11:10–23
- Eaton-Rye JJ, Tripathy BC, Sharkey TD (2012) *Photosynthesis – Plastid Biology, Energy Conversion and Carbon Assimilation*. *Advances in Photosynthesis and Respiration*, Volume 34. Springer, Dordrecht
- Engel GS, Calhoun TR, Read EL, Ahn T-K, Mancal T, Cheng Y-C, Blankenship RE, Fleming GR (2007) Evidence for wavelike energy transfer through quantum coherence in photosynthetic systems. *Nature* 446:782–786
- Englman R, Jortner J (1970) The energy gap law for radiationless transitions in large molecules. *Mol Phys* 18:145–164
- Fassioli F, Dinshaw R, Arpin PC, Scholes GD (2014) Photosynthetic light harvesting: excitons and coherence. *J R Soc Interface* 11:20130901
- Ferguson J (1958) Absorption and fluorescence spectra of crystalline pyrene. *J Chem Phys* 28:765–768
- Fidder H, Knoester J, Wiersma DA (1991) Optical properties of disordered molecular aggregates: a numerical study. *J Phys Chem* 95:7880–7890
- Förster T (1946) Energiewanderung und Fluoreszenz. *Naturwissenschaften* 33:166–175
- Förster T (1948) Zwischenmolekulare Energiewanderung und Fluoreszenz. *Ann Phys* 2:55–75
- Förster T (1963) Formation and dissociation of excited dimers. *Pure Appl Chem* 7:73–78
- Förster T (1965) Delocalized excitation and excitation transfer. In: Sinanoglu O (ed) *Modern Quantum Chemistry*. Academic, New York, pp 93–137
- Förster T (1967) Mechanisms of energy transfer. In: Florkin M, Stotz EH (eds) *Comprehensive Biochemistry*. Elsevier, Amsterdam, pp 61–80
- Förster T (1975) Excimers and exciplexes. In: Gordon M, Ware WR (eds) *The Exciplex*. Academic, New York, pp 1–21
- Förster T (2012) Energy migration and fluorescence. 1946. *J Biomed Opt* 17:011002

- Frank HA, Cogdell RJ (1996) Carotenoids in photosynthesis. *Photochem Photobiol* 63:257–264
- Frank HA, Polivka T (2008) Energy transfer from carotenoids to bacteriochlorophylls. In: Hunter NC, Daldal F, Thurnauer MC, Beatty TJ (eds) *The Purple Phototrophic Bacteria. Advances in Photosynthesis and Respiration, Volume 28*. Springer, Dordrecht, pp 213–230
- Frank HA, Cua A, Chynwat V, Young A, Gosztola D, Wasielewski MR (1994) Photophysics of the carotenoids associated with the xanthophyll cycle in photosynthesis. *Photosynth Res* 41:389–395
- Frank HA, Desamero RZB, Chynwat V, Gebhard R, van der Hoef I, Jansen FJ, Lugtenburg J, Gosztola D, Wasielewski MR (1997) Spectroscopic properties of spheroidene analogs having different extents of π -electron conjugation. *J Phys Chem A* 101:149–157
- Frank HA, Young AJ, Britton G, Cogdell RJ (eds) (1999) *The Photochemistry of Carotenoids. Advances in Photosynthesis and Respiration, Volume 8*. Kluwer Academic Publishers, Dordrecht
- Gilmore AM (1997) Mechanistic aspects of xanthophyll cycle-dependent photoprotection in higher plant chloroplasts and leaves. *Physiol Plant* 99:197–209
- Gilmore AM, Govindjee (1999) How higher plants respond to excess light: energy dissipation in photosystem II. In: Singhal GS, Renger G, Sopory SH, Irrgang KD, Govindjee (eds) *Concepts in Photobiology: Photosynthesis and Photomorphogenesis*. Narosa Publishing House, New Delhi, pp 513–548
- Gilmore AM, Hazlett TL, Govindjee (1995) Xanthophyll cycle-dependent quenching of photosystem II chlorophyll a fluorescence: formation of a quenching complex with a short fluorescence lifetime. *Proc Natl Acad Sci USA* 92:2273–2277
- Gorbunov MY, Falkowski PG, Kolber ZS (2000) Measurement of photosynthetic parameters in benthic organisms in situ using a SCUBA-based fast repetition rate fluorometer. *Limnol Oceanogr* 45:242–245
- Gouterman M (1961) Spectra of porphyrins. *J Mol Spectrosc* 6:138–163
- Govindjee (1995) Sixty-three years since Kautsky: chlorophyll a fluorescence. *Austr J Plant Physiol* 22:131–160
- Govindjee (1999) Carotenoids in photosynthesis: an historical perspective. In: Frank HA, Young AJ, Britton G, Cogdell RJ (eds) *The Photochemistry of Carotenoids. Advances in Photosynthesis and Respiration, Volume 8*. Kluwer Academic Publishers (now Springer), Dordrecht, pp 1–19
- Govindjee (2004) Chlorophyll a Fluorescence: a bit of basics and history. In: Papageorgiou GC, Govindjee (eds) *Chlorophyll a Fluorescence: A Signature of Photosynthesis. Advances in Photosynthesis and Respiration, Volume 19*. Springer, Dordrecht, pp 1–42
- Govindjee, Amesz J, Fork DC (eds) (1986) *Light Emission by Plants and Bacteria*. Academic, Orlando
- Gradinaru C, van Stokkum I, Pascal A, van Grondelle R, van Amerongen H (2000) Identifying the pathways of energy transfer between carotenoids and chlorophylls in LHCII and CP29. A multicolor, femtosecond pump-probe study. *J Phys Chem B* 104:9330–9342
- Green BR, Parson WW (eds) (2003) *Light-harvesting Antennas in Photosynthesis. Advances in Photosynthesis and Respiration, Volume 13*. Kluwer Academic Publishers (now Springer), Dordrecht
- Greenfield SR, Seibert M, Wasielewski MR (1997) Direct measurement of the effective rate constant for primary charge separation in isolated photosystem II reaction centers. *J Phys Chem B* 101:2251–2255
- Grimm B, Porra RJ, Rüdiger W, Scheer H (Eds) (2006) *Chlorophylls and Bacteriochlorophylls. Advances in Photosynthesis and Respiration, Volume 25*. Springer, Dordrecht
- Hansson O, Wydrzynski T (1990) Current perceptions of photosystem II. *Photosynth Res* 23:131–162
- Herzberg G, Teller E (1933) Fluctuation structure of electron transfer in multiatomic molecules. *Z Phys Chem B* 21:410–446
- Holt NE, Zigmantas D, Valkunas L, Li X-P, Niyogi KK, Fleming GR (2005) Carotenoid cation formation and the regulation of photosynthetic light harvesting. *Science* 307:433–436
- Holzwarth AR, Müller MG, Reus M, Nowaczyk M, Sander J, Rögner M (2006) Kinetics and mechanism of electron transfer in intact photosystem II and in the isolated reaction center: pheophytin is the primary electron acceptor. *Proc Natl Acad Sci USA* 103:6895–6900
- Horton P, Ruban AV, Walters RG (1996) Regulation of light harvesting in green plants. *Annu Rev Plant Physiol Plant Mol Biol* 47:655–684
- Horton P, Ruban AV, Wentworth M (2000) Allosteric regulation of the light-harvesting system of photosystem II. *Philos Trans R Soc Lond B Biol Sci* 355:1361–1370
- Hsu C-P, Walla PJ, Head-Gordon M, Fleming GR (2001) The role of the S1 state of carotenoids in photosynthetic energy transfer: the light-harvesting complex II of purple bacteria. *J Phys Chem B* 105:11016–11025
- Hu X, Schulten K (1997) How nature harvests sunlight. *Phys Today* 50:28–34
- Ilioaia C, Johnson MP, Liao P-N, Pascal AA, van Grondelle R, Walla PJ, Ruban AV, Robert B (2011) Photoprotection in plants involves a change in lutein 1 binding domain in the major light-harvesting complex of photosystem II. *J Biol Chem* 286:27247–27254

- Ishizaki A, Fleming GR (2009) Theoretical examination of quantum coherence in a photosynthetic system at physiological temperature. *Proc Natl Acad Sci USA* 106:17255–17260
- Ishizaki A, Fleming GR (2012) Quantum coherence in photosynthetic light harvesting. *Annu Rev Condens Matter Phys* 3:333–361
- Jean JM, Chan C-K, Fleming GR, Owens TG (1989) Excitation transport and trapping on spectrally disordered lattices. *Biophys J* 56:1203–1215
- Jimenez R, Dikshit SN, Bradforth SE, Fleming GR (1996) Electronic excitation transfer in the LH2 complex of *Rhodobacter sphaeroides*. *J Phys Chem* 100:6825–6834
- Jursinic P, Govindjee (1977) Temperature dependence of delayed light emission in the 6 to 340 microsecond range after a single flash in chloroplasts. *Photochem Photobiol* 26:617–628
- Kaplanová M, Parma L (1984) Effect of excitation and emission wavelength on the fluorescence lifetimes of chlorophyll a. *Gen Physiol Biophys* 3:127–134
- Kasajima I, Takahara K, Kawai-Yamada M, Uchimiya H (2009) Estimation of the relative sizes of rate constants for chlorophyll de-excitation processes through comparison of inverse fluorescence intensities. *Plant Cell Physiol* 50:1600–1616
- Kasha M (1950) Characterization of electronic transitions in complex molecules. *Discuss Faraday Soc* 9:14
- Ke B (2001a) Role of Carotenoids in Photosynthesis, a Chapter in “Photosynthesis: Photobiochemistry and Photobiophysics”. *Advances in Photosynthesis and Respiration*, Volume 10. Springer, Dordrecht, pp 229–250
- Ke B (2001b) Photosynthesis: Photobiochemistry and Photobiophysics. *Advances in Photosynthesis and Respiration*, Volume 10. Kluwer Academic Publishers (now Springer), Dordrecht
- Kennis JTM, Streltsov AM, Permentier H, Aartsma TJ, Amez J (1997) Exciton coherence and energy transfer in the LH2 antenna complex of *Rhodospseudomonas acidophila* at low temperature. *J Phys Chem B* 101:8369–8374
- Kirstein S, Bourbon S, Gao M, De Rossi U (2000) Layer-by-layer deposition of J-aggregates and polyelectrolytes for electroluminescence applications: a spectroscopic study. *Isr J Chem* 40:129–138
- Knox RS (2012) Förster’s resonance excitation transfer theory: not just a formula. *J Biomed Opt* 17:011003
- Kobayashi M, Akiyama M, Kano H, Kise H (2006) Spectroscopy and structure determination. In: Grimm B, Porra RJ, Rüdiger W, Scheer H (eds) *Chlorophylls and Bacteriochlorophylls*. *Advances in Photosynthesis and Respiration*, Volume 25. Springer, Dordrecht, pp 79–94
- Kolber ZS, Prášil O, Falkowski PG (1998) Measurements of variable chlorophyll fluorescence using fast repetition rate techniques: defining methodology and experimental protocols. *Biochim Biophys Acta* 1367:88–106
- Kosumi D, Yanagi K, Fujii R, Hashimoto H, Yoshizawa M (2006) Conjugation length dependence of relaxation kinetics in β -carotene homologs probed by femtosecond Kerr-gate fluorescence spectroscopy. *Chem Phys Lett* 425:66–70
- Krause GH, Weiss E (1991) Chlorophyll fluorescence and photosynthesis: the basics. *Annu Rev Plant Physiol Plant Mol Biol* 42:313–349
- Krikunova M, Kummrow A, Voigt B, Rini M, Lokstein H, Moskalenko A, Scheer H, Razjivin A, Leupold D (2002) Fluorescence of native and carotenoid-depleted LH2 from *Chromatium minutissimum*, originating from simultaneous two-photon absorption in the spectral range of the presumed (optically “dark”) S1 state of carotenoids. *FEBS Lett* 528:227–229
- Krueger BP, Scholes GD, Fleming GR (1998) Calculation of couplings and energy-transfer pathways between the pigments of LH2 by the ab initio transition density cube method. *J Phys Chem B* 102:5378–5386
- Krüger TPJ, Novoderezhkin VI, Iliaia C, van Grondelle R (2010) Fluorescence spectral dynamics of single LHCII trimers. *Biophys J* 98:3093–3101
- Krüger TPJ, Iliaia C, Johnson MP, Ruban AV, Papagiannakis E, Horton P, van Grondelle R (2012) Controlled disorder in plant light-harvesting complex II explains its photoprotective role. *Biophys J* 102:2669–2676
- Lakowicz JR (1999) *Principles of Fluorescence Spectroscopy*. Kluwer Academic Publishers, New York
- Liao M-S, Scheiner S (2002) Electronic structure and bonding in metal porphyrins, metal=Fe, Co, Ni, Cu, Zn. *J Chem Phys* 117:205–219
- Liao P-N, Bode S, Wilk L, Hafi N, Walla PJ (2010a) Correlation of electronic carotenoid–chlorophyll interactions and fluorescence quenching with the aggregation of native LHC II and chlorophyll deficient mutants. *Chem Phys* 373:50–55
- Liao P-N, Holleboom C-P, Wilk L, Kühlbrandt W, Walla PJ (2010b) Correlation of Car S(1) \rightarrow Chl with Chl \rightarrow Car S(1) energy transfer supports the excitonic model in quenched light harvesting complex II. *J Phys Chem B* 114:15650–15655
- Livingston R (1960) Physicochemical aspects of some recent work on photosynthesis. *Q Rev Chem Soc* 14:174–199
- MacPherson AN, Arellano JB, Fraser NJ, Cogdell RJ, Gillbro T (2001) Efficient energy transfer from the carotenoid S-2 state in a photosynthetic light-harvesting complex. *Biophys J* 80:923–930
- Malkin S, Armond PA, Mooney HA, Fork DC (1981) Photosystem II photosynthetic unit sizes from fluorescence induction in leaves: correlation to photosynthetic capacity. *Plant Physiol* 67:570–579
- Marian CM (2012) Spin-orbit coupling and intersystem crossing in molecules. *WIREs Comput Mol Sci* 2:187–203

- May V, Kühn O (2011) Dynamics of Isolated and Open Quantum Systems. Charge and Energy Transfer Dynamics in Molecular Systems. Wiley-VCH Verlag GmbH, Weinheim, pp 81–194
- McDermott G, Prince SM, Freer AA, Hawthornthwaite-Lawless AM, Papiz MZ, Cogdell RJ, Isaacs NW (1995) Crystal structure of an integral membrane light-harvesting complex from photosynthetic bacteria. *Nature* 374:517–521
- McGlynn SP, Armstrong AT, Azumi T (1965) Interaction of molecular excitons, charge resonance states, and excimer luminescence. In: Sinanoglu O (ed) *Modern Quantum Chemistry: Istanbul Lectures. Part III, Action of Light and Organic Crystals*. Academic, New York, pp 203–228
- Melkozernov AN, Kargul J, Lin S, Barber J, Blankenship RE (2004) Energy coupling in the PSI–LHCI supercomplex from the green alga *Chlamydomonas reinhardtii*. *J Phys Chem B* 108:10547–10555
- Miloslavina Y, Szczepaniak M, Müller MG, Sander J, Nowaczyk M, Rögner M, Holzwarth AR (2006) Charge separation kinetics in intact photosystem II core particles is trap-limited. A picosecond fluorescence study. *Biochemistry* 45:2436–2442
- Miloslavina Y, Wehner A, Lambrev PH, Wientjes E, Reus M, Garab G, Croce R, Holzwarth AR (2008) Far-red fluorescence: a direct spectroscopic marker for LHCII oligomer formation in non-photochemical quenching. *FEBS Lett* 582:3625–3631
- Mimuro M, Nagashima U, Takaichi S, Nishimura Y, Yamazaki I, Katoh T (1992) Molecular structure and optical properties of carotenoids for the in vivo energy transfer function in the algal photosynthetic pigment system. *Biochim Biophys Acta* 1098:271–274
- Monshouwer R, van Grondelle R (1996) Excitations and excitons in bacterial light-harvesting complexes. *Biochim Biophys Acta* 1275:70–75
- Monshouwer R, Abrahamsson M, van Mourik F, van Grondelle R (1997) Superradiance and exciton delocalization in bacterial photosynthetic light-harvesting systems. *J Phys Chem B* 101:7241–7248
- Montroll EW (1969) Random walks on lattices. III. Calculation of first-passage times with application to exciton trapping on photosynthetic units. *J Math Phys* 10:753–765
- Moya I, Sebban P, Haehnel W (1986) Lifetime of excited states and quantum yield of chlorophyll *a* fluorescence in vivo. In: Govindjee, Ames J, Fork DD (eds) *Light Emission by Plants and Bacteria*. Academic Press, Orlando (now available from Elsevier), pp 161–400
- Mukai K, Abe S, Sumi H (1999) Theory of rapid excitation-energy transfer from B800 to optically-forbidden exciton states of B850 in the antenna system LH2 of photosynthetic purple bacteria. *J Phys Chem B* 103:6096–6102
- Müller MG, Lambrev P, Reus M, Wientjes E, Croce R, Holzwarth AR (2010) Singlet energy dissipation in photosystem II light-harvesting complex does not involve energy transfer to carotenoids. *Chem Phys Lett* 11:1289–1296
- Mullineaux CW, Pascal AA, Horton P, Holzwarth AR (1993) Excitation-energy quenching in aggregates of the LHC II chlorophyll-protein complex: a time-resolved fluorescence study. *Biochim Biophys Acta* 1141:23–28
- Murrell JN, Tanaka J (1964) The theory of the electronic spectra of aromatic hydrocarbon dimers. *Mol Phys* 7:363–380
- Nemykin VN, Hadt RG (2010) Interpretation of the UV-vis spectra of the meso(ferrocenyl)-containing porphyrins using a TDDFT approach: is Gouterman's classic four-orbital model still in play? *J Phys Chem A* 114:12062–12066
- Noomnarm U, Clegg RM (2009) Fluorescence lifetimes: fundamentals and interpretations. *Photosynth Res* 101:181–194
- Novoderezhkin VI, van Grondelle R (2010) Physical origins and models of energy transfer in photosynthetic light-harvesting. *Phys Chem Chem Phys* 12:7352–7365
- Novoderezhkin V, Monshouwer R, van Grondelle R (1999a) Exciton (de)localization in the LH2 antenna of *Rhodobacter sphaeroides* as revealed by relative difference absorption measurements of the LH2 antenna and the B820 subunit. *J Phys Chem B* 103:10540–10548
- Novoderezhkin V, Monshouwer R, van Grondelle R (1999b) Disordered exciton model for the core light-harvesting antenna of *Rhodospseudomonas viridis*. *Biophys J* 77:666–681
- Nuijs AM, van Gorkom HJ, Plijter JJ, Duysens LNM (1986) Primary-charge separation and excitation of chlorophyll *a* in photosystem II particles from spinach as studied by picosecond absorbance-difference spectroscopy. *Biochim Biophys Acta* 848:167–175
- Owens T (1994) Excitation energy transfer between chlorophylls and carotenoids. A proposed molecular mechanism for non-photochemical quenching. In: Baker NR, Bowyer JR (eds) *Photoinhibition of Photosynthesis from Molecular Mechanisms to the Field*. Bios Scientific Publishers, Oxford, pp 95–109
- Papageorgiou GC (2012) Fluorescence emission from the photosynthetic apparatus. In: Eaton-Rye JJ, Tripathy BC, Sharkey TD (eds) *Photosynthesis: Plastid Biology, Energy Conversion and Carbon Assimilation. Advances in Photosynthesis and Respiration, Volume 34*. Springer, Dordrecht, pp 415–443
- Papageorgiou GC, Govindjee (eds) (2004) *Chlorophyll *a* Fluorescence: A Signature of Photosynthesis. Advances in Photosynthesis and Respiration, Volume 19*. Kluwer Academic Publishers (now Springer), Dordrecht

- Pascal AA, Liu Z, Broess K, Van Oort B, van Amerongen H, Wang C, Horton P, Robert B, Chang W, Ruban AV (2005) Molecular basis of photoprotection and control of photosynthetic light-harvesting. *Nature* 436:134–137
- Pearlstein RM (1982) Chlorophyll singlet excitons. In: Govindjee (ed) *Photosynthesis: Energy Conversion by Plants and Bacteria*. Academic Press, New York, pp 293–331
- Pearlstein RM (2005) Photosynthetic exciton theory in the 1960s. In: Govindjee, Beatty JT, Gest H, Allen JF (eds) *Discoveries in Photosynthesis. Advances in Photosynthesis and Respiration, Volume 20*. Springer, Dordrecht, pp 147–154
- Peterman EJ, Dukker FM, van Grondelle R, van Amerongen H (1995) Chlorophyll a and carotenoid triplet states in light-harvesting complex II of higher plants. *Biophys J* 69:2670–2678
- Polívka T, Sundström V (2004) Ultrafast dynamics of carotenoid excited states-from solution to natural and artificial systems. *Chem Rev* 104:2021–2071
- Polívka T, Sundström V (2009) Dark excited states of carotenoids: consensus and controversy. *Chem Phys Lett* 477:1–11
- Polívka T, Herek JL, Zigmantas D, Åkerlund H-E, Sundström V (1999) Direct observation of the (forbidden) S1 state in carotenoids. *Proc Natl Acad Sci USA* 96:4914–4917
- Pullerits T, Freiberg A (1992) Kinetic model of primary energy transfer and trapping in photosynthetic membranes. *Biophys J* 63:879–896
- Pullerits T, Visscher KJ, Hess S, Sundström V, Freiberg A, Timpmann K, van Grondelle R (1994) Energy transfer in the inhomogeneously broadened core antenna of purple bacteria: a simultaneous fit of low-intensity picosecond absorption and fluorescence kinetics. *Biophys J* 66:236–248
- Rabinowitch E, Govindjee (1969) *Photosynthesis*, Wiley, New York (see <http://www.life.illinois.edu/govindjee/g/Books.html>, and <http://www.life.illinois.edu/govindjee/photosynBook.html>)
- Raszewski G, Renger T (2008) Light harvesting in photosystem II core complexes is limited by the transfer to the trap: can the core complex turn into a photoprotective mode? *J Am Chem Soc* 130:4431–4446
- Razi Naqvi K (1998) Carotenoid-induced electronic relaxation of the first excited state of antenna chlorophylls. In: Garab G (ed) *Photosynthesis: Mechanisms and Effects*. Kluwer Academic Publishers, Dordrecht, pp 265–270
- Renger G (1999) Basic principles of photophysics and photochemistry. In: Singhal GS, Renger G, Sopory SK, Irrgang K-D, Govindjee (eds) *Concepts in Photobiology*. Springer, Dordrecht, pp 52–90
- Renger G (2008a) Overview of primary processes of photosynthesis. In: Renger G (ed) *Primary Processes of Photosynthesis*. The Royal Society of Chemistry, Cambridge, pp 5–35
- Renger T (2008b) Absorption of light, excitation energy transfer and electron transfer reactions. In: Renger G (ed) *Primary Processes of Photosynthesis*. The Royal Society of Chemistry, Cambridge, pp 39–97
- Renger T, Holzwarth AR (2008) Theory of excitation energy transfer and optical spectra of photosynthetic systems. In: Aartsma TJ, Matysik J (eds) *Biophysical Techniques in Photosynthesis II. Advances in Photosynthesis and Respiration, Volume 26*. Springer, Dordrecht, pp 421–443
- Rijgersberg CP, van Grondelle R, Amesz J (1980) Energy transfer and bacteriochlorophyll fluorescence in purple bacteria at low temperature. *Biochim Biophys Acta* 592:53–64
- Robert B (2008) Spectroscopic properties of antenna complexes from purple bacteria. In: Hunter NC, Daldal F, Thurnauer MC, Beatty TJ (eds) *The Purple Phototrophic Bacteria. Advances in Photosynthesis and Respiration, Volume 28*. Springer, Dordrecht, pp 199–212
- Romero E, Mozzo M, van Stokkum IHM, Dekker JP, van Grondelle R, Croce R (2009) The origin of the low-energy form of photosystem I light-harvesting complex Lhca4: mixing of the lowest exciton with a charge-transfer state. *Biophys J* 96:L35–L37
- Ross RT, Calvin M (1967) Thermodynamics of light emission and free-energy storage in photosynthesis. *Biophys J* 7:595–614
- Ruban AV, Berera R, Ilioaia C, van Stokkum IHM, Kennis JTM, Pascal A, van Amerongen H, Robert B, Horton P, van Grondelle R (2007) Identification of a mechanism of photoprotective energy dissipation in higher plants. *Nature* 450:575–578
- Ruban AV, Johnson MP, Duffy CDP (2011) The photoprotective molecular switch in the photosystem II antenna. *Biochim Biophys Acta* 1817:167–181
- Rubio M, Roos BO, Serrano-Andrés L, Merchán M (1999) Theoretical study of the electronic spectrum of magnesium-porphyrin. *J Chem Phys* 110:7202–7209
- Schatz GH, Brock H, Holzwarth AR (1988) Kinetic and energetic model for the primary processes in photosystem II. *Biophys J* 54:397–405
- Scheer H (2006) An overview of chlorophylls and bacteriochlorophylls: biochemistry, biophysics, functions and applications. In: Grimm B, Porra RJ, Rüdiger W, Scheer H (eds) *Chlorophylls and Bacteriochlorophylls. Advances in Photosynthesis and Respiration, Volume 25*. Springer, Dordrecht, pp 1–19
- Scherer P, Fischer SF (2010) *Theoretical Molecular Biophysics*. Springer, Dordrecht

- Schmidt M, Tavan P (2012) Electronic excitations in long polyenes revisited. *J Chem Phys* 136: 124309
- Scholes GD (2003) Long-range resonance energy transfer in molecular systems. *Annu Rev Phys Chem* 54:57–87
- Scholes GD, Fleming GR (2000) On the mechanism of light harvesting in photosynthetic purple bacteria: B800 to B850 energy transfer. *J Chem Phys B* 104:1854–1868
- Scholes GD, Fleming GR (2005) Energy transfer and light harvesting. In: Berry RS, Jortner J (eds) *Adventures in Chemical Physics: A Special Volume of Advances in Chemical Physics, Volume 132*. Wiley, Hoboken, pp 57–129
- Scholes GD, Rumbles G (2006) Excitons in nanoscale systems. *Nat Mater* 5:683–696
- Scholes GD, Ghiggino KP, Wilson GJ (1991) Excimer geometries in bichromophoric molecules: a theoretical and experimental study of 1,2-(bis-9-anthryl)-ethane. *Chem Phys* 155:127–141
- Scholes GD, Fleming GR, Olaya-Castro A, van Grondelle R (2011) Lessons from nature about solar light harvesting. *Nat Chem* 3:763–774
- Schreiber U (2004) Pulse-Amplitude-Modulation (PAM) fluorometry and saturation pulse method: an overview. In: Papageorgiou GC, Govindjee (eds) *Chlorophyll *a* Fluorescence: A Signature of Photosynthesis. Advances in Photosynthesis and Respiration, Volume 19*. Springer, Dordrecht, pp 279–319
- Seely GR, Connolly JS (1986) Fluorescence of photosynthetic pigments in vitro. In: Govindjee A, Ames J, Fork DD (eds) *Light Emission by Plants and Bacteria*. Academic, Orlando, pp 99–136
- Sharkov AV, Kryukov IV, Khoroshilov EV, Kryukov PG, Fischer R, Scheer H, Gillbro T (1992) Femtosecond energy transfer between chromophores in allophycocyanin trimers. *Chem Phys Lett* 191:633–638
- Shevela D, Pishchalnikov RY, Eichacker LA, Govindjee (2013) Oxygenic photosynthesis in cyanobacteria. In: Srivastava AK, Rai AN, Neilan BA (eds) *Stress Biology of Cyanobacteria*. CRC Press, Taylor&Francis Group, Boca Raton, pp 3–40
- Shipman LL (1980) A theoretical study of excitons in chlorophyll *a* photosystems on a picosecond timescale. *Photochem Photobiol* 31:157–167
- Shipman LL (1982) Electronic structure and function of chlorophylls and their pheophytins. In: Govindjee (ed) *Photosynthesis: Energy Conversion by Plants and Bacteria*. Academic, New York, pp 275–293
- Somsen OJ, van Mourik F, van Grondelle R, Valkunas L (1994) Energy migration and trapping in a spectrally and spatially inhomogeneous light-harvesting antenna. *Biophys J* 66:1580–1596
- Song PS, Koka P, Prézélin BB, Haxo FT (1976) Molecular topology of the photosynthetic light-harvesting pigment complex, peridinin-chlorophyll *a*-protein, from marine dinoflagellates. *Biochemistry* 15:4422–4427
- Strasser RJ, Tsimilli-Michael M, Srivastava A (2004) Analysis of the chlorophyll *a* fluorescence transient. In: Papageorgiou GC, Govindjee (eds) *Chlorophyll *a* Fluorescence: A Signature of Photosynthesis. Advances in Photosynthesis and Respiration, Volume 19*. Springer, Dordrecht, pp 321–362
- Sumi H (1999) Theory on rates of excitation-energy transfer between molecular aggregates through distributed transition dipoles with application to the antenna system in bacterial photosynthesis. *J Phys Chem B* 103:252–260
- Sundström V, Pullerits T, van Grondelle R (1999) Photosynthetic light-harvesting: reconciling dynamics and structure of purple bacterial LH2 reveals function of photosynthetic unit. *J Phys Chem B* 103:2327–2346
- Tavan P, Schulten K (1986) The low-lying electronic excitations in long polyenes: a PPP-MRD-CI study. *J Chem Phys* 85:6602
- Telfer A, Pascal A, Gall A (2008) Carotenoids in photosynthesis. In: Britton G, Liaaen-Jensen S, Pfander H (eds) *Carotenoids, Volume 4: Natural Functions*. Birkhauser-Verlag, Basel, pp 265–308
- Truscott GT, Edge R (2004) Carotenoid radicals and the interaction of carotenoids with active oxygen species. In: Frank HA, Young A, Britton G, Cogdell RJ (eds) *The Photochemistry of Carotenoids. Advances in Photosynthesis and Respiration, Volume 8*. Kluwer (now Springer), Dordrecht, pp 223–234
- Valkunas L, Liuolia V, Dekker JP, van Grondelle R (1995) Description of energy migration and trapping in photosystem I by a model with two distance scaling parameters. *Photosynth Res* 43: 149–154
- van Amerongen H, Croce R (2013) Light harvesting in photosystem II. *Photosynth Res* 116:251–263
- van Amerongen H, van Grondelle R (2001) Understanding the energy transfer function of LHCII, the major light-harvesting complex of green plants. *J Phys Chem B* 105:604–617
- van Amerongen H, Valkunas L, van Grondelle R (2000a) *Photosynthetic Excitons*. World Scientific Publishers, London
- van Amerongen H, Valkunas L, van Grondelle R (2000b) Migration of localized excitons: Förster excitation transfer and trapping by reaction center. *Photosynthetic Excitons*. World Scientific Publishers, London, pp 401–448
- van Amerongen H, Valkunas L, van Grondelle R (2000c) Exciton energy transfer and trapping.

- Experiments. Photosynthetic Excitons. World Scientific Publishers, London, pp 449–478
- van Grondelle R (1985) Excitation energy transfer, trapping and annihilation in photosynthetic systems. *Biochim Biophys Acta* 811:147–195
- van Grondelle R, Gobets B (2004) Transfer and trapping of excitations in plant photosystems. In: Papageorgiou GC, Govindjee (eds) *Chlorophyll a Fluorescence: A Signature of Photosynthesis*. Advances in Photosynthesis and Respiration, Volume 19. Springer, Dordrecht, pp 107–132
- van Grondelle R, Novoderezhkin VI (2006) Energy transfer in photosynthesis: experimental insights and quantitative models. *Phys Chem Chem Phys* 8:793–807
- van Grondelle R, Dekker JP, Gillbro T, Sundström V (1994) Energy transfer and trapping in photosynthesis. *Biochim Biophys Acta* 1187:1–65
- Van Oort B, Alberts M, de Bianchi S, Dall’Osto L, Bassi R, Trinklunas G, Croce R, van Amerongen H (2010) Effect of antenna-depletion in photosystem II on excitation energy transfer in *Arabidopsis thaliana*. *Biophys J* 98:922–931
- van Zandvoort MAMJ, Wróbel D, Lettinga P, van Ginkel G, Levine YK (1995) Chlorophylls in polymers. I. State of chlorophyll a in unstretched polymer systems. *Photochem Photobiol* 62:279–289
- Vasil’ev S, Orth P, Zouni A, Owens TG, Bruce D (2001) Excited-state dynamics in photosystem II: insights from the x-ray crystal structure. *Proc Natl Acad Sci USA* 98:8602–8607
- Visscher KJ, Bergstrom H, Sundström V, Hunter CN, van Grondelle R (1989) Temperature dependence of energy transfer from the long wavelength antenna BChl-896 to the reaction center in *Rhodospirillum rubrum*, *Rhodobacter sphaeroides* (w.t. and M21 mutant) from 77 to 177K, studied by picosecond absorption spectroscopy. *Photosynth Res* 22:211–217
- Vredenberg WJ (2004) System analysis and photoelectrochemical control of chlorophyll fluorescence in terms of trapping models of photosystem II: a challenging view. In: Papageorgiou GC, Govindjee (eds) *Chlorophyll a Fluorescence: A Signature of Photosynthesis*. Advances in Photosynthesis and Respiration, Volume 19. Springer, Dordrecht, pp 133–172
- Wahadoszamen M, Berera R, Ara AM, Romero E, van Grondelle R (2012) Identification of two emitting sites in the dissipative state of the major light harvesting antenna. *Phys Chem Chem Phys* 14:759–766
- Wasielewski MR, Johnson DG, Seibert M, Govindjee (1989) Determination of the primary charge separation rate in isolated photosystem II reaction centers with 500-fs time resolution. *Proc Natl Acad Sci USA* 86:524–528
- Weis E, Berry JA (1987) Quantum efficiency of photosystem II in relation to “energy”-dependent quenching of chlorophyll fluorescence. *Biochim Biophys Acta* 894:198–208
- Weiss C (1978) Electronic absorption spectra of chlorophylls. In: Dolphin D (ed) *The Porphyrins*, Volume 3: Physical Chemistry, Part A. Academic, New York, pp 211–223
- Wientjes E, van Amerongen H, Croce R (2013) Quantum yield of charge separation in photosystem II: functional effect of changes in the antenna size upon light acclimation. *J Phys Chem B* 117:11200–11208
- Willock D (2009) *Molecular Symmetry*. Wiley, Chichester
- Wohlleben W, Buckup T, Hashimoto H, Cogdell RJ, Herek JL, Motzkus M (2004) Pump-deplete-probe spectroscopy and the puzzle of carotenoid dark states. *J Chem Phys B* 108:3320–3325
- Wraight CA, Clayton RK (1974) The absolute quantum efficiency of bacteriochlorophyll photooxidation in reaction centres of *Rhodospseudomonas spheroides*. *Biochim Biophys Acta* 333:246–260
- Yang M, Damjanović A, Vaswani HM, Fleming GR (2003) Energy transfer in photosystem I of cyanobacteria *Synechococcus elongatus*: model study with structure-based semi-empirical Hamiltonian and experimental spectral density. *Biophys J* 85:140–158
- Yoshizawa M, Kosumi D, Komukai M, Hashimoto H (2006) Ultrafast optical responses of three-level systems in beta-carotene: resonance to a high-lying n(1)a(g)(-) excited state. *Laser Phys* 16:325–330
- Zahlan AB (1967) *The Triplet States*. Cambridge University Press, New York
- Zgierski MZ (1974) Herzberg-Teller interaction and vibronic coupling in molecular crystals. II. The simple model with inclusion of a frequency change in the non-totally symmetric vibration. *Phys Status Solidi (B)* 62:51–55
- Zubik M, Luchowski R, Grudzinski W, Gospodarek M, Gryczynski I, Gryczynski Z, Dobrucki JW, Gruszecki WI (2011) Light-induced isomerization of the LHCII-bound xanthophyll neoxanthin: possible implications for photoprotection in plants. *Biochim Biophys Acta* 1807:1237–1243
- Zucchelli G, Brogioli D, Casazza AP, Garlaschi FM, Jennings RC (2007) Chlorophyll ring deformation modulates Qy electronic energy in chlorophyll-protein complexes and generates spectral forms. *Biophys J* 93:2240–2254