

PHYSICAL BIOLOGY: EN ROUTE TO QUANTUM BIOLOGY?

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Complex relationships between the quantum realms of molecular processes and the classical world of living organisms are outlined. Recent validation of the survival of photosynthetic excitons at functional temperatures is yet another example bridging the gap between quantum physics and biology.

INTRODUCTION

Not long ago, a physicist and a layman alike would think: “Physical biology, what a nonsense!” Indeed, from the middle- and high-school experience, nothing seems to be farther away from each other than these two disciplines. Physics deals with inanimate matter, while biology, with the living world. Biology is wet, warm, and messy, functioning at ambient temperatures; physics is solid, cold, and neat, rather studied at cryogenic temperatures. Last, but not least, to understand physics one needs math, while biology can be figured out ‘naturally’. This confusion is an unfortunate result of the extreme specialization of modern science. Once called ‘natural philosophy’, the scientific study of all of nature, the science began to divide into separate disciplines with increasing pace since the Renaissance. Great advantages of the narrow specialism of various scientific disciplines are obvious. As a result of that, hidden secrets of nature at the scales of both the very large and the very small have been revealed with unprecedented details. At the same time, pointless barriers have been raised which largely prevent scientists from different fields from acknowledging common grounds of their subject matters and from recognizing broader perspectives. Yet science develops cyclically and this state of affairs is bound to change. Surfacing of the various mixed two-word subjects such as the one used in the title of this treatise precisely reflects this trend. The reductionist approach has been enormously successful and literally dominated research in biology for over the last 50 years. However, complexity of cellular processes reinforces an opposite, integrated method that has not long ago materialized in a discipline called systems biology. The physical biology, known also as biological physics or biophysics, combines essential knowledge from physics

and biology of the living state of matter. One of the lessons taught by the physical biology is that on the very basic level the biology is as quantitative a science as the physics is. The emerging fields of physical and systems biology are clearly among the most exciting frontiers of the 21st century science (Zewail 2008).

Physics has fundamentally a quantum origin. Photosynthesis, on the other hand, is a basic biological process, a major source of life on Earth. However, since photosynthesis begins with absorption of solar quanta, it can be considered a subject at the borderline of classical and quantum realms. The physical concept of 'exciton', a collective electronic excitation of condensed matter introduced by J.Frenkel almost exactly eighty years ago, is by now well established in photosynthesis (Van Amerongen et al. 2000). It is believed that absorption of a solar photon by chlorophyll and other chromophores in light-harvesting (LH) antenna complexes creates a coherent exciton that transfers its energy very efficiently to the photochemical reaction centres (RC), where it is subsequently transformed via vectorial electron transfer steps into potential chemical energy. Yet this insight has primarily arisen from interpretations of the low-temperature spectroscopic measurements. Experimental evidence for the excitons at physiological temperatures is scarce at best. Thorough spectroscopic studies of *in vitro* and *in vivo* (bacterio) chlorophyll molecules performed in our laboratory over a broad temperature range from liquid helium to ambient temperatures provided first clear proof for the presence of excitons, hence of long-lasting (stationary) quantum coherences, in photosynthetic systems at functional temperatures.

RECENT ADVANCES IN UNDERSTANDING QUANTUM EXCITATIONS OF *IN VITRO* (BACTERIO) CHLOROPHYLLS

Chlorophylls are ubiquitous in plants and algae, similar to bacteriochlorophylls in bacterial photosynthetic systems (Blankenship 2002). The useful functioning of the (bacterio) chlorophyll molecules is based on their unique quantum-optical and redox properties. Being the lowest-energy electronic transitions in the visible range, the Q_y singlet electronic transitions are instrumental in all photophysical and -chemical processes these chromophores are involved in. Their properties in dependence on solvent polarity, axial coordination of the central magnesium atom, pigment aggregation, temperature, external pressure and electric field, etc. have

experimentally been studied using almost all conceivable spectroscopic techniques. Despite this intensive scrutiny, optical spectroscopy of the (bacterio) chlorophyll molecules is still not wholly understood, neither experimentally nor theoretically.

The couplings between a molecular electronic transition and intramolecular nuclear vibrations determine the vibronic structure of optical spectra of individual chromophores. The chromophore-host bath interactions present in the solvent phase give rise to the electron-phonon structure of the spectra. Phonons are collective vibrational modes (standing waves) of the surrounding matrix in which all the matrix particles simultaneously participate. The electron-phonon and intramolecular vibronic interactions together shape the so-called homogenous spectra of impurity molecules. Avarmaa and co-workers (Avarmaa, Rebane 1985) were the first to obtain high-resolution spectra related to the Q_y electronic state of the impurity chlorophyll *a* (Chl *a*) and bacteriochlorophyll *a* (Bchl *a*) molecules. Yet these low-temperature results had rather qualitative than quantitative value, since experimental limitations prohibited determination of the coupling strengths for both the lattice phonons and individual intramolecular vibrations. Zazubovich et al. (2001) achieved this task for the excited electronic state using hole burning in a triethylamine glass. Comparable results for the ground electronic state have been published by us only recently (Rätsep et al. 2009a, 2011).

Fig. 1 demonstrates the homogeneous spectral profiles revealing detailed phonon and vibronic structures of the fluorescence spectra of Chl *a* and Bchl *a* molecules in low-temperature glass matrices. The high-resolution spectra, which comprise a narrow zero-phonon line (ZPL) at the spectral origin, a phonon sideband (PSB) attached to the ZPL from the low-energy side, and up to 50 vibronic replicas distributed over a wide frequency range between ~ 80 and 1700 cm^{-1} are obtained using the novel difference fluorescence-line narrowing method (Δ FLN). The coupling between the Q_y electronic transition and the intramolecular vibrational degrees of freedom is weak; therefore, the replicas represent single-quantum band profiles for the local vibrational modes. Δ FLN as developed in our laboratory (Rätsep, Freiberg 2003) is a highly selective technique, which combines the strengths of both hole burning and traditional fluorescence-line narrowing methods.

Whether or not asymmetry exists between the Q_y absorption and emission spectra of the Chl *a* and Bchl *a* molecules was another unresolved question. The experimental data concerning this problem were not only scarce but also controversial.

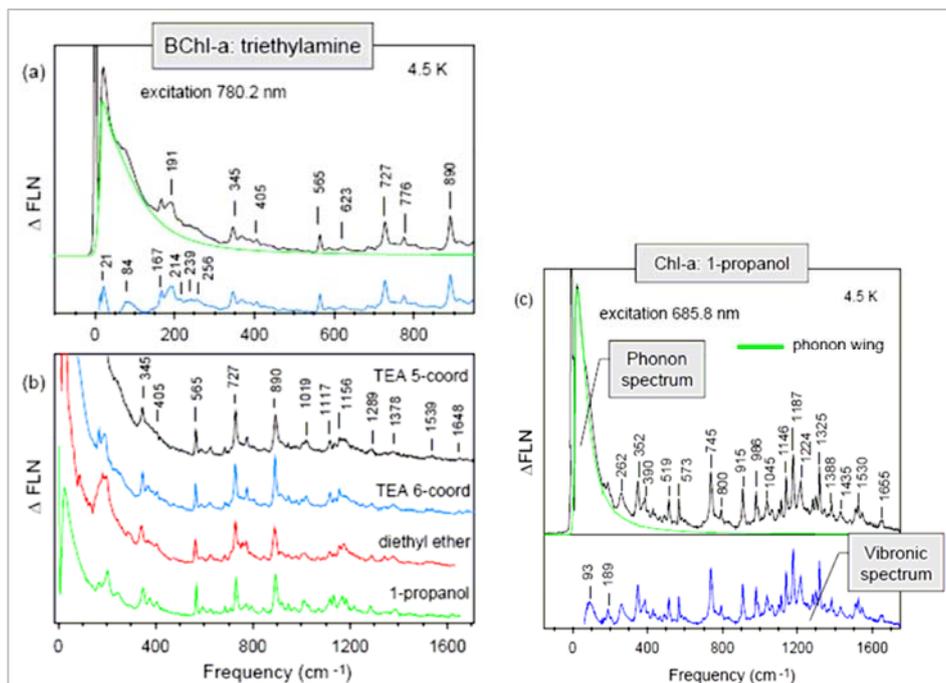


Figure 1.

Δ FLN spectra of BChl *a* in triethylamine (a) and Chl *a* in 1-propanol (c) at 4.5 K. The narrow ZPL at spectral origins (780.2 nm for BChl *a* and 685.8 nm for Chl *a*) is cut off at \sim 5% intensity level to amplify the sideband structure. The numbers indicate the main vibronic frequencies in wave numbers. The modeled phonon sidebands are shown with green curves. The lower blue curves are the difference between the black and green curves offset for clarity. (b) Vibronic region of the Δ FLN spectra of BChl *a* in various five fold- and six fold-coordinated matrices at 4.5 K.

Mirror symmetry between the conjugate absorption and emission spectra is theoretically expected within the model framework of the crude adiabatic, harmonic, linear electron-phonon and vibronic coupling, and Condon approximations (also known as the basic model (Rebane 1970)), together with an assumption of a fast excited state relaxation (the Kasha-Vavilov rule). While quantitative quantum chemical modelling of the absorption and emission spectra should reveal the nature of the electronic transitions and the origin of any spectral asymmetry, this is a challenging task for the chlorophyll type molecules owing to their rather large size and extended conjugation.

We have experimentally studied the deviations from mirror symmetry in optical absorption and fluorescence emission spectra of Chl *a* and Bchl *a* molecules using different solvents to induce either penta- or hexa-coordination of the central Mg atom (Rätsep et al. 2009a, 2011). Various semi-empirical, density-functional, and *ab initio* methods to find a reliable scheme for the *a priori* prediction of absorption and emission spectra of the (bacterio) chlorophylls have also been investigated. Based on the data obtained at different temperatures, one can safely conclude that mirror symmetry does not hold at any temperature between 4.5 K and ambient temperature neither in case of the Chl *a* nor Bchl *a* molecules. In the Bchl *a* emission, for example, the low-resolution vibronic sideband structure is centred on modes at $\sim 900\text{ cm}^{-1}$, while in absorption this band moves out to $1100\text{--}1200\text{ cm}^{-1}$. In environments with six fold-coordinated Mg, the emission sideband is considerably stronger, resulting in the corresponding reorganization energy λ_E slightly larger than the reorganization energy λ_A in absorption. In five fold coordinated environments, however, λ_A is 50% larger than λ_E . Most computational methods predict the spectral asymmetry to be very much larger than that actually observed; only the long-range corrected density functional CAM-B3LYP reproduced detailed experimental results for Bchl *a* and predicted asymmetry of the correct magnitude. These simulations also revealed that the spectral asymmetry arises from subtle consequences of the Duschinsky rotation on the high-frequency modes, allowing these vibrations to maintain their form on excitation and yet have very different normal-mode displacements through correctly phased minor contributions coming from a large number of low-frequency vibrational modes. In the case of the Chl *a* molecule, breakdown of the Condon approximation in inducing asymmetry appears to be significant. This work has thus established the first complete description of the Q_y absorption and fluorescence spectra of the (bacterio) chlorophyll molecules.

Inhomogeneous broadening of the optical spectra of impurity molecules in solids is a well-known phenomenon. The static spatial fluctuations of the environment can be formally looked at as a certain kind of a random noise that modulates the transition energies, i.e., the spectral positions of ZPL. Possible modifications of the homogenous shapes of the spectra, which are related to electron-phonon and/or vibronic couplings, are ignored in this approach. This turns out to be oversimplification. Fig. 2 demonstrates a significant increase of the linear electron-phonon coupling strength as a function of the excitation wavelength through the inhomogeneously broadened origin bands in the low-temperature

absorption spectra of the glassy samples of Chl *a* and Bchl *a* (Rätsep et al. 2009b; Renge et al. 2011). The linear electron-phonon coupling strength is associated with the displacement of the equilibrium positions of the nuclei upon a photoexcitation of the chromophore.

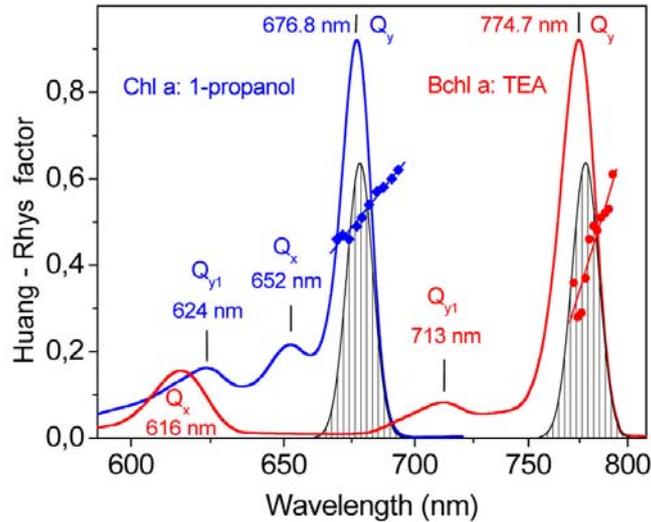


Figure 2.

Wavelength dependence of the electron-phonon coupling strength S for the Chl *a*-doped 1-propanol (blue rombs) and for the Bchl *a*-doped triethylamine (red rings) solutions at 4.5 K. The lines connecting the scattered data points present linear fits of the data. Plotted on the background with continuous coloured curves are the corresponding absorption spectra. The areas with drop lines denote inhomogeneous distribution functions of the ZPL related to the Q_y electronic transitions. A reciprocal (linear in energy) presentation of the wavelength scale is used. Q_y and Q_x refer to the two lowest-energy singlet electronic transitions; Q_{y1} indicates vibronic sidebands related to Q_y transitions.

Information about this parameter can be obtained from the so-called Huang-Rhys factor, S , which may be experimentally evaluated as: $\exp(-S) = I_{ZPL} / (I_{ZPL} + I_{PSB})$. Here, I_{ZPL} and I_{PSB} refer to the integrated intensities of the ZPL and the PSB, respectively. Physically, the Huang-Rhys factor is a measure of the average number of phonons that accompany a particular electronic transition. The observed quasi-linear increase of the Huang-Rhys factor with the excitation wavelength suggests a strong correlation between the electron-phonon coupling strength and the solvent shift. Such correlation has been rationalized within a two-particle Lennard-

Jones model of intermolecular interactions (Renge et al. 2011). This model also allows changes of the quadratic couplings, which show up in the widths of the ZPL. The latter prediction still waits to be proven experimentally.

QUANTUM COHERENCE SETS THE STAGE FOR EFFICIENT SOLAR ENERGY HARNESSING BY PHOTOSYNTHETIC ANTENNA NANOSTRUCTURES

Nanoscale systems, including molecular aggregates of biological origin, provide a transition between single molecules and bulk materials. A natural question then arises “Are their inner workings also intermediate to those of the individual molecules and bulk systems?” This fundamental query, although addressed in the past (Kasha 1963), attracted relatively little attention until the last decade. Since then, there has been an explosive wake of interest in small quantum systems due to the advent of refined experimental procedures with which to fabricate and study the molecular nanostructures of great potential technical importance. Notable examples include plastic electronics and photonics, organic solar cells, fluorescent (bio) markers, and artificial photosynthetic complexes. A well-founded general prospect is that in nanoscale the solid-state materials acquire molecular-like features, first of all discrete spectroscopic transitions. The aggregate formation is also often accompanied by rather spectacular changes in optical spectra whose origin is coherent excitation extending over many molecular sites – an exciton.

Since the inner biological machinery uniquely controls the composition and size of the biological nano-aggregates, they are ideally suited for investigations of the basic spectral characteristics of the excitons in nano-scale systems. Great variety of such aggregates are available that include from a few to ~100000 chromophores. Diversity of available genetic manipulation techniques only adds to the attractiveness of these samples. As an example, herein, we deal with a peripheral light-harvesting 2 (LH2) pigment-protein complex from purple photosynthetic bacteria. The high-resolution crystal structure of the LH2 complex from *Rhodospseudomonas (Rps.) acidophila* (strain 10050) is shown in Fig. 3 (McDermott et al. 1995). It reveals a cyclic array of nine transmembrane α , β – polypeptide pairs. Each polypeptide pair binds two Bchl *a* molecules at the outer membrane surface and one molecule on its intracytoplasmic side, forming two concentric molecular circles of nearly C_9 -symmetry. The inner ring of 6-7-nm diameter

consists of 18 strongly coupled Bchl *a* molecules, which feature intermolecular distances of less than 1 nm. The molecules in this ring give rise to the intense absorption band around 850-870 nm (B850 band) in the various species of purple bacteria. The other ring consists of 9 largely monomeric Bchl *a* molecules (separation > 2 nm) that absorb around 800 nm (B800 band).

While free in organic solvents, the Q_y transition of the Bchl *a* molecules is at ~775 nm (see Fig. 2). The related absorptions of the B800 and, especially, the B850 chromophores appear thus strongly red-shifted with respect to the transition of an individual molecule. This is because the spectral positions of the B850 and B800 bands are determined not only by the transition energies of the specific Bchl *a* sites but also by the dipolar exciton interactions between all the chromophores.

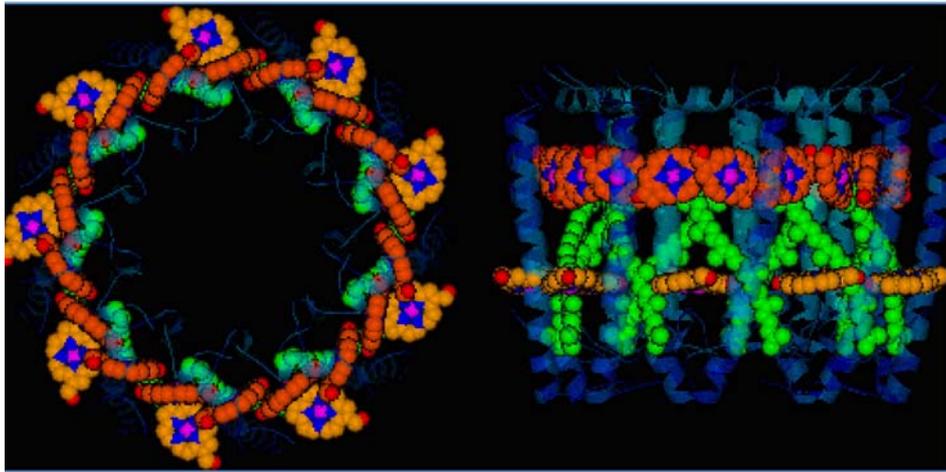


Figure 3.

Molecular structure of the LH2 light-harvesting complex from *Rps. acidophila* viewed top-down (left) and sidewise (right) with respect to the imaginable photosynthetic double-layer membrane. The red-colored Bchl *a* molecules of the B850 arrangement in the top circle of the sidewise view are squeezed between the two protein walls formed by the membrane-spanning α -helix ribbons. The top-down view reveals dimerization of the pigments in this ring. The Bchl *a* molecules of the B800 system located at opposite side of the membrane are shown in yellow. The green carotenoid molecules make close contact simultaneously with both the B850 and B800 chromophores. Here and on successive figures, only the porphyrin macrocycles of the Bchl *a* pigments are exposed for clarity. (Figure courtesy of R. Cogdell.)

The latter interactions sensitively depend on distances and mutual orientations of the molecules with respect to each other. The site energies of the chromophores can be fine-tuned by subtle interactions with the surrounding protein matrix. The main differences in the absorptions of the Bchl *a* molecules that are organized in the B800 ring and those assembled in the B850 ring stem from the strong (300-400 cm⁻¹, depending on the species (Freiberg, Trinkunas 2009; Pajusalu et al. 2011)) resonant coupling between the transition dipole moments of the closely packed B850 Bchl *a* molecules, which lead to the formation of the exciton states. For the B800 molecules the inter-chromophore interactions are much weaker, in the order of 20-30 cm⁻¹, and can be neglected in most applications. In the following, we will predominantly talk about the B850 transitions where the quantum coherence effects cannot be overlooked.

In the simplest approximation, disregarding the symmetry-lowering disorders of both static (inhomogeneous) and dynamic (homogeneous) origin, the excited-state manifold for the C₉-symmetric assembly of the 18 Bchl *a* sites features two non-degenerate (denoted as $k=0$ and $k=9$) and eight pairwise degenerate ($k=\pm 1, k=\pm 2, \dots, k=\pm 8$) exciton states (Fig. 4). Because of the dimeric sub-structure of the Bchl *a* molecular chain imposed by the surrounding protein scaffold, these exciton states are divided into two so-called Davydov sub-groups, 9 states in each sub-group (Davydov 1971). In solid-state theory, the splitting between the Davydov sub-bands is considered a measure of the exciton coupling strength. Due to the circular assembly of the sites only the exciton states $k=0, k=\pm 1, k=\pm 8$, and $k=9$ can be photo-excited. Since the transition-dipole moments of the individual B850 pigments are oriented mainly in the plane of the ring, just a small part of the total oscillator strength is associated with the non-degenerate exciton states $k=0$ and $k=9$. Given the head-to-tail arrangement of the transition dipole moments of the adjacent Bchl *a* molecules nearly all the oscillator strength is concentrated in the $k=\pm 1$ exciton states, being reflected in a strong electronic absorption band at about 850 nm. The upper exciton components, $k=\pm 8$, carry less than 3% of the total oscillator strength and give rise to very weak absorptions in the spectral range from 750 nm to 780 nm. Furthermore, in the systems with strong exciton coupling an additional contribution that determines the exact spectral position of the absorption band is given by the displacement energy (Davydov 1971).

The displacement energy accounts for the difference of the Coulomb interaction between neighbouring pigments in the electronically excited state and in the ground state.

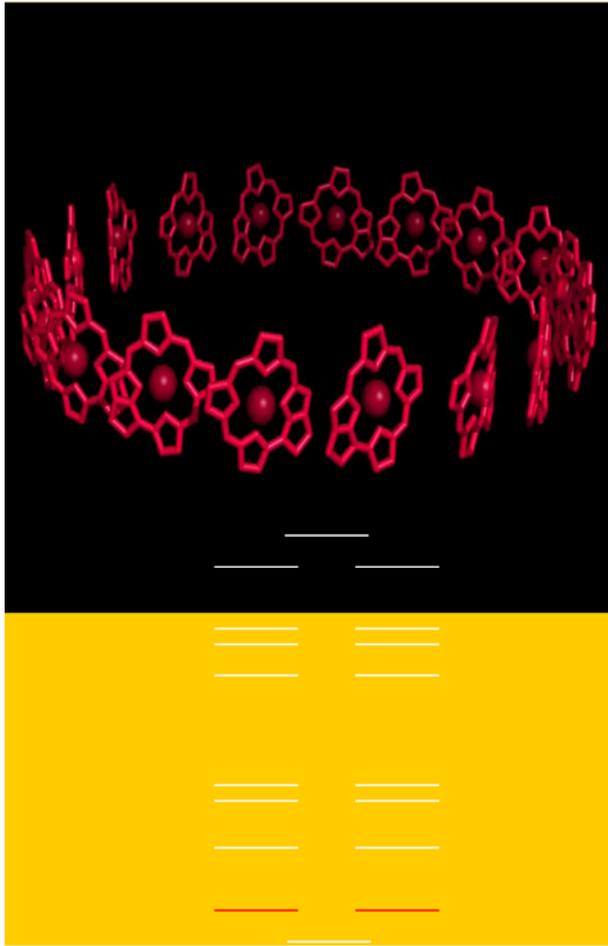


Figure 4.
Exciton level structure (white and red bars) for the 18 Bchl *a* pigments of the B850 arrangement shown on top. The two $k=\pm 1$ exciton states, which possess nearly all the oscillator strength for the transitions to the ground state are highlighted with red. (Figure courtesy of D.Rutkauskas.)

An *ab initio* estimation of the displacement energy for multi-chromophore systems is still a demanding theoretical task. From these considerations it follows that changes of the spectral position of the B800 band, in the first approximation, just reveal the interactions that involve site energies of the chromophores, whereas the origin of the B850 band spectral position change is more complex and reflects contributions from interactions that affect both the site energies and the coherent exciton interactions between the chromophores.

When suitably combined with the inevitable static energetic disorder, the coherent exciton theory briefly introduced above qualitatively explains all the major spectroscopic features of the LH2 light-harvesting complexes (Sundström et al. 1999; Van Amerongen et al. 2000; Cogdell et al. 2006). The only problem is that ‘the numbers don’t work’. Indeed, a detailed

comparison of the absorption and emission spectra of ensembles of the LH2 complexes measured at low temperature with the predictions of the established disordered Frenkel exciton model reveals several serious discrepancies (Freiberg et al. 1999; Timpmann et al. 2001; Freiberg, Trinkunas 2009).

PHOTOSYNTHETIC EXCITON POLARONS IN SOFT PROTEIN ENVIRONMENT

Quantum coherence is all about phase relationships. The idealized exciton states in deformable lattices interact with the local vibrations of the molecular units themselves as well as with the collective phonons of the surroundings, leading (like in individual molecules albeit more complex ways) to dissipation of excitations as well as to vibronic and electron-phonon spectral structures, respectively. The same interactions, which increase with temperature, also govern decoherence and transport dynamics of excitons. To envision the latter effects, one could picture a moving exciton dragging behind a cloud of phonons, an exciton polaron. The thicker the cloud, the slower moves the entity exciton plus cloud. In the limiting scenario the exciton may stop moving altogether localizing on a small region of the lattice. This phenomenon is called exciton self-trapping.

A strong ($S \geq 2$) exciton-phonon coupling in the fluorescence emission spectra of the B850 Bchl *a* aggregates has been discovered (Freiberg et al. 2003a, 2009; Timpmann et al. 2004b), several times greater than observed for monomeric chromophores in similar protein environment (Rätsep, Freiberg 2007). Visible consequences of such a strong interaction are broad multi-phonon sidebands, as demonstrated in Fig. 5. This is unexpected, since coupling for the exciton should be several times smaller than that for the respective localized excitation due to ‘motional reduction’ (Toyozawa 2003).

The Frenkel model as well as its various modifications such as the Redfield theory, which assume negligible exciton-lattice interactions cannot capture strong coupling effects. This is also the most obvious source of the above-noted inconsistencies between theory and experiment. A theoretical model involving dynamic exciton polaron formation in the spirit of Holstein, Heeger and others has, therefore, been developed by us to explain the conflicting observations in case of the LH2 complexes (Freiberg et al. 2003b; Trinkunas, Freiberg 2006; Freiberg, Trinkunas 2009; Pajusalu et al.

2011). The fundamental nature of this model is that due to strong exciton-phonon interaction a structural reorganization of the immediate environment is induced by the exciton, thereby lowering the exciton energy and causing its trapping. In one-dimensional electronic systems this kind of exciton self-trapping is taking place at any non-vanishing coupling (Sumi, Sumi 1994), being thus unavoidable in any real structure. The situation is rather different in higher dimensional organizations, where the free and self-trapped excitons are separated by an energetic barrier. Since the B850 ring can be considered with certain reservations a one-dimensional crystal made from the Bchl *a* molecules that strongly couple with their environment, exciton polarons rather than Frenkel excitons are generic photoexcitations of the LH2 antenna complex.

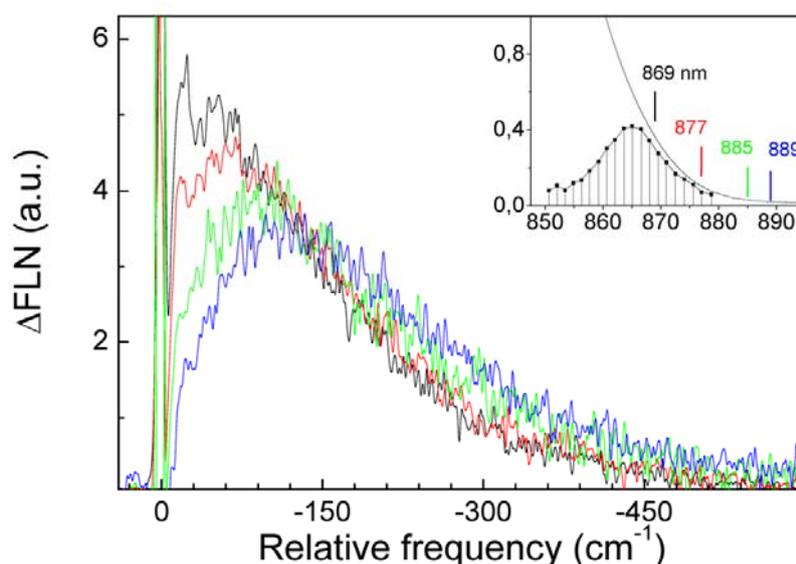


Figure 5.

Δ FLN spectra of isolated LH2 complexes from *Rhodospirillum rubrum* (*Rb.*) *sphaeroides* at 4.5 K. For better analysis of the phonon sideband changes, the spectra measured at different excitation wavelengths (as indicated with the color-coded vertical lines in the inset) are shifted together at the ZPL origin and normalized by the sideband area. The narrow ZPL are cut off at a few percent intensity levels. The inset shows the inhomogeneous distribution function of the lowest-energy exciton states (drop lines) and the long-wavelength tail of the absorption spectrum (solid line).

A configuration coordinate diagram (Fig. 6) illustrates this issue. Shown in the figure are the adiabatic potential energy surfaces that correspond to the ground state (GS, gray), the self-trapped exciton state (STE, green), and the three lowest ($k = 0, \pm 1$) light-absorbing Frenkel exciton states (Exciton, blue of different shades). The transitions between the ground and excited states are linearly coupled via two interaction coordinates. The horizontal Q_2 coordinate accounts for the diagonal (energetic) coupling, while the Q_1 , for the non-diagonal (structural) interaction. The straight and curly arrows represent the optical transitions and relaxation channels, respectively. The reorganization energy related to the diagonal exciton-phonon bath couplings in the LH2 complexes from *Rps. acidophila* is 285 cm^{-1} . The same parameter for the off-diagonal couplings is 73 cm^{-1} (Pajusalu et al. 2011).

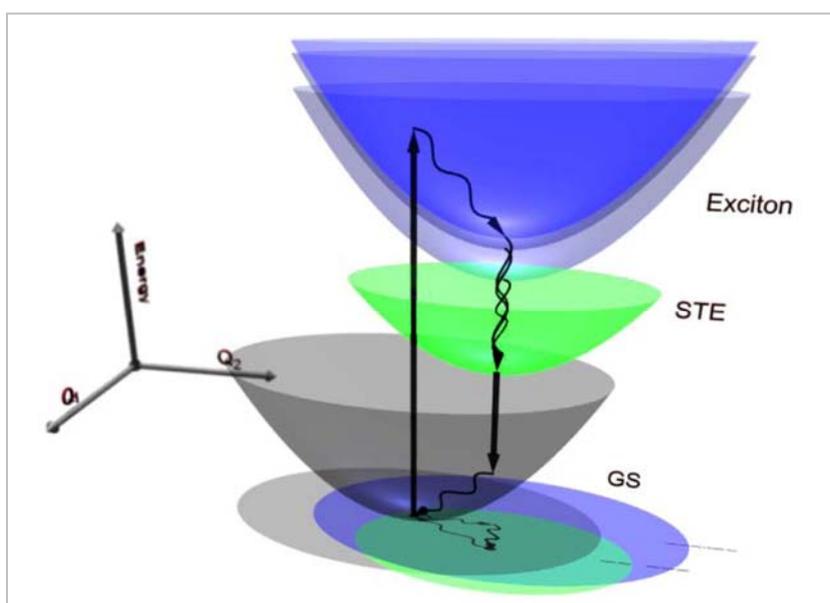


Figure 6.

The configuration coordinate model with two (off-diagonal Q_1 and diagonal Q_2) interaction coordinates for the excitons in the B850 aggregate of LH2. The colored surfaces counted from the bottom of the figure towards the growing potential energy (not in scale) correspond to the ground state (GS, gray), self-trapped exciton (STE, green), and Frenkel exciton states $k = 0$ and $k = \pm 1$ (Exciton, blue of different shades). Upward and downward bold arrows indicate the photon absorption and fluorescence emission transitions, respectively. The curly arrows designate relaxation channels.

Since just the ground state and the Frenkel exciton states from one side and the self-trapped exciton state and the ground state from another side are directly coupled by photon transitions, it should be clear from Fig. 6 that the absorption and emission spectra in such system deliver different information about the excited states of the matter. While the absorption spectra characterize the exciton immediately after its creation, the emission spectra describe the exciton behaviour immediately before its annihilation (Toyozawa 2003). This situation is qualitatively different from that for localized impurities in solids.

Fig. 7 demonstrates major effects of the polaronic coupling on the fluorescence spectral response of the LH2 antenna complexes. Compared in this figure at two temperatures are the simulated fluorescence emission spectra of LH2 complexes using either a Frenkel exciton model or the exciton polaron model. As one can see, the spectra according to the two models significantly deviate from each other at all temperatures both in terms of the fluorescence band position and its shape. The exciton polaron spectrum is generally broader as well as redder. These differences grow larger with temperature due to thermal occupation of the higher-energy states. Only the exciton polaron picture is in agreement with experiment (Pajusalu et al. 2011).

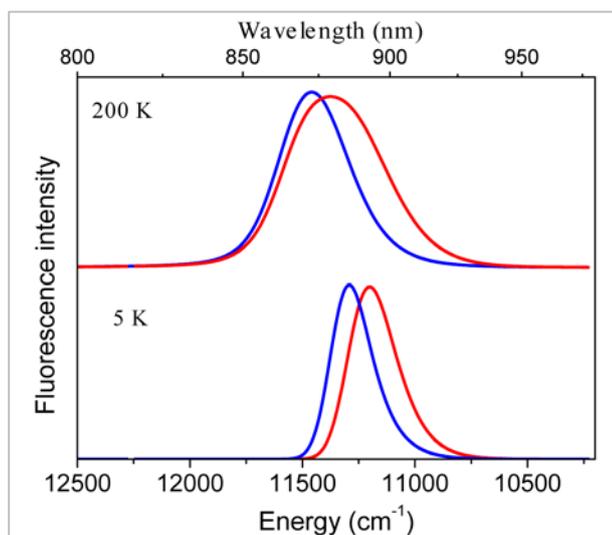


Figure 7. Simulated fluorescence emission spectra for LH2 complexes from *Rps. acidophila* according to Frenkel exciton (blue line) and exciton polaron (red line) models. The spectra calculated at two indicated temperatures are normalized by peak intensity. (Figure adapted from (Pajusalu, Rätsep et al. 2011).)

We have noted above (see Fig. 2) that the Huang-Rhys factors (consequently, the homogeneous spectral shapes) that correspond to the impurity centres of Bchl *a* depend on excitation energy. As can be seen in Fig. 5, similar behaviour is characteristic for the LH2 antenna exciton polarons as well (Timpmann et al. 2004b; Freiberg et al. 2009). With increasing excitation wavelength the peak of the phonon sideband in the emission spectrum shifts toward higher energies. The shift is accompanied with the sideband broadening and loss of its fine structure. All these changes point to progressively increasing exciton-phonon coupling toward lower-energy systems. Quantitative evaluation of the exciton-phonon coupling strength supports this conclusion; the Huang-Rhys factor grows from ~ 1.9 to ~ 3.0 between 869 and 889 nm. The excitations in the sub-group of the LH2 complexes that absorb high-energy light thus appear more exciton-like, while they are rather exciton polaron/self-trapped exciton-type when absorbing lower-energy light. In the recent single molecule experiments (paper under preparation) the fluorescence excitation and emission spectra from individual antenna complexes were measured. These experiments notably confirm the above insight.

PHOTOSYNTHETIC EXCITONS AT FUNCTIONAL TEMPERATURES

As already noted in Introduction, the exciton concept in photosynthesis heavily relies on spectroscopic measurements carried out at low temperatures. Except for circular dichroism data, which are sometimes demanding to interpret, there is not much experimental evidence for the relevance of excitons in functional photosynthesis that operates at ambient temperatures. Therefore, we shall next dwell on the following fundamental question “Do the excitons in the photosynthetic light-harvesting complexes survive functional temperatures?” The essence of this problem is that temperature by virtue of inducing noise into the system tends to smear out the firm relationships between the phases of the wavefunctions that correspond to photoexcitations on individual molecular sites of the light-harvesting complex. Broadly, it might be expected that at $k_B T \gg V$ (where $k_B T$ is the average thermal energy of environmental vibrations, and V is the nearest-neighbour exciton coupling energy) the phases of the site excitations may become randomised by thermal noise and the excitons cease to exist. More rigorously, however, V should be compared with the thermal energy

due to the coupling to the environment (reorganization energy λ) rather than with $k_B T$.

To check what really happens, one would like to investigate the bandwidth of the B850 excitons as a function of temperature over the whole range from liquid He to ambient temperatures. For that, it would be sufficient to measure energetic separation of the two light-absorbing exciton states ($k=\pm 1$ and $k=\pm 8$), which can be considered as markers of the exciton band edges. Yet the $k=\pm 8$ absorption is so small that it is totally covered by the much stronger absorption tail of the B800 molecules. This obstruction can be overcome by exploiting the polarized fluorescence excitation spectroscopy (Timpmann et al. 2004a; Trinkunas, Freiberg 2006). Since the transition dipole moments within the pairs $k=\pm 1$ and $k=\pm 8$ are mutually orthogonal, there exists an energy for each pair of states where they are excited with equal probability. The fluorescence anisotropy will then feature a minimum at those energies, effectively designating spectral positions of the respective pairs of the exciton states (Pajusalu et al. 2011). The fluorescence anisotropy, r , is defined as: $r = (I_{vv} - I_{vh}) / (I_{vv} + 2I_{vh})$, where I_{vv} and I_{vh} are the emission intensities that are polarized parallel (vv) and perpendicular (vh) with respect to the vertical orientation of the electric field vector of the linearly polarized excitation light.

The fluorescence anisotropy excitation spectra of the LH2 complexes from *Rps. acidophila* at the low (4.5 K) and high (263 K) temperature limits are shown in Fig. 8. At both temperatures the anisotropy is low at short wavelengths and rises across the B850 absorption band toward the theoretical limit of 0.4 at the long-wavelength boundary of the spectra. The most striking feature of the anisotropy curves, however, is the two distinct minima, one visible between 760 and 770 nm, another around 860 nm. The minima at high temperatures are similar albeit much shallower than at low temperatures. The dip wavelengths very well correlate with the expected positions of the $k = \pm 1$ and $k = \pm 8$ exciton states, thus marking the search for edges of the B850 exciton state manifold. The energetic separation, ΔE , of the dips is 1447 cm^{-1} at 4.5 K and 1259 cm^{-1} at 263 K. Increasing temperature from deep cryogenic to physiological temperatures thus results in only $\sim 13\%$ narrowing of the exciton bandwidth. Providing definitive evidence for survival of excitons at functional temperatures, the present data strongly suggest that the collective coherent electronic excitations may indeed play profound role in the functional photosynthetic light-harvesting process.

The single most obvious explanation for the observed robustness of light-harvesting excitons against temperature is the strong inter-molecular

coupling in the B850 ring. A straightforward estimate of the nearest-neighbour coupling energy from the exciton bandwidth yields $V \approx \Delta E/4 = 362 \text{ cm}^{-1}$, while a more elaborate theory (Pajusalu et al. 2011) returns $V = 374 \text{ cm}^{-1}$. These numbers are greater than $k_B T$ at ambient temperature amounting to $\sim 205 \text{ cm}^{-1}$. However, the reorganization energy, $\lambda = 358 \text{ cm}^{-1}$, is bigger and practically equals V . Proposals have been made that the protein scaffold is somehow special in protecting electronic state coherences. This is unlikely. A more sound idea came around only recently (Galve et al. 2010). According to this study the common relationship between V and λ is only valid if the system is in thermal equilibrium with its environment. If not, the temperature no longer provides the relevant energy scale against which to compare quantum behaviour of the system. This role is taken over by an effective temperature, which can be much lower than the absolute temperature. Similar magnitude of electronic and exciton-lattice couplings imply that exciton processes in light-harvesting antennas might indeed take place in non-equilibrium conditions, at least partially explaining the above conundrum.

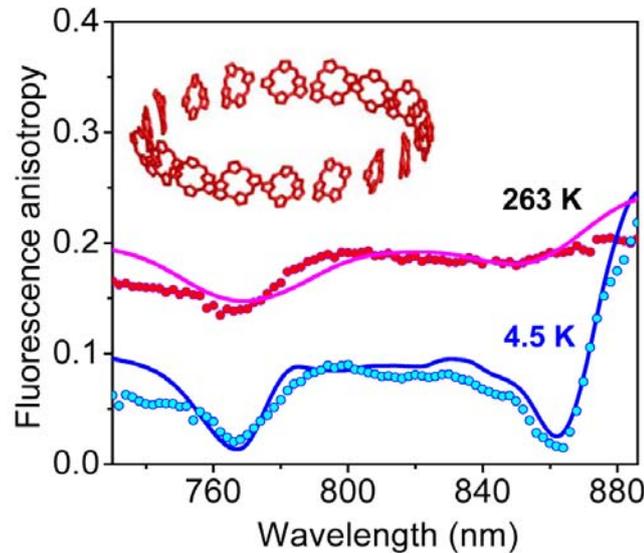


Figure 8.

Effect of temperature on the fluorescence anisotropy excitation spectra of the LH2 light-harvesting complexes from *Rps. acidophila*. Scattered dots represent experimental data at the two indicated temperatures, while their theoretical fits are drawn with continuous lines. The two curves are vertically shifted with respect to each other for better observation. The inset shows the B850 pigment ring supporting coherent excitons. (Figure adapted from (Pajusalu, Rätsep et al. 2011).)

Two groups have recently reported recording the quantum coherence in photosynthetic systems at ambient temperature, one group dealing with the bacterial LH complexes similar to those discussed in this work (Panitchayangkoon et al. 2010), another with the cryptophyte algae (Collini et al. 2010). Using femtosecond pulse laser excitation electronic coherences that lasted a few hundred femtoseconds were demonstrated, proving an old theoretical prediction (Nedbal, Szöcs 1986). It is still not clear, however, whether these oscillations are long enough to be useful for photosynthetic energy transfer, which predominantly takes place in picosecond range. Our experiments detect exciton eigenstates, which by definition are stationary states. This is a definitive distinction. Since steady-state exciton states are present, there must be no doubt that quantum coherence is involved with functional photosynthesis.

NEW LIGHT ON LIGHT-HARVESTING: COHERENT STATES IN NATIVE PHOTOSYNTHETIC MEMBRANES

Photosynthetic chromatophore vesicles of nearly spherical shape of 50-60 nm diameter found in some purple bacteria (Fig. 9) constitute one of the simplest light-harvesting membrane systems in nature. The overall architecture of the chromatophore vesicles and the vesicle function remain poorly understood despite structural information being available on individual constituent proteins. The knowledge about relative spatial arrangement of the LH and RC complexes has historically mainly obtained by spectroscopic methods, more recently by atomic force microscopy, and notably in these days computationally. An all-atom computational structure model for an entire chromatophore vesicle of *Rb. sphaeroides* was lately presented in (Sener et al. 2010). The model improved upon earlier models by taking into account the stoichiometry of core RC-LH1 and peripheral LH2 complexes in intact vesicles, as well as the curvature-inducing properties of the dimeric core complex. The absorption spectrum of low-light-adapted vesicles was shown to correspond to a LH2 to RC ratio of 3:1. Confirmed also by massive computations of exciton properties was the hitherto assumed principle that the success of photosynthesis depends on ultrafast events, in which up to hundreds of membrane proteins are cooperating in a multi-step process of solar excitation energy funnelling to the RC (Freiberg 1995).

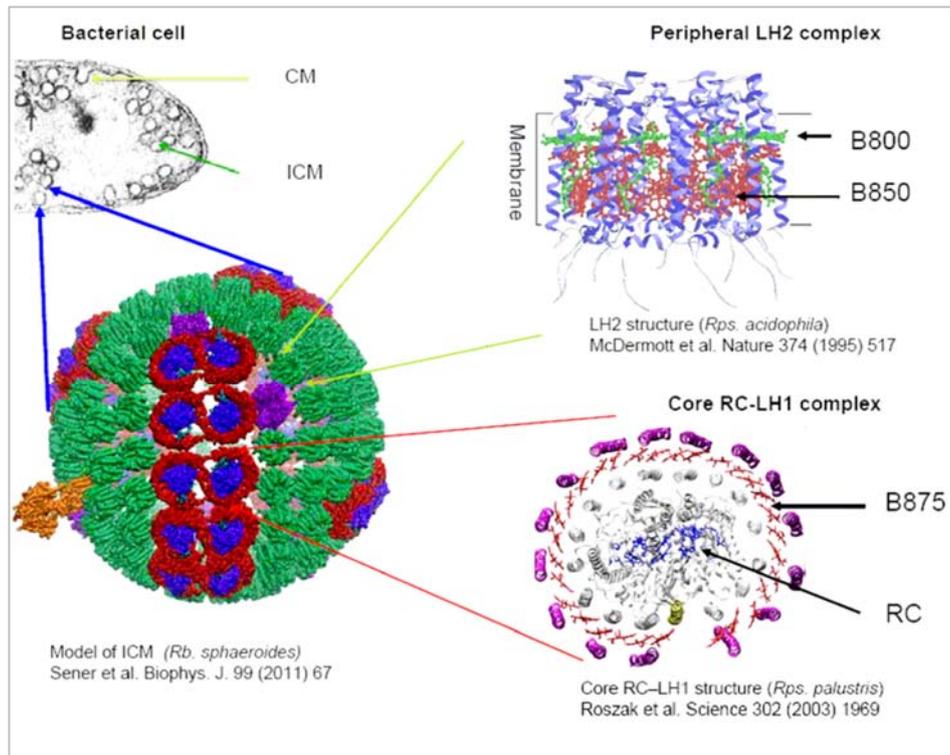


Figure 9.

In *Rb. sphaeroides*, the photosynthetic apparatus is organized into 50-60 nm diameter intracytoplasmic chromatophore vesicles (a spherical structure shown on the lower left corner), which are mainly populated by the peripheral LH2 light-harvesting complexes (drawn in green), along with a smaller number of core LH1 (red) and RC complexes (blue). The core antennas may appear in variable shapes, encircling either single (as in *Rps. palustris*) or double (wild type *Rb. sphaeroides*) RC. CM denotes the cytoplasmic membrane; ICM, the intracytoplasmic membrane; B875, the assembly of Bchl *a* molecules in LH1.

Quantum coherence requires great deal of order to manifest measurably. The biological membranes carrying photosynthesis machinery, on contrary, are highly disordered and dynamic structures. It wouldn't be too surprising if the coherent quantum effects apparent in individual light-harvesting complexes do not materialize in the native membranes. Therefore, we next assess, again using fluorescence anisotropy excitation

spectroscopy, to what extent the coherent exciton concept holds in fully developed photosynthetic membranes.

Fig. 10 compares the anisotropy excitation spectrum of isolated LH2 complexes with this for the native membrane of *Rps. acidophila* complete with LH and RC complexes. In order to disentangle individual contributions to the anisotropy from the LH2 and LH1 complexes, the emission were detected either around 890-910 nm (the predominant emission range of LH2) or 920-950 nm (LH1). Thereby, clear signals corresponding to the exciton band edges of the LH2 and LH1 complexes have been observed.

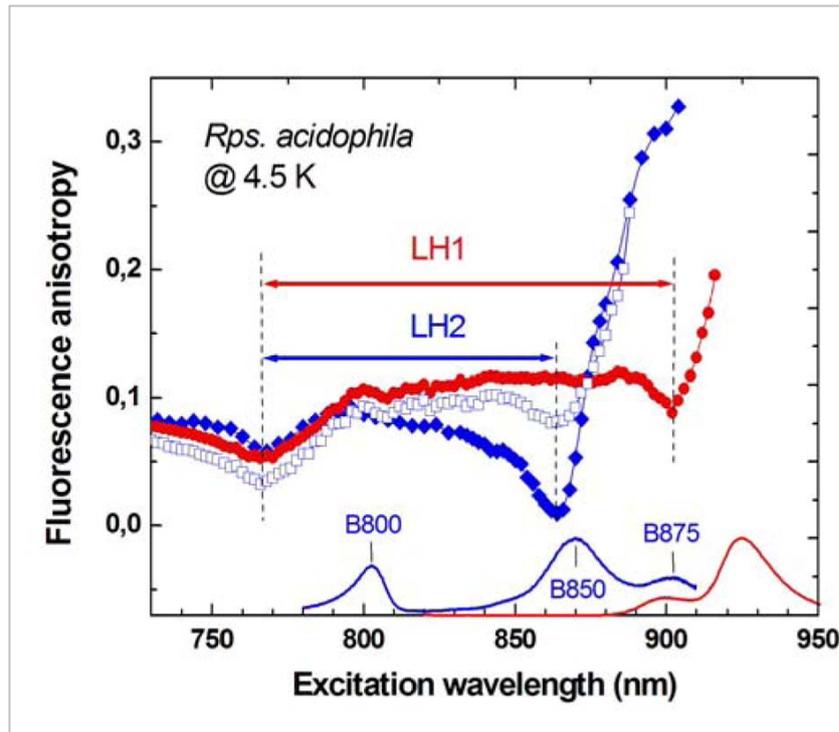


Figure 10.

Fluorescence anisotropy excitation spectra for native membranes (red dots and open blue squares) and isolated LH2 complexes (filled blue diamonds) from *Rps. acidophila* at 4.5 K. The reference fluorescence emission and fluorescence excitation spectra shown at the bottom of the figure are drawn with red and blue lines, correspondingly. The two peaks of the fluorescence spectrum at ~900 and ~925 nm relate, respectively, to the B850 and B875 bands in the excitation spectrum. The double arrows mark the bandwidths for the B850 and B875 exciton manifolds.

Most remarkably, however, exciton bandwidths of the LH2 complexes in membranes and in detergent-isolated complexes appear the same, meaning that excitons in these two rather different environments are basically identical. This is very good news for protein crystallographers, indicating that the crystals of detergent-isolated membrane proteins may indeed represent native membrane conformation and provide relevant structural information. Notable also is that the high-energy limits of exciton state manifolds in the LH1 and LH2 antennas are tuned together, so that the broader LH1 exciton band ($\Delta E = 1951 \text{ cm}^{-1}$) totally engulfs the narrower LH2 band. Similar situation is observed in the membranes of *Rb. sphaeroides*. The perfect overlap of the high-energy exciton states of the peripheral and core antennas must be advantageous for efficient tunneling of the excitation energy toward the RC. This is a wonderful example of optimization of solar light harnessing by natural selection!

WHAT IS THE ANSWER?

The single essential quality that distinguishes quantum theory from classical mechanics is the phase of the wavefunction. The validation of stationary coherent photosynthetic excitations at ambient temperatures is yet another piece of evidence that helps to bridge the gap between quantum physics and biology. Other more or less established examples are vision, bioluminescence, tunneling of electrons and protons in enzymatic reactions, magnetoreception of birds and animals, and weak van der Waals interactions between the molecules. The ubiquity of the quantum biological phenomena appears to suggest that biological systems might exploit the wave properties of matter for optimization of their functionality. This hint should be taken seriously in expectation of a future society powered mostly by solar energy.

Do we indeed live in the quantum world? This question dates back to the influential work of Schrödinger, one of the founders of quantum mechanics, for more than half a century ago (Schrödinger 1944). Discussion goes on (Davydov 1982; Abbott et al. 2008) but there is little doubt that at the end this as well as the title question should be answered affirmatively. This is not to say that all problems have been solved. The major challenge still is to understand how our everyday classical world emerges from the counterintuitive realm of quantum mechanics (Ball 2008). A non-trivial revelation of the recent studies is that the quantum-classical transition is not

so much a matter of size, but of time. The quantum laws manage equally well the phenomena at the scales of both the very small such as atoms and molecules and the very large such as black holes and, according to modern cosmology (Carroll 2011), even the whole universe.

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REFERENCES

- Abbott, D., C. W., et al. (eds.) 2008. *Quantum Aspects of Life*. Imperial College Press, London.
- Avarmaa, R. A., Rebane, K. K. 1985. High-resolution optical spectra of chlorophyll molecules. *Spectrochimica Acta A*, 41, 1365-1380.
- Ball, P. 2008. Quantum all the way. *Nature*, 453, 22-25.
- Blankenship, R. E. 2002. *Molecular Mechanisms of Photosynthesis*. Blackwell Science, Oxford.
- Carroll, S. 2011. *From Eternity to Here*. Oneworld Publications, Oxford.
- Cogdell, R. J., et al. 2006. The architecture and function of the light-harvesting apparatus of purple bacteria: from single molecules to in vivo membranes. *Quarterly Review of Biophysics*, 39, 227-324.
- Collini, E., et al. 2010. Coherently wired light-harvesting in photosynthetic marine algae at ambient temperature. *Nature*, 463, 644-648.
- Davydov, A. S. 1971. *Theory of Molecular Excitons*. Plenum Press, New York.
- Davydov, A. S. 1982. *Biology and Quantum Mechanics*. Pergamon, New York.
- Freiberg, A. 1995. Coupling of antennas to reaction centres. Blankenship, R. E., Madigan, M. T., Bauer, C. E. (eds.) *Anoxygenic Photosynthetic Bacteria*. Kluwer Academic Publishers, Dordrecht, 2, 385-398.
- Freiberg, A., et al. 1999. Disordered exciton analysis of linear and nonlinear absorption spectra of antenna bacteriochlorophyll aggregates: LH2-only

- mutant chromatophores of *Rhodobacter sphaeroides* at 8 K under spectrally selective excitation. *Journal of Physical Chemistry B*, 103, 45, 10032-10041.
- Freiberg, A., et al. 2003a. Self-trapped excitons in circular bacteriochlorophyll antenna complexes. *Journal of Luminescence*, 102-103, 363-368.
- Freiberg, A., et al. 2003b. Self-trapped excitons in LH2 antenna complexes between 5 K and ambient temperature. *Journal of Physical Chemistry B*, 107, 11510-11519.
- Freiberg, A., et al. 2009. Excitonic polarons in quasi-one-dimensional LH1 and LH2 bacteriochlorophyll a antenna aggregates from photosynthetic bacteria: A wavelength-dependent selective spectroscopy study. *Chemical Physics*, 357, 102-112.
- Freiberg, A., Trinkunas, G. 2009. Unraveling the hidden nature of antenna excitations. Laisk, A., Nedbal, L., Govindjee (eds.) *Photosynthesis in Silico. Understanding Complexity From Molecules to Ecosystems*. Springer, Heidelberg, 55-82.
- Galve, F., et al. 2010. Bringing entanglement to the high temperature limit. *Physical Review Letters*, 105, 180501.
- Kasha, M. 1963. Energy transfer mechanisms and the molecular exciton model for molecular aggregates. *Radiation Research*, 20, 55-71.
- McDermott, G., S. et al. 1995. Crystal structure of an integral membrane light-harvesting complex from photosynthetic bacteria. *Nature*, 374, 6522, 517-521.
- Nedbal, L., Szöcs, V. 1986. How long does excitonic motion in the photosynthetic unit remain coherent? *Journal of Theoretical Biology*, 120, 411-418.
- Pajusalu, M., et al. 2011. Davydov splitting of excitons in cyclic bacteriochlorophyll a nanoaggregates of bacterial light-harvesting complexes between 4.5 and 263 K. *European Journal of Chemical Physics and Physical Chemistry*, 12, 634-644.
- Panitchayangkoon, G., et al. 2010. Long-lived quantum coherence in photosynthetic complexes at physiological temperature. *Proceedings of the National Academy of Sciences of the USA*, 107, 12766-12770.
- Rebane, K. K. 1970. *Impurity Spectra of Solids*. Plenum Press, New York.

- Renge, I., et al. 2011. Intermolecular repulsive-dispersive potentials explain properties of impurity spectra in soft solids. *Journal of Luminescence*, 131, 262-265.
- Rätsep, M., et al. 2009a. Mirror symmetry and vibrational structure in optical spectra of chlorophyll a. *Journal of Chemical Physics*, 130, 194501.
- Rätsep, M., et al. 2009b. Wavelength-dependent electron-phonon coupling in impurity glasses. *Chemical Physics Letters*, 479, 140-143.
- Rätsep, M., et al. 2011. Demonstration and interpretation of significant asymmetry in the low-resolution and high-resolution Qy fluorescence and absorption spectra of bacteriochlorophyll a. *Journal of Chemical Physics*, 134, 024506.
- Rätsep, M., Freiberg, A. 2003. Resonant emission from the B870 exciton state and electron-phonon coupling in the LH2 antenna chromoprotein. *Chemical Physics Letters*, 377, 371-376.
- Rätsep, M., Freiberg, A. 2007. Electron-phonon and vibronic couplings in the FMO bacteriochlorophyll a antenna complex studied by difference fluorescence line narrowing. *Journal of Luminescence*, 127, 251-259.
- Schrödinger, E. 1944. *What is Life?* Cambridge University Press, Cambridge.
- Sener, M., et al. 2010. Photosynthetic vesicle architecture and constraints on efficient energy transfer. *Biophysical Journal*, 99, 67-75.
- Sumi, H., Sumi, A. 1994. Dimensionality dependence in self-trapping of excitons. *Journal of the Physical Society of Japan*, 63, 637-657.
- Sundström, V., et al. 1999. Photosynthetic light-harvesting: Reconciling dynamics and structure of purple bacterial LH2 reveals function of photosynthetic unit. *The Journal of Physical Chemistry B*, 103, 13, 2327-2346.
- Timpmann, K., et al. 2001. Exciton self-trapping in one-dimensional photosynthetic antennas. *The Journal of Physical Chemistry B*, 105, 12223-12225.
- Timpmann, K., et al. 2004a. Bandwidth of excitons in LH2 bacterial antenna chromoproteins. *Chemical Physical Letters*, 398, 384-388.
- Timpmann, K., et al. 2004b. Emitting excitonic polaron states in core LH1 and peripheral LH2 bacterial light-harvesting complexes. *The Journal of Physical Chemistry B*, 108, 10581-10588.
- Toyozawa, Y. 2003. *Optical Processes in Solids*. Cambridge University Press, Cambridge.

Trinkunas, G., Freiberg, A. 2006. A disordered polaron model for polarized fluorescence excitation spectra of LH1 and LH2 bacteriochlorophyll antenna aggregates. *Journal of Luminescence*, 119-120, 105-110.

Van Amerongen, H., et al. 2000. *Photosynthetic Excitons*. World Scientific, Singapore.

Zazubovich, V., et al. 2001. Bacteriochlorophyll a Frank-Condon factors for the S₀ S₁(Q_y) transition. *The Journal of Physical Chemistry B*, 105, 12410-12417.

Zewail, A. H. (ed.) 2008. *Physical Biology. From Atoms to Medicine*. Imperial College Press, Singapore.