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Biocomplexity Faculty Search Committee
c/o Prof. Rob de Ruyter van Steveninck
Biocomplexity Institute
Indiana University
Swain Hall West 117
Bloomington IN, 47405-7105

Dear Professor de Ruyter van Steveninck, dear Search Committee Members,

I am writing to apply for the Assistant Professor position in the Biocomplexity Institute, as advertised on the Institute web site, with the primary focus on the area of Biophysics. Currently I am the Assistant Scientist II at the Ames Laboratory (USDOE) and Iowa State University, in the group of Gerald J. Small, Distinguished Professor of physical chemistry (deceased) and Professor Ryszard Jankowiak, working in the field of optical spectroscopy of photosynthetic pigment-protein complexes. My Ph.D. is in solid-state physics and was obtained in 1999 from the University of Tartu, Estonia, under the supervision of Professor Jaak Kikas; my graduate research was devoted to optical spectroscopy of impurity centers in glasses and molecular crystals, including one incommensurate system, biphenyl.

I am particularly interested in a faculty position that would allow me to continue my research on photosynthetic complexes. The issues I am planning to address include details of energy transfer within and between the complexes and primary charge separation, with the ultimate goals being a) to determine the relationship between the organization of the pigment network (embedded into protein environment) and its function and to uncover the reasons for extreme effectiveness of energy conversion achieved by Nature in photosynthesis and b) to develop molecular electronics devices incorporating photosynthetic complexes. Such a research, I believe, can complement current scientific efforts of the Biocomplexity Institute. As mentioned in my statement of research interests, photosynthesis research, in addition to being an interesting field by itself, has a large potential from the viewpoint of developing highly efficient photovoltaic devices mimicking or incorporating natural photosynthetic complexes. Photosynthetic complexes may be also integrated into herbicide/pesticide sensors. On the other hand,

the methodology of high-resolution optical spectroscopy, especially single entity spectroscopy, can be successfully applied to a broad range of problems in biophysics and condensed matter physics.

As a physicist working among chemists on biology-related problems, I developed the ability to collaborate successfully with researchers having various educational backgrounds. Within the five years I have been working at the ISU / Ames Laboratory, I have grown into the key experimentalist of the photosynthesis project of our group. I was involved in setup design, experiments, data processing, computer simulations and manuscript writing; as well as in finding new directions of research, writing proposals, establishing collaborations and supervising the work of graduate students. The experience I gained working with Professor Small and his colleagues and collaborators as well as with groups of Professors Kikas (University of Tartu) and Friedrich (TU Munich) will allow me to develop an internationally competitive research program.

As I have progressed through my scientific career, I have grown more and more interested in teaching. My experiences with various teaching styles as a student, supervising and training students as a postdoctoral associate, presenting the results of my work at various conferences and seminars, as well as giving private lessons, provided a lot of chances to become acquainted with and to think through different aspects of teaching and facilitating students' learning. Participating in the ISU Preparing Future Faculty program allowed me to gain additional valuable experiences, which definitely will help me to succeed within the American educational system. Due to my background, I would be most interested in and most comfortable with teaching optics, spectroscopy, or condensed matter physics, as well as some special course on photosynthesis.

It is probably important to mention that I am a citizen of Estonia, currently working in the United States in the H1B status. That status can be extended until May of 2009.

Enclosed is my CV including the list of publications and the names and addresses of distinguished scientists who will provide the letters of recommendation, as well as the description of my research and teaching interests and plans. If you have any questions or wish to obtain any additional information (reprints, transcripts, etc), please feel free to contact me. You can also visit my web page (www.fi.tartu.ee/~valter), which contains interactive version of my CV, all but one of my publications in pdf format, as well as some personal information. Thank you for your consideration.

With best regards,

Valter Zazubovich

Teaching philosophy and interests.

I deeply believe that primary responsibility of a teacher is to create an environment which is both challenging and supportive and which facilitates learning in the broader meaning of that word. The latter includes not just getting acquainted with a certain set of facts and acquiring hands-on experience directly related to the subject taught, but also acquiring the learning skills and the taste for learning, as well as the capacity for critical thinking and intellectual bravery. In order to achieve such an ambitious goal, it is important to encourage students to be the participants in the learning process, rather than just the recipients of knowledge.

Expanding on the general statements above, I would like to achieve the balance between the following aspects of teaching:

- Providing sufficient information on the subjects I will teach, taking into account that it will become the necessary part of the foundation for future learning. It also will help students to make informed decisions about their future education and career plans. That information includes both the fundamental "textbook" facts and the information about the recent developments in the field. I believe that in order to be aware of those recent developments, the teacher must be an active researcher as well.
- Allowing sufficient time for discussions and actively encouraging them, since that would facilitate development of students' critical and analytical skills. My goal is to help students to feel reasonably comfortable questioning teacher's opinion as well as the opinions of other authorities in the field.
- Being sufficiently sensitive and accommodating to varying needs of different personalities and learning styles. That includes providing variety of resources, including lecture outlines, handouts, worksheets, as well as homework assignments that supplement lectures and labs. The Internet offers an ideal opportunity for making these resources available on a 24/7 basis. I also believe that getting experiences with various experimental techniques (via labs and the experimental presentations by the teacher during lecture time) is extremely useful for students and I plan to devote significant attention to this aspect of teaching.
- Constantly learning from my own experiences with teaching as well as from experiences of my fellow teachers.

The successful teacher is responsible for setting reasonable standards of achievement and fairly evaluating the progress of the students according to these standards. Thus, it is very important to determine which standards are not subject to compromise and which could be somewhat altered in order to make the learning experience more pleasurable for students. Although I expect students to exhibit interest, motivation and willingness to work, I consider it a teacher's responsibility to support and nurture these attitudes throughout the learning process.

Given my background, I would prefer to teach Optics, Spectroscopy, or Condensed Matter Physics as well as some special course on photosynthesis.

High-resolution Frequency-domain Optical Spectroscopy of Photosynthetic Pigment-protein Complexes: Exploring the Relationship between Organization and Function of Chlorophyll Networks.

Contents:

Summary:

Excited state electronic structure of chlorophyll-protein photosynthetic complexes and initial steps of photosynthesis, including excitation energy transfer (EET) within and between the complexes and electron transfer processes will be studied combining **Spectral Hole Burning (SHB)** [1,2] and **Single Molecule / Complex Spectroscopy (SMS)** [3]. This is a powerful frequency-domain approach promising to improve understanding the primary events of photosynthesis and the ways in which Nature achieved remarkable, almost perfect effectiveness of energy funneling and transformation. In particular, research will be initially focused on the following systems: a) **Peripheral antenna complexes Lhca1...4 of Photosystem I of higher plants** [4]. The goals include exploring properties of the lowest-energy states, matching various chlorophyll molecules or groups of molecules with their spectral signatures (i.e. experimental determination of Chl excitation energies and inter-pigment couplings) as well as finding out if there are mixed Chl *a* / Chl *b* sites in the Lhca complexes. b) Different photosynthetic complexes extracted from the strains of recently discovered cyanobacterium ***Prochlorococcus marinus*** adapted to different illumination levels (depths) by means of varying their Chl a_2 to Chl b_2 content ratio [5]. Of particular interest will be to find out if and how the adaptive variations in the above ratio affect EET pathways and rates, as well as the CS rates. Research will also focus on finding the specific sites within the complexes where natural substitution of Chl a_2 by Chl b_2 occurs. c) **Photosystem I of various cyanobacteria** not containing Chl *b* or its derivatives. Key issues include finding the correspondence between various chlorophyll molecules known from structure data and their spectral bands, not only in the red antenna state region (>700 nm) but also at higher energies, as well as determining which hole burning mechanism is actually responsible for non-resonant and, probably, also resonant hole-burning for states at >680 nm [6,7]. In case the photochemical nature of this non-resonant hole-burning mechanism will be proven, I intend to look for the evidence of similar mechanisms in other complexes. The possibility of reversible photochemical processes in pigments not belonging to the electron transfer chain is unexplored so far.

Additionally, I am planning to apply my expertise with photosynthesis research to development of **photovoltaic devices incorporating intact natural photosynthetic complexes**. The main problems to be solved are a) creating monolayers of densely packed oriented photosynthetic complexes; b) determination of factors influencing stability of the complexes incorporated into photovoltaic devices; c) finding external donors, acceptors and linkers for minimization of electron transfer times to and from photosynthetic complexes; and d) development of multi-layer architectures for more efficient light-harvesting. Spectroscopic studies of samples with uniform orientation of photosynthetic complexes may prove very useful also from the viewpoint of basic science. Polarization-sensitive measurements on oriented bulk samples may allow resolving the bands overlapping in the samples without preferential complex orientation, which would facilitate understanding of the relationship between structure and function in photosynthetic complexes. In addition to orienting complexes on the surfaces, orientation in/by liquid crystals will be employed.

Introduction: Significance of photosynthesis research and methods of high-resolution frequency-domain optical spectroscopy.

It is hard to overestimate the importance of photosynthesis for sustaining life on Earth. In photosynthesis light energy is utilized to drive the reactions producing complex organic substances needed for functioning of plants and bacteria, with molecular oxygen as a by-product. These complex substances serve as building blocks and energy source for plants and bacteria themselves, as well as for higher organisms. Natural hydrocarbon fuels, still the main source of energy for the mankind, also originate from the processes of photosynthesis, which occurred millions of years ago. The excess of carbon dioxide released into the atmosphere is believed to cause global warming and related effects. Thus, carbon fixation, performed by photosynthetic organisms, has important implications for the dynamics of the climate changes.

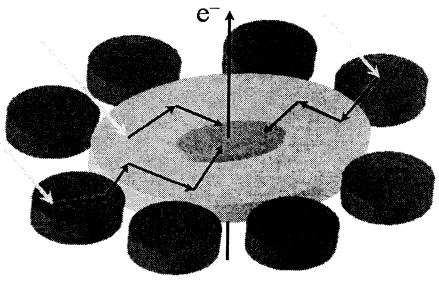

Figure 1:Schematic representation of energy conversion in photosynthetic unit. Light energy is absorbed by antenna pigments and funneled into the reaction center, where the primary charge separation takes place; eventually electron is transported through the thylakoid membrane. Green: peripheral antenna complexes; yellow: core antenna; orange: reaction center.

Recently much effort has been invested into research on nanoscale. Splitting water with sunlight for hydrogen production and high efficiency harvesting of solar energy are just a few examples of proposed applications of nanostructures [8]. Note that the processes of light harvesting and energy conversion in photosynthetic complexes are essentially nanoscale processes, which may either be integrated with solid-state devices [9] or mimicked by totally artificial systems in order to produce efficient photovoltaic devices. Photosystems may also be employed in herbicide sensors [10]. It makes perfect sense to uncover the basic physical principles of photosynthesis and apply them to the development of nanoscale technologies, especially taking into account that the effectiveness of energy funneling and transformation achieved by Nature in photosynthetic complexes has yet to be matched by that of artificial devices.

Since the methods of optical spectroscopy focus on interactions of photosynthetic complexes with light, they offer the most straightforward way to obtain relevant information. **Spectral Hole Burning (SHB)** involves spectral selection of molecules from the inhomogeneously broadened bands by narrow laser and inducing either photo-transformation of the molecule itself or the rearrangement of molecule's environment. It has been used successfully by the group of Professor G. J. Small at ISU (as well as the group of Professor S. Völker in Leiden and some other groups) for studying excited state electronic structure, excitation energy transfer and electron transfer dynamics in photosynthetic complexes at low temperatures. (Since zero-phonon line (ZPL) widths are

temperature-dependent, low (liquid helium) temperatures will be used to achieve the highest spectral resolution.) It is important to note that the behavior of the protein environment of chlorophyll molecules in photosynthetic complexes is very similar to that of amorphous solids. From the viewpoint of a spectroscopist, this has two important consequences. First, optical spectra of photosynthetic complexes exhibit notable inhomogeneous broadening and, second, the spectra are subject to non-photochemical spectral hole burning (NPSHB) [11], meaning that the resonant decrease in the absorption spectrum (spectral hole) is due to slight rearrangement of the local environment of the pigment, rather than to any photo-transformation of the chlorophyll molecule itself, i.e. the functionality of the complex remains preserved.

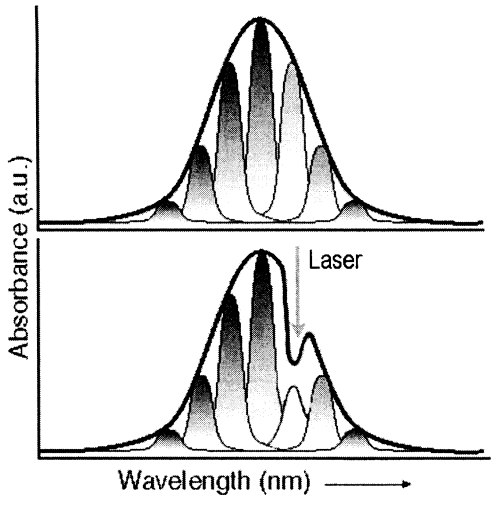

Figure 2: Schematic representation of inhomogeneous broadening and spectral hole burning

As mentioned above, the information about the rates of EET, charge separation or pure dephasing is contained in the widths of ZPH (or ZPL in case of SMS) and their temperature dependences. Information on electron-phonon coupling can be obtained from the shape of the spectra, including ZPH (or ZPL) and phonon sideband. Satellite hole structures can yield information either on vibration dynamics or on the energy transfer pathways. Delocalized exciton states originating from pigment molecules with strong electron exchange contribution to the coupling can be identified by spectroscopic measurements on samples under pressure or in electric field. Such states are characterized by large pressure-induced shifts of ZPH, large difference in permanent dipole moment in ground and excited states and large electron-phonon coupling.

Note that I propose to use a combination of techniques, which is rarely applied by a single group. These techniques are complementary to each other: while SHB is technically simpler (simple enough for last year undergraduate students) but provides ensemble (average) data, **Single Complex Spectroscopy (SMS)** (See [12] for a recent review on the spectroscopy of single photosynthetic complexes.) is more difficult but allows investigation of the complexes one by one. This can yield distributions of various parameters of interest, which contain more information on static and dynamic properties of the system being investigated than just the average values do. (Every attempt will be made to compare and correlate data obtained with SHB and SMS.) Proposed research is outstanding also because SHB and SMS will be combined with application of electric field and hydrostatic pressure. These external fields can be used to fine-tune the inter-pigment and pigment-protein interactions within photosynthetic complexes. "Bulk" and single complex spectroscopy on the arrays of uniformly oriented photosynthetic

complexes promise to facilitate development of novel photovoltaic devices and herbicide sensors. Additionally, they also promise to yield more precise fundamental understanding of primary events in photosynthesis, since the effects due to random orientation of complexes typically observed in spectra of bulk samples should be suppressed. Finally, I would like to note that frequency-domain techniques I intend to use are complimentary to time-domain techniques such as pump-probe spectroscopy and time-resolved fluorescence measurements, and often are the preferred way to go, especially when high spectral resolution is required.

Specific targets:

1. Lhca1...4 peripheral antenna complexes of Photosystem I of higher plants.

Relevant preliminary knowledge:

Lhca1...4 subunits comprise the outer antenna (LHC-I) of the PS-I of higher plants. Eight Lhca polypeptides (most likely organized in dimers) are located at one side of the PS-I core in the PS-I/LHC-I complex. Their main function is to capture and deliver energy to the photochemically active part of the PS-I. The structures of these peripheral subunits recently became available [13]. According to biochemical data [4 and refs. therein] Lhca1...4 complexes bind 10 Chls *a* and *b* and 2-3 carotenoids each, with Chl *a/b* ratio depending on complex, while according to crystallographic data [13] there are 14-15 Chls per polypeptide. Of particular interest are the properties of the lowest-energy states of Lhca1...4 complexes. In Lhca-3 and -4 the lowest states absorb lower in energy than the reaction center does and in all the Lhca-type complexes the lowest states exhibit strong electron-phonon coupling (see Figure 3). It is believed that these lowest states belong to strongly coupled chlorophyll dimers. It is also believed that the remaining chlorophylls are weakly coupled to each other. Therefore, corresponding electronic states are highly localized. The energy transfer between most of those weakly coupled chlorophylls occurs on a timescales from 0.5 to several ps

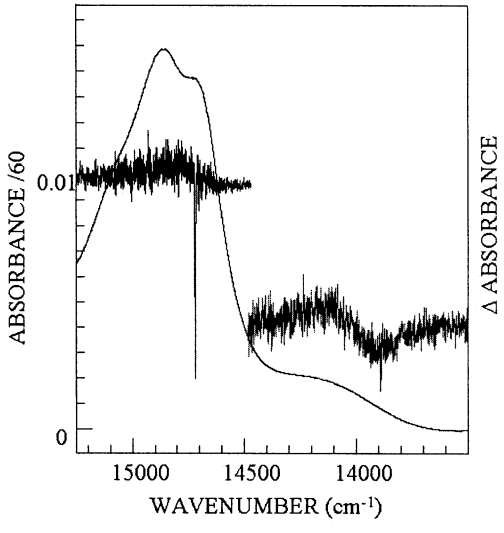

Figure 3: Q_y absorption band and sample spectral holes for reconstituted Lhca-4 complex. The shape of the hole burned at 720 nm (red) indicates that (unlike at 680 nm) electron-phonon coupling is strong. [V. Zazubovich, R. Croce et al., unpublished.]

[14,15]. Consequently, and also due to the relatively small total number of chlorophyll molecules in the complex (10-15), single-complex spectra are expected to contain a limited number of sufficiently well-resolved lines with the ZPL widths of up to 10 cm^{-1}.

Proposed research:

Samples (native and reconstituted) will be obtained from Dr. R. Croce, Institute of Biophysics, CNR, Trento, Italy.

Different variations of SHB will be applied to Lhca1...4, providing information on energy transfer rates and pathways. SHB will also be applied to explore vibration dynamics, and, combined with high pressure and electric field, will be used to verify that the lowest-energy states of the Lhca complexes are indeed the lowest exciton states of certain chlorophyll aggregates, and that these states possess significant charge-transfer character. Relevant parameters (electron-phonon coupling, permanent dipole moment difference between excited and ground states, inter-pigment coupling) will be determined quantitatively.

I am also planning to compare in detail the properties of the lowest-energy states of Lhca complexes with those of "red antenna states" of PS-I cores. One of the goals of this comparison will be to explore the reasons for the surprising lack of splitting of spectral holes in the electric field, observed for red states of cyanobacterial PS-I cores [16].

Polarization-sensitive SMS (and SHB) measurements will be performed in order to determine relative orientations of the chlorophyll molecules in the complex, to match different chlorophylls with their spectral signatures, and to gain insight on energy transfer pathways within the complexes, between the components of Lhca dimers (Lhca1/Lhca4 and Lhca2/Lhca 3), and within LHC system in general.

SMS can also directly answer the question if there are mixed Chl *a* / Chl *b* sites in the Lhca complexes, which is currently a topic of debate. Mixed sites can actually serve as internal markers facilitating assignments of spectral features to particular chlorophylls.

Possible observation of variations in the lifetime of the same state from complex to complex (using SMS) would serve as an indication that kinetics of ET from that state is dispersive, which would be a natural consequence of site energy disorder. Thus, it is possible to access the effects of energy disorder on variations from complex to complex in rates of EET from given chlorophyll. Disentangling contributions of diagonal and off-diagonal disorder may become possible, by analyzing single-complex Hamiltonians constructed using experimental data.

2. Various subunits of photosynthetic apparatus of strains of cyanobacterium *Prochlorococcus marinus* adapted to different illumination levels.

Relevant preliminary knowledge:

It is estimated that different strains of *Prochlorococcus marinus* may comprise up to 50% of the photosynthetic biomass in the Ocean. This makes *Prochlorococcus* an

organism of unprecedented significance from the viewpoint of oxygen production and carbon fixation. *Prochlorococcus* also has the smallest genome among cyanobacteria and may therefore serve as a model system for other cyanobacteria and higher plants. One of the remarkable properties of *Prochlorococcus*, setting it apart from other photosynthetic organisms, is that it achieved adaptation to drastically different illumination levels (at different depths) by means of varying the Chl b_2 to Chl a_2 ratio [5]. Since it was discovered only recently (!!!), *Prochlorococcus* is practically unexplored with spectroscopic methods. Although it has been extensively studied by biochemical methods as well as electron microscopy, the spectroscopic data published so far is limited to intact cells, thylakoids and Photosystem I (PS-I) of a few strains and were obtained at low-resolution and at ambient, or, at best, liquid nitrogen, temperatures [17,18]. To the best of my knowledge, no spectroscopic data is available for Photosystem II (PS-II) of any *Prochlorococcus* strain.

The photosynthetic apparatus of *Prochlorococcus* exhibits similarities as well as differences, compared to that of other cyanobacteria. Instead of regular chlorophylls *a* and *b* (Chl *a* and Chl *b*), *Prochlorococcus* contains divinyl derivatives, Chl a_2 and Chl b_2, as well as carotenoids and small amount of phycoerythrin. There is evidence suggesting that, unlike in other cyanobacteria and higher plants, not only the peripheral antenna complexes but also the PS-I core contain Chl b_2 [17]. By varying the Chl b_2 to Chl a_2 ratio *Prochlorococcus* achieved remarkable adaptation to illumination levels varying by 3 orders of magnitude, surviving at depths from surface to 200 m. Low-light strains exhibit quite unusual arrangement of PS-I, where the core PS I trimer is surrounded by up to 18 copies of the *pcbB* antenna complexes genetically similar to the CP43 core antenna complexes of PS-II of higher plants [19]. Concerning PS-II, it is only known that for MIT9313 strain the PS-II core dimers are in contact with 4 or 8 copies of CP43-like *pcbA* antenna complex [20]. Higher-resolution structures, usually determined by X-ray diffraction, of various photosystems from Prochlorococcus are not available yet. However, those structures are expected to be similar to the structures of respective photosystems of other cyanobacteria, at least from the viewpoint of the positions and orientations of the pigment molecules.

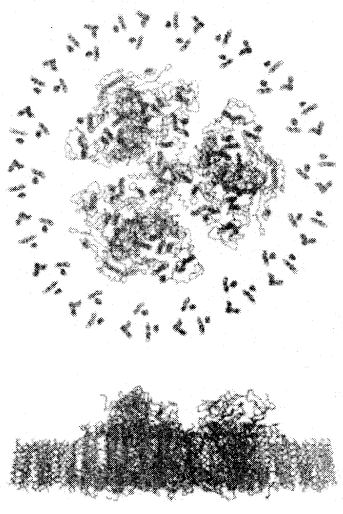

Figure 4: PS-I of *Prochlorococcus* strain SS120. (From [19]).

Proposed research:

I have reached initial agreement to obtain samples from the groups of Drs. S. Chisholm / Z. Johnson (MIT/University of Hawaii) and/or Dr. F. Partensky (France).

I intend to compare systematically the spectroscopic properties of various complexes (intact PS-I and PS-II, PS-I and PS-II cores, peripheral CP43-like antenna proteins *pcbA*

and *pcbB* as well as isolated components of the PS-II cores – RC, CP43 and CP47) extracted from the strains of *Prochlorococcus* adapted to different illumination levels (depths), as well as to compare the properties of photosystems from *Prochlorococcus* with their counterparts from other cyanobacteria and higher plants.

Of particular interest is finding out to which subunits the adaptive variations in Chl a_2/b_2 ratio are confined and how these variations affect energy transfer pathways and rates. It is unknown yet if surrounding the PS I core with increasing number of copies of a small CP43-like Chl b_2-containing antenna protein is the main mechanism responsible for varying Chl b_2 to Chl a_2 ratio or if that ratio varies from strain to strain both within those peripheral subunits and within PS I cores (and if similar mechanisms are at work for PS II cores as well). If the latter is the case, that natural substitution of chlorophylls in particular binding sites within PS-I (and PS-II) core should lead to changes in the shape of absorption spectra and in energy transfer rates and pathways, observable by spectroscopic methods.

All possible variations of SHB will be applied to *Prochlorococcus* when necessary. The elimination of inhomogeneous broadening in SMS experiments will provide the most direct insight into the electronic structure of the complexes and allow one to access additional information, including information on distributions of site excitation energies for different states and of EET rates. Using polarized excitation and/or detection in SMS experiments will allow making conclusions about relative orientations of different pigments (connected by EET) in the complex. Combining information gained using the SHB and SMS will allow matching chlorophylls or groups of them known from structure data to their spectroscopic signatures (See [7] for an example of using SHB for such matching for PS-I of *Synechococcus elongatus*.)

Just like in the case of Lhca antenna complexes of higher plants (see previous subsection), the investigation of mixed Chl a_2 / Chl b_2 sites (if such sites will be found) will facilitate the process of structural/spectral matching. Since the structures of photosystems from *Prochlorococcus* likely are quite similar to those of other cyanobacteria, the results obtained for *Prochlorococcus* may prove helpful in determining structure-function relationship for photosystems of other cyanobacteria and higher plants, which do not contain Chl *b* (or Chl b_2).

3: Photosystem I of cyanobacteria.

Relevant preliminary knowledge:

Recently, significant progress has been achieved in understanding properties of Photosystem I, one of two main photosynthetic complexes of green plants, algae and cyanobacteria. Structures of cyanobacterial [21] and plant [13] PS-I were obtained by means of X-ray diffraction with resolution sufficiently high to determine not only positions but also orientations of chlorophyll molecules. According to [13,21] the structure of PS-I core of higher plants is very similar to the structure of monomeric

cyanobacterial PS-I. (In vivo cyanobacterial PS-I exists in trimeric form.) Therefore, cyanobacterial PS-I, which (with some exceptions, like low-illumination strains of *Prochlorococcus* above) lacks peripheral antenna complexes, can serve as a good model for the core of PS-I of higher plants.

One of the intriguing properties of PS-I is that it contains chlorophylls absorbing at lower energy than the strongly coupled special pair (P700) of the reaction center (RC). These "red antenna states" of PS-I play an important role in the energy transfer dynamics and possibly in protecting complexes in excessive illumination conditions. It was determined, by means of SHB combined with high pressure and electric field that the red antenna states of *Synechocystis* PCC 6803 and *Synechococcus elongatus* are associated with Chl *a* aggregates with the coupling between the monomers being strong [6,7]. The properties of the lowest-energy red states of *Synechocystis* and *Synechococcus* are very similar, and are determined by very strong coupling between Chl *a* molecules, with significant contribution from electron exchange, as evidenced by a large permanent dipole moment change observed for these states of both species. Very strong coupling is also consistent with the large linear pressure shift rate for the optical transition frequency and strong linear electron-phonon coupling.

Various groups proposed different contradictory assignments of the red antenna states to strongly coupled Chl *a* aggregates known from structure data [7, 22-25]. Currently there is no agreement on this issue. Also, two different sets of Chl *a* site energies are available [23,24]. The most likely reason for this is the inability to calculate with sufficient accuracy the site excitation energies of Chl *a* molecules and the excitonic coupling energies associated with Chl *a* aggregates. Thus, which antenna aggregates are responsible for the red absorbing states remains an unanswered question. There is even less experimental data, which would allow matching higher-energy (<700 nm) antenna states with respective chlorophylls. Thus, good understanding of the relation between structure and function of PS-I antenna has not been achieved yet.

Proposed research:

Samples will be obtained from groups of Drs. Jankowiak (ISU/KSU) and P. Fromme (ASU). Most of the samples are in fact already available.

I am intending to combine my experimental expertise with expertise of several theory groups (Schulten, Ritz, Fleming, Damjanovic) in an iterative process where theoretical predictions for the match between various bands and Chl *a* molecules or aggregates will be tested against the experimental results. (Experimental research on red antenna states will be a collaborative effort with the group of Dr. Jankowiak, ISU/KSU.) The way to perform this iterative procedure would be to compare the EET rates determined from the widths of spectral holes (SHB) or lines (SMS) at different wavelengths with the rates theoretically predicted for different molecules or aggregates. In this respect it is important to mention that according to recent simulations by Fleming's group (Berkeley), certain chlorophyll molecules absorbing at high energies (<700 nm) serve as quasi-traps even at room temperature [26], i.e. the EET from those chlorophylls is relatively slow. I am planning to investigate if the site excitation energies of those chlorophyll molecules

are correlated with the wavelengths of preferential location of sharp lines in single-complex excitation (absorption) spectra and/or with the locations of satellite holes emerging upon non-resonant excitation (Figure 5). If necessary, monomeric or mutant PS-I will be investigated.

Concerning Figure 5, the origin of the non-resonant satellite holes is actually not clear. In [6,7] those holes were attributed to non-photochemical spectral hole burning following the downward EET. However, while the hole spectrum is conservative in the vicinity of the resonant hole ("positive" photoproduct to the blue from the narrow hole at 670 nm compensates for the "negative" hole, including ZPH and pseudo-PSB), in agreement with NPSHB hypotheses, at lower energies the spectrum is clearly non-conservative, suggesting that some photochemical processes are taking place. In this case, some features in the intermediate spectral region (680-700 nm) may be due to the electrochromic shifts. I am planning to explore the possibility that both non-resonant and resonant hole burning of the lowest antenna states in PS-I is photochemical in nature, using spectroscopic monitoring of the formation of Chl radicals as well as monitoring the formation of higher-energy satellite holes and/or derivative-like features upon lower-energy excitation. Results of SHB experiments will be compared with data on (possible) permanent bleaching of certain lines in SMS spectra; correlations between permanent bleaching and shifts of different lines will be sought.

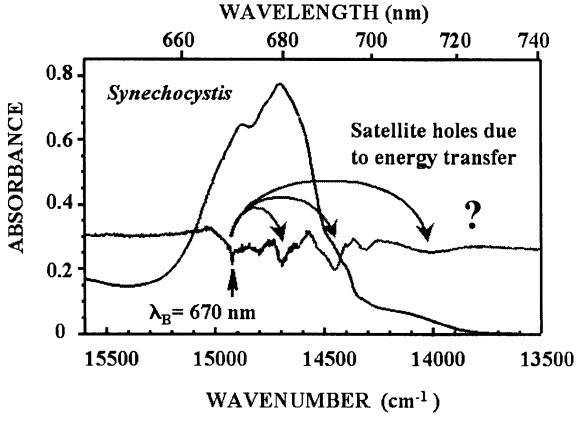

Figure 5: Absorption spectrum (blue) and hole spectrum obtained as a result of irradiation at λ_B= 670 nm (red). Modified figure from [6].

In case photochemical nature of this hole-burning mechanism will be verified, I intend to look for the manifestations of similar mechanisms in other complexes. (Similar non-conservative non-resonant burning seems to occur for CP-43 core antenna complex of Photosystem II [27]. Here it is important to remember, that while red antenna states of PS-I are localized on strongly coupled chlorophyll aggregates, the lowest energy states of CP-43 are supposedly localized on single chlorophyll molecules [27]. The latter assumption may prove to be unjustified.) In general, to the best of my knowledge, the possibility of reversible photochemical processes in pigments not belonging to the electron transfer chain is not systematically explored so far.

4. Photovoltaic devices incorporating photosynthetic complexes and spectroscopy of the arrays of oriented complexes.

Preliminary knowledge:

Several methods for producing densely-packed arrays of uniformly oriented photosynthetic complexes have been developed recently [9,28]. The authors of [9] achieved the energy conversion effectiveness of more than 10% in dry photovoltaic devices based on spinach Photosystem I and Bacterial Reaction Centers (BRC). The orientation of the complexes has been achieved by binding polyhistidine tags, genetically engineered into photosynthetic complexes, to Ni^{+}-NTA monolayer on gold electrode. In [28], Photosystem I particles were oriented on gold surface covered with 2-mercaptoethanol. Proper determination of the theoretical maximal effectiveness of photovoltaic devices based on photosynthetic complexes requires knowledge of the maximal throughput, i.e. the number of charge separation acts per second the photosystem can actually perform. To the best of my knowledge, this issue was never addressed in the literature on such devices. In the first approximation, the throughput is limited by the time constant of the longest electron transfer process: For BRC the maximal throughput is 5000 e/sec and is limited by the 200 μs time if Q_A to Q_B electron transfer. With packing density as described in [9], this results in the maximal achievable current of about 1 mA/cm2 for BRC monolayer. For PS-I the maximal throughput is >30000 e/s and is limited by the 30 μsec time if F_A to F_B electron transfer. In the most recent work [29] even smaller time of 500 ns was reported for that process (Figure 6). For PS II the maximal throughput is 500-1000 e/sec only, due to very long time of Q_A to Q_B electron transfer, ~1-2 ms. It is quite possible that PS-II without qiunones and water-splitting machinery might be a much better material for photovoltaic devices than intact ones.

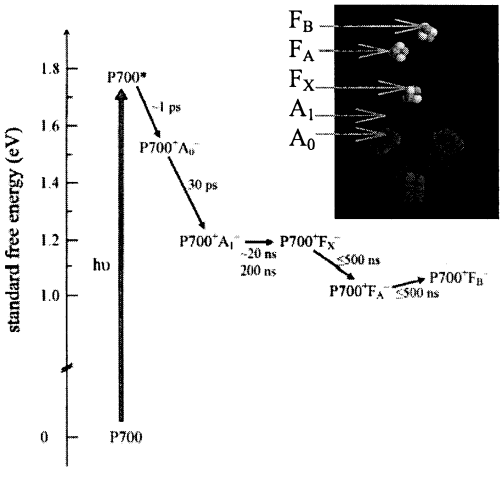

Figure 6. Electron transfer processes in PS-I. From [29]. Green: P700 special pair. Blue: other RC chlorophylls. Red: phylloquinone A_1, orange: FeS clusters.

Proposed research:

Although the energy conversion effectiveness and device lifetime (several weeks) achieved in [9] are remarkable and design is truly ingenious, the necessity to grow genetic engineered complexes makes the production of such devices somewhat impractical. Additionally, peptide suffractants used to stabilize photosystems embedded into dry solid-state devices are quite expensive. Therefore, I am planning to adopt initially the much simpler approach similar to that by Lee et al. [28] who oriented

photosynthetic complexes on the gold surface covered with 2-mercaptoethanol. Unlike Lee et al., who used spinach PS-I complexes with 40 Chl *a* molecules per primary donor / special pair (PS-I-40), i.e. damaged monomeric PS-I, I am planning to use intact cyanobacterial PS-I. As mentioned in the previous subsections, cyanobacterial PS-I exists in trimeric form. PS-I trimer is a much more disk-like entity than PS-I-40 and therefore is much more likely to orient on the substrate in one certain way. Only ~70% of the complexes were oriented in a way advantageous for electron transfer between the electrodes in [28].

The processes limiting maximal electron throughput listed above are "internal" processes. Maximizing the effectiveness of a photovoltaic device would require minimization of the time of "external" processes, i.e. of the reduction of the primary donor of the photosystem by the outside donor and of the electron transfer from the final "internal" acceptor to the outside acceptor. Time constants of these processes should be smaller than the time constant of the longest "internal" process. (In *in-vivo* PS-I these processes take tens of μs.) Provided these conditions are satisfied, the devices based on the PS-I promise to yield much higher maximal currents, since PS-I-based devices may process 7-20 times larger number of photons per mono-layer than BRC-based ones. One of the main factors influencing the time constants of any electron transfer process is the distance between donor and acceptor. Thus, the lengths of the linkers connecting the photosystems to electrodes should be minimized in order to increase the effectiveness of photovoltaic devices. The relatively large length of the NTA-His linkers employed in [9] might be the factor limiting the electron throughput and be one of the reasons why only 10% effectiveness was achieved in that work. Adhesion of photosystems directly to negatively charged electrodes will be tested.

Absorption (at the wavelength of the respective absorption spectrum maximum) of a densely-packed monolayer of photosynthetic complexes ranges from 0.2 % for BRC to 1% for PS-I. Thus, developing multi-layer device architectures would greatly increase the effectiveness of light-harvesting. Note that in these multi-layered structures the photosystem layers should be connected in series from the optical viewpoint but in parallel from the electrical viewpoint.

Spectroscopic methods are very suitable for testing intactness and orientation of the photosynthetic complexes incorporated into photovoltaic devices. Additionally, spectroscopic studies of samples with uniform orientation of photosynthetic complexes may prove very useful from the viewpoint of basic science. Polarization-sensitive measurements on oriented bulk samples may allow resolving the bands overlapping in the samples without preferential complex orientation, which would facilitate understanding of the relationship between structure and function in photosynthetic complexes. One promising way to achieve preferential orientation of the photosystems in the bulk sample is to mix them into liquid crystal solutions. After uniform orientation will be obtained using electric or magnetic field, the sample will be "frozen", either literally (highest spectral resolution is achieved at low temperatures) or using polymerization of photocrosslinkable polymers added to solution. Several liquid crystal substances have been identified, which are water-soluble and have particle size comparable to that of photosynthetic complexes, for example suspensions of cellulose crystals and tobacco

mosaic virus (TMV) and solutions of poly-(N-2-[2-(methoxyethoxy) ethoxy]acetyl-lysine) [30] and alanine dipeptide aggregates [31]. The latter two are the most promising candidates. Poly-(N-2-[2-(methoxyethoxy)ethoxy]acetyl-lysine) may be synthesized by the group of Professor Deming (UCLA) in various sizes from ~55 to ~120 kDa. Alanine dipeptide aggregates are disk-like entities 22 Å thick and 80 Å in diameter.

Conclusions:

I propose to apply a unique combination of techniques of high-resolution frequency-domain optical spectroscopy to improve understanding of the relationship between structure and details of the energy and electron transfer processes occurring in photosynthetic complexes. This combination includes Spectral Hole Burning and Single Complex Spectroscopy; additional competitive edge will be acquired by adding the possibility to perform measurements in the electric field or under hydrostatic pressure. Research will also involve development of photovoltaic devices and, possibly, herbicide sensors and water splitting devices incorporating photosynthetic complexes. The first steps down this path will be developing simple and reliable methods of orienting photosynthetic complexes on various substrates and of prolonging lifetime of the photosynthetic complexes incorporated into various nanodevices. Spectroscopic methods are very suitable for testing intactness and orientation of the photosynthetic complexes in such devices. Polarization-sensitive measurements on oriented bulk samples may prove extremely useful also from the viewpoint of basic science since they will allow resolving the bands overlapping in the samples without preferential complex orientation, thereby facilitating understanding of the relationship between structure and function in photosynthetic complexes.

I can envision several ways of expanding my research beyond the area of photosynthesis, which may be considered in the more distant future. They are based on the fact that fluorescent entities (molecules and/or nanoparticles) are very sensitive probes for structure and dynamics of their local environment. Obviously, impurity center spectroscopy can be used to resolve many important issues in physical chemistry and condensed matter physics. Provided that efficient methods of site-selective labeling of cells, organelles and large biomolecules of interest with fluorescent probes (molecules or quantum dots) will be developed, single-entity spectroscopy can be applied to a broad range of biophysical problems, including protein folding and unfolding, motion in biomolecular motors, cancer research, etc.

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- DOE
- NSF

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Appendix: Equipment

Note that the following list contains only major pieces of equipment and therefore is incomplete.

Absorption and fluorescence excitation spectra of the bulk samples will be measured using frequency-stabilized tunable dye laser, such as Coherent 899-29, with solid-state (preferred) or Argon-ion laser as a pump; the same laser system will be used to burn spectral holes. Photomultiplier with photon counter will serve as a detector. Additionally, for measurements in the broader spectral range than the laser dye band, spectrometer such as Varian Cary 4000 will be needed. Since the measurements will be performed at liquid helium temperatures, cryostat such as Janis SVT 200 or 300 with accessories (vacuum pumps, temperature sensor/controller, etc) will be required. Pressure and SHB Stark cells are relatively simple and may be ordered at the local machine shop.

Single complex spectroscopy system will be based on a home-built confocal microscope. Experiences of our group and other groups show that relatively inexpensive small achromatic high NA microscope objectives available from Newport or Microthek work reliably even when immersed into liquid helium below 2 K, and achieve diffraction-limited performance. Precision motorized mirror (Thorlabs) will be used to focus the excitation beam at (and collect the fluorescence from) different points in the plane of the sample. The same laser system as used in the spectral hole burning experiments will be employed as the excitation source. Fluorescence excitation spectra of single complexes will be measured using an avalanche photodiode with the lowest possible dark count (Perkin-Elmer offers avalanche photodiodes with the dark count of less than 25 s^{-1}). Emission spectra of single complexes will be dispersed using imaging spectrometer (Jobin Yvon Triax 320, for example) and collected with a Princeton Instruments Gen III PI-MAX ICCD camera. The above spectrometer and camera may be used also for measuring emission spectra of bulk samples for resonant (Fluorescence Line Narrowing) and non-resonant excitation. Polarizers and polarization plane rotators, as well as polarization-preserving optical fibers will be required. The sample substrate chips with electrodes for single complex Stark experiments may be either obtained from Dr. L. Kador (University of Bayreuth, Germany) or made according to specifications available in [32]. Finally, for regular SMS sample (thin film) preparation I will need a spin-coater and a plasma-cleaner.

Cost estimates are available upon request.