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Supramolecular Energy Transfer in Self-Assembled Biomimetic Polymer Nanocomposites Based upon Green Bacterial Antenna Complexes

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Photosynthetic organisms use complex and regulated multichromophore assemblies, called light-harvesting (LH) antennas, to capture, concentrate and direct solar radiation to reaction centers that then carry out concomitant chemistry[1]. Nature's LH antennas are remarkable, operating with high efficiency in fluctuating environmental and photic conditions as well as being assembled with nanoscale precision thus, they often serve as inspiration in material design[2]. The presented work was inspired by a natural LH antenna. We show that a diblock copolymer amphiphile enables the generation and integration of optically dense chromophore arrays, within a biomimetic polymer membrane. The entire construct is solution-processable, scalable and exhibits intra and inter-supramolecular energy transfer in a completely noncovalent design. This work demonstrates the potential of polymer membrane materials in generating spatial-energetic landscapes for photonic applications.

Photosynthesis is the most prolific biological process on the planet with net primary production of biomass exceeding 100 Gt C yr^{-1} [3]. Photosynthesis is initiated by efficient LH antennas that are diverse in structure and chromophore composition, but have ubiquitous function: to provide a network of light-absorbing molecules that move electronic excitation energy to a "trap"[1] that catalyzes endergonic electron transfer. However, biological systems have accumulated structural complexity through evolution and natural selection that is often superfluous to basic functioning[4] and obfuscates the key design principles for optimizing solar light harvesting in artificial systems. Thus, bio-inspired materials should possess the desirable properties of biology such as function, regulation or repair, while not necessarily mimicking the atomic level details of natural systems. In this vein, we sought inspiration from a more archaic LH system; the green bacterial photosynthetic antenna. Green bacteria use enormous membrane extrinsic antenna called chlorosomes to gather and focus light excitation to a fleetingly thin lipid bilayer membrane that houses energy acceptors. This is in contrast to most other photosynthetic organisms that use intracytoplasmic membranes[5] or multilamellar stacks of thylakoids[6] to increase the effective absorption cross-section of the antenna. Chlorosomes are supramolecular structures containing 10-100's of thousands of bacteriochlorophyll (BChl) *c* (*d*,*e*, or *f*), which are self-assembled through H-bonding and π- π stacking

interactions, and are bounded by a single membrane leaflet containing lipid and proteins (Fig 1A). This organization scheme is starkly dissimilar to all other LH antennas where intricate protein coordination of chromophores is a requisite. Chlorosomes have an enormous absorption cross section, owing to their large size (~150×~50×~25 nm) and considerable BChl concentration that results in effective harvesting of photonic energy while also being efficient at transferring energy to membrane-associated acceptors[7,8]. Thus, from a biomaterials perspective, the spectroscopic and physical attributes of chlorosomes are very attractive. In addition, integration of a dense, peripheral light-harvester such as the chlorosome is an intriguing design attribute for bio-inspired assembly.

Bchl *c* and its analogs are preprogrammed to form long-range, ordered, photonic aggregates under idealized conditions such as the interior of the chlorosome or suitable solvents [9] Attempts to reassemble chlorosomes from purified pigments and lipids have resulted in bchl *c* aggregates that mimic some of the spectral attributes of chlorosomes. However, such constructs exhibit limited functional capacity and no observed control of assembly or demonstrated integration into hierarchical complexes. Diblock copolymers and in particular, Poly(ethylene oxide)-block-poly(butadiene) (PEO-*b*-PBD), represent an alternative molecular amphiphile that can also self-assemble and have been explored as a biomimetic membrane[10,11]. 1.3:1.2kDa PEO-*b*-PBD forms micelles in solution on the order of ~20 nm that spontaneously organize into molecularly thin monolayer/bilayer films (~4-5nm diameter) on solid supports, thus demonstrating the morphological flexibility of the polymer. Further, chromophore donor-acceptor pairs capable of efficient light-harvesting and energy transfer could be self-assembled into polymer micelles and membrane-like films [10]. The inherent flexibility of PEO-*b*-PBD as a membrane material useful for chromophore encapsulation led us to investigate the polymer as a matrix to overcome lipid limitations for self-assembling photonic biomaterials inspired by the green bacteria antenna system.

Supramolecular structures that we are calling polymer-chlorosome nanocomposites (PCNs), physically and spectrally resembling natural chlorosomes were formed through self-assembly of BChl *c* and the copolymer amphiphile PEO-*b*-PBD (Fig. 2). Initially 30 nmols of BChl *c* and various amounts of

PEO-*b*-PBD (0-30 nmols) were dissolved in 50 µl of tetrahydrofuran and 1 ml of 20 mM Tris buffer was added slowly while the sample was continuously mixed. Thus, as the solvent quality was changed, the propensity for BChl *c* and polymer to self-associate and associate with each other increased. Cosolvent processing of copolymers is a routine method to create well-defined nanostructures [12] and is used in the loading of lipid and polymer micelles/vesicles with hydrophobic cargo such as therapeutic agents [13]. In the current work, the 'cargo' can be considered the BChl *c* pigments which self-assemble in the polymer interior.

After buffer infusion, the samples were purified on continuous sucrose density gradients resulting in 3 unique fractions (Fig. 2A). Unlike most natural tetrapyrrole macrocycle pigments, BChl *c* is relatively soluble in aqueous buffer [14] and in the absence of PEO-b-PBD, a dark green, loose pellet was observed at the bottom of the gradient. When BChl c was infused in the presence of PEO-b-PBD, two bands were observed; a dark green fraction banding around 25% sucrose and a light green band at 10% sucrose. As the amount of PEO-b-PBD increased in the sample, the prevalence of the upper band increased. The gradients were subsequently fractionated and analyzed further. In the absence of copolymer, BChl *c* had a broad absorbance spectrum superimposed with substantial scatter (Fig 2B). Absorbance spectra of the fractions banding around 25% sucrose gave a red-shifted Q_Y band at 730 nm, characteristic of aggregated BChl *c* [14]. The full width half max (FWHM) of the Q_Y band is 50 nm and is wider than the 30 nm FWHM of the chlorosome from which the BChl *c* pigments were purified [9]. The upper gradient fraction had absorbance maxima characteristic of BChl *c* monomers and dimers [14,15] with absorbance maxima of 670 and 714 nm, respectively.

Samples prepared in the absence of PEO-b-PBD were large and highly polydisperse as measured by dynamic light scattering (DLS) implying no defined size or structure. Atomic force microscopy (AFM) topographs of these samples support this observation (Fig. S1). Contrasting this, the fractions purified around 25% and 10 % sucrose had hydrodynamic diameters of approximately 140 nm and 20 nm (Fig 2C), respectively, with the former having a narrow polydispersity index of less than 0.15. The 20 nm

diameter of the upper band is consistent with a micellar description for these fractions in which a few BChl *c* are distributed, consistent with spectral data of Fig 2B and previous reports[11]. AFM topographs of the 25% sucrose fractions on a mica support revealed triaxial ellipsoids, very similar in shape to natural chlorosomes[16] but were generally longer and wider with dimensions approximately of 250nm x 80nm x 40nm (*l* x *w* x *h*) (Fig. 2D). In order to generate data on a larger number of particles for statistical analysis, we used a low energy electron microscope that enabled length and width measurements in the absence of staining. Analysis of 126 PCNs gave an average length and width of 243 ± 55 nm and 80 ± 15 nm, respectively (Fig S2).

These results imply several important aspects of the system. First, the critical micelle concentration (CMC) for PEO-b-PBD used in this study is approximately 1.5 μM (Fig S3). When the concentration of PEO-b-PBD exceeds the CMC in the preparation, micelles containing BChl *c* in uncontrolled amounts are observed in the density gradients (upper band). However, if the concentration of polymer in the preparation is optimized, then PCNs can be formed without the need for gradient purification (Fig S4), removing a labor intensive and yield limiting process step important to scalability. Secondly, as evidenced by DLS, AFM and electron microscopy, the resulting PCNs have a narrow polydispersity demonstrating that the cosolvent processing strategy is highly controllable and reproducible.

Morphological similarities between natural chlorosomes and the nanocomposites created here likely reflects a similarity in the long-range ordering of the BChl *c* however we emphasize that for our purposes these similarities are not critical. Our aim was to incorporate a flexible scaffold that will allow the integration of PCNs with a substrate while maintaining the optical properties of BChl *c* for photon capture and energy transfer. In order to focus excitation energy absorbed by PCNs, an asymmetric energy acceptor should be designed into the construct. We exploited the fluorescent dye 1,1'-Dioctadecyl-3,3,3',3'-Tetramethylindotricarbocyanine Iodide (DiR) and the propensity of PEO-*b*-PBD to spontaneously form bilayer films on hydrophilic substrates [11]. The absorbance of DiR has suitable spectral

overlap with the emission from PCNs (Fig. 3B) which should enable resonant energy transfer if the donor and acceptor are spatially close.

A schematic representation of the assembly procedure is presented in Fig 3A. First, PCNs were incubated on piranha-cleaned (hydrophilic) glass. This resulted in assemblies that were immobilized on the substrate, likely due to the strong affinity for PEO for hydrophilic surfaces [11]. After washing away non-bound PCNs, micelles of PEO-b-PBD containing a non-covalently bound DiR were subsequently incubated. Finally, unbound and loosely associated micelles were removed by washing and the sample was interrogated with hyperspectral confocal fluorescence microscopy (HCFM).[17] HCFM records an entire emission spectrum (500-800 nm) at every spatial voxel of an image. To enable analysis, multivariate curve resolution (MCR) algorithms [18,19] were used to unmix the total signal into a sum of individual fluorescence contributions (Supplemental information). These methods were first applied to independent images of PCNs on glass and to films of PEO-b-PBD containing DiR (Fig S5 and S6) to generate spectral signatures of the PCNs and PEO-b-PBD containing DiR.

Once a spectral model was established for both sets of images, the models were combined, and applied to images containing both PCNs and DiR using a constrained classical least squares prediction [20]. This method enables independent localization of PCN and DiR fluorescence, despite their high degrees of spectral overlap. Figure 3C-E shows the distribution of PCN and PEO-b-PBD by the described method. The size of individual PCNs is near the diffraction-limited resolution for the microscope (240 nm, lateral resolution), however most of the observed bright regions are larger ranging from approximately 250 - 600 nm (Fig 3C). These regions likely reflect small clusters of PCNs similar to those observed from AFM and TEM images (Figs 2D, S2). In the DiR image (Fig 4D), emission is enhanced in the same spatial regions as PCNs. Further, a nonzero signal is observed everywhere suggesting a membrane continuum of PEO-b-PBD forms in the space between PCNs. This can be seen more clearly in the merged image (Fig4E). Finally, Fig 3F displays the intensity profile of BChl *c* and DiR along the indicated line which highlights the correlation of enhanced DiR emission in the same spatial pixels as PCNs. Energy transfer efficiency

(ETE) can be estimated by the extent of donor quenching in the presence and absence of acceptors[21] following ETE = 1-(F_{DA}/F_D). As we have spectral images of both PCNs alone and PCNs in the presence of the DiR collected under identical conditions and analyzed with a common spectral model, we can estimate the ETE by simply masking images to include only regions of BChl *c* emission and calculating the mean intensity. The mean intensities for PCNs alone and in the presence DiR were 754 and 414 counts, respectively and resulted in a calculated ETE of approximately 55%. If we assume that each image pixel possessing BChl *c* emission represents a single PCN, then this was done for 13,021 and 7,206 PCNs in the absence and presence of DiR. The calculated ETE should be treated cautiously, as there are associated uncertainties. For instance, chlorosomes show redox dependent quenching of fluorescence even in the absence of mediators such as quinones [22]. We made no effort to control the redox state of the sample however, the quenching of BChl *c* emission in the presence of acceptor and the enhanced emission of DiR gives solid evidence for directional energy transfer.

To summarize, the flexibility and self-assembly properties of short-block length PEO-b-PBD were exploited for two different aspects of the presented work. First, the ability of the amphipathic copolymer to adopt morphologies far from equilibrium allowed the appropriate environment for long-range Bchl c organization. These supramolecular structures were prepared by cosolvent processing to reliably produce photonic materials of consistent parameters without the need for additional purification. Secondly, the propensity of the polymer to form biomimetic membranes on solid supports enabled the creation of a spatial-energetic landscape where immobilized PCNs could be backfilled with a polymer bilayer housing non-covalent acceptor chromophores. In this case, the bio-similar membrane thickness is of critical importance in placing chromophores within sufficient distance to perform efficient energy transfer. The resulting assembly is optically dense and exhibited energy transfer into the polymer membrane. Importantly, the entire construct was prepared through self-assembly and non-covalent interactions of chromophores resulting in a highly modular and scalable system with physical and optical

properties that are potentially tunable by switching or adding different chromophores and adaptable polymers.

Methods

BChl *c* was purified as describe in the electronic supplement accompanying this article. PEO-*b*-PBD with block weights of 1.3 and 1.2 kDa and a polydispersity index of 1.1 was purchased from Polymer Source Inc. (Quebec, Canada). PCNs were prepared by slowly infusing polymer and pigments dissolved in tetrahydrofuran (THF) with tris(hydroxymethyl)aminomethane (Tris) (buffer (pH=8.0). PEO-b-PBD and BChl c, were mixed from stock solution in THF to give final concentrations of 30 μM BChl c and various amounts of PEO-b-PBD after infusion. 20 mM Tris buffer (pH = 8) was added at 2ml/hr to samples while stirring. The final concentration of THF was 5% of the total solution volume. Samples were purified on continuous sucrose (10-30%) gradients containing 20mM Tris (pH=8.0) at 250,000 x g for 15 hours and 4°C. PEO-b-PBD micelles containing 0.75% DiR were made via solvent exchange as described previously[10] to give a final polymer concentration of 1 mM. Absorbance spectra were acquired on a Cary 6000i (Agilent Technologies, Santa Clara, CA) spectrometer. Fluorescence data were acquired on a Photon Technology International (Edison, NJ) fluorometer. Dynamic light scattering was measured on a Zetasizer Nano (Malvern Instruments Ltd, Malvern UK) on samples that were dialyzed to remove sucrose prior to analysis.

PCNs were incubated on piranha-cleaned coverslip for 20 minutes before being washed with copious amounts of 20 mM tris buffer. In some experiments, the coverslip was subsequently incubated for 20 minutes with 200μM PEO-b-PBD micelles that were loaded with 0.75% DiR. The solution was finally washed with 20 mM Tris. Images were acquired on a custom-built hyperspectral confocal fluorescence microscope.[17] Spectral images from HCFM were preprocessed by the procedure outlined in the electronic supplement. Additionally, PCNs were drop cast on freshly cleaved mica and dried under vacuum prior to image acquisition on an atomic force microscope (MFP-3D-SA system, Asylum Research, Santa Barbara,

CA). Topographs were generally 512 x 512 pixels and acquired at 1Hz scan speed using a Si-tip (AC240TS,). Image analysis was performed using Gwyddion 28. Transmission electron microscopy was performed on a low energy electron microscope (LVEM5, Delong America, Montreal, Canada). Samples were drop cast on onto copper grids (Cu-300, Pacific Grid-Tech, San Francisco, CA) and dried in a vacuum desiccator overnight before analysis.

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Figure legends.

Fig. 1 (A) Model organization in natural and nanocomposite systems. In natural chlorosomes , a single membrane leaflet containing lipids and protein provides a hydrophobic environment for BChl c aggregation in the interior. Chlorosomes are attached to the cytoplasmic membrane through the baseplate protein that contains BChl a and serves as a mediator of energy transfer from BChl c in the chlorosome interior and BChl a in the membrane associated photosystem. The interior of the chlorosome also contains carotenoid molecules that aid light harvesting as well as photoprotection (not shown). (B) In the nanocomposite, the lipid and protein envelop of the natural system is replaced by an amphipathic diblock copolymer.

Fig. 2 - Characterization and morphology of PCNs. (A) Linear sucrose gradients (10-30%) for samples prepared with decreasing ratios of BChl *c* to polymer. The concentration of BChl *c* in each preparation was 30 μM. (B) Absorbance spectra of the circled bands off of the gradient in (A). The spectra have been offset for clarity. (C) Dynamic light scattering intensity distribution of the samples in (B). D - AFM topograph of PCNs. Data are displayed in 3D.

Fig 3. Three dimensional energy transfer in a spatial-energetic landscape. (A) Representation of the assembly process. PCNs are drop cast on hydrophilic glass and become immobilized on the substrate. The sample is then washed to remove loosely-bound PCNs and then backfilled with PEO-b-PBD micelles containing DiR acceptor chromophores. PEO-b-PBD forms a continuous bilayer film between and around the deposited PCNs. With selective excitation in to BChl c, emission from DiR is observed demonstrating energy transfer. (B) Spectral overlap of PCN emission (green) and DiR absorbance (red). The data have been normalized to the peak of each trace and the overlap region is shaded. (C-E) fluorescence images showing the distribution and location fluorescence intensity from PCNs (C) and DiR (D) and the merged image (E). The images are scaled from zero to maximum intensity within the image. (F) Zoomed in view of the boxed area in (E) along with the intensity profile of the PCN and DiR components along the indicated line. The scale bar in (C) represents 5 μm.