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Efficient Energy Transfer within Self-Assembling Peptide Fibers: A Route to Light-Harvesting Nanomaterials

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There is currently a great deal of interest in the development of self-assembling materials for a variety of purposes.[1−3] Small peptides offer an attractive starting point for the development of these materials, since they are relatively simple to produce and can be tailored to provide an expansive range of chemical functionality. One application is the use of biological molecules to drive the self-organization of chromophores to perform a light-harvesting function analogous to that of the light-harvesting complexes (LHCs) that enable efficient photosynthesis in bacteria and green plants. Soft materials such as organogels[4] and dendrimers[5] have previously been demonstrated to perform light-harvesting functions, and peptide scaffolds offer an alternative mechanism to drive the self-assembly of chromophores.[6]

Proteins and polypeptides readily self-assemble into multimolecular fibrillar architectures via nonspecific hydrogen-bonding interactions.[7−10] The resulting fibrils (designated "amyloid-like" for historical reasons) are generally on the order of 10 nm in diameter, have lengths of a few micrometers and an unbranched appearance;[11] these characteristics tend to be independent of the size and composition of the originating protein or peptide sequence. Amyloid-like fibrils are also found to be stable in a wide range of physiochemical environments that are not ordinarily tolerated by protein-based structures.[12−14] This stability and morphological regularity of amyloid-like fibrils makes them an attractive starting point for the development of peptide-based self-assembling bionanomaterials.

We have previously shown that the self-assembly of a fragment of transthyretin ($TTR_{105-115}$, amino acid sequence YTIAALLSPYS) into amyloid-like fibrils is able to drive the coassembly of a "cargo" species and that the polypeptide backbone dictates the structure of the resulting aggregate.[15] We now show that this process can be extended to drive the coassembly of two independent luminescent moieties and that the resulting complex performs a light-harvesting function.

In the system presented here, the fluorescence characteristics of the two precursors were chosen to promote resonance energy transfer (RET) following self-assembly. We have previously demonstrated energy migration in fibrils decorated with a single fluorescent species;[15] in a binary system, excitation of donor species should result in rapid transfer of excitation energy to acceptor sites and observation of emission from acceptors (Figure 1, bottom). The starting mixture comprises a large excess of donor species to facilitate energy migration from excited donors to red-shifted traps (the acceptor species). In view of the (quasi-one-dimensional) fibrillar architecture, it is likely that many donor species will service relatively few acceptor molecules and that each acceptor will be rapidly excited by the closest excited donor. If the donor and acceptor have comparable lifetimes, then in the time required for the acceptor to relax from the excited state, most of the donors will also have relaxed, releasing the harvested energy. It is thus preferential to use a donor with a long excited-state lifetime. The donor constituent was formed by conjugation of the long-lived transition-metal complex bis(2,2′-bipyridine)-4′-methylcarboxybipyridineruthenium (RuBiPy), purchased as a succinimidyl ester (Sigma-Aldrich), to the N-terminus of the peptide via an aminohexanoic acid linker. The lumiphore comprises ∼34% of the mass of the peptide conjugate, which is designated as RBY-ahxTTR. The acceptor component, Alexa 647 (Molecular Probes), was conjugated to the N-terminus of a different sample of the same peptide, again via an aminohexanoic acid linker. The fluorophore comprises ∼50% of the mass of the acceptor conjugate, which is designated as X647-ahxTTR.

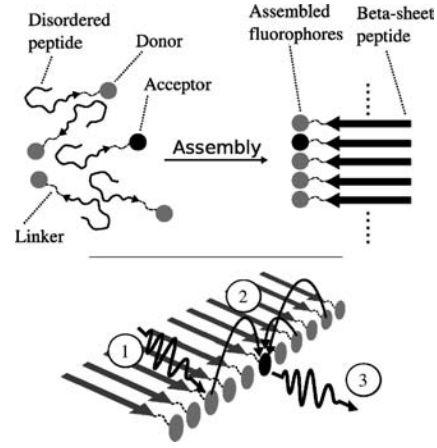

Figure 1. (top) Each synthetic precursor comprises a peptide component (arrow) and an organic fluorophore (disk). Two types of precursor were synthesized: donors (gray discs) and acceptors (black disks). Donors and acceptors are mixed and self-assemble to form a fiber (right). (bottom) Light harvesting occurs in fibrils via (1) absorption of a photon by the donor and (2) nonemissive transfer to an acceptor via RET. The energy is released by the acceptor as an observed photon (3), allowing confirmation of light-harvesting in the system.

Fibrils were prepared by dissolving RBY-ahxTTR into dimethylformamide (DMF) and separately dissolving X647-ahxTTR into a 50% (v/v) acetonitrile (MeCN)/water solution. The X647-ahxTTR solution was then added to the RBY-ahxTTR solution in a X647-ahxTTR/RBY-ahxTTR molar ratio of 1:160 and then further diluted with H_2O/HCl (pH 2) to a final DMF concentration of 5% (v/v) and a combined peptide concentration of 5 mM. Samples were incubated at 40 °C for 24 h and then stored at room temperature. Aliquots were taken from the solution after 24 h and after 1 week for analysis by transmission electron microscopy (TEM) and fluorescence spectroscopy.

After 24 h, fibrils could readily be seen by TEM (Figure 2). The morphology of the fibrils conformed closely to that of most amyloid-

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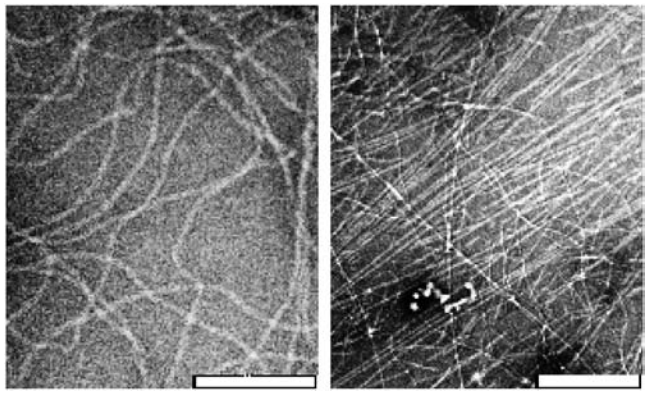

Figure 2. (left) Bright-field TEM image of fibrils incubated for 1 day (scale bar = 200 nm). (right) TEM image of the same fibrils after further incubation for 1 week at room temperature (scale bar = 500 nm).

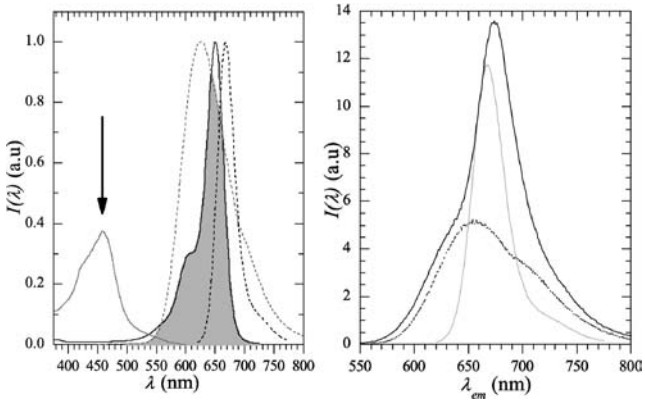

Figure 3. (left) Fluorescence excitation (solid) and emission (dashed) spectra of RuBiPy (donor, gray) and Alexa 647 (acceptor, black). The arrow indicates the wavelength of excitation used to demonstrate light harvesting in the fiber. The shaded area illustrates the overlap between donor emission and acceptor excitation. (right) Emission spectra of light-harvesting fibrils (solid, black) at $\lambda_{ex} = 465$ nm. Also shown are emission from an equivalent but unincubated mixture of donor and acceptor (dotted, black) and emission from direct excitation of the acceptor (gray) at $\lambda_{ex} = 645$ nm.

like fibrils imaged by TEM, with a width of 14 ± 2 nm and lengths on the scale of hundreds of nanometers. Interestingly, the fibrils apparently undergo significant morphological changes with time. After 24 h at 40 °C (Figure 2, left), the fibrils appeared to have a very low persistence length. However, after 1 week of incubation at room temperature, the fibrils appeared to have stiffened greatly (Figure 2, right) and remained virtually unbent for some micrometers.

The energy transfer behavior of the fibrils was investigated using fluorescence spectroscopy (Figure 3). Samples were diluted 300-fold into H_2O and transferred to a quartz cuvette for analysis. Excitation of fibrils at the donor absorption maximum (465 nm; Figure 3 left) resulted in significant emission from the acceptor. This effect was confirmed to be due to energy transfer within fibrils, and not simply between solution components, by mixing the two precursors at concentrations equivalent to those present in spectroscopic fiber samples without incubation. Under those conditions, no significant emission from the acceptor was observed upon excitation at the donor excitation maximum (Figure 3, right). Maliwal et al.[16] have shown that in the case of a long-lived donor in close proximity to a high-quantum-yield acceptor, the resulting emission intensity is determined by the product of the extinction coefficient of the donor and the quantum yield of the acceptor. According to this model, emission from the acceptor should be on the order of 40% of that from direct excitation (also shown in Figure 3). We found that the actual emission intensity was almost twice this expected value, indicating that the acceptors harvest energy from more than one donor.

A challenging task in the design of artificial light-harvesting systems is controlling the relative distance and orientation of the donor and acceptor species. We have previously demonstrated that in cases where fibrils are formed from a mixture of labeled and unlabeled peptides, the two components are included within the same fibril with populations that depend on the molar ratio of precursors.[17] Thus, the average relative distance between donors and acceptors can be controlled. Moreover, the filamentous architecture fixes the orientation of the chromophores. Self-assembly driven by peptide aggregation therefore provides a level of control over the distribution and orientation of lumiphores intermediate between that of organogels, where the acceptor species is encapsulated within the gel and therefore randomly oriented and distributed, and that of dendrimers, where the location and orientation of the light-harvesting species are fully prescribed.

In summary, we have demonstrated that it is possible to use the self-assembly of a fibril-forming peptide to drive the coassembly of two nonassembling functional groups. This has allowed us to produce a macromolecular system capable of capturing light energy over a large area and transporting it to a number of spatially defined points, a process that mimics natural light-harvesting structures. It is striking that the peptide controls the architecture of the complex despite the size of the lumiphores, suggesting this is a robust system with applicability to a wide range of cargo species. In principle, the technique used here should be applicable to many peptide and protein systems as well as polypeptides that self-assemble into alternative fibrillar architectures.

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