Light Harvesting Hot Paper

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G-Quartet-Based Nanostructure for Mimicking Light-Harvesting Antenna**

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Abstract: *Artificial light-harvesting systems have received great attention for use in photosynthetic and optoelectronic devices. Herein, a system involving G-quartet-based hierarchical nanofibers generated from the self-assembly of guanosine 5′-monophosphate (GMP) and a two-step Förster resonance energy transfer (FRET) is presented that mimics natural light-harvesting antenna. This solid-state property offers advantages for future device fabrication. The generation of photocurrent under visible light shows it has potential for use as a nanoscale photoelectric device. The work will be beneficial for the development of light-harvesting systems by the self-assembly of supramolecular nanostructures.*

G-quartets are formed by the self-assembly of four guanosine residues into planar tetramers, assisted by Hoogsteen-type hydrogen bonds and specific metal cations.[1] Many nucleosides, oligonucleotides, and synthetic derivatives can form a rich array of G-quartets, depending on their environments. For many years, there was great interest in the role of G-quartets in biological processes, such as aging and cancer, as well as their potential as therapeutic targets for cancer.[2] The G-quartet architecture has also been proposed as a promising building block for functional nanostructures.[3] Guanosine 5′-monophosphate (GMP), a guanosine derivative with self-complementary hydrogen-bonding edges and aromatic surfaces, has emerged in recent years as a key player because of its ability to self-associate to form ordered structures.[4] Despite great progress, the strict conditions required for its assembly limited the fabrication of nanomaterials. Recently, we demonstrated a novel strategy to construct G-quartet-based supramolecular nanowires.[5] However, the creation of G-quartet-based nanostructures with specific properties and functions still remains a big challenge.

Artificial light-harvesting hybrids have received intense attention as they mimic natural photosynthetic systems, in which chlorophyll is organized to form photoactive, highly ordered supramolecular structures. Many systems were developed to obtain more insight into the photochemical processes, which involve Förster resonance energy transfer (FRET).[6] Among them, DNA-based light-harvesting hybrids were considered as an excellent platform to organize arrays of chromophores with precise control.[7] However, most of these DNA-based strategies involve the design of DNA sequences, synthesis of chromophore–DNA conjugates, separation of products, and annealing, which add to the complexity, cost, and time. In addition, they were commonly performed only in aqueous solutions, which is a serious concern for their processability for applications in the ultimate device. To address these limits, a light-harvesting system involving G-quartet-based hierarchical nanofibers generated from the self-assembly of GMP has been constructed for the first time. The system mimics natural light-harvesting antenna without any laborious synthesis or modification. Moreover, the material can be separated from aqueous solution and processed into a solid substrate, which is a significant feature from the point of view of application in a device.

A schematic illustration of the G-quartet-based light-harvesting system is shown in Scheme 1. GMP was chosen as the building block of the nanostructures. Three dyes thioflavin T (ThT), thiazole orange (TO), and pyronin Y (PY) were used as the donor, intermediate, and acceptor, respectively. The addition of Sr^{2+} ions to a mixture of GMP and the dyes results in the formation of G-quartet structures, which gradually grow into long nanofibers. The three dyes are confined simultaneously in the nanofibers and brought into proximity, thus allowing for a two-step FRET from ThT to PY mediated by TO. In the experiment, $SrCl_2$ (8 mM) was added to solutions of GMP (20 mM) containing dyes. After stirring the mixtures for a few seconds, they were stored at 4 °C for three days. Solid products formed gradually, with the dyes trapped within the supramolecular structures (see Figure S1 a–g in the Supporting Information). Adenosine 5′-monophosphate (AMP) and uridine 5′-monophosphate (UMP) were used as controls under the same experimental conditions, and no precipitation was formed upon the addition of $SrCl_2$ (see Figure S1 i–n in the Supporting Information). Scanning electron microscopy (SEM) images of the samples obtained with GMP indicate that the solid products were nanofibers with an average diameter of 100 nm and lengths up to several micrometers (Figure 1, and see

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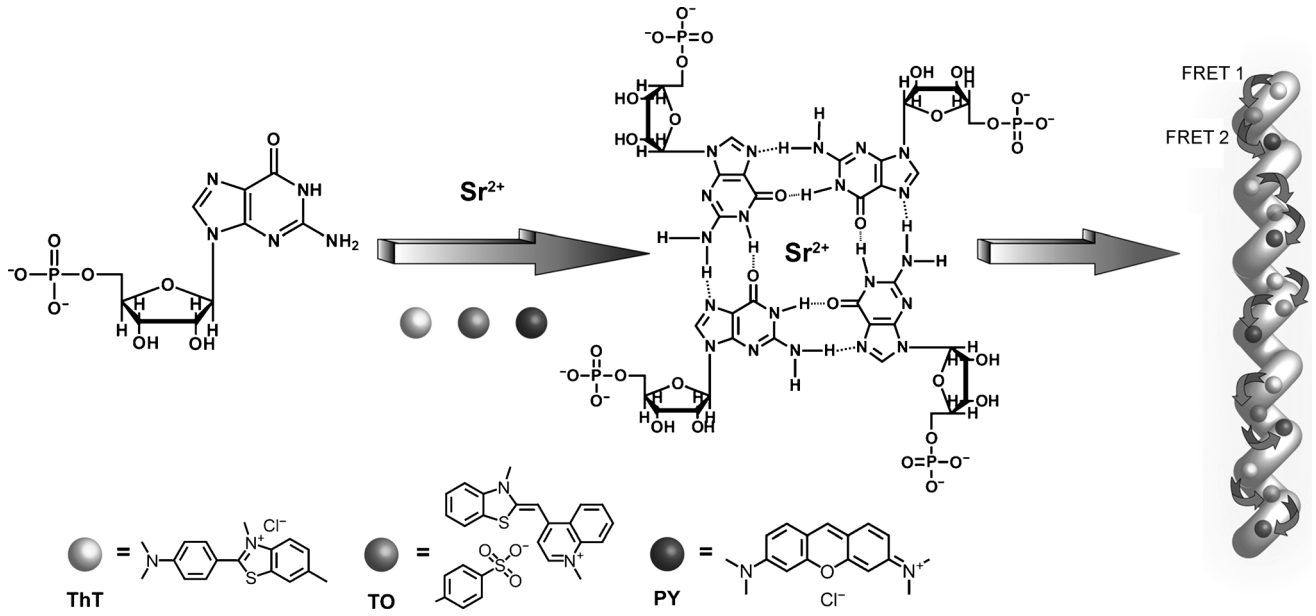

Scheme 1. Schematic representation of a nanostructured light-harvesting system based on G-quartet stacks.

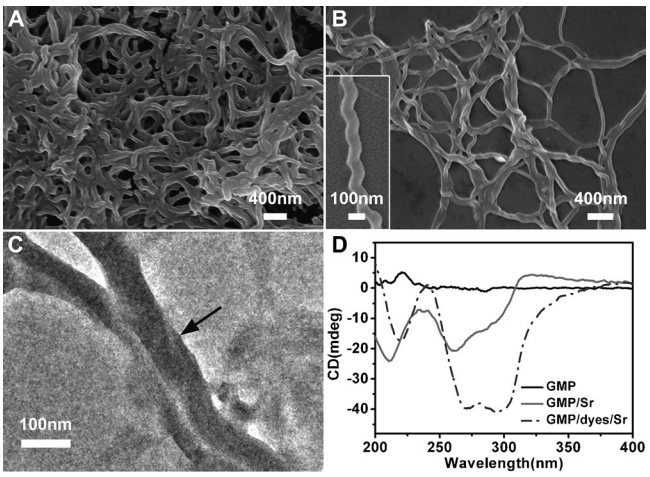

Figure 1. SEM images of A) GMP/Sr and B) GMP/ThT/TO/PY/Sr nanofibers. C) TEM image of GMP/ThT/TO/PY/Sr nanofibers. D) Circular dichroism (CD) spectra of GMP, GMP/Sr, and GMP/ThT/TO/PY/Sr nanofibers.

Figure S2 in the Supporting Information). Moreover, the nanofibers were right-handed helical structures (Figure 1 B (inset), Figure 1 C). The size of the helical pitches is 80–200 nm. Elemental analysis revealed the presence of C, N, P, O, and Sr (see Figure S3 in the Supporting Information). The FTIR spectra suggest the formation of quadruplex structure through hydrogen bonding of the guanine units (see Figure S4 in the Supporting Information). Circular dichroism (CD) spectroscopy was used to obtain evidence that the GMP formed stacked G-quartets. As shown in Figure 1 D, GMP has a weak positive band at 220 nm. The GMP/Sr complex showed a positive CD Cotton effect at 240 nm as well as a negative band at about 260 nm with a shoulder at approximately 290 nm followed by a positive long-wavelength tail. The bisignate CD signal was characteristic of a G-quartet.[3c,8] The encapsulation of the dyes induced an increase in the magnitude of the characteristic CD amplitude of the GMP/Sr and a slight red-shift of the band at 260 nm, thus implying the stacked G-quartet structures persisted. The enhanced CD signal at about 295 nm and the band at about 270 nm exhibited a double-hump feature, which suggests that the aggregate had right-handed chirality.[9]

Recently, ThT was demonstrated to be an efficient inducer and selective fluorescent sensor for DNA with a G-quadruplex structure.[10] TO is another dye commonly used to stain nucleic acids.[11] Free ThT and TO are non-emissive in aqueous solution. After being encapsulated in the GMP/Sr nanofibers, the emissions of ThT at 500 nm and TO at 530 nm were increased remarkably on excitation at 440 nm and 490 nm, respectively (see Figure S5 A in the Supporting Information). A notable red-shift in the ThT emission was observed relative to the emission of ThT in the presence of G-quadruplex DNA (see Figure S5 B in the Supporting Information). Restriction of intramolecular rotation might be the main cause for the generation of fluorescence. However, the phenomena were not observed for AMP or UMP, since no ensemble was formed (see Figure S6 in the Supporting Information). PY is a dye that intercalates and stains RNA.[12] After being encapsulated, PY emitted at 573 nm upon excitation at 546 nm (see Figure S5A in the Supporting Information). The three dyes were chosen because of the good overlap of the emission band of ThT with the absorption band of TO, as well as of the emission band of TO with the absorption band of PY (Figure 2 A, and see Table S1 in the Supporting Information), which are desirable for efficient FRET. When the three dyes were confined in the nanofibers simultaneously, they were brought into proximity (see Table S1 in the Supporting Information), which satisfies the distance requirement for FRET.

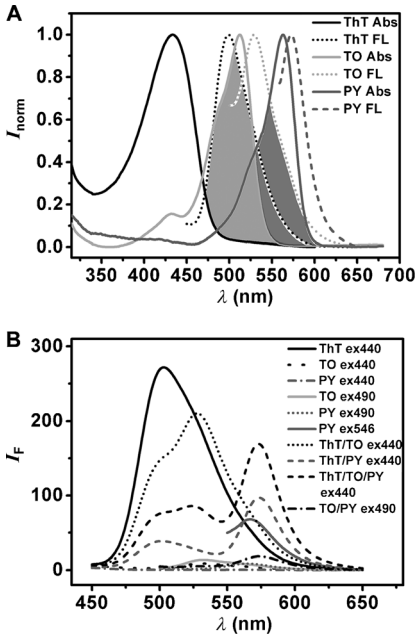

Figure 2. A) Normalized absorption and fluorescence spectra of GMP/ThT/Sr, GMP/TO/Sr, and GMP/PY/Sr nanofibers. B) Fluorescence spectra of GMP/Sr nanofibers containing different dyes on excitation at 440, 490, and 546 nm. [ThT] = 1.1×10^{-4} M, [ThT]/[TO]/[PY] = 25:4:1.

G-quartet-based nanofibers containing different dyes with an optimized molar ratio were prepared to test the energy transfer. The binding of one dye or two dyes did not perturb the binding of the other dyes in the concentration range used in this study (see Figure S7 in the Supporting Information). As shown in Figure 2B for GMP/ThT/TO/Sr, the strong emission of ThT at 500 nm decreased and the emission of TO at 530 nm increased upon excitation at 440 nm. Compared to the excitation of GMP/TO/Sr at 490 nm, the emission of TO was strongly enhanced on excitation at 440 nm, thus indicating the energy was transferred from ThT to TO. Moreover, the ratio of the emission intensity at 530 and 500 nm (I_{530}/I_{500}) gradually increased as the concentration of TO increased (see Figure S8 in the Supporting Information). Similarly, for GMP/TO/PY/Sr, a decrease in the emission of TO at 530 nm and an increase in the emission of PY at 573 nm could be observed under excitation at 490 nm, which corresponds to energy transfer from TO to PY (see Figure S9 in the Supporting Information). The I_{573}/I_{530} ratios increased as the concentration of PY increased (see Figure S10 in the Supporting Information). The emission spectrum of triple-dye-doped nanofibers was then compared with that of GMP/ThT/TO/Sr under the same excitation wavelength of 440 nm (Figure 2B). A decrease in the emissions of ThT and TO, as well as an increase in the emission of PY, was observed. The fluorescence intensity of PY was higher than that obtained after direct excitation of GMP/PY/Sr at 546 nm. These results suggest a FRET cascade took place and energy was transferred from ThT to TO and then to PY. In the two-step FRET process, ThT, TO, and PY played the roles of energy donor, intermediate, and terminal acceptor, respectively. In addition, as a result of partial overlap of the emission band of ThT and the absorbance band of PY, FRET also took place from ThT to PY in GMP/ThT/PY/Sr when excited at 440 nm. However, the emission of PY at 573 nm was lower than that of GMP/ThT/TO/PY/Sr. This finding implies TO acts as an intermediate and facilitates energy transfer from ThT to PY. The concentrations of ThT and PY were then fixed and the variation in the fluorescence intensity with the amount of TO was studied (see Figure S11 in the Supporting Information). The complexes containing more TO resulted in higher I_{573}/I_{500} ratios, which indicated the promotional effect of TO on energy transfer.

The FRET effect was also supported by the excitation spectra of GMP/dyes/Sr (see Figure S12 in the Supporting Information). GMP/ThT/Sr and GMP/TO/Sr had little or no emission at 573 nm and, therefore, no band in the excitation spectrum was observed. However, the excitation spectrum of GMP/ThT/TO/PY/Sr with an emission at 573 nm corresponded to the excitation spectra of GMP/ThT/Sr with an emission at 500 nm and GMP/TO/Sr with an emission at 530 nm. These spectra demonstrated that both ThT and TO contributed to the acceptor emission, and the energy was transferred from ThT to PY through TO. FRET-mediated multicolor nanofibers could be observed when illuminated with a UV lamp (see Figure S13 in the Supporting Information). The emission from GMP/ThT/Sr was bright green, while GMP/TO/Sr and GMP/PY/Sr showed weak emissions. Bright yellow light was observed for GMP/ThT/PY/Sr as a consequence of FRET from ThT to PY. The brightness of the emission from GMP/ThT/TO/PY/Sr was higher than that from GMP/ThT/PY/Sr, which suggests TO can facilitate FRET from ThT to PY. These observations strongly supported the results of the fluorescence spectroscopy measurements.

The light-harvesting ability can be determined by evaluating the antenna effect (AE), a widely used empirical parameter which is defined as the ratio of the fluorescence intensity of the acceptor upon excitation of the donor to that from direct excitation of the acceptor.[7b] The AE for GMP/ThT/TO/PY/Sr with an optimized molar ratio (25:4:1) was 2.7 (see Table S2 in the Supporting Information). In the absence of both ThT and TO, the value was only 0.0082, since light with a wavelength of 440 nm can not be absorbed efficiently by PY. The AE for GMP/ThT/PY/Sr (25:0:1) was 1.5, while that for GMP/ThT/TO/PY/Sr (25:2:1) was 2.1, which suggests that the intermediate TO enhances the energy transfer from ThT to PY. Reducing the amount of ThT resulted in a decrease in the AE, since the initial amount of light absorbed by the donor was reduced and less energy was transferred.

The above data demonstrate that a nanostructured light-harvesting system based on G-quartet stacks could be constructed. Furthermore, we tested the FRET between the three dyes in the presence of DNA with a G-quadruplex structure. A significant reduction in the intensity of ThT, a weak increase in the emission of TO, and a negligible increase in the emission of PY were observed upon excitation at 440 nm (see Figure S14 in the Supporting Information). The AE for G-quadruplex DNA containing the three dyes (25:4:1) was 0.14. In the absence of both ThT and TO, the AE was only 0.031. The results suggest that energy could not be

transferred from ThT to PY efficiently, which might be attributed to their different affinities to DNA and steric hindrance. Only a small number of dyes were usually incorporated per DNA sequence, which dramatically affected the AE. Therefore, the light-harvesting ability of the GMP-based system was higher than that of the system based on G-quadruplex structure DNA. It was also higher than that of the system based on a 7-helix DNA bundle nanoscaffold.[7b] Moreover, the large-scale preparation of GMP-based light-harvesting material is feasible because of the relatively low price of GMP compared to DNA-based systems.

Coordination polymer nanoparticles (CPNs) that showed efficient fluorescence enhancement were recently reported to form during the self-assembly of nucleotides, lanthanide ions, and dyes.[13] We also fabricated an artificial light-harvesting material based on CPNs.[14] Herein, we replaced Sr^{2+} ions with Eu^{3+} ions and compared the morphologies and optical properties of the two materials. G-quartet stacks did not form upon addition of Eu^{3+} (see Figure S15 and S16 in the Supporting Information). Upon excitation, the CPNs displayed band shifts (see Figure S17 in the Supporting Information). The spectra overlap could still be observed, and FRET from ThT to PY mediated by TO occurred (see Figure S18 in the Supporting Information). However, the AE of the CPNs system was 0.61, which was lower than that of the nanofibers with the same dye ratio. These results show that the AE strongly depends on the assembly state. Although our system did not show the highest AE,[6d,7c] it is a promising candidate for mimicking light-harvesting antenna because of the advantages of facile preparation.

Various organic aggregates have been developed for light-harvesting applications. However, fluorescence quenching often occurs when they are in the solid form because of aggregate formation in the condensed phase. In our case, though, the fluorescence properties of the dye-encapsulated nanofibers were preserved when they were separated from the aqueous solution by centrifugation and then freeze-dried. Excitation of solid GMP/ThT/TO/PY/Sr at 440 nm led to a decrease in the fluorescence at 507 and 530 nm and an enhancement at 571 nm (see Figure S19 in the Supporting Information). Irradiation of GMP/ThT/Sr powder under a UV lamp resulted in the emission of bright green light, while GMP/TO/Sr and GMP/PY/Sr were only weakly emissive. GMP/ThT/TO/PY/Sr powder showed bright yellow fluorescence arising from two-step FRET. This finding implies that the self-assembly of the GMP prevented the dyes from forming aggregates. This property offers great potential for application in the solid state.

To further examine the behavior of the photogenerated electrons in the nanofibers, photoelectrochemical measurements were carried out using a three-electrode system. The irradiation of GMP/ThT/TO/PY/Sr nanofibers on an ITO electrode with light led to a photocurrent (Figure 3). When the light was turned off, the photocurrent dropped instantly. GMP/ThT/Sr nanofibers also showed photocurrent activity. However, GMP/Sr, GMP/TO/Sr, and GMP/PY/Sr did not generate photocurrent upon illumination under the same light. These results implied that the photocurrents originate from the excitation of ThT in the nanofibers. All of these

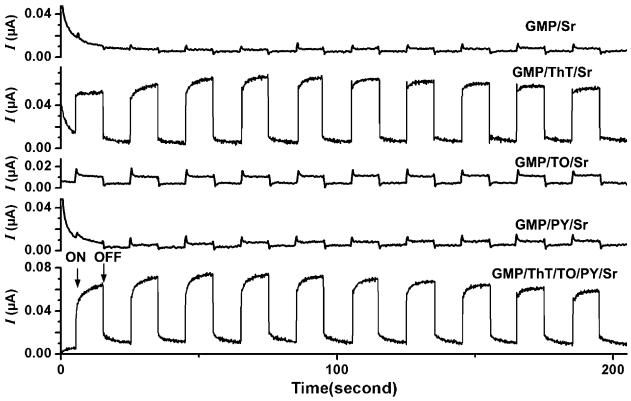

Figure 3. The photocurrent response as a function of time of GMP/Sr nanofibers containing different dyes in 100 mM $NaNO_3$ solution upon irradiation with light (450 nm, 35 $mW cm^{-2}$). "ON" represents the electrode under light irradiation, while "OFF" means the light was turned off.

properties make our material highly appealing for the development of photoenergy-harvesting and -conversion materials.

In summary, we have constructed a nanostructured light-harvesting hybrid based on G-quartet stacks generated by the Sr^{2+}-templated self-assembly of GMP. The system favors a two-step FRET from the donor to acceptor molecules confined in the right-handed helical nanofibers. Compared to systems with inefficient and costly covalent synthesis processes, the major merits of this system include facile preparation, absence of organic solvents, and low cost. Furthermore, these nanofibers showed light-harvesting properties both in solution and in the solid state, thus offering an advantage from the viewpoint of device processing. The generation of photocurrent was induced by visible light, which provides the material with the potential for application as a nanoscale photoelectric device. Therefore, the study will open up new possibilities in the fabrication of functional nanomaterials based on G-quartets.

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