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Nonlinear fluorescence response driven by ATP-induced self-assembly of guanidinium-tethered tetraphenylethene†

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Nonlinear fluorescence response, which is particularly important to attain the high signal-to-background ratio, was realized by the aggregation-induced fluorescence increase of guanidinium-tethered tetraphenylethene with ATP.

The development of fluorescent sensing systems that recognize biologically important metals, molecules and polymers has currently been an active research area because the fluorescence technique is highly simple and sensitive, facilitating their potential applications as bioprobes, and in imaging and monitoring.[1] As fluorophores, small organic dyes have been mainly used, in which recognition sites specific to concerned targets are integrated. The organic dyes are allowed therein to optimize their wavelength window, brightness and photostability by appropriate chemical modifications.[2] However, most of the organic dyes are intrinsically emissive and quenched at the higher concentration due to their aggregation (self-quenching).[3] On the basis of this basic principle, various fluorescent probes have been designed.[4] However, this approach (*i.e.* turn-off switching) inevitably causes low response as well as low signal-to-background contrast in the fluorescence detection because simultaneous satisfaction of the efficient emission and the efficient quenching is rather difficult. As an alternate approach, a few examples have demonstrated target-specific molecular probes, in which the recognition-induced disassembling of the probes switches them "turn-on".[5] In contrast, fluorophores exhibiting aggregation-induced emission (AIE) have been increasingly attracting much attention because AIE molecules behave in an opposite manner to intrinsic self-quenching dye molecules.[6] The design of AIE molecules can be realized by a modular-type structure composed of an emissive moiety, a spacer and a recognition site, and the aggregation is triggered by the target-specific association. Most importantly, the AIE molecules are endowed to show the "turn-on" switching in response to self-assembly with a target molecule. Despite their remarkable advantages, a limited number of examples have so far been reported to apply the AIE molecules as sensors for DNA,[7] protein[8] and heparin[9] and as a chiral recognition host.[10] It is clear that the wide challenging field still remains toward development of a new class of AIE-based bioprobes.

In order to elicit full potential of AIE properties, we have designed a novel fluorescent molecule, that is, the marriage of AIE with an allosteric function attained by a self-assembly system. This strategy was inspired from our previous work on the highly selective molecular recognition attainable *via* the allosteric control.[11] Since the allosteric control realizes nonlinear, sigmoidal information transduction, a small change in the input signal can be amplified into a large change in the output signal.[12] If such a system is accomplished in the fluorescent sensing, a steep turn-on fluorescent transition will be realized in a nonlinear fashion, leading to a significant enhancement of the signal-to-background contrast. In this context, the present system is intrinsically different from the linearly transmitted examples generally observed for conventional fluorescent sensing systems. We have thus focused on tetraphenylethene known as an AIE molecule, whose emission mechanism is rationalized in terms of the restriction of C–C bond rotation.[13] Fig. 1 shows our probe design for target ATP, one of the most significant biomolecules involving a universal energy source as well as an extracellular signalling mediator.[4] The designed probe, TPE, has the following three unique features: (1) multiple recognition sites to be required

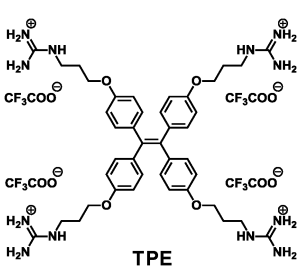

Fig. 1 Chemical structure of guanidinium-tethered tetraphenylethene (TPE) for ATP detection.

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for a nonlinear response under the allosteric control, (2) guanidinium groups[14] as a recognition site which is known to bind with phosphate anions in a 1 : 1 molar ratio *via* the specific hydrogen-bond-assisted electrostatic interaction in water and (3) short spacers consisting of the propyl group between TPE and guanidinium to avoid the background fluorescence arising from micelle formation of TPE itself in water. We have confirmed that our TPE probe realizes selective fluorescent detection of ATP through the cooperative self-assembly that is characterized by the sensitive, nonlinear fluorescence response together with the high signal-to-background ratio.

The core skeleton of TPE was synthesized by McMurry coupling,[15] which was decorated by introduction of the guanidine groups.[16] The TPE structure was confirmed by ESI-MS and elemental analysis. We first examined the fluorescence response of TPE upon addition of nucleotides (AMP, ADP, and ATP) keeping the guanidinium/phosphate ratio at 1 : 1 (Fig. 2a). No significant fluorescence enhancement was observed upon addition of AMP and ADP ([TPE] = 6.0 μM, [AMP] = 24 μM, [ADP] = 12 μM). In contrast, a strong blue emission was observed at 463 nm only when TPE was mixed with ATP ([ATP] = 8.0 μM). The emission intensity was increased by 60 fold compared with that in the absence of ATP, and the bright emission was detectable even by our naked eyes (Fig. 2b). In the excitation spectrum, the maximum appeared at 335 nm (Fig. S1a, ESI†), which is in good agreement with the absorption maximum of the TPE–ATP mixture (Fig. S1b, ESI†), indicating that the blue emission is originated from TPE. This result indicates that TPE can act as an ATP-selective turn-on fluorescent probe. The most important point of this finding is that the three sequential phosphate groups as in ATP are essential for the fluorescence enhancement. This suggests that the cooperative self-assembly takes place in the aggregation process of TPE and ATP.

The relationship between the self-assembly properties and fluorescence emission was further investigated under the various measurement conditions. Fig. 3 shows the effect of pH on the fluorescence intensity of the TPE/ATP complex. The fluorescence intensity at 463 nm decreased gradually with lowering of the pH value of aqueous media, and showed a sharp decrease at pH 6.5. This pH value corresponds to the pK_{a2} of a phosphate group (pH ~ 6.8). Below pK_{a2}, the triphosphate group of ATP mainly exists as its partially protonated species, which is unable to interact

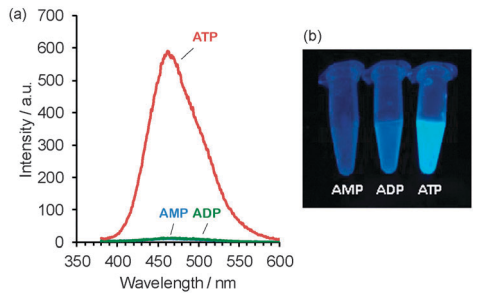

Fig. 2 (a) Fluorescence spectra (λ_{ex} = 335 nm) of TPE (6.0 μM) in HEPES buffer (5.0 mM, pH 7.4) in the presence of AMP (24 μM), ADP (12 μM) and ATP (8.0 μM). (b) Photograph of TPE (6.0 μM) in the presence of the corresponding nucleotides in HEPES buffer (5.0 mM, pH 7.4); left: AMP (24 μM), middle: ADP (12 μM) and right: ATP (8.0 μM). The image was obtained under UV irradiation (λ_{ex} = 365 nm).

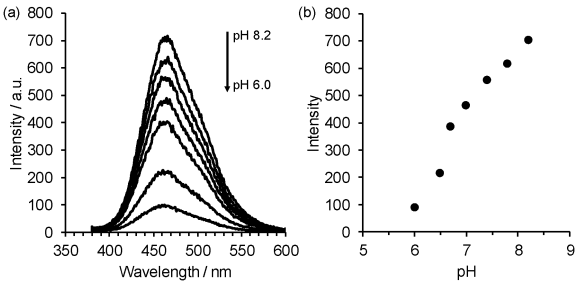

Fig. 3 (a) pH-dependent fluorescence spectra (λ_{ex} = 335 nm) of TPE (6.0 μM) in the presence of ATP (8.0 μM) in HEPES (5.0 mM). (b) Plot of the fluorescence intensity (463 nm) *versus* the medium pH value.

with the cationic guanidinium group in an electrostatic complementary fashion. The fluorescence enhancement is observed only at the pH region where the phosphate group of ATP is deprotonated, indicating that the electrostatic interaction is the primary force for the association. The UV-Vis spectra showed that the addition of ATP to TPE (final concentration: [TPE] = 6.0 μM and [ATP] = 8.0 μM) shifted the absorption maximum from 310 to 335 nm and significantly broadened the peak (Fig. S1b, ESI†). This change is indicative of the aggregate formation. This fact was further confirmed by the measurements of dynamic light scattering (DLS) and scanning electron microscopy (SEM). In DLS measurements, the aqueous dispersion showed the presence of aggregates with a mean diameter of 674 ± 252 nm (Fig. 4a). The SEM measurements confirmed the formation of cohesive aggregates with a bulk size of *ca.* 1 μm (Fig. 4b), which is comparable with that obtained from the DLS measurements. The SEM energy dispersive X-ray (EDX) microanalysis showed the presence of phosphine atoms, indicative of the formation of a TPE–ATP complex (Fig. S2, ESI†). To visualize the origin of the fluorescence emission, the aqueous dispersion was subjected to fluorescence microscopy. As shown in Fig. 4c, fluorescent aggregates with a mean size of *ca.* 1 μm were directly observed, and their image was well overlapped with that obtained from differential interference contrast microscopy (Fig. 4d). These results consistently support the view that the fluorescence enhancement is induced by ion-pairing-based

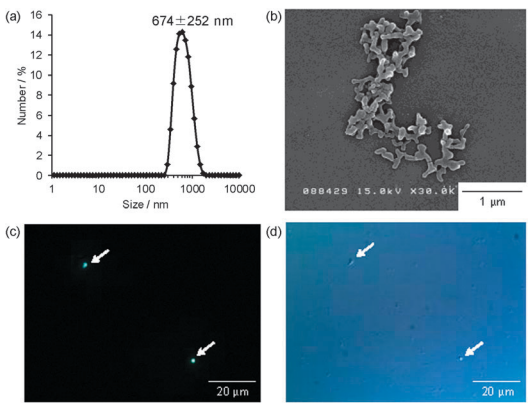

Fig. 4 (a) Size distribution of the aggregates measured by DLS. (b) SEM image of the aggregates prepared by mixing TPE and ATP. (c) Fluorescence and (d) differential interference contrast image of the solution obtained by the addition of ATP to TPE. Arrows indicate the fluorescent aggregates. Conditions: [TPE] = 6.0 μM, [ATP] = 8.0 μM, [HEPES] = 5.0 mM (pH 7.4), 25 °C.

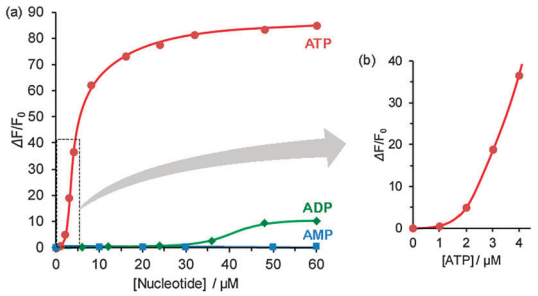

Fig. 5 (a) Fluorescence titration curve (λ_{ex} = 335 nm) of TPE (6.0 μM) upon the addition of AMP (blue), ADP (green) and ATP (red) in HEPES buffer (5.0 mM, pH 7.4) at 25 °C. (b) Magnified figure at the lower ATP concentration region (0–5 μM). $\Delta F/F_0$ means the fluorescence intensity change ($\Delta F = F - F_0$) and F_0 is the fluorescence intensity of TPE in the absence of nucleotides.

complexation of TPE and ATP followed by the formation of the larger aggregates (Fig. S3, ESI†).

Next, we performed the fluorescence titration of TPE by AMP, ADP and ATP. The addition of AMP or ADP exhibited no significant fluorescence increase. On the other hand, the fluorescence intensity was increased by the incremental addition of ATP (Fig. 5 and Fig. S4, ESI†). A change in the fluorescence intensity was saturated at around 16 μM. Most importantly, the plot of fluorescence intensity against ATP concentration showed a sigmoidal curve, which means that the ATP recognition monitored by fluorescence proceeds according to the nonlinear relationship. Such a nonlinear response has been reported for the conversion of molecular information,[17] and utilized to improve the chiral recognition efficiency: that is, the high enantioselectivity can be attained through the combination with the allosteric effect even though the enantioselectivity obtained in the conventional 1 : 1 stoichiometric system is low.[11] In the present fluorescence system, the nonlinear response realized by a steep turn-on switching leads to the high signal-to-background ratio, which is the most critical point in a sensing system.

As mentioned above, the effect of ATP on the fluorescence increase was the most conspicuous. It is undoubted that the electrostatic interaction between guanidinium and triphosphate groups plays the primary role in the aggregation leading to the fluorescence enhancement (Fig. S5, ESI†). Then, does the adenine moiety play any role to facilitate the aggregation? To answer this question, the effects of triphosphate and nucleobase structures on the fluorescence enhancement were investigated and compared with that of ATP. Triphosphate gave the fluorescence intensity comparable with that of ATP at the high concentration (60 μM), whereas at the low concentration (0–16 μM) the fluorescence intensities were generally lowered and the sigmoidal dependence was rather indistinct (Fig. S5, ESI†). This difference is in contrast to the effect of ATP which afforded a steep transition of the fluorescence response even at the low concentration region. This result indicates that the collective π–π stacking in and the hydrophobic interaction with the adenine moiety facilitate cooperative self-assembly to produce the nonlinearly steep fluorescence response. More remarkably, the difference of nucleobase structures was manifested by the fluorescence response (Fig. S6 in ESI†, GTP ≥ ATP > CTP > UTP). It is generally known that a subtle balance of noncovalent intermolecular interactions predominantly impacts on the molecular self-assembly. Here, the subtle balance (the difference of nucleobase structures) was embodied with the fluorescence responses. The recognition of triphosphate along with the discrimination between nucleobase structures could be a striking feature of AIE-based bioprobes that utilize the molecular self-assembly.

In conclusion, we have demonstrated that the AIE-based guanidinium bioprobe shows the nonlinear fluorescence response accompanying self-assembly of TPE with the aid of ATP. The modular design of the AIE-based probe will open wide possibilities for the detection of other biomolecules and polymers. We now envisage that this system would be applicable to sensitive detection of DNA sequences, as expected from the favourable binding of purine-rings over pyrimidine-rings. Also, applications to other biologically essential molecules bearing carboxylate and sulfate anionic groups would be possible. We consider that these future potentials will be realized by the synergistic marriage of molecular self-assembly with AIE-based bioprobes.

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