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PAPER

Functionalization of methyl orange using cationic peptide amphiphile: colorimetric discrimination between ATP and ADP at pH 2.0†

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A solvatochromic and non-fluorescent acid–base indicator, methyl orange (MO) was applied to colorimetric discrimination between adenosine triphosphate (ATP) and the corresponding diphosphate (ADP) at pH 2.0 in the presence of L-glutamic acid-derived cationic peptide amphiphile **1**. This method is based on the fact that the amphiphile **1** can prevent MO from protonation even at pH 2.0. No similar colour change was observed when ADP was added instead of ATP under the same conditions. The effect of the molecular structure of several peptide amphiphiles and dyes was also investigated.

Introduction

We herein demonstrate colorimetric discrimination between ATP and ADP using MO, a common anionic azo dye often used as an acid–base indicator and/or a positively solvatochromic microenvironmental probe, in collaboration with a cationic peptide amphiphile **1** in water at pH 2.0.

We are interested in how commercially available dyes as existing resources are highly functionalized through a supramolecular approach using oppositely charged self-assembling peptide amphiphiles. This methodology would be simple, readily accessible and versatile, because the supramolecular approach using self-assembling acyclic hosts requires much less complicated synthetic procedures and/or laborious synthetic efforts as compared with the preparation of conventional macrocyclic hosts. Furthermore, a range of combinations of self-assembling peptide amphiphiles and commercially available dyes are possible depending on the purpose. Recently, we have demonstrated that appropriately designed self-assembling peptide amphiphiles could induce novel functions in commercially available dyes. For example, anionic peptide amphiphiles that can form extremely hydrophobic sites induced intense fluorescence in very weakly fluorescent cationic dyes in water,[1] and a certain kind of amphiphile–dye complex showed increased sensitivity to alkali metal ions based on Hofmeister's/lyotropic series.[1b] This amphiphile–dye complex could be applicable to turn-on fluorescence sensor for Li^+. Also, self-assembled nonionic peptide lipids containing Gly, β-Ala, sarcosine (*N*-methylglycine) and/or ethylenediamine residues introduced as receptor moieties could induce considerable blue-shift in visible absorption bands of negatively solvatochromic betaine dyes, *i.e.*, Reichardt's Dye and its analogues, by capturing them through cooperation of complementary hydrogen bonding and ion–dipole interactions in nonpolar aprotic chlorobenzene.[2] The absorption bands were remarkably blue-shifted as if the dyes were dissolved in much more polar organic solvents than chlorobenzene. In fact, the large blue-shift could not be attained even by H-aggregation of the dyes under saturated conditions in chlorobenzene. All these unprecedented phenomena in water[1] and nonpolar chlorobenzene[2] could not be attained without the self-assembled peptide amphiphiles/lipids as supramolecular hosts.

As a part of supramolecular functionalizations of a range of commercially available dyes using self-assembling peptide amphiphiles, we would like to demonstrate a new methodology for colorimetric discrimination between ATP and ADP using MO in conjunction with cationic peptide amphiphile **1**. MO is known as a common anionic azo dye that is used as an acid–base indicator and a positively solvatochromic microenvironmental probe. To date, considerable efforts have been devoted to the development of fluorometric detection of ATP because of its high sensitivity.[3] Consequently, only a few reports have been published on colorimetric detection of ATP.[3q,4] In the present study, we have succeeded in supramolecular functionalization of MO as an acid–base indicator with the help of a self-assembling cationic peptide amphiphile **1** in terms of colorimetric discrimination between ATP and ADP at pH 2.0, although fluorometric detection could not be applicable due to non-fluorescent nature of MO. Also, the **1**-MO solution at pH 2.0 exclusively enabled colorimetric discrimination between ATP and ADP. The detection at pH 2.0 does not have any particular advantages over detection at physiological pH since ATP sensors are generally designed to work at physiological pH.[3,4] However, we have also found that local negative charge (*i.e.*, triphosphate moiety) of ambipolar ATP molecule at pH 2.0 has only to be considered for regulation of polyion-complex (PIC) formation with polycations (*i.e.*, self-assembled **1**), without taking

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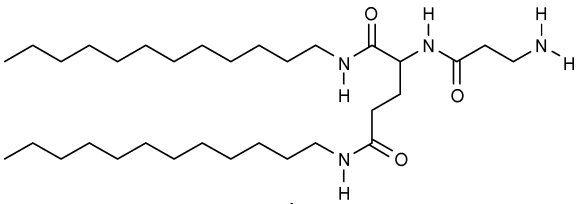
positive charge of local adenine moiety of the ATP molecule into account under such highly acidic pH conditions.



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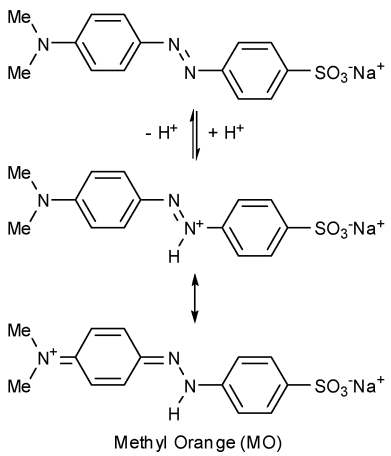
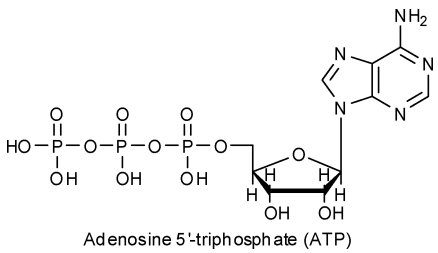




Results and discussion

Spectral behaviour of MO in water at pH 2.0 in the presence of self-assembled 1

It is known that MO shows positive solvatochromism, *i.e.*, the nature of having a red-shift in the absorption maximum wavelength (λ_{max}) with increasing solvent polarity. In fact, the λ_{max}s of MO (0.05 mM) in water and methanol were 465 nm and 426 nm, respectively (Fig. S1 in ESI†). Therefore, MO was used in this study not only as an acid–base indicator but also as a solvatochromic probe in order to confirm that MO is incorporated in the hydrophobic region of self-assembled **1**. When the amphiphile **1** was added to the aqueous solution of MO at pH 6.4 ([**1**] = 1.0 mM, [MO] = 0.05 mM, [**1**]/[MO] = 20, temp.: 20 °C), the original λ_{max} of 465 nm blue-shifted to 417 nm, indicating that MO is incorporated in the inner hydrophobic region of self-assembled **1**.[5] The local polarity of self-assembled **1** in which MO is incorporated is assumed to be slightly lower than that of methanol (λ_{max} = 426 nm in MeOH).

It has been reported that the λ_{max} of the MO species at 465 nm in water (orange colour, pH >4.4) is converted to that at 507 nm (red colour), assigned to the protonated MO species, under highly acidic pH condition below its pK_a (3.46).[6] It is noted that although the pH of the aqueous solution of MO in the presence of self-assembled **1** was lowered to 2.0, the absorption band of the protonated MO species (λ_{max} = 507 nm) was hardly detected, and the λ_{max} of 417 nm (yellow colour) was still maintained as shown in Fig. 1. This indicates that self-assembled **1** prevented MO from protonation even at pH 2.0 which is much lower than the original pK_a (3.46)[6] of the conjugate acid (azonium) form of MO. In fact, the apparent pK_a of MO incorporated in the hydrophobic region of self-assembled **1** was estimated to be 1.2 (Fig. S2†). The remarkable pK_a shift from 3.46 to 1.2 is consistent with the fact that the incorporated MO could remain unprotonated even under highly acidic pH conditions as low as pH 2.0. It has been reported that cyclodextrin,[7] dendrimers[8] and surfactant assemblies[9] can lower the pK_a value of the azonium species of MO as a result of incorporating the free MO species into their hydrophobic cavities. Similar phenomena were reported for lysine residue in protein where the deprotonation of the side chain ε-ammonium group is promoted when buried in hydrophobic core of the protein,[10] and for organic aliphatic carboxylic acids such as lauric acid incorporated in the hydrophobic cavity of cyclodextrin.[11] Therefore, it may stand to reason that the pK_a of the conjugate acid of MO is lowered by being incorporated in the hydrophobic site of self-assembled **1**. The pH value 2.0 coincides with the pH at which the local negative charges of oligophosphate moieties of ATP and ADP in water at 20 °C are estimated to be *ca.* –2.0 and *ca.* –1.0, respectively. This prompted us to investigate the specific polyion-complex (PIC) formation between self-assembled polycationic **1** and ATP at pH 2.0.

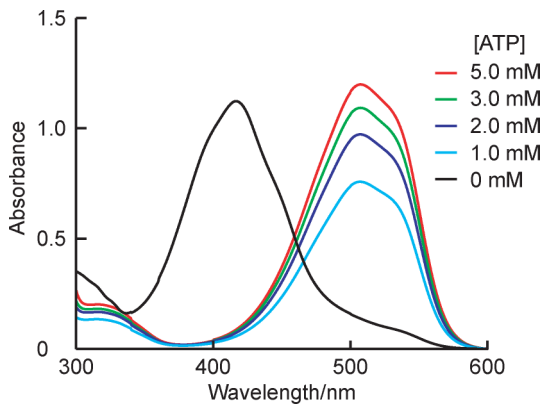


Fig. 1 UV–vis absorption spectra of aqueous solutions of MO in the presence of self-assembled **1** and varying concentrations of ATP; [MO] = 0.05 mM, [**1**] = 1.0 mM, [ATP] = 0, 1.0, 2.0, 3.0 and 5.0 mM, pH 2.0, temp.: 20 °C.

Colorimetric discrimination between ATP and ADP at pH 2.0 using MO and self-assembled 1

The finding that MO incorporated in the hydrophobic region of self-assembled **1** remained unprotonated at pH 2.0 was found to be applicable to colorimetric discrimination between ATP and ADP through regulation of PIC formation with self-assembly of **1**. It is noted that when ATP or ADP was added to the aqueous **1**–MO systems and the pH was adjusted to pH 2.0, only ATP induced a colour change in MO. This indicates that MO was released from the hydrophobic region of self-assembled **1** immediately after PIC formation between self-assembled polycationic **1** and ATP. The

release of MO was promoted effectively by heat treatment in a water bath (85 °C) for *ca.* 20 s. Within 5 min of the heat treatment, the solution (supernatant) was subjected to UV–vis absorption spectral measurements at 20 °C. It was found that the percentage of the released and protonated MO molecule depended on the concentration of ATP as shown in Fig. 1. At the concentrations above 0.75 mM of ATP, the precipitation of the PIC of **1**–ATP could be visually observed.‡ When the concentration of ATP was 5.0 mM, the absorbance at 507 nm was *ca.* 1.2, corresponding to *ca.* 60% of the initial concentration (0.05 mM) of MO. The rest was found to remain incorporated in the precipitate as **1**–MO–ATP.

The colour changes in the aqueous solutions of MO (0.05 mM) in the presence of self-assembled **1** (1.0 mM) at pH 2.0 after the addition of 2.0 mM of biologically important phosphate anions (ATP, ADP, adenosine 5′-monophosphate (AMP), pyrophosphate and phosphate) are shown in Fig. 2. It is noted that when ADP was added to the aqueous **1**–MO system at pH 2.0, the colour change from yellow to red and a precipitate of PIC were not observed. This indicates that ADP cannot form PIC with self-assembled **1** at pH 2.0. From these results, it was found possible to discriminate between ATP and ADP colorimetrically. As shown in Fig. 3 and 4, the sensitivity towards the anions is in the following order: ATP > pyrophosphate > ADP > phosphate ≈ AMP. In particular, ratiometric plots showed remarkably enhanced selectivity for ATP (Fig. 4). From these results, it is suggested that the negative charge of the triphosphate moiety of the ambipolar ATP molecule plays an important role in the PIC formation rather than the net charge of ATP because the above order of sensitivity is consistent with the order of negative charges of the oligophosphate moieties or total negative charges of the anions. The negative charges of the oligophosphate moieties of ATP, ADP, AMP, pyrophosphate and phosphate at pH 2.0 are calculated to be *ca.* −2.01, −1.07, −0.93, −1.43 and −0.56, respectively, based on the pK_a values.§ Since

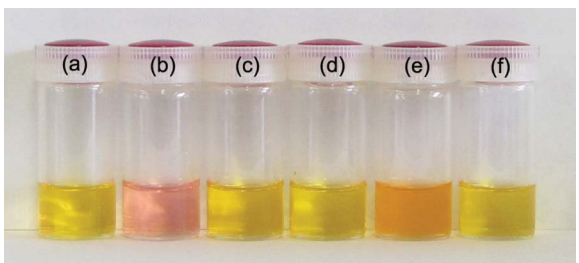


Fig. 2 Digital photograph of aqueous solutions of MO in the presence of self-assembled **1** after addition of various anions; [MO] = 0.05 mM, [**1**] = 1.0 mM, [anion] = 2.0 mM, pH 2.0, temp.: 20 °C; (a) none, (b) ATP, (c) ADP, (d) AMP, (e) pyrophosphate, (f) phosphate.

‡ DSC thermogram showed that the PIC of **1**–ATP still maintained highly-oriented lipid bilayer structure after being allowed to stand at room temperature because obvious crystalline-to-liquid crystalline phase transition phenomena were observed with transition temperature (T_c) of 37 °C and transition enthalpy (ΔH) of 12 kcal mol^{-1} (Fig. S5†), which suggests the existence of densely packed assembly of **1** even in the precipitates of the PIC of **1**–ATP.

§ Sum of negative charges of the oligophosphate moieties of ambipolar ATP, ADP and AMP and total negative charges of anionic pyrophosphate (diphosphate) and phosphate at selected pH were calculated based on the equation $-1 \times [A^-]/([A^-] + [HA]) = -1 \times K_a/(K_a + [H^+])$ using respective pK_a values that appeared in the literature: 0.9, 1.5, 2.3 and 7.7 for ATP;[12] 0.9, 2.8 and 6.8 for ADP;[12] 0.9 and 6.1 for AMP;[13] 0.9, 2.0, 6.6 and 9.4 for pyrophosphoric acid;[12] 1.9, 6.7 and 12.4 for phosphoric acid.[12]

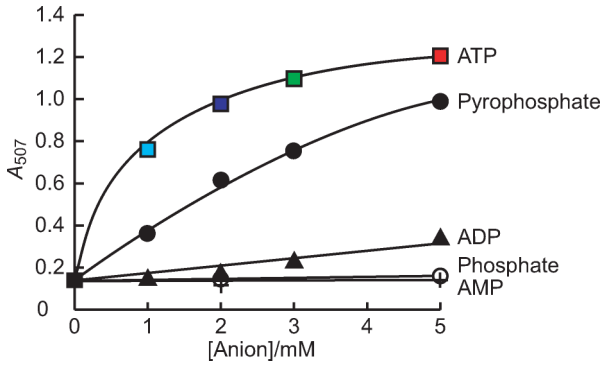


Fig. 3 Relationships between absorbance at 507 nm (A_{507}) of aqueous **1**–MO solutions and different anions at various concentrations. The data for ATP were taken from Fig. 1.

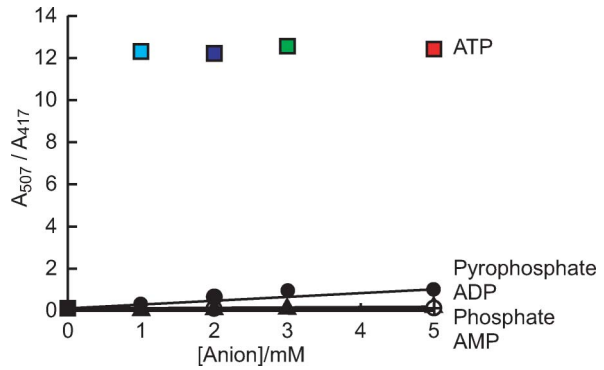


Fig. 4 The dependence of the relative absorbance at 507 and 417 nm (A_{507}/A_{417}) of **1**–MO on the different anions at various concentrations in water; [MO] = 0.05 mM, [**1**] = 1.0 mM, pH 2.0, temp.: 20 °C. The data for A_{507} were taken from Fig. 3.

the net charge of pyrophosphate is obviously more negative than that of ambipolar ATP possessing the protonated adenine moiety (*N*1: pK_a 3.8)[14] under highly acidic pH conditions (at pH 2.0), the preference for ATP over pyrophosphate is inconsistent with the order of the net negative charges. Therefore, these results indicate that whether PIC between nucleotides and self-assembled polycationic **1** can be formed or not could be predicted only from the local negative charges of the nucleotides.

As mentioned above, when ADP was added to the aqueous **1**–MO system at pH 2.0, the precipitation of the PIC and the release of MO were not observed due to the insufficient local negative charge, namely, monoanionic charge. However, it was found that increasing the pH value above 2.0 led to the precipitation of the PIC of **1**–ADP and the release of MO. This means that the diphosphate moiety of ADP could be no longer regarded as monoanionic with increasing the pH value. On the other hand, lowering the pH value below 2.0 resulted in reddish solution even without addition of ATP. In view of these results, the colorimetric discrimination between ATP and ADP by the present method is possible specifically at pH 2.0 by suppressing the degree of the ionization of the oligophosphate moiety. AMP induced no PIC formation of **1**–AMP even at pH 3.0.

The influence of cations that can be typically found in biological fluids (except for Li^+) has also been investigated to make sure that the analytical signal does not change in the presence of these species, and the results were summarized in Table S1.† It was

found that 2 mM of alkaline cations (Li^+, Na^+, K^+), alkaline earth cations (Mg^{2+}, Ca^{2+}), and heavy metal cations (Cu^{2+}, Fe^{2+}) slightly affected the matrix. Addition of excess amount of NaCl (200 mM) led to precipitation of **1**–MO complex due to salting-out effect and MO turned red in the supernatant. These results indicate that interference from even small amounts of these metal cations abundant in any biological fluids makes this detector unsuitable for real applications. It is also noted that tripolyphosphate (2 mM), chondroitin sulfate C (2 unit-mM) and salicylic acid (2 mM) affected more or less the **1**–MO matrix (Table S1†). Consequently, MO turned red even without addition of ATP in the presence of these three anionic species studied. Therefore, it may be concluded that this sensor would be unsuitable for practical applications.

Effect of molecular structure of peptide amphiphiles

It is noted that the kind of cationic peptide amphiphiles that show such specific behaviour is highly restricted as shown by investigations using structurally related cationic peptide amphiphiles **2–4** and DL-**1**. Amphiphiles **2** with one more spacer methylene, **3** with one less spacer methylene and **4** with one less side chain methylene than **1** could not retain unprotonated MO at pH 2.0 before addition of ATP (Fig. S3a–c†). Although the UV–vis absorption spectrum of aqueous **3**–MO before addition of ATP is similar to that of **1**–MO shown in Fig. 1, the solution was not suitable for colorimetric detection of ATP. This is due to the existence of a shoulder band at around 500 nm that affords reddish color ascribed to small amount of protonated MO species. The racemic amphiphile DL-**1** corresponding to **1** exhibited insufficient release of MO when ATP was added to the aqueous DL-**1**–MO system at pH 2.0 (Fig. S3d†). Consequently, the amphiphiles **2–4** and DL-**1** were inferior to **1** for the colorimetric discrimination between ATP and ADP by the present method.

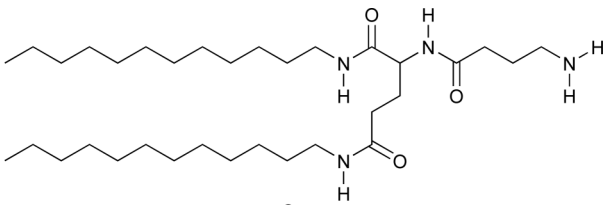
On the other hand, a conventional simple cationic amphiphile, dihexadecyldimethylammonium bromide (**5**),[15] formed a ternary yellow PIC of **5**–MO–ATP at pH 2.0 (Fig. S3e†). Also, hexadecyltrimethylammonium bromide (**6**) could not precipitate with ATP as PIC (Fig. S3f†). In other words, the amphiphiles **5** and **6** exhibited insufficient ATP-triggered release of MO. These results suggest that the side chain of the L-glutamic acid residue of **1** plays an important role in the ATP-triggered release of MO.

Mechanism of colorimetric discrimination between ATP and ADP

In general, cationic/anionic amphiphile assemblies can precipitate when PIC is formed with oppositely charged polyelectrolytes possessing at least divalent anionic/cationic charges in water. At pH 2.0, the negative charge of the diphosphate moiety of ADP is estimated to be *ca.* −1.07 based on the pK_a values (0.9, 2.8 and 6.8)[12] Therefore, it is natural that ADP hardly forms the PIC with self-assembled polycationic **1** at pH 2.0. On the other hand, the negative charge of the triphosphate moiety of ATP is estimated to be *ca.* −2.01 at pH 2.0 based on the pK_a values (0.9, 1.5, 2.3 and 7.7).[12] Therefore, it is natural that ATP forms the PIC and coprecipitate with self-assembled polycationic **1** at pH 2.0.

The influence of the molecular structure of the dyes was also investigated. It is noted that *p*-Methyl Red (*p*-MR) with a carboxylic acid whose pK_a is *ca.* 4 was not applicable to the purpose because a host–guest complex between **1** and *p*-MR could not be maintained at pH 2.0. Therefore, the sulfonate group of MO was found to play an important role in the formation of ionic bond with the ammonium headgroup of **1** at pH 2.0. On the other hand, when fluorescent dansyl acid possessing a sulfonic acid was used instead of MO under the same conditions (at pH 2.0), the dye turned non-fluorescent due to the protonation of the dimethyamino group (pK_a = *ca.* 4[16]). Consequently, among the dyes investigated in this study, only MO could be applied to the colorimetric discrimination of ATP and ADP with the help of self-assembled **1** at pH 2.0.

The present method has the following drawbacks as a colorimetric sensor for quantitative analysis of ATP: (i) this method is applicable only at pH 2.0, not at physiological pH (at *ca.* pH 7.4); (ii) the detection limit as a colorimetric ATP sensor could



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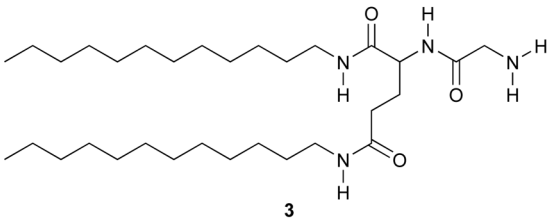

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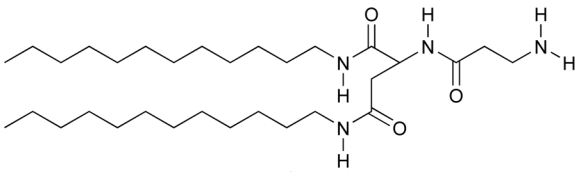

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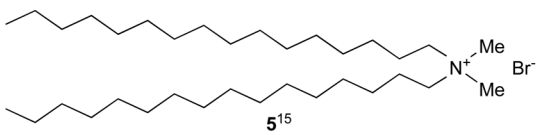

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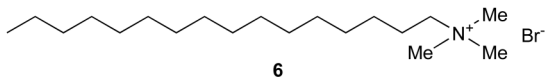

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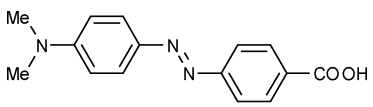
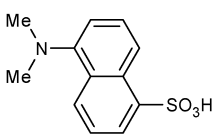


p-Methyl Red (*p*-MR)



Dansyl Acid

be, in principle, somewhat higher than those of the colorimetric molecular sensors reported so far[3q,4] because the present method is based on interaction of MO with the supramolecular assembly of **1** that possesses a critical aggregation concentration (9×10^{-5} M) below which MO incorporated in hydrophobic region of self-assembled **1** would be no longer protected from proton in the bulk water at pH 2.0; (iii) the amphiphile assembly of **1** was not sensitive to small amounts of ATP due to insufficient PIC formation between ATP and **1**. In other words, release of MO from the hydrophobic region of self-assembled **1** was not triggered by ATP under the condition of a high molar ratio of **1** to ATP.

Conclusions

We have demonstrated that a common acid–base indicator, MO, could be functionalized supramolecularly using a cationic peptide amphiphile **1** in terms of colorimetric discrimination between ATP and ADP at in water at pH 2.0. When ATP was added to the aqueous **1**–MO system at pH 2.0, ATP induced a colour change in MO from yellow to red. The drastic change in the colour of the **1**–MO solution from yellow to red was attributed to the protonation of MO released from hydrophobic region of self-assembled **1** to the bulk water/supernatant at pH 2.0. The release of MO was triggered by the PIC formation between self-assembled **1** and the negatively charged triphosphate moiety of the ambipolar ATP molecule. No similar release behaviour was observed for dihexadecyldimethylammonium bromide ($2C_{16}N^+2C_1Br^-$) and hexadecyltrimethylammonium bromide (CTAB), suggesting that the side chain of the L-glutamic acid residue of **1** plays an important role in the ATP-triggered release of MO at pH 2.0. pH 2.0 was found to be a critical pH not only because the yellow MO species incorporated in the hydrophobic region of self-assembled **1** could still remain unprotonated but also because ADP could not form a PIC with the self-assembly of **1** due to insufficient negative charge of the local diphosphate moiety. These ATP-triggered events, *i.e.*, PIC formation, precipitation, release and protonation, happened successively to lead to the colorimetric discrimination between ATP and ADP. Although the detection limit of the present supramolecular system is not so low as those of the state-of-the-art colorimetric sensory systems for ATP,[3q,4] the present methodology would provide a potential application to the construction of nucleotide-triggered release systems for negatively charged water-soluble functional molecules from assemblies of appropriately designed cationic amphiphiles. The present results also provide insight into development of nucleotide-based novel functional materials.

Experimental

Materials and methods

All the peptide amphiphiles were newly synthesized. The amphiphiles derived from α-amino acids denote L-isomers unless otherwise specified. All the dyes (MO, *p*-MR and dansyl acid) were commercially available and used as received.

Preparations of peptide amphiphiles

The peptide amphiphile **1** derived from L-glutamic acid was newly synthesized according to the method reported previously.[2,5] *N*′,*N*″-didodecyl-*N*$^{\alpha}$-[3-(*N*‴-benzyloxycarbonyl)amino]propanoyl-L-glutamide (L-**7**)[2] (1.82 g, 2.65 mmol) was dissolved in ethanol (200 cm^3) with heating and Pd black (1 g) was added to the solution. H_2 gas was bubbled slowly into the solution for 6 h. After confirming the removal of benzyloxycarbonyl group by FTIR measurement, Pd black was removed by filtration. The filtrate was concentrated and dried *in vacuo* to give white solid (**1**): yield 1.46 g (100%); mp 124–128 °C; FTIR (ATR):/cm^{-1} 3287, 2955, 2919, 2851, 1631, 1545, 1466, 1448, 1375 and 715; ^{1}H NMR (400 MHz; $CDCl_3$; Me_4Si): δ 0.86–0.90 (6 H, t, $CH_3 \times 2$), 1.25 (36 H, s, $CH_3(CH_2)_9 \times 2$), 1.43–1.51 (4 H, m, $CH_2CH_2NH \times 2$), 1.96–2.11 (2 H, m, *CHCH_2), 2.30–2.41 (2 H, m, *CHCH_2CH_2), 2.49–2.60 (2 H, m, CH_2CH_2NH_2), 2.93–2.96 (2 H, q, CH_2NH_2), 3.17–3.24 (4 H, m, CH_2NH × 2), 4.33–4.37 (1 H, m, *CH), 6.44 (1 H, t, NH), 7.05–7.08 (1 H, t, NH), 8.64–8.63 (1 H, d, NH); ^{13}C NMR (400 MHz; $CDCl_3$; Me_4Si): δ 14.1 (CH_3), 22.7 (CH_2), 26.9 (CH_2), 27.0 (CH_2), 28.8 (CH_2), 29.31 (CH_2), 29.35 (CH_2), 29.55 (CH_2), 29.63 (CH_2), 29.7 (CH_2), 31.9 (CH_2), 33.1 (CH_2), 35.8 (CH_2), 39.6 (CH_2NH_2), 39.8 (CH_2NH), 52.9 (*CH), 171.1 (C═O), 172.6 (C═O), 172.8 (C═O) (for details of ^{1}H NMR and ^{13}C NMR spectra, see ESI†); (Found: C, 68.70; H, 11.21; N, 9.27. Calc. for $C_{32}H_{64}N_4O_3$: C, 69.51; H, 11.67; N, 10.14%).

N′,*N*″-Didodecyl-*N*$^{\alpha}$-[(3-amino)propanoyl]-DL-glutamide (DL-**1**) was prepared by debenzyloxycarbonylation of the corresponding DL-isomer *N*′,*N*″-didodecyl-*N*$^{\alpha}$-[3-(*N*‴-benzyloxycarbonyl)amino]propanoyl-DL-glutamide (DL-**7**):[2] DL-**1**: yield 0.57 g (100%); mp 101–104 °C; FTIR (ATR):/cm^{-1} 3287, 2958, 2920, 2851, 1632, 1545, 1466, 1451, 1374 and 718; ^{1}H NMR (400 MHz; $CDCl_3$; Me_4Si): δ 0.86–0.90 (6 H, t, $CH_3 \times 2$), 1.25 (36 H, s, $CH_3(CH_2)_9 \times 2$), 1.46–1.52 (4 H, m, CH_2CH_2NH × 2), 1.91–2.02 (1 H, m, *CHCH_2), 2.05–2.13 (1 H, m, *CHCH_2), 2.26–2.31 (2 H, m, *CHCH_2CH_2), 2.38–2.42 (2 H, m, CH_2CH_2NH_2), 2.73–2.76 (2 H, m, CH_2NH_2), 3.17–3.26 (4 H, m, CH_2NH × 2), 4.33–4.38 (1 H, q, *CH), 6.24 (1 H, s, NH), 6.77–6.79 (1 H, t, NH), 8.95–8.97 (1 H, d, NH); ^{13}C NMR (400 MHz; $CDCl_3$; Me_4Si): δ 14.1 (CH_3), 22.7 (CH_2), 26.9 (CH_2), 27.0 (CH_2), 29.2 (CH_2), 29.30 (CH_2), 29.35 (CH_2), 29.56 (CH_2), 29.60 (CH_2), 29.63 (CH_2), 31.9 (CH_2), 33.0 (CH_2), 33.1 (CH_2), 39.6 (CH_2NH_2), 39.7 (CH_2NH), 52.4 (*CH), 171.1 (C═O), 172.5 (C═O), 173.1 (C═O) (for details of ^{1}H NMR and ^{13}C NMR spectra, see ESI†); (Found: C, 69.99; H, 11.45; N, 8.86. Calc. for $C_{32}H_{64}N_4O_3$: C, 69.51; H, 11.67; N, 10.14%).

All the other cationic peptide amphiphiles were prepared by debenzyloxycarbonylation of the corresponding various *N*-protected lipophilic lipids.[2] *N*′,*N*″-didodecyl-*N*$^{\alpha}$-[(4-amino)butanoyl]-L-glutamide (**2**): yield 1.09 g (100%); mp 106–111 °C; FTIR (KBr):/cm^{-1} 3298, 3100, 2958, 2922, 2852, 1638, 1562, 1466, 1450, 1379 and 721; ^{1}H NMR (400 MHz; $CDCl_3$; Me_4Si): δ 0.86–0.90 (6 H, t, $CH_3 \times 2$), 1.20–1.34 (36 H, m, $CH_3(CH_2)_9 \times 2$), 1.43–1.50 (4 H, m, CH_2CH_2NH × 2), 1.79–2.09 (4 H, m, *CHCH_2, CH_2CH_2NH_2), 2.28–2.41 (4 H, m, C(═O)CH_2 × 2), 2.75–2.81 (2 H, m, CH_2NH_2), 3.01 (2 H, br s, NH_2), 3.20–3.23 (4 H, q, CH_2NH × 2), 4.30–4.35 (1 H, q, *CH), 4.33–4.38 (1 H, q, *CH), 6.65 (1 H, m, NH), 7.02–7.04 (1 H, m, NH), 7.83–7.84 (1 H, d, NH); ^{13}C NMR (400 MHz; $CDCl_3$; Me_4Si): δ 14.1 (CH_3), 22.7 (CH_2), 26.9 (CH_2), 27.0 (CH_2), 29.1 (CH_2), 29.4 (CH_2), 29.5 (CH_2), 29.58 (CH_2), 29.64 (CH_2), 31.9 (CH_2), 33.0 (CH_2), 33.8 (CH_2), 39.7 (CH_2NH), 39.8 (CH_2NH), 43.5 (CH_2NH), 52.9 (*CH), 171.5 (C═O), 172.7 (C═O), 173.0 (C═O) (for details of ^{1}H NMR and ^{13}C NMR spectra, see ESI†); (Found: C, 66.55;

H, 11.15; N, 8.48. Calc. for $C_{33}H_{66}N_4O_3$: C, 66.91; H, 11.74; N, 9.89%). *N′*,*N″*-didodecyl-*N*$^{\alpha}$-[(2-amino)ethanoyl]-L-glutamide (**3**): yield 0.61 g (100%); mp 100–107 °C; FTIR (ATR):/cm^{-1} 3286, 2956, 2919, 2851, 1636, 1548, 1466, 1453, 1376 and 720; ^{1}H NMR (400 MHz; $CDCl_3$; Me_4Si): δ 0.86–0.90 (6 H, t, $CH_3 \times 2$), 1.25 (36 H, s, $CH_3(CH_2)_9 \times 2$), 1.48–1.50 (4 H, m, $CH_2CH_2NH \times 2$), 1.90–2.02 (1 H, m, *CHCH_2), 2.06–2.16 (1 H, m, *CHCH_2), 2.25–2.38 (2 H, m, C(═O)CH_2CH$_2$), 2.74–2.80 (2 H, m, NH_2), 3.16–3.26 (4 H, m, $CH_2NH \times 2$), 3.42–3.49 (2 H, m, CH_2NH_2), 4.37–4.43 (1 H, m, *CH), 6.55–6.61 (1 H, m, NH), 7.26 (1 H, m, NH), 8.20–8.25 (1 H, m, NH); ^{13}C NMR (400 MHz; $CDCl_3$; Me_4Si): δ 14.1 (CH_3), 22.7 (CH_2), 26.97 (CH_2), 27.04 (CH_2), 29.4 (CH_2), 29.46 (CH_2), 29.55 (CH_2), 29.6 (CH_2), 29.7 (CH_2), 29.8 (CH_2), 31.9 (CH_2), 33.0 (CH_2), 39.69 (CH_2NH), 39.74 (CH_2NH), 44.0 (CH_2NH_2), 52.5 (*CH), 171.0 (C═O), 172.6 (C═O) (for details of ^{1}H NMR and ^{13}C NMR spectra, see ESI†); (Found: C, 65.99; H, 10.92; N, 9.09. Calc. for $C_{31}H_{62}N_4O_3$: C, 69.09; H, 11.60; N, 10.40%). *N′*,*N″*-didodecyl-*N*$^{\alpha}$-[(3-amino)propanoyl]-L-aspartamide (**4**): yield 0.419 g (69.7%); mp 150–154 °C; FTIR (ATR):/cm^{-1} 3280, 2955, 2917, 2872, 2849, 1688, 1640, 1543, 1465, 1375 and 721; ^{1}H NMR (400 MHz; $CDCl_3$; Me_4Si): δ 0.86–0.90 (6 H, t, $CH_3 \times 2$), 1.25 (36 H, s, $CH_3(CH_2)_9 \times 2$), 1.43–1.47 (4 H, m, $CH_2CH_2NH \times 2$), 1.79 (2 H, br s, NH_2), 2.36–2.51 (4 H, m, C(═O)$CH_2 \times 2$), 2.85–3.07 (2 H, m, CH_2NH_2), 3.15–3.23 (4 H, m, $CH_2NH \times 2$), 4.68–4.71 (1 H, m, *CH), 6.21–6.23 (1 H, m, NH), 7.70–7.73 (1 H, t, NH), 8.14–8.16 (1 H, d, NH); ^{13}C NMR (400 MHz; $CDCl_3$; Me_4Si): δ 14.1 (CH_3), 22.7 (CH_2), 26.9 (CH_2), 29.3 (CH_2), 29.36 (CH_2), 29.42 (CH_2), 29.56 (CH_2), 29.58 (CH_2), 29.63 (CH_2), 31.9 (CH_2), 36.0 (CH_2), 36.2 (CH_2), 37.3 (CH_2NH_2), 39.7 (CH_2NH), 39.8 (CH_2NH), 50.3 (*CH), 170.6 (C═O), 171.2 (C═O), 172.3 (C═O) (for details of ^{1}H NMR and ^{13}C NMR spectra, see ESI†); (Found: C, 68.29; H, 11.26; N, 9.69. Calc. for $C_{31}H_{62}N_4O_3$: C, 69.09; H, 11.60; N, 10.40%).

Characterization of peptide amphiphiles

The chemical structures of all the compounds synthesized were confirmed by Fourier transform infrared spectroscopy (FTIR) measurement with a JASCO FT/IR-7000 or PerkinElmer Spectrum 100, by ^{1}H NMR and ^{13}C NMR measurements with JEOL ECS-400 FT-NMR, and by elemental analysis with a Yanaco CHN Corder MT-3.

Preparation of aqueous dispersions of amphiphiles

The amphiphiles were suspended in water (pH 6) and quickly sonicated in a water bath using Ultrasonic Cleaner US-2R produced by AS ONE Co. Ltd., followed by heating in hot water. The procedure was repeated several times until homogeneous dispersions were obtained. Then the pH was adjusted with hydrochloric acid and measured using a Standard Glass Combination Electrode with Ag/AgCl (Fisher Scientific) attached to an Accumet AR50 pH meter (Fisher Scientific).

Preparation of aqueous amphiphile–dye mixtures

All the solutions of amphiphile–dye complexes were prepared by addition of stock solutions of the dyes to the aqueous dispersions of the amphiphiles and by subsequent sonication. After adjusting to pH 2.0 at 20 °C, the solutions were heated for *ca.* 1 min and allowed to stand at room temperature for 5 min in the dark. The solutions were subjected to UV–vis absorption and fluorescence spectral measurements at 20 °C.

Preparation of aqueous amphiphile–dye–anion mixtures

The aqueous mixtures of **1** (1.0 mM)–MO (0.05 mM) and 5.0 mM of anions (ATP, ADP, AMP, pyrophosphate and phosphate) were prepared by addition of 1 cm^3 of stock solutions (20 mM) of anions to 3 cm^3 of the aqueous amphiphile (1.33 mM)–MO (0.067 mM) solutions, respectively. Aqueous mixtures with lower anion concentrations were prepared by addition of the diluted stock solutions to the aqueous amphiphile–MO solutions. After adjusting to pH 2.0, the aqueous mixtures were sonicated and heated. After being allowed to stand at room temperature for 5 min in the dark, the aqueous mixtures were subjected to UV–vis absorption spectral measurements. Other amphiphiles (**2–6** and DL-**1**) and dyes (*p*-MR and dansyl acid) were also used instead of **1** and MO under the same conditions.

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