

# Intense fluorescence-inducing amphiphile in cationic dyes and its applicability†

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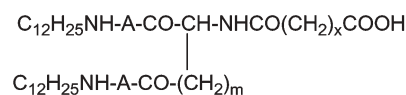
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An anionic amphiphile has been found to form extremely hydrophobic sites in water and specifically incorporate stilbazolium-based compact hemicyanine dyes as monomeric species, resulting in induction of intense fluorescence emission.

Fluorescent dyes are known to be very useful materials for sensory systems,<sup>1a</sup> probing microenvironments,<sup>1b</sup> fluorescence resonance energy transfer (FRET),<sup>1b</sup> and also dye-sensitized solar cells,<sup>2,3</sup> etc. However, the fluorescence property is, in general, largely dependent on and limited by the chemical structure of the dye itself. This can be improved to some extent by lowering the polarity of organic solvents. However, many kinds of ionic dyes are too polar to dissolve in non-polar organic solvents. Also, the molecular design of dyes for improved properties generally causes problems in terms of a complicated synthesis procedure, etc. It is known that those fluorescent dyes that exhibit intramolecular charge transfer (ICT) character are strongly solvatochromic and generally show low fluorescence efficiency in water.<sup>4</sup> Therefore, increasing local hydrophobicity in an aqueous amphiphile assembly system would be a promising method in order not only to circumvent such problems but also to readily utilize numerous kinds of commercially available, water-soluble, ionic dyes as fluorescent ones. Tightly packed self-assembly of appropriately designed amphiphiles in water could be a powerful candidate for this purpose. However, since there has been a limitation to lower the polarity of local hydrophobic region in aqueous molecular assemblies using conventional amphiphiles, development of a novel self-assembling compound that can provide unprecedentedly highly specific/hydrophobic environment for water-soluble dyes in water is desired. In this study, we developed an anionic amphiphile **1** that induces molecular recognition-based intense fluorescence emission in incorporated monomeric stilbazolium dyes that originally fluoresce very weakly in water.

Anionic peptide amphiphiles (**1–5**) with strong self-assembling nature in water due to complementary intermolecular hydrogen bonding<sup>5</sup> were prepared according to ordinary peptide synthesis procedures. Anionic amphiphiles **1**, **2** and **5** were designed for further increase in intermolecular hydrogen bonding moieties over those of **4**.<sup>5</sup> Amphiphile **3** was designed for increased side-chain hydrophobicity as compared with that of **4**. Amphiphile **5** was designed for increased hydrophobicity over that of **1** and **2** using

$\gamma$ -amino butyric acid (GABA) instead of  $\beta$ -alanine ( $\beta$ -Ala). All the dyes were commercially available and used without further purification. Aggregate morphologies of **1–5** in water were observed using transmission electron microscopy (TEM): tubules and ribbons for **1** (see ESI†), tubules and ribbons for **2**, tubules for **3** (see ESI), tubules and helices for **4** and long tubules for **5**.



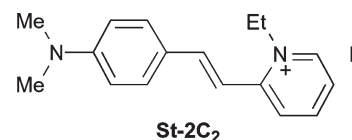
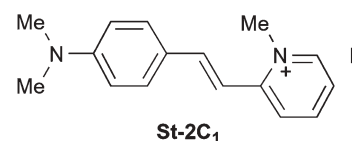
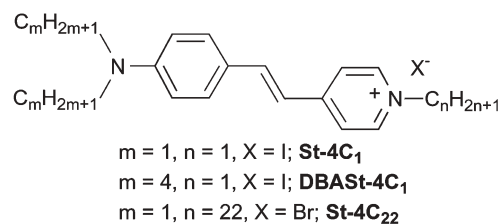
**1**: A =  $\beta$ -Ala, m = 2, x = 3

**2**: A =  $\beta$ -Ala, m = 2, x = 2

**3**: A = -, m = 3, x = 3

**4**<sup>5</sup>: A = -, m = 2, x = 3

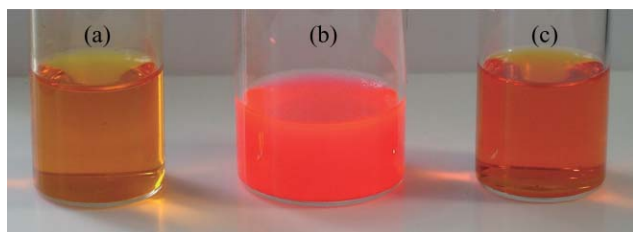
**5**: A = GABA, m = 2, x = 3



To investigate the inner hydrophobicity of self-assembly of **1** in water, solvatochromic stilbazolium dye, *trans*-4-[4-(dimethylamino)styryl]-1-methylpyridinium iodide (abbreviated as St-4C<sub>1</sub> hereafter) was used as a probe. The St-4C<sub>1</sub> is known to exhibit negative solvatochromism, that is, the nature of red-shift of the absorption maximum wavelength ( $\lambda_{\text{max}}$ ) with decreasing solvent polarity. The  $\lambda_{\text{max}}$  of St-4C<sub>1</sub> (0.15 mM) in water and in methanol (containing 10 vol% water) are 448 and 475 nm,<sup>5</sup> respectively, as shown in Fig. 1 and Fig. 2 (a). When 20 mM of sodium dodecyl sulfate (SDS) was added to the aqueous solution, the  $\lambda_{\text{max}}$  shifted from 448 nm to 475 nm. This indicates that the local polarity of SDS micelles in water is comparable to that in methanol. Similar results have already been obtained for several peptide amphiphiles,<sup>5</sup> indicating that the polarity in a range of conventional

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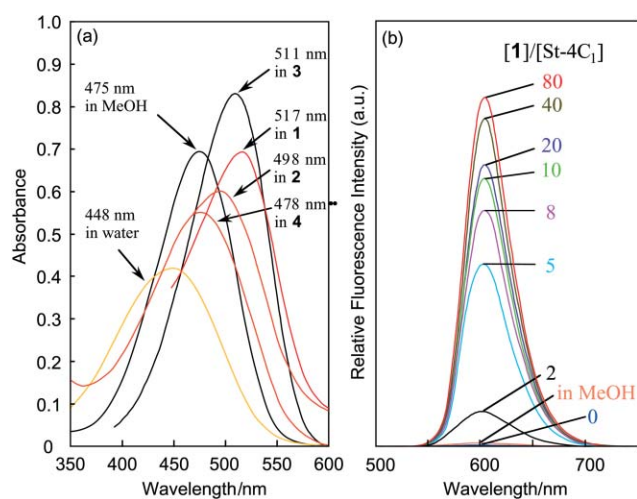
† Electronic supplementary information (ESI) available: Transmission electron micrographs of **1** and **3** and full chemical structure and supposed interaction mode. See DOI: 10.1039/b517483j



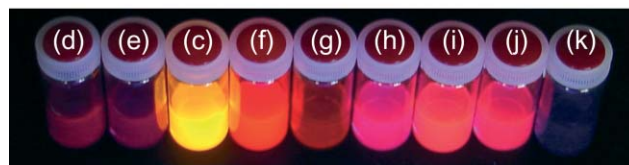
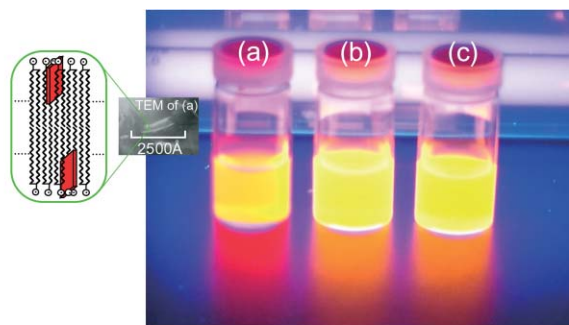
**Fig. 1** Photograph of aqueous solutions of St-4C<sub>1</sub>: pH 10, [St-4C<sub>1</sub>] = 0.15 mM = const., [I] = 3.0 mM. (a) St-4C<sub>1</sub> alone in water, (b) St-4C<sub>1</sub> in water in the presence of 3 mM of amphiphile **1**, (c) St-4C<sub>1</sub> alone in methanol.

amphiphile assemblies in water is at most comparable to that of the polar organic solvent, methanol. It is noted that, when **1** is added to the aqueous solution of St-4C<sub>1</sub>, the  $\lambda_{\text{max}}$  shifted up to 517 nm as shown in Fig. 1 and Fig. 2 (a). Similarly, the  $\lambda_{\text{max}}$  of St-4C<sub>1</sub> were 498 nm and 511 nm for **2** and **3**, respectively, as shown in Fig. 2 (a). The  $\lambda_{\text{max}}$  of St-4C<sub>1</sub> in chlorobenzene was *ca.* 517 nm although its solubility was considerably limited. This indicates that the polarities of inner regions of assemblies of **1** and **3** are comparable to those of nonpolar aprotic solvents such as chlorobenzene. It is also noted that the  $\lambda_{\text{max}}$  of 498 nm for **2** indicates that decreased spacer methylene number (*x*) compared with that of **1** is not suitable for the formation of a specific cavity for St-4C<sub>1</sub>. On the other hand, when  $\beta$ -Ala residues (**1**) were replaced by GABA residues (**5**), no appreciable  $\lambda_{\text{max}}$  shift from 448 nm was observed, indicating that St-4C<sub>1</sub> was not incorporated into the hydrophobic region and existed as a monomer in contact with bulk water in the aqueous **5** system. These results clearly indicate that the molecular design of the inner hydrophobic region of amphiphiles plays a crucial role in the specific incorporation of St-4C<sub>1</sub> as a monomeric one, resulting in induction of intense fluorescence emission.

Next, molar ratio dependence on the  $\lambda_{\text{max}}$  was investigated to confirm the assumption that the highly red-shifted absorption



**Fig. 2** (a) UV spectra of aqueous solutions of St-4C<sub>1</sub>: temperature 20 °C, pH 10, [St-4C<sub>1</sub>] = 0.15 mM = const., [I] = [2] = [3] = [4] = 3.0 mM. (b) Fluorescence spectra of St-4C<sub>1</sub> in the presence of **1** in water:  $\lambda_{\text{ex}}$  = 570 nm in the presence of **1**,  $\lambda_{\text{ex}}$  = 534 nm in the absence of **1** in water,  $\lambda_{\text{ex}}$  = 546 nm in methanol : water = 9 : 1 (v/v), [St-4C<sub>1</sub>] = 0.15 mM = constant, [I]/[St-4C<sub>1</sub>] = 2, 5, 8, 10, 20, 40, 80, temperature 20 °C, pH 10.



**Fig. 3** Photographs of aqueous solutions of **1** and various cationic dyes under UV irradiation: pH 10, [I] = 3.0 mM, [cationic dye] = 0.15 mM. (a) St-4C<sub>1</sub>, (b) St-2C<sub>1</sub>, (c) St-2C<sub>2</sub>, (d) Quinaldine Red, (e) 2-[4-(dimethylamino)styryl]-1-methylquinolinium iodide, (f) *trans*-4-[4-(dibutylamino)styryl]-1-methylpyridinium iodide, (g) 1,1'-diethyl-2,2'-cyanine iodide, (h) 3,3'-dipropylthiobarbitone iodide, (i) 3,3'-diethyl-9-methylthiobarbitone iodide, (j) 3,3'-diethyl-9-ethylthiobarbitone iodide, (k) 4-[4-(diethylamino)phenylazo]-1-methylpyridinium iodide. All the solutions were visually non-fluorescent without **1** and became fluorescent except for (k) on addition of **1**.

band of St-4C<sub>1</sub> is not due to J-aggregates<sup>6</sup> but incorporated monomeric species. In general, aggregated dye species are converted to monomeric species with increasing molar ratio of amphiphile to dye.<sup>5,7</sup> The visible absorption bands of St-4C<sub>1</sub> did not convert to any other bands in the presence of **1–3** with increasing molar ratio of amphiphile to dye up to 80 under the condition of constant dye concentration (0.15 mM). This indicates that the  $\lambda_{\text{max}}$  of St-4C<sub>1</sub> (517, 498, and 513 nm) in the presence of **1–3** are ascribed to incorporated monomeric species. These results indicate that the highly red-shifted  $\lambda_{\text{max}}$  of St-4C<sub>1</sub> is due to specifically incorporated ones as monomer into the inner hydrophobic region of **1–3**. Such red-shifted  $\lambda_{\text{max}}$  have never been attained by using conventional amphiphile assemblies such as SDS ( $\lambda_{\text{max}}$ : 475 nm) and **4** ( $\lambda_{\text{max}}$ : 478 nm).<sup>5</sup> It is noted that the red-shifted bright color for **1**-St-4C<sub>1</sub> and **2**-St-4C<sub>1</sub> systems were hardly changed up to the temperatures near 100 °C, because the crystalline-to-liquid crystalline phase transition temperatures (*T<sub>c</sub>*) of **1** and **2** are around the boiling point of water.‡ The bright color of **3**-St-4C<sub>1</sub> system was reconverted to the original color of St-4C<sub>1</sub> alone in pure water due to low *T<sub>c</sub>* (45 °C).‡ The color of the solution of **1**-St-4C<sub>1</sub> system has been maintained for three years at room temperature. Fig. 2 (b) shows fluorescence spectra of various aqueous **1**-St-4C<sub>1</sub> systems with different amphiphile concentrations. Relative fluorescence intensities of St-4C<sub>1</sub> alone in water and methanol are much smaller than those for aqueous **1**-St-4C<sub>1</sub> systems. This indicates that fluorescence of St-4C<sub>1</sub> in water is anomalously enhanced (*ca.* 450 times at molar ratio of 80) in the presence of **1**.

Next, analogues of St-4C<sub>1</sub> were investigated to clarify the dye specificity towards **1**. It is noted that St-2C<sub>1</sub> and St-2C<sub>2</sub> exhibited the same  $\lambda_{\text{max}}$  at 500 nm in the presence of **1** at 20 °C. These same

$\lambda_{\text{max}}$  strongly suggest that the red-shifted species of St-2C<sub>1</sub> and St-2C<sub>2</sub> are not J-aggregates but incorporated monomeric species, respectively, not only because all these three dyes (St-4C<sub>1</sub>, St-2C<sub>1</sub>, and St-2C<sub>2</sub>) red-shifted by ca. 40 nm, respectively, from the  $\lambda_{\text{max}}$  of dyes alone in methanol ( $\lambda_{\text{max}}$  change: 475 nm→517 nm for St-4C<sub>1</sub>, 460 nm→500 nm for St-2C<sub>1</sub>, and 460 nm→500 nm for St-2C<sub>2</sub>), but also because almost the same differences of  $\lambda_{\text{max}}$  ( $\Delta\lambda_{\text{max}}$ : ca. 40 nm) suggest the limit of lowered polarity in water attained by assembly of **1**. The  $\lambda_{\text{max}}$  of J-aggregates are known to depend on the angle between aggregated dyes with head-to-tail orientation.<sup>6</sup> Therefore, it is unnatural to ascribe the species of the 500 nm of St-2C<sub>1</sub> and St-2C<sub>2</sub> to J-aggregates. In fact, J-aggregated St-4C<sub>1</sub> exhibited  $\lambda_{\text{max}}$  of around 550 nm.<sup>5</sup> From these results, it may safely be concluded that the present  $\lambda_{\text{max}}$  (497–517 nm) of St-4C<sub>1</sub> in the presence of lipids in Fig. 2 (a) are ascribed to those of monomeric species existing in respective highly hydrophobic and specific local microenvironments.

Various cationic dyes were also investigated using amphiphiles **1–5**. Fig. 3 shows representative photographs of aqueous solutions of various cationic dyes in the presence of 3 mM of **1** under UV irradiation. It is noted that **1** induces fluorescence emission in most of the dyes used except for azo dye (k) 4-[4-(diethylamino)phenylazo]-1-methylpyridinium iodide. However, dye species existing in the solutions (f), (g), (h), (i), and (j) are found to be not only incorporated monomeric species but also J-aggregates. It is noted that no similar versatility in terms of fluorescence induction in various cationic dyes was observed by amphiphiles **2–5**, suggesting that the self-assembly of **1** is specific and exhibits molecular recognition phenomena based on, especially, a stilbazolium moiety with relatively small substituents. The following results from visible absorption spectra are consistent with the conclusion. (i) Although solutions (d) and (e) containing quinolinium-containing dyes that correspond to pyridinium-containing dyes St-2C<sub>2</sub> and St-2C<sub>1</sub>, respectively, existed as incorporated monomers ( $\lambda_{\text{max}}$ : 570 nm and 567 nm), these dyes exhibited less intense fluorescence as compared with St-2C<sub>2</sub> and St-2C<sub>1</sub>. (ii) Stilbazolium dye, DBASt-4C<sub>1</sub> (solution (f)) with bulky *N,N*-dibutylamino group was less incorporated into the hydrophobic region than St-4C<sub>1</sub> in spite of its increased hydrophobicity. (iii) The dye (St-4C<sub>22</sub>) with *N*-docosyl group was hardly incorporated and practically non-fluorescent. These results strongly suggest that amphiphile **1** can recognize stilbazolium-based compact dyes to incorporate as monomeric species, resulting in molecular recognition-based induction of intense fluorescence emission.

Amphiphile **1** would provide potential application to fluorescent sensory systems because **1** could afford many other cationic dyes enhanced fluorescence that enables sensing by reduced fluorescence intensity. Also, it may be promising for a novel anode photosensitizer of dye-sensitized solar cell (DSSC),<sup>8,9</sup> provided that the cationic dye bound to one carboxylate of amphiphile can be immobilized onto/near the TiO<sub>2</sub> surface through dye-free carboxylates of adjacent amphiphiles in their self-assemblies as shown in Fig. 3. The preliminary DSSC experiments using

commercially available DSSC kit (Nishinoda Electronics Co. Ltd, Japan) showed that DSSC prepared using an aqueous solution of **1**-St-4C<sub>1</sub> (Fig. 1 (b)) at boiling point under liquid crystalline conditions<sup>‡</sup> gave short-circuit current density ( $J_{\text{sc}}$ ) twice as large as that for St-4C<sub>1</sub> alone in water, although its specifications in terms of solar energy-to-electricity conversion efficiency ( $\eta$ ) is limited. This suggests that constructing thermally stable<sup>2b</sup> amphiphile-dye nanohybrid such as **1**-St-4C<sub>1</sub> is a promising methodology for potential application to DSSC, because numerous kinds of commercially available highly fluorescent cationic dyes such as Rhodamine 6G<sup>8</sup> are incapable of effectively adsorbing onto TiO<sub>2</sub> nanocrystals due to a lack of appropriate coordinating functional groups<sup>2,3,9</sup> such as carboxylate or hydroxyl groups, and therefore, only limited classes of metal-free organic dyes have been investigated to date.<sup>3,9</sup>

In summary, amphiphile **1** was found to form highly specific hydrophobic sites enough to induce intense fluorescence emission in solvatochromic stilbazolium dyes with compact push-pull substituents and other related cationic hemicyanine, thiacyanine, and thiacyanocyanine dyes except for the corresponding azo dye.

## Notes and references

‡ Crystalline-to-liquid crystalline phase transition temperature ( $T_c$ ) and transition enthalpy ( $\Delta H$ ) of lipid bilayer-based aggregates estimated by differential scanning calorimetry (DSC) measurement were as follows:  $T_c$ , 89 °C ( $\Delta H$ ; 2.6 kcal mol<sup>-1</sup>) and  $T_c$ , 102 °C ( $\Delta H$ ; 5.4 kcal mol<sup>-1</sup>) for **1**;  $T_c$ , 83 °C ( $\Delta H$ ; 3.9 kcal mol<sup>-1</sup>) and  $T_c$ , 107 °C ( $\Delta H$ ; 5.1 kcal mol<sup>-1</sup>) for **2**;  $T_c$ , 45 °C ( $\Delta H$ ; 2.9 kcal mol<sup>-1</sup>) for **3**;  $T_c$ , 49 °C ( $\Delta H$ ; 5 kcal mol<sup>-1</sup>) for **4**;  $T_c$ , 45 °C, 66 °C, ( $\Delta H$ ; 10 kcal mol<sup>-1</sup>) for **5**.

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