Amphiphilic *p*-sulfonatocalix[4]arene-coated CdSe/ZnS quantum dots for the optical detection of the neurotransmitter acetylcholine†

Takashi Jin,*[a] Fumihiko Fujii,[b] Hiroshi Sakata,[b] Mamoru Tamura[a] and Masataka Kinjo[a]

Water-soluble CdSe/ZnS (core–shell) semiconductor quantum dots surface-modified with tetrahexyl ether derivatives of *p*-sulfonatocalix[4]arene were synthesized for the optical detection of the neurotransmitter acetylcholine.

Colloidal quantum dots (QDs) have recently attracted considerable attention as a new class of fluorophores for chemical and biological applications.[1] QDs are semiconductor nanoparticles that have all three dimensions confined to the 2–15 nm length scale.[2] The optical properties of QDs have significant advantages compared with traditional organic fluorescent dyes. QDs are highly bright fluorophores, and they are highly resistant to photobreaching, making them useful for continuous monitoring of fluorescence intensity.[1,2] Besides, QDs have size-tunable narrow emission spectra (typical full width at half maximum <30 nm) in addition to broad excitation spectra.[3] This unique optical property allows a single-light source excitation for multi-colour emission of QDs. Since the first reports[4] of the use of QDs as fluorescent bioprobes in 1998, many applications of QDs have been reported in the field of cellular and biomedical imaging.[3,5]

In contrast, less attention has been paid to the use of QDs as fluorescent probes for chemical and biochemical sensing. So far, several reports of QD-based ion probes have appeared for the fluorescence sensing of metal ions, such as Ag^+, Cu^{2+}, Cd^{2+}, Co^{2+}, and Zn^{2+}.[6] A recent paper has shown the highly sensitive determination of cyanide ions by using CdSe QDs in aqueous solutions.[7] However, possible applications of the QD-based fluorescent probes of biologically important ions or molecules are virtually unexplored. It is well known that the fluorescence efficiency of QDs is sensitive to the presence and nature of adsorbates at the surface of QDs.[1a] Therefore, it is expected that a chemical sensing system based on QDs can be developed using fluorescence changes induced by molecular recognition at the surface of QDs.

Here, we report an optical detection system for the neurotransmitter acetylcholine (ACh) using water-soluble CdSe/ZnS quantum dots surface-modified with tetrahexyl ether derivatives of *p*-sulfonatocalix[4]arene **3** (Scheme 1).

[a]*Section of Intelligent Materials and Devices, Research Institute for Electronic Science, Hokkaido University, Sapporo, 060-0812, Japan. E-mail: jin@imd.es.hokudai.ac.jp; Fax: +81-11-706-4964; Tel: +81-11-706-2886*
[b]*Innovation Plaza Hokkaido, Japan Science and Technology Agency, Sapporo, 060-0819, Japan. Fax: +81-11-708-1614; Tel: +81-11-708-1614*
† Electronic supplementary information (ESI) available: experimental details for the preparation of QDs and tetrahexyl ether derivatives of *p*-sulfonatocalix[4]arene **3**, ^{1}H NMR data for **3**, and the surface-coating method using **3**. See http://dx.doi.org/10.1039/b506608e

CdSe/ZnS QDs were prepared in a two-step process (ESI†). First, CdSe QDs were synthesized using a method reported by Qu and Peng[8] with a high temperature reaction in a trioctylphosphine oxide (TOPO)–hexadecylamine mixture. Then, the CdSe core was overcoated with a ZnS shell using TOPO as a solvent according to previously published methods.[9] The resulting CdSe/ZnS QDs were capped with TOPO molecules, and they were highly hydrophobic and insoluble in aqueous media.
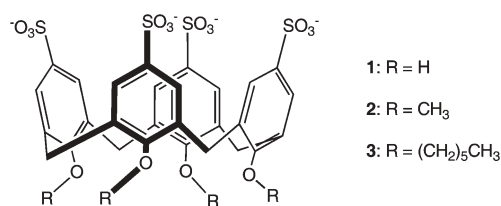
To prepare water-soluble CdSe/ZnS QDs, we used amphiphilic *p*-sulfonatocalix[4]arenes **1–3** (Scheme 1) as surface-coating agents for the TOPO capped QDs. *p*-Sulfonato[4]calixarene **1** is known to have the ability to bind the quaternary ammonium cation moiety of ACh by its hydrophobic cavity of the aromatic rings.[10] On the basis of this finding, we expected that if TOPO capped QDs could be coated by amphiphilic *p*-sulfonatocalix[4]arenes to form water-soluble QDs, the resulting QDs would have complexing abilities towards ACh at the surface of the QDs.

p-Sulfonatocalix[4]arenes **1** was purchased from Tokyo Kasei Chemicals (Japan) and its derivatives, **2** and **3** were prepared according to the literature method (ESI†).[11] The surface-coating of TOPO capped CdSe/ZnS QDs was performed by mixing the hydrophobic QDs and **1–3** in tetrahydrofuran at room temperature (ESI†). When **1** and **2** were used as the surface-coating agents, the resulting QDs showed very week emission, and they precipitated after several hours in water. In the case of **3**, highly fluorescent and stable, water-soluble QDs were obtained.

Fig. 1 shows the fluorescence spectrum of **3**-coated CdSe/ZnS QDs in water. For comparison, the fluorescence spectrum of water-soluble CdSe/ZnS QDs coated by mercaptoacetic acid (MAA) is also shown. MAA is one of the widely used surface-coating agents used to prepare water-soluble monodisperse QDs.[1] The emission efficiency of the **3**-coated QDs is higher than that of the MAA-coated QDs by a factor of about 3. The quantum yield was estimated as 0.1 using Rhodamine 6G as standard. The spectral width (28 nm) of the **3**-coated QDs is almost the same as that of MAA-coated QDs, indicating that the **3**-coated QDs are monodisperse particles similar to the case of MAA-coated QDs.

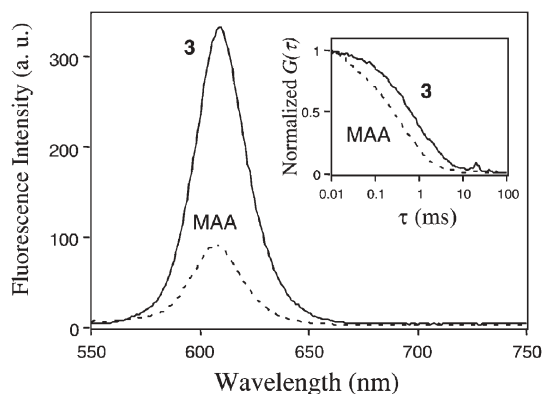**Scheme 1** *p*-Sulfonatocalix[4]arene (**1**) and its derivatives (**2**, **3**).

Fig. 1 Fluorescence emission spectra of **3**- and MAA-coated CdSe/ZnS QDs in water. Inset shows fluorescence correlation curves for the QDs in tetraborate buffer (pH = 9.2). The absorbance at 480 nm (excitation wavelength) was adjusted to be 0.05.

To estimate the hydrodynamic size of the QDs, fluorescence correlation spectroscopy (FCS)[12] was used. FCS measures the fluctuation of the fluorescence intensity of fluorophores at the single molecule level in solution. From the analysis of the fluorescence autocorrelation function $G(\tau)$,[13] the diffusion times of the fluorophores can be determined. The $G(\tau)$ curves were measured by using a compact FCS system (C9413, Hamamatsu Photonics, Japan).

The inset in Fig. 1 shows the normalized $G(\tau)$ curves for **3**- and MAA-coated QDs in aqueous solution. The $G(\tau)$ curves were analyzed using a simple one-component diffusion model.[14] The diffusion time of the **3**- and MAA-coated QDs was found to be 0.65 and 0.25 ms, respectively. By using the value of the diffusion time (0.74 ms) measured for 14 nm fluorescent latex beads (Molecular Probes, Inc. USA), the hydrodynamic sizes were calculated to be 12 and 4.7 nm in diameter for **3**- and MAA-coated QDs, respectively.[15]

In the cases of the surface-coating with thiol compounds, it has been shown that the TOPO molecules passivating the QD surface are exchanged with the thiol compounds[1b,c] (Fig. 2). Assuming a molecular length of MAA as 0.35 nm,[16] the size of the semiconductor core of the MAA-coated CdSe/ZnS QDs is
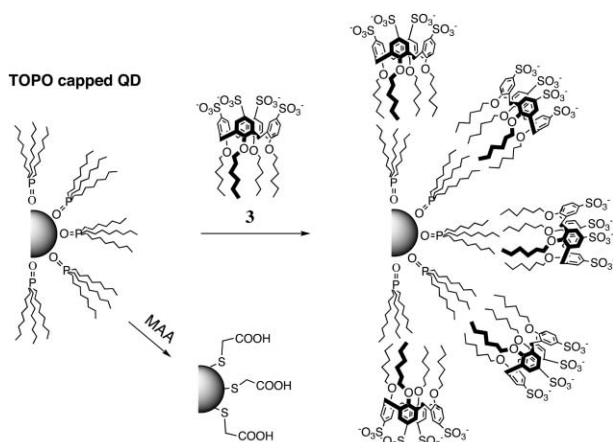
estimated as at least 4.0 nm in a diameter. Thus, in the **3**-coated CdSe/ZnS QDs, the length of the surface-coating layer should be 3.6–4 nm, suggesting that the amphiphilic *p*-sulfonatocalix[4]arene molecules form a bilayer structure[17,18] with the TOPO molecules surrounding the QDs (Fig. 2). The higher emission efficiency observed for **3**-coated QDs in comparison with MAA-coated QDs may be explained by a higher barrier towards the access of water molecules to the QD surface. In fact, it has been shown that a surface coating of thiol compounds with longer alkyl chains results in water-soluble CdSe/ZnS QDs with higher emission efficiencies.[18]

In the **3**-coated CdSe/ZnS QDs, the QD surface is covered with the negative charges of the sulfonyl groups. Thus, we first examined the effect of metal cations such as Na^+ and K^+ on the fluorescence spectrum of **3**-coated QDs. The addition of these cations to the colloidal solution (1 mg/20 mL) of **3**-coated QDs did not cause any changes in the fluorescence spectrum, even at a high concentration of 100 mM. Ammonium cations (100 mM) also did not affect the fluorescence spectrum of the QDs. The addition of ACh caused significant changes in the fluorescence spectrum of **3**-coated QDs. Fig. 3 shows the effect of increasing concentrations of ACh on the fluorescence spectrum of the QDs, where its absorbance at 480 nm (excitation wavelength) is adjusted to be 0.05. As can be seen, the fluorescence of the QDs is strongly quenched by ACh. In the presence of 1×10^{-3} M of ACh, fluorescence quenching of *ca.* 50% was observed. It should be noted that the addition of ACh does not change the spectral widths and the emission maximum of the QDs. This finding indicates that ACh does not cause surface deterioration or aggregation in the **3**-coated QDs.

To get insight of the quenching mechanism by ACh, the temperature dependence of the fluorescence quenching was examined (inset in Fig. 3). The results show upward-curving Stern–Volmer plots at 25 and 50 °C, indicating the quenching process contains both a static and dynamic mechanism.[19] It is observed that the quenching efficiency at 50 °C is much larger than that of the lower temperature of 25 °C. This temperature dependence suggests that the quenching is mainly caused by a dynamic mechanism: ACh molecules bound in **3** at the water–QD interface diffuse into the organic layer of the coating, and they may
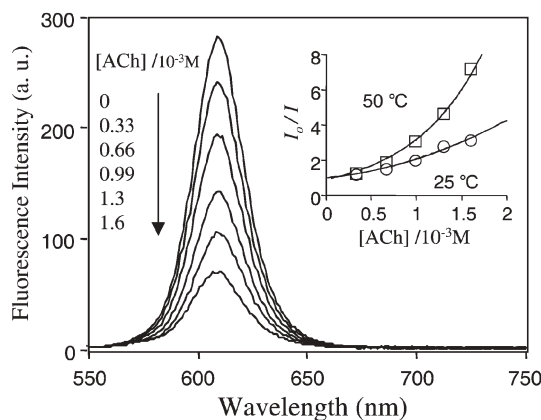

Fig. 2 A schematic representation of the surface-coating of TOPO capped CdSe/ZnS QDs with **3** and MAA.

Fig. 3 Quenching of the fluorescence emission of **3**-coated CdSe/ZnS QDs by addition of ACh. Inset shows the Stern–Volmer plots at 25 and 50 °C.
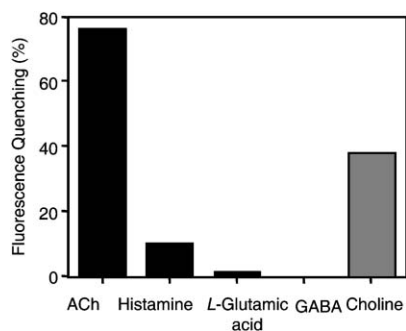
Fig. 4 Fluorescence quenching (I/I_0) of **3**-coated CdSe/ZnS QDs (PBS solution, pH = 7.4) in the presence of neurotransmitter compounds and choline (1.6 mM).

interact with the semiconductor surface to reduce the core electron–hole recombination.

Fluorescence quenching of **3**-coated CdSe/ZnS QDs was examined for several neurotransmitter compounds and choline that is the hydrolyzed product of ACh (Fig. 4). The fluorescence quenching of the **3**-coated QDs is quite selective to ACh among the neurotransmitter compounds tested. The anionic and neutral neurotransmitters (L-glutamic acid and GABA) do not affect the fluorescence spectra of **3**-coated QDs. These neurotransmitters may not have access to the QD surface due to the anionic charges of the sulfonyl groups of **3**. In contrast, the monoamine neurotransmitter histamine with a cationic charge causes a fluorescence quenching of *ca.* 10%, and choline causes a fluorescence quenching of *ca.* 40%. The fact that choline acts as an effective quencher indicates that **3** recognizes the quaternary ammonium cation moiety of choline as well as ACh. The higher quenching efficiency of ACh in comparison with choline can be attributed to its higher hydrophobicity which increases the affinity of ACh towards **3**.[10c]

In summary, we have presented water-soluble CdSe/ZnS QDs surface-modified with amphiphilic *p*-sulfonatocalix[4]arene **3** for the optical detection of the neurotransmitter ACh. To the best of our knowledge, this is the first use of QDs for the detection of neurotransmitter compounds. In this work, the amphiphilic *p*-sulfonatocalix[4]arenes are used as not only surface-coating agents of QDs, but also as host molecules towards ACh which act at the water–QD interface. We believe that the surface architectures of QDs on introducing the calixarene-based host–guest recognition will afford a variety of molecular sensing systems for chemical and bioanalytical applications.[20]

Notes and references

1 (*a*) C. J. Murphy, *Anal. Chem.*, 2002, **74**, 520A; (*b*) J. Riegler and T. Nann, *Anal. Bioanal. Chem.*, 2004, **379**, 913; (*c*) A. M. Smith and S. Nie, *Analyst*, 2004, **129**, 672; (*d*) P. Alivisatos, *Nat. Biotechnol.*, 2004, **22**, 47.

2 (*a*) A. P. Alivisatos, *J. Phys. Chem.*, 1996, **100**, 13226; (*b*) X. Zhong, Y. Feng, W. Knoll and M. Han, *J. Am. Chem. Soc.*, 2003, **125**, 13559; (*c*) C. B. Murray, S. Sun, W. Gascher, H. Doyle, T. A. Betley and C. R. Kagan, *IBM J. Res. Dev.*, 2001, **45**, 47.

3 A. Watson, X. Wu and M. Bruchez, *BioTechniques*, 2003, **34**, 296.

4 (*a*) M. Bruchez, Jr., M. Moronne, P. Gin, S. Weiss and A. P. Alivisatos, *Science*, 1998, **281**, 2013; (*b*) W. C. W. Chan and S. Nie, *Science*, 1998, **281**, 2016.

5 J. V. Frangioni, *Curr. Opin. Chem. Biol.*, 2003, **7**, 626.

6 (*a*) D. E. Moore and K. Patel, *Langmuir*, 2001, **17**, 2541; (*b*) Y. Chen and Z. Rosenzweig, *Anal. Chem.*, 2002, **74**, 5132; (*c*) K. M. Gattás-Asfura and R. M. Leblanc, *Chem. Commun.*, 2003, 2684; (*d*) J.-G. Liang, X.-P. Ai, Z.-K. He and D.-W. Peng, *Analyst*, 2004, **129**, 619; (*e*) H.-Y. Xie, J.-G. Liang, Z.-L. Zhang, Y. Li, Z.-K. He and D.-W. Peng, *Spectrochim. Acta, Part A*, 2004, **60**, 2527.

7 W. J. Jin, M. T. Fernández-Arguelles, J. M. Costa-Fernández, R. Pereiro and A. Sanz-Medel, *Chem. Commun.*, 2005, 883.

8 L. Qu and X. Peng, *J. Am. Chem. Soc.*, 2002, **124**, 2049.

9 (*a*) M. A. Hines and P. Guyot-Sionnest, *J. Phys. Chem.*, 1996, **100**, 468; (*b*) B. O. Dabbousi, J. Rodriguez-Viejo, F. V. Mikulec, J. R. Heine, H. Mattoussi, R. Ober, K. F. Jensen and M. G. Bawendi, *J. Phys. Chem. B*, 1997, **101**, 9463.

10 (*a*) J.-M. Lehn, R. Meric, J.-P. Vigneron, M. Cesario, J. Guilhem, C. Pascard, Z. Asfari and J. Vicens, *Supramol. Chem.*, 1995, **5**, 97; (*b*) K. N. Koh, K. Araki, A. Ikeda, H. Otsuka and S. Shinkai, *J. Am. Chem. Soc.*, 1996, **118**, 755; (*c*) T. Jin, *J. Inclusion Phenom.*, 2003, **45**, 195.

11 S. Shinkai, S. Mori, H. Koreishi, T. Tsubaki and O. Manabe, *J. Am. Chem. Soc.*, 1986, **108**, 2409.

12 R. Rigler and E. S. Elson, *Fluorescence Correlation Spectroscopy*, Springer-Verlag, Berlin Heidelberg, 2001.

13 $G(\tau)$ is defined as $G(\tau) = \langle \delta I(\tau)\delta I(0)\rangle/\langle I(\tau)\rangle^2$, where $I(\tau)$ is the fluorescence intensity at the time τ, and $\delta I(\tau) = I(\tau) - \langle I(\tau)\rangle$. The brackets denote the ensemble average.

14 (*a*) S. Björling, M. Kinjo, Z. Földes-Papp, E. Hagman, P. Thyberg and R. Rigler, *Biochemistry*, 1998, **37**, 12971; (*b*) M. Kinjo, G. Nishimura, T. Koyama, Ü. Mets and R. Rigler, *Anal. Biochem.*, 1998, **260**, 166; (*c*) N. Yoshida, M. Kinjo and M. Tamura, *Biochem. Biophys. Res. Commun.*, 2001, **280**, 312.

15 Assuming a spherical body of fluorophores, the size of the fluorophores can be evaluated by the following relationship: $r_1/r_2 = \tau_1/\tau_2$, where r_i and τ_i mean the radius and diffusion time of the fluorophore i.

16 For estimation of the molecular length of MAA, the CPK (Corey–Pauling–Koltun) molecular model is used.

17 The molecular lengths of TOPO and **3** along with the alkyl chains are estimated as 1.3 nm and 1.6 nm, respectively.

18 T. Jin, F. Fujii, H. Sakata, M. Tamura and M. Kinjo, *Chem. Commun.*, 2005, 2829.

19 J. R. Lakowicz, *Principles of Fluorescence Spectroscopy*, Kluwer Academic/Plenum Publishers, New York, 1999, ch. 8, pp. 238–264.

20 Recently, β-cyclodextrin-modified CdS QDs for the control of complexation-induced fluorescence change were reported: K. Palaniappan, S. A. Hackney and J. Liu, *Chem. Commun.*, 2004, 2704.