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Journal Name

COMMUNICATION

Multi-charged Bis(p-calixarene)s/Pillararenes functionalized Gold nanoparticles for ultra-sensitive sensing of butyrylcholinesterase

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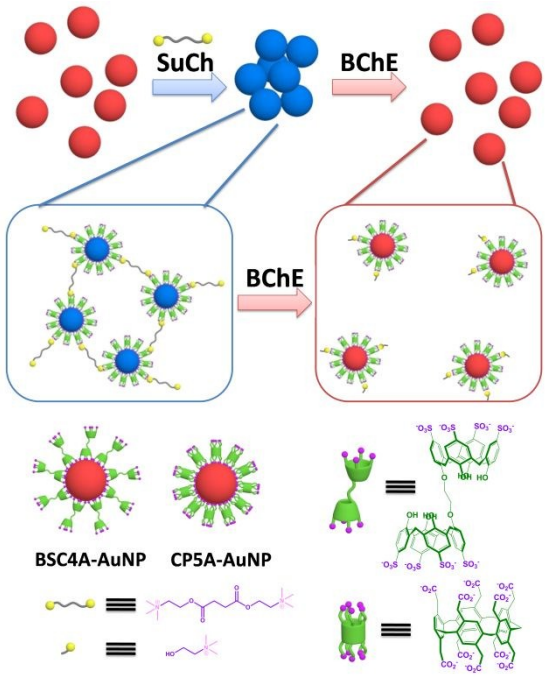
Pei-yu Li [a], Yong Chen [a], Chang-hui Chen[a], and Yu Liu*[a]

A series of supramolecular assembly based on multi-charged calixarenes (SC4A), bis(p-calixarene)s (BSC4A) and pillararenes (CP5A) modified gold nanopartices (AuNP) was constructed to realize **colorimetric sensing of both succinylcholine (SuCh) and butyrylcholinesterase (BChE). With the highly binding affinity of BSC4A and CP5A towards SuCh, BSC4A-AuNPs and CP5A-AuNPs could assemble with micromolar level SuCh as SuCh-BSC4A/CP5A-AuNPs. More interestingly, the enzymatic hydrolysis of SuCh by BChE could lead the disassembly of SuCh-BSC4A/CP5A-AuNPs and provide a sensitive time-dependent color change from blue to red which could be observed by naked eyes and used to monitor BChE activity. As BChE activity is an important biomarker for diseases and poor healthy conditions, this novel supramolecular tandem colorimetric sensing strategy may have potential enlightenment for early diagnosis of diseases.**



Scheme 1 A schematic representation of the supramolecular assembly for colorimetric sensing of succinylcholine (SuCh) and butyrylcholinesterase (BChE).

Butyrylcholinesterase (BChE), one of the nonspecific cholinesterase synthesized in the liver, can hydrolyze ester-containing drugs and scavenges cholinesterase inhibitors.[1, 2] The monitoring of BChE activity has great prognostic and diagnostic importance as the abnormal BChE activity related to many diseases and poor physical conditions.[3] Meanwhile, succinylcholine (SuCh), a specific substrate of BChE and a choline receptor blocker, is widely used as a neuromuscular blocking agent. The inappropriate use of SuCh in clinic may cause respiratory depression and cardiac arrest.[4, 5] SuCh is also a common crime tool used by lawbreakers and lead to intoxication or death of victims.[6] A convenient and efficient method for sensing SuCh concentration and mornitoring BChE activity is in great demand.

Among the supramolecular macrocycle hosts, SC4A and CP5A are known receptors for trimethylammonium-containing bioactivity molecules especially neurotransmitter (choline) with high affinity. Fluorescent sensing of acetylcholine was realized through indicator displacement assay (IDA) with the host–guest complexes between SC4A and dyes as reporting pair.[7-13] The supramolecular tandem assay for monitoring BChE activity was also achieved by Liu.[14] All these works confirmed the important role of host-guest interactions in the sensing of cholinergic bioactive molecules and their associated enzymatic reaction.

However, all of these supramolecular sensing strategies were based on fluorescent which need relatively complex equipment and professional operation. Gold nanoparticle

[a] College of Chemistry, State Key Laboratory of Elemento-Organic Chemistry, Nankai University, Tianjin 300071, P. R. China.

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(AuNPs) was a kind of widely used biosensors through a convenient and efficient colorimetric sensing method.[15-17] Compared with fluorescence sensing, colorimetric sensing by naked eyes plays an important role in the early diagnosis of diseases due to its convenience and ease of operation. The modification of AuNPs with macrocycle compound to construct stimuli-responsive assembly is a hot-spot in material chemistry. Yang reported a pillararene-modified AuNPs for sensing of viologen.[18] Huang reported the supramolecular hybrid nanostructures based on pillar[6]arene modified gold nanoparticles for pH-controlled release.[19] Versatile functional supramolecular assembly based on macrocycles molecules were widely reported in the medical, biochemical fields.[20-26] Based on all of these, a new sensing method combined the strong host-guest complexation with the colorimetric sensing method of AuNPs assembly for monitoring SuCh hydrolysis and BChE activity is of our interest.

Herein, we wish to report a novel supramolecular assembly based on BSC4A/CP5A modified AuNPs for colorimetric sensing of both succinylcholine (SuCh) and butyrylcholinesterase (BChE)**.** SuCh with two quaternary ammonium moieties as binding sites towards BSC4A/CP5A was used to aggregate BSC4A/CP5A-AuNPs to form assembly (SuCh-BSC4A/CP5A-AuNPs). The colour of this solution changed from red to blue caused by the host-guest complexation of SuCh and BSC4A at the surface of AuNPs, and this color change was also used to realize a sensitive colorimetric sensing of SuCh. The hydrolysis of SuCh by BChE lead to the disassembly of the SuCh-BSC4A-AuNPs and also provided a time-resolved visible colour change for monitoring the BChE activity. Accordingly, the strong supramolecular host-guest interaction increased the sensitivity of both the colorimetric sensing of SuCh and the assessment of BChE activity.

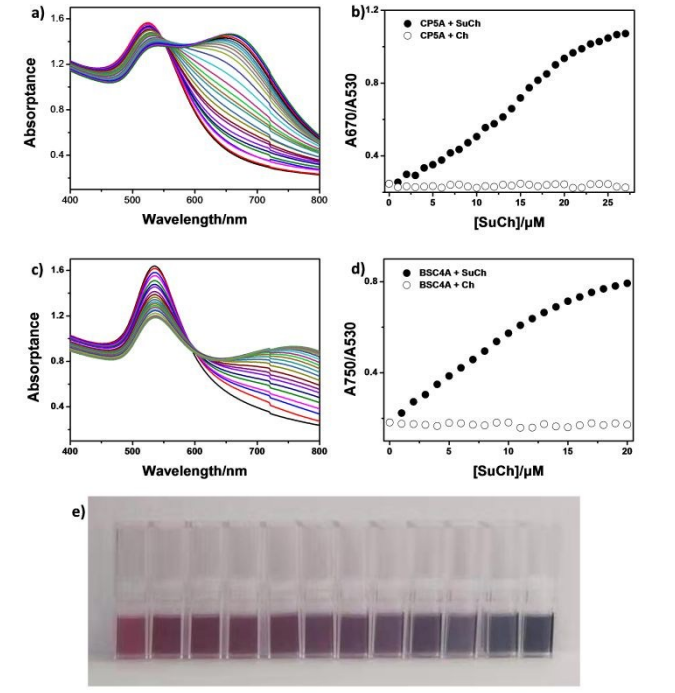

Figure 1 (a) Uv-vis absorption spectra of CP5A-AuNPs solution with different concentrations of SuCh from 0μM to 30μM. (b) Dependence of the ratio of the absorbance values at 670nm and 530nm on SuCh/Ch concentration. (c) Uv-vis absorption spectra of BSC4A-AuNPs solution with different concentrations of SuCh from 0μM to 20μM. (d) Dependence of the ratio of the absorbance values at 750nm and 530nm on SuCh/Ch concentration. (e) The corresponding photos of CP5A-AuNPs solution with different concentrations of SuCh from 0μM to 30μM.

BSC4A-AuNPs and CP5A-AuNPS were first characterized by FT-IR, UV-Vis spectroscopy and Transmission Electron Microscopy (TEM). Fig. S1 showed the FT-IR spectra of SC4A-AuNPs, BSC4A-AuNPs and CP5A-AuNPS. From the FT-IR of BSC4A, the peaks at 1176 and 1040 cm^{-1} were observed which belonged to the SO_3^- groups of BSC4A and these proved the modification of AuNPs with BSC4A. From the FT-IR of CP5A-AuNPs, the peak at 1600 cm^{-1} was observed arising from the C=C stretching of the benzene ring of CP5A. The corresponding Uv-vis absorption spectra of CP5A-AuNPs, BSC4A-AuNPs and SC4A-AuNPs were presented in Fig. S2. All of the three kinds of AuNPs possessed a characteristic absorption peak at 530nm originated from the surface plasmon absorption of dispersed AuNPs. This absorption at 530nm also represented a red color macroscopically visible to naked eye. The UV-vis absorption spectrum of both BSC4A-AuNPs CP5A-AuNPs remained almost unchanged after the solution was kept a week at room temperature because BSC4A and CP5A increased the stability of AuNPs as a stabilizing agent. From the TEM image (Fig. S3), BSC4A-AuNPs presented the morphology as highly dispersed spherical nanoparticles with an average diameter of about 5nm which in accordance with the Uv-vis experiments.

Next, we use the CP5A-AuNPs and BSC4A-AuNPs to assemble with SuCh to realize the colorimetric sensing of SuCh. The assembly was first investigated by UV-vis absorption spectra (Fig. S4, S5). When SuCh was added into the CP5A-AuNPs solution, the absorption peak at 530nm greatly decreased and a new absorption peak at 670nm appeared, indicating the formation of SuCh-CP5A-AuNPs assembly (Fig. S4). The colour change from red (dispersed AuNPs) to blue (assembled AuNPs) was also observed by naked eyes. The corresponding Uv-vis spectra of BSC4A-AuNPs showed a similar result. Choline (Ch), the product of the hydrolysis of SuCh with only one binding site towards CP5A/BSC4A was also added to the CP5A-AuNPs and BSC4A-AuNPs solution as a control group. No change of the UV-vis absorption spectra was observed, indicating that no large AuNPs assembly was formed (Fig. S7, S8). Then, different concentrations of SuCh was added into the CP5A-AuNPs solution to investigate the relationship between the concentration of SuCh and the aggregation extent of CP5A-AuNPs (Fig.1). The absorption at 670nm gradually enhanced while the peaks at 530nm decreased with the increasing of SuCh concentration From 0-30 μM. Fig 1b the line of black points showed the relationship between concentration of SuCh and the ratio of I_{670nm}/I_{530nm} (dependence of the ratio of the absorbance values at 630 nm and 520 nm on SuCh concentration). The control experiment with different concentration of Ch instead of SuCh was also presented in Fig.

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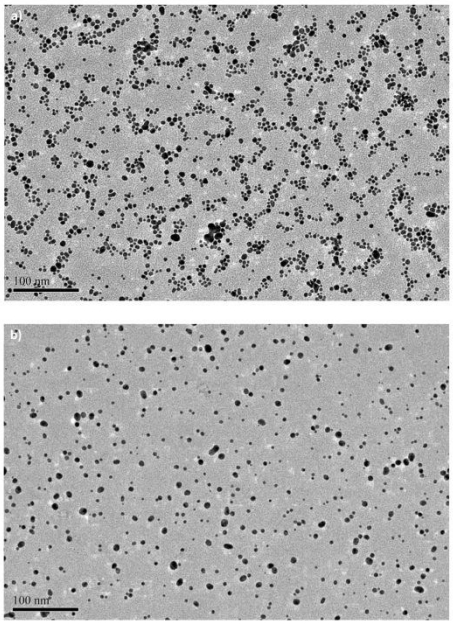

Figure 2 TEM images of (a) bisSC4A-AuNPs with SuCh, (b) bisSC4A-AuNPs with Ch.

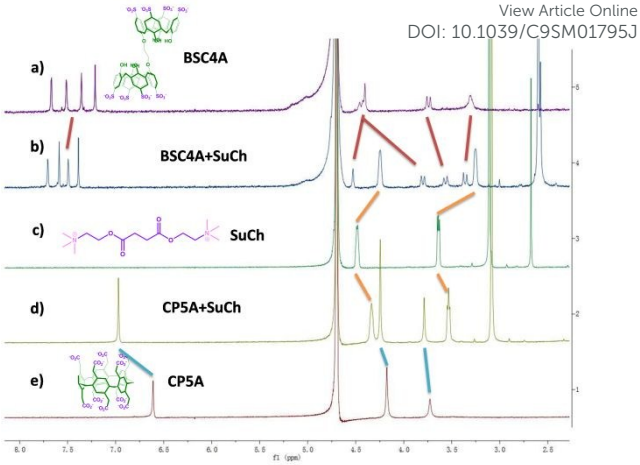

Figure 3 ^{1}H NMR spectra (400 MHz, D_2O, 298 K) of (a) 1 mM BSC4A. (b) 1 mM BSC4A with 1 mM SuCh. (c) 1 mM SuCh. (d) 1 mM CP5A with 1 mM SuCh. (e) 1 mM CP5A

1b the line of white points. No obvious color change was observed which prove that Ch as the hydrolyse product of SuCh cause no assembly of CP5A-AuNPs. The zeta potential of the CP5A-AuNPs also increased with the addition of SuCh (Fig. S11). The concentration of SuCh was set as 20 μM for the further enzymatic hydrolysis experiments. A similar result was also observed for BSC4A-AuNPs with SuCh or Ch added. The only difference was that the absorption peak of assembled BSC4A-AuNPs was at 750nm and the color change is not as obvious as that of that assembled CP5A-AuNPs. The TEM images (Fig. 2) also presented the consistency result with the UV-vis absorption spectral experiment. Many irregular clusters of AuNPs could be seen and almost no dispersive AuNPs existed for the sample of CP5A-AuNPs solution with SuCh added (Fig. 2a). Meanwhile, in the TEM images of CP5A-AuNPs with the addition of Ch (Fig. 2b), no assembled AuNPs were observed. Dynamic light scattering (DLS) data showed that the average size of SuCh-CP5A-AuNPs is about 36 nm (Fig. S10). All of these proved that the CP5A-AuNPs and BSC4A-AuNPs could be used to colorimetric sensing of SuCh through a supramolecular assemble process.

Next, the sensing mechanism of SuCh by CP5A-AuNPs and BSC4A-AuNPs was studied. It was reported that the dicholine could be captured by BSC4A with quaternary ammonium moieties deeply immersed into the cavity, while the substrate spacer was located outside.[27] The binding constant between BSC4A and dicholine was $(1.2 \pm 0.2) \times 10^4$ M^{-1}. The binding behaviour of CP5A or BSC4A with SuCh was investigated by ^{1}H NMR as shown in Fig. 3. Due to the host-guest interaction, all of the hydrogen signals on the aromatic ring of CP5A and BSC4A moved to high field. Meanwhile, both BSC4A and CP5A had two ends with negative charge, which coordinated to the surface of AuNPs with one end while the other one remained the binding affinity towards SuCh and Ch. SC4A with only one ends of negative charge was chosen as a control compound to further proved the sensing mechanism of SuCh. Sulfonatocalixarene (SC4A) modified AuNPs was prepared and after the addition of SuCh, no colour change was observed, and only the absorption peak at 530 nm appeared in the UV-vis spectra (Fig. S6). This indicated that after binding to AuNPs with all of the SO_3^- groups, SC4A lost its affinity towards SuCh. Compared to SC4A-AuNPs, BSC4A-AuNPs had an unoccupied sensing end remained the binding affinity towards SuCh. Therefore, the sensing mechanism could be illustrated as follow: SuCh with two quaternary ammonium moieties as binding sites towards CP5A and BSC4A could assemble CP5A-AuNPs and BSC4A-AuNPs and provide a visible color change to determine the concentration of SuCh (Fig. S22). To further comfirm the selectivity of this sensing system, the sensing of other positively charged linkers with two binding sites existed in biological environment as Arginine and Lysine, and other choline species as Acetylcholine and Butylcholine was also measured (Fig. S16-S19). Compared with the high sensable SuCh, other linkers and possible disturbances could not cause clear siganal change of CP5A-AuNPs in UV-vis spectra at the corresponding low concentration. The sensing of SuCh in Serum was also studied which proved the availability of CP5A-AuNPs in biological environment.

As SuCh was a specific substrate of BChE, the hydrolyze of SuCh by BChE could lead the disassembly of SuCh-CP5A-AuNPs. These decreasing of SuCh concentration in the hydrolyzing process could bring a reversed time-resolved color change signal from blue to red which could be used to realized a BChE activity assay. SuCh-CP5A-AuNPs was prepared in phosphate buffer, and BChE ($1UmL^{-1}$) was added to the solution. The time-dependent UV-vis absorption was presented in Fig. 4a. The I_{670nm}/I_{530nm} decreased in time from 0 to 30 min

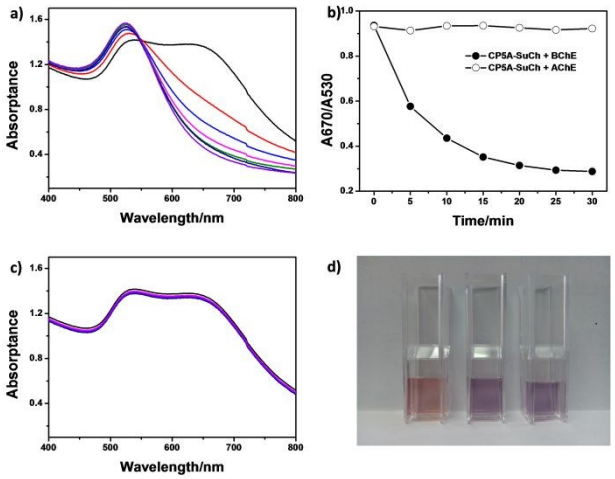

Figure 4 Colorimetric BChE assay using CP5A-AuNPs: (a) Uv-vis absorption spectra of CP5A-AuNPs solution with 20 μM SuCh and 1U/mL BChE with a time of 0-30 min. (b) time-dependent UV-vis absorption at I_{670nm}/I_{530nm}. (c) Uv-vis absorption spectra of CP5A-AuNPs solution with 20 μM SuCh and 1U/mL AChE with a time of 0-30 min. (d) The corresponding photos of the two assays: (left) CP5A-AuNPs solution with 20 μM SuCh and 1U/mL BChE after 30 min, (middle) CP5A-AuNPs solution with 20 μM SuCh, (right) CP5A-AuNPs solution with 20 μM SuCh and 1U/mL AChE after 30 min. All the experiments were done at 37°C in phosphate buffer at PH 8.0.

until the final plateau region (Fig. 4b, line of black points). To verify the specificity of the colorimetric sensing of BChE, a control experiment using AChE was also carried out. With AChE added to the SuCh-CP5A-AuNPs solution, no change was found in the UV-vis spectra in time from 0 to 30min (Fig. 4c). All these results prove that the hydrolysis of SuCh by BChE caused the disassembly of SuCh-CP5A-AuNPs along with the time-resolved colour change from blue to red to be used to sensitively and selectively monitor BChE activity (Fig. 4d). The applicability of this colorimetric system to monitoring the BChE inhibitors was also investigated. SuCh assemble CP5A-AuNPs were prepared in phosphate buffer solution with fixed concentration of BChE and Tacrine (an approved Alzheimer's drug). The expected results could be found from the time-dependent UV-vis absorption spectra at I_{670nm}/I_{530nm} (Fig. S15). With the addition of BChE inhibitors, the rate of enzymatic reaction reduced.

In summary, a novel and facile enzyme-responsive supramolecular assembly based on CP5A-AuNPs for colorimetric sensing of SuCh and monitoring BChE activity was constructed. The high binding affinity of CP5A towards SuCh was a key factor to enhance the sensing sensitivity. The modification of AuNPs with CP5A/BSC4A enriched the surface of AuNPs with sensing specificity. The macroscopic visible colour change caused by the BChE hydrolyzing provided a convenient and easy colorimetric sensing method for BChE activity assessment. This colorimetric sensing strategy based on supramolecular assembly of CP5A-AuNPS or BSC4A-AuNPS may provide a sensitive and efficient analytical platform for early diagnose of disease and may had potential enlightenment for supramoleculer chemistry being applied in biomedical and clinical fields.

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Conflicts of interest

There are no conflicts to declare.

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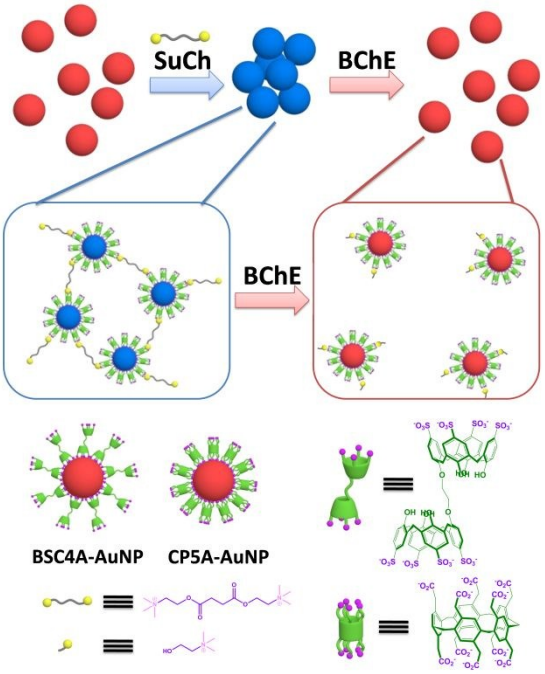

A novel supramolecular assembly based on BSC4A/CP5A modified AuNPs for colorimetric sensing of both succinylcholine (SuCh) and butyrylcholinesterase (BChE)