Supporting Information

For

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

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Materials and Measurements

Bis(p-sulfonatocalix[4]arene) (BSC4A) and Carboxylpillar[5]arenes (CP5A) was synthesized and purified according to the literature process.\(^1\) \(^2\) Succinylcholine chloride (SuCh), chloroaauric acid, sodium borohydridecholine chloride (Ch), acetylcholine and butyrylcholine were purchased from TCI. Acetylcholinesterase (AChE, from human erythrocytes, 2712.50 U mg\(^{-1}\)), butyrylcholinesterase (BChE, from equine serum, 246 U mg\(^{-1}\)) and tacrine (9-amino-1, 2, 3, 4-tetra-hydroacridine hydrochloride hydrate) were purchased from Sigma–Aldrich. Fetal bovine serum (FBS) was purchased from GIBCO. All of the compounds were used without further purification. The phosphate buffer solution (pH 8.0) was prepared by dissolving sodium dihydrogen phosphate in distilled deionized water to make a 10 mM stock solution, which was then adjusted to pH 8.0 by the addition of NaOH. The reagent solutions were freshly prepared daily. The pH values of the buffer solutions were verified by using a pH meter that was calibrated with two standard buffer solutions.

Preparation of SC4A/BSC4A/CP5A modified AuNPs

The SC4A/BSC4A/CP5A modified AuNPs were prepared by reacting chloroaauric acid (AuCl\(_3\)-HCl·4H\(_2\)O) 1mL (1g in 100mL) with 1mL 0.1M sodium borohydride in 96mL deionized water with 2mL 1mM SC4A/BSC4A/CP5A at room temperature for 5 min. After centrifuging the reaction solution, SC4A/BSC4A/CP5A-AuNPs were obtained and washed with deionized water for three times.
**FT-IR spectra.** Infrared spectroscopy was performed on a Thermo Nicolet iS10 with a diamond ATR attachment.

**UV-vis spectra.** UV-Vis spectra were recorded in a quartz cuvette (light path 10 mm) on a Shimadzu UV-2450 UV-Vis spectrophotometer equipped with a dual cuvette peltier accessory and a temperature controller (TCC-240A).

**The \(^1\)H NMR spectra.** \(^1\)H and \(^{13}\)C NMR data were recorded on a Bruker AV400 spectrometer.

**Transmission Electron Microscopy (TEM) measurements.** The sample solutions were dropped onto a copper grid and air-dried. The samples were examined by a high-resolution TEM (Tecnai G2 F20 high-resolution TEM) operating at an accelerating voltage of 200 kV.

**Dynamic light-scattering (DLS) measurements.** The samples were performed on a laser light scattering spectrometer (BI-200SM) equipped with a digital correlator (BI-9000AT) at 532 nm at 298 K. All DLS measurements were performed at a scattering angle of 90°.

**Zeta potential measurements.** Zeta potential values were determined on a Brookheaven ZetaPALS (Brookheaven Instrument, USA) at 25 °C. The instrument utilizes phase analysis light scattering to provide an average over multiple particles. Doubly distilled water was used as the background electrolyte for zeta potential measurements.
The characterization of BSC4A-AuNPs and CP5A-AuNPs

**Figure S1.** FT-IR spectra of SC4A-AuNPs (black line), BSC4A-AuNPs (red line) and CP5A-AuNPs (blue line).
Figure S2. Uv-vis absorption spectra of CP5A/BSC4A/SC4A-AuNPs solution in phosphate buffer at pH 8.0.

Figure S3. TEM images of BSC4A-AuNPs.
The colorimetric sensing of SuCh

Figure S4. Uv-vis absorption spectra of CP5A-AuNPs solution with and without 20 μM SuCh in phosphate buffer at pH 8.0.

Figure S5. (a) Uv-vis absorption spectra of BSC4A-AuNPs solution with and without 20 μM SuCh (blue line) in phosphate buffer at pH 8.0.
Figure S6. Uv-vis absorption spectra of SC4A-AuNPs solution with and without 20 μM SuCh (blue line) in phosphate buffer at pH 8.0.

Figure S7. Uv-vis absorption spectra of CP5A-AuNPs solution with different concentrations of Ch from 0μM to 30μM.
Figure S8. Uv-vis absorption spectra of BSC4A-AuNPs solution with different concentrations of Ch from 0μM to 20μM.

Figure S9. Uv-vis absorption spectra of SC4A-AuNPs solution with different concentrations of Ch from 0μM to 20μM.
Figure S10. Hydrodynamic diameter distribution of (left) CP5A-AuNPs; (right) CP5A-AuNPs with 20μM SuCh, respectively.
Zeta potential measurements

<table>
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<tr>
<th>SuCh(μM)</th>
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<td>0</td>
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<tr>
<td>5</td>
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<td>-41.37</td>
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<tr>
<td>20</td>
<td>-32.52</td>
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</tbody>
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**Figure S11.** Zeta potential measurement of the AuNPs solution with different concentration of SuCh from 0 – 20 μM added.
Figure S12. $^1$H NMR spectra (400 MHz, D$_2$O, 298 K) of (a) 1 mM SuCh; (b) 1 mM BSC4A with 1 mM SuCh; (c) 1 mM BSC4A.

Figure S13. $^1$H NMR spectra (400 MHz, D$_2$O, 298 K) of (a) 1 mM SuCh; (b) 2 mM SC4A with 1 mM SuCh; (c) 2 mM SC4A.
Figure S14. $^1$H NMR spectra (400 MHz, D$_2$O, 298 K) of (a) 1 mM CP5A; (b) 1 mM CP5A with 1 mM SuCh; (c) 1 mM SuCh.
The BChE activity assessment

**Figure S15.** Colorimetric assay using CP4A-AuNPs: 20 μM SuCh, 1 U/mL BChE, with or without Tacrine; time-dependent UV-vis absorption at I$_{670nm}$/I$_{530nm}$.

**Figure S16.** Uv-vis absorption spectra of CP5A-AuNPs solution with and without 60 μM acetylcholine (blue line) in phosphate buffer at pH 8.0.
Figure S17. Uv-vis absorption spectra of CP5A-AuNPs solution with and without 60 μM butyrylcholine (blue line) in phosphate buffer at pH 8.0.

Figure S18. Uv-vis absorption spectra of CP5A-AuNPs solution with and without 30 μM arginine (blue line) in phosphate buffer at pH 8.0.
Figure S19. Uv-vis absorption spectra of CP5A-AuNPs solution with and without 30 μM lysine (blue line) in phosphate buffer at pH 8.0.

Figure S20. Dependence of the ratio of the absorbance values at 670nm and 530nm on SuCh concentration and the linear fit curve. The sensitivity was calculated as 0.0347 mM⁻¹ and the limit of detection was determined as 1μM.
**Figure S21.** Uv-vis absorption spectra of CP5A-AuNPs with different concentrations of SuCh from 0μM to 25μM in Fetal bovine serum (FBS), the reference was also FBS.

**Figure S21.** Dependence of the ratio of the absorbance values at 670nm and 530nm on SuCh concentration in FBS.
**Figure S22.** The schematic illustration of BSC4A-AuNPs and SC4A-AuNPs to explain the sensing mechanism.
References
