An enzyme-responsive supra-amphiphile constructed by pillar[5]arene/acetylcholine molecular recognition

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1. Materials and methods

Methyl 6-bromohexanoate, 1-pyrenol, $N,N$-dimethylethanolamine and other reagents were commercially available and used as received. Solvents were either employed as purchased or dried according to procedures described in the literature. $^1$H NMR and $^{13}$C NMR spectra were recorded on a Bruker Avance III-400 spectrometry. The 2D NOESY NMR spectrum was collected on a Bruker Avance DMX-500 spectrometer with internal standard TMS. Mass spectra were obtained on a Bruker Esquire 3000 plus mass spectrometer (Bruker-Franzen Analytik GmbH Bremen, Germany) equipped with an ESI interface and an ion trap analyzer. HRMS were obtained on a WATERS GCT Premier mass spectrometer. UV-vis spectra were taken on a Shimadzu UV-2550 UV-vis spectrophotometer. The melting points were collected on a SHPSIC WRS-2 automatic melting point apparatus. The fluorescence experiments were conducted on a RF-5301 spectrofluorophotometer (Shimadzu Corporation, Japan). Scanning electron microscopy investigations were carried out on a JEOL 6390LV instrument operating at an energy of 15 Kev or 20 Kev. Transmission electron microscopy (TEM) investigations were carried out on a HT-7700 instrument. X-ray diffraction (XRD) was recorded on a Rigaku Ultima IV instrument. The critical aggregation concentration (CAC) values were determined on a DDS-307 instrument. The ITC experiment was performed on a VP-ITC micro-calorimeter (Microcal, USA). Energy-minimized structure of WP5$\rightarrow$M was calculated using PM3 semiempirical molecular orbital methods (Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. Gaussian 03, revision D.01; Gaussian, Inc.: Wallingford, CT, 2005).
2. Synthesis of PyCh

Fig. S1 $^1$H NMR spectrum (400 MHz, chloroform-$d$, room temperature) of 1.

Fig. S2 $^{13}$C NMR spectrum (100 MHz, chloroform-$d$, room temperature) of 1.
Fig. S3 Electrospray ionization mass spectrum of 1. Assignment of the main peak: $m/z$ 346.9 [M + H]$^+$ (100%).

Fig. S4 $^1$H NMR spectrum (400 MHz, DMSO-$d_6$, room temperature) of 2.

Fig. S5 $^{13}$C NMR spectrum (100 MHz, DMSO-$d_6$, room temperature) of 2.
**Fig. S6** Electrospray ionization mass spectrum of 2. Assignment of the main peak: m/z 330.9 [M-H] (100%).

**Fig. S7** $^1$H NMR spectrum (400 MHz, chloroform-$d$, room temperature) of 3.
Fig. S8 $^{13}$C NMR spectrum (100 MHz, chloroform-$d$, room temperature) of 3.

Fig. S9 Electrospray ionization mass spectrum of 3. Assignment of the main peak: $m/z$ 404.0 [M + H]$^+$ (100%).
Fig. S10 $^1$H NMR spectrum (400 MHz, DMSO-$d_6$, room temperature) of PyCh.

Fig. S11 $^{13}$C NMR spectrum (100 MHz, DMSO-$d_6$, room temperature) of PyCh.
Fig. S12 Electrospray ionization mass spectrum of PyCh. Assignment of the main peak: m/z 418.0 [M – I]^+ (100%).

3. Host–guest complexation between WP5 and M

Fig. S13 2D NOESY NMR spectrum (500 MHz, D$_2$O, 295 K) of WP5 (10.0 mM) and M (10.0 mM).
**Fig. S14** Microcalorimetric titration of M with WP5 in water at 298.15 K. (Top) Raw ITC data for 29 sequential injections (10 µL per injection) of a M solution (2.00 mM) into a WP5 solution (0.100 mM). (Bottom) Net reaction heat obtained from the integration of the calorimetric traces.

**Fig. S15** Energy-minimized structure of WP5⇌M by the PM3 semiempirical molecular orbital method: (a) top view, (b) side view.
4. Host–guest complexation between WP5 and PyCh

Further evidence for the formation of a stable host–guest complex WP5⊃PyCh was obtained from UV-vis absorption spectroscopy. When WP5 and PyCh (molar ratio = 1:1) were mixed in water, a notable red-shift was observed, confirming the presence of electronic communication between WP5 and PyCh.

![Fig. S16 UV-vis spectra of (a) PyCh and (b) WP5⊃PyCh in water.](image)

![Fig. S17 Partial 1H NMR spectra (400 MHz, D2O, 295 K): (a) WP5 (2.00 mM) and PyCh (6.00 mM); (b) WP5 (2.00 mM).](image)
5. Critical aggregation concentration determination of PyCh and WP5⇒PyCh

Fig. S18 The concentration-dependent conductivity of PyCh. The critical aggregation concentration was determined to be $1.25 \times 10^{-6}$ M.

Fig. S19 The concentration-dependent conductivity of WP5⇒PyCh. The critical aggregation concentration was determined to be $1.52 \times 10^{-4}$ M.

Fig. S20 DLS result of the nanoparticles formed by WP5⇒PyCh.
6. Packing mode investigation of PyCh before and after treating with AChE

Fig. S21 XRD scans of (a) nanosheets and (b) nanoribbons.

Fig. S22 UV-vis absorption spectra of aqueous solutions of PyCh at different concentrations.
Fig. S23 TEM images of (a) the intermediate state from nanoparticles to nanoribbons, (b) PyCh treated with AChE, (c) nanosheets formed by PyCh, and (d) multilayer nanoribbons.

Fig. S24 Electrospray ionization mass spectrum of PyCh in the absence of AChE.

Fig. S25 Electrospray ionization mass spectrum of PyCh after treatment with AChE for 5 h.
7. Preparation of supramolecular hybrid materials

**Fig. S26** Electrospray ionization mass spectrum of PyCh after treatment with AChE for 12 h.

**Fig. S27** Size histogram of gold nanoparticles corresponding to the hybrid AuNPs@nanosheets based on 100 particles.

**Fig. S28** UV-vis spectra of (a) PyCh, (b) WP5→PyCh, (c) AuNPs@nanosheets, and (d) AuNPs@particles. Inset: i, AuNPs@nanoparticles after treating with AChE; ii, AuNPs@nanoparticles; iii, AuNPs; iv, AuNPs@nanosheets; v, AuNPs@nanosheets after treating with AChE.
Fig. S29 Fourier transform IR spectra of (a) PyCh, (b) Au@nanosheets, (c) bare AuNPs, (d) Au@nanoparticles and (e) WP5.

Fig. S30 EDS study of the Au@nanosheets.

Fig. S31 EDS study of the Au@nanoparticles.
8. Catalytic reduction of 4-nitroanilin

In our reaction system, the concentration of BH$_4^-$ greatly exceeded that of 4-nitroaniline, so it is reasonable to consider its concentration to be constant during the reaction. As expected, a good linear correlation of ln(A) versus time was obtained, and the kinetic reaction rate constant was estimated.$^\text{S1}$

**Fig. S32** (a) The successive UV-vis absorption of the reduction of 4-nitroaniline and (b) plot of ln(A) against time with excess NaBH$_4$ in the presence of Au@nanoparticles. The kinetic reaction rate constant was calculated to be $(2.61 \pm 0.28) \times 10^{-3}$ S$^{-1}$.

**Fig. S33** (a) The successive UV-vis absorption of the reduction of 4-nitroaniline and (b) plot of ln(A) against time with excess NaBH$_4$ in the presence of Au@nanosheets. The kinetic reaction rate constant was calculated to be $(1.85 \pm 0.15) \times 10^{-3}$ S$^{-1}$.

**Fig. S34** (a) The successive UV-vis absorption of the reduction of 4-nitroaniline and (b) plot of ln(A) against time with excess NaBH$_4$ in the presence of Au@nanoparticles after treatment with AChE. The kinetic reaction rate constant was calculated to be $(1.78 \pm 0.11) \times 10^{-3}$ S$^{-1}$. 
**Fig. S35** (a) The successive UV-vis absorption of the reduction of 4-nitroaniline and (b) plot of ln(A) against time with excess NaBH₄ in the presence of Au@nanosheets after treatment with AChE. The kinetic reaction rate constant was calculated to be $(1.01 \pm 0.09) \times 10^{-3}$ S⁻¹.

**References:**