Electronic Supplementary Information

CAMP sensitive nanochannels driven by conformational transition of a tripeptide–based smart polymer

Shengyan Ji,[ab] Yuting Xiong,[*b] Wenqi Lu,[b] Minmin Li,[b] Xue Wang,[ab] Cunli Wang,[b] Dongdong Wang,[b] Jie Xiao,[c] Zhichao Zhu,[c] Lihua Chen,[a] Yahui Zhang*[b] and Guangyan Qing*[bc]

[a]State Key Laboratory of Advanced Technology for Materials Synthesis and Processing, Wuhan University of Technology, 122 Luoshi Road, Wuhan 430070, P. R. China.

[b]Key Laboratory of Separation Science for Analytical Chemistry, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, 457 Zhongshan Road, Dalian 116023, P. R. China.

[c]College of Chemistry and Chemical Engineering, Wuhan Textile University, 1 Sunshine Road, Wuhan 430200, China

Corresponding authors. E-mail: qinggy@dicp.ac.cn; zhangyh@dicp.ac.cn; xiongyt@dicp.ac.cn

Table of Contents

1. Materials and Instruments

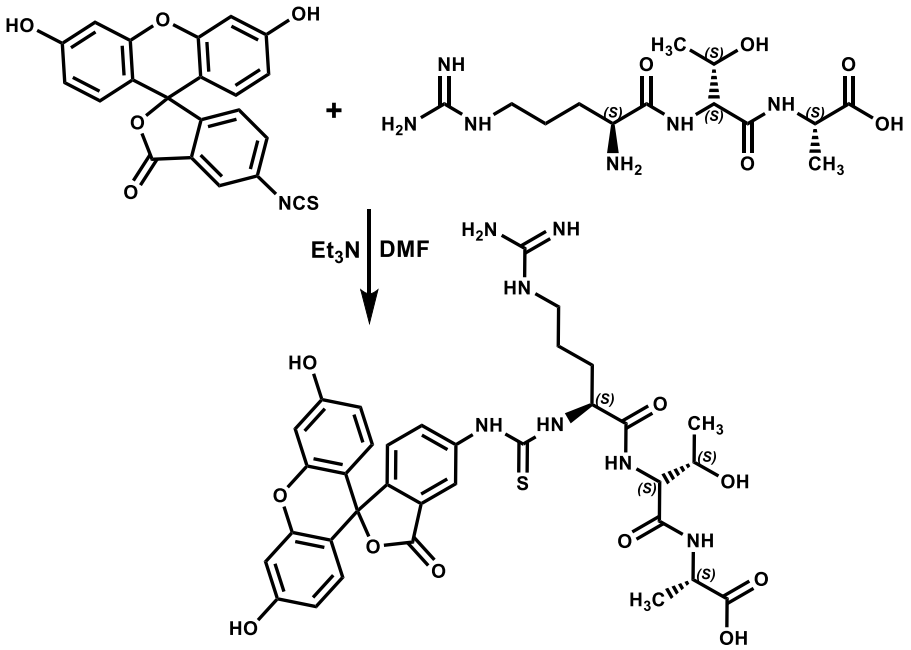
Materials: Tripeptide Arg-Thr-Ala (RTA) was purchased from Synpeptide Co., Ltd (China) with high purity (>95%) and used without further purification. *N*-isopropyl acrylamide (NIPAAm) was purchased from Sigma-Aldrich Corp. and then recrystallized four times in *n*-hexane prior to use. 2-Bromoisobutyryl bromide, 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride (EDCI·HCl), *N*,*N*,*N*',*N*'',*N*''-pentamethyldiethylenetriamine, cyclic adenosine monophosphate salt (cAMP), adenosine mono-phosphate salt (AMP, 97%), adenosine 5'-diphosphate salt (ADP, 97%), adenosine 5'-triphosphate salt (ATP), adenosine, 2-mercapto-ethylamine, fluorescein isothiocyanate (FITC), *N*-hydroxysuccinimide (NHS), 3-aminopropyl trimethoxysilane (ATMS), copper bromide (CuBr) were purchased from Alfa Aesar (Germany). Triethylamine (Et_3N), *N*,*N*'-dimethyl formide (DMF), methanol, ethanol, hydrochloric acid, hydrogen peroxide and deuterated dimethyl sulfoxide (DMSO-d_6) were all available commercially and used as received. Toluene was dried by conventional procedures and distilled under nitrogen prior to use. Anodic aluminum oxide (AAO) membrane (with average pore size of 80 nm, thickness of 60 μm) were commercially obtained from Puyuan Nano (Hefei, China). Experimental double distilled water was obtained from Milli-Q system (18.2 MΩ·cm^{-1}, Bedford, MA, USA).

Instruments: Hydrogen and carbon nuclear magnetic resonance (^{1}H and ^{13}C NMR) spectra were recorded on a BRUKER AVANCE III 400–MHz spectrometer. ESI-HRMS spectra were recorded on a HPLC/Q-Tof 6540 mass spectrometer. Fluorescent titration experiments were conducted by using a Perkin-Elmer FL-6500 fluorescence spectrometer. Electrochemical impedance spectroscopy (EIS) measurements were carried out on a CHI 760E electrochemical workstation. Quartz crystal microbalance (QCM) measurements were performed using a Sweden Q-Sense QCM-D E4 system. Lower critical solution temperature (LCST) experiments were measured on a PerkinElmer Lambda 365 UV-Vis spectrophotometer. Isothermal titration calorimetry (ITC) experiments were performed on a Malvern MicroCal iTC200 system. Current-voltage curves were recorded on a Keithley 6487 picoammeter/voltage source. Atomic force microscope (AFM) images were obtained from Bruker Multimode 8 AFM. Scanning electron microscope (SEM) images were obtained from

S4 JSM-7800F (Japan) SEM. Helium ion microscope (HIM) images were obtained from ORION NANOFAB (Germany). X-ray photoelectron spectroscopy (XPS) data were recorded on an ESCALAB 250Xi instrument.

2. Experimental procedures

2.1 Synthesis of fluorescein isothiocyanate-labelled Arg-Thr-Ala (FITC-labelled RTA)

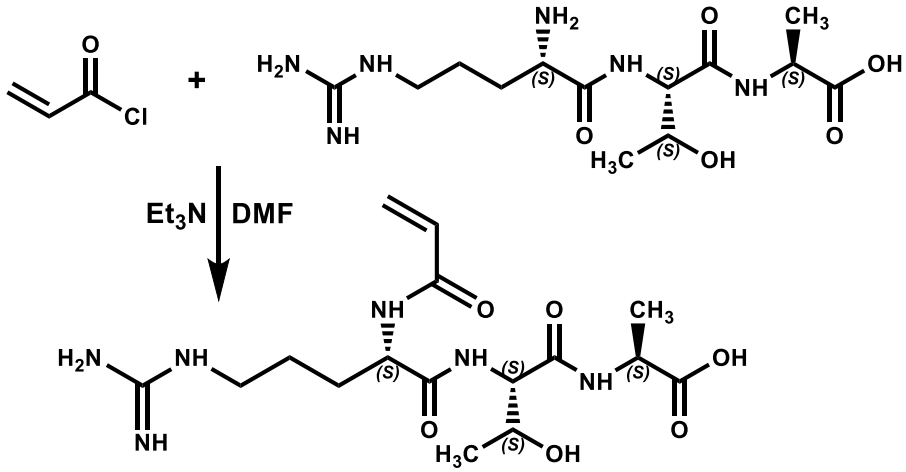


Scheme S1 Preparation of FITC-labelled RTA

Et_3N (8 μL, 0.06 mmol) was added to a solution of RTA (52 mg, 0.12 mmol) in anhydrous DMF (2 mL), then a solution of FITC (47 mg, 0.12 mmol) in anhydrous DMF (2 mL) was added dropwise to the mixture, continuous stirring for 24 hours under ambient temperature. After rotary evaporation and freeze-drying of the solvent, the crude product was purified on the purification system of Shimadzu UFLC 20A with C18 column (particle size: 10 μm, inner pore size: 100 Å, 10 mm×250 mm). Flow rate was maintained at 3 mL min^{-1} and column temperature was set as 20 ℃. Mobile phase A was water and mobile phase B was acetonitrile. Gradient condition was: 0.0–20.0 min, 10-90% B. Through rotary evaporation and freeze-drying, the product was obtained as yellow powder (46 mg, 45%). ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm): 10.20 (s, 2H, ArO*H*), 8.71 (d, *J*=8.9 Hz, 1H, CON*H*), 8.47 (s, 1H, CSN*H*), 8.21 (s, 1H, Ar*H*), 7.78 (d, *J*=8.6 Hz, 1H, CSN*H*), 7.56 (d, *J*=6.0 Hz, 1H, CON*H*), 7.09-7.18 (m, 4H, guanidine-N*H*), 6.54-6.66 (m, 8H, Ar*H*), 5.15 (d, *J*=6.6 Hz, 1H, *CO*H*),

4.87 (m, 1H, *C*H*), 4.13-4.17 (m, 1H, *C*H*), 4.05-4.08 (m, 1H, *C*H*), 3.71-3.75 (m, 1H, *C*H*), 3.07-3.10 (m, 2H, NCH_2), 1.57-1.61 (m, 2H, *CCH_2), 1.18-1.24 (m, 5H, *CCH_3, CH_2). 1.07 (d, *J*=6.1 Hz, 3H, *CCH_3). ESI-HRMS (m/z): calcd for $C_{34}H_{37}N_7O_{10}S$: 735.2318; found: 736.2391 [$M+H^+$].

2.2 Synthesis of 4-acrylamide-Arg-Thr-Ala (ARTA)

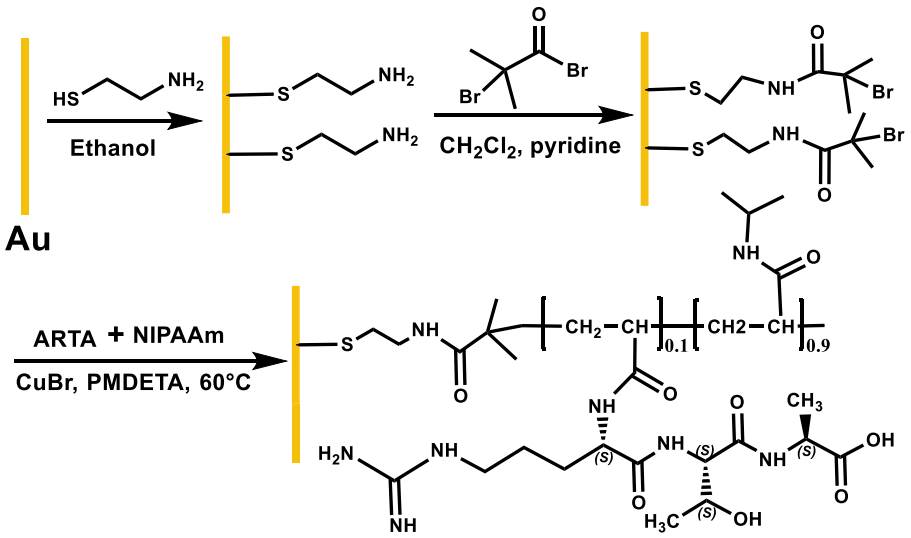


Scheme S2 Preparation of 4-acrylamide-Arg-Thr-Ala (ARTA)

RTA (250 mg, 0.72 mmol) and Et_3N (150 μL, 1.08 mmol) were dissolved in anhydrous DMF (2 mL) in an ice water bath, acryloyl chloride (59 μL, 0.72 mmol) was added dropwise to the solution and keep at 0 ℃ for about 30 min. Then the mixture was stirred for 24 hours at room temperature. After the removal of the solvent by rotary evaporator, the crude product was purified on the purification system of Shimadzu UFLC 20A with X Amide column (particle size: 10 μm, inner pore size: 100 Å, 10 mm×250 mm). Flow rate was maintained at 3 mL·min^{-1} and column temperature was set as 20 ℃. Mobile phase A was water and mobile phase B was acetonitrile. Gradient condition was: 0.0–20.0 min, 80-60% B. Through rotary evaporation and freeze-drying, the product was obtained as white powder (128 mg, 39%). ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm): 8.46 (d, *J*=9.0 Hz, 1H, CON*H*), 8.27 (d, *J*=7.5 Hz, 1H, CON*H*), 7.67-7.71 (m, 1H, guanidine-N*H*), 7.60 (d, *J*=6.1 Hz, 1H, CON*H*) 7.05-7.31 (m, 3H, guanidine-N*H*), 6.36, 6.40 (dd, J_1=10.2 Hz, J_2=10.1 Hz, 1H, C=C*H*), 6.08, 6.12 (dd, J_1=2.1 Hz, J_2=2.0 Hz, 1H, C=C*H*), 5.59, 5.61 (dd, J_1=2.2 Hz, J_2=2.0 Hz, 1H, C=C*H*), 4.59 (d, *J*=4.3 Hz, 1H, *CO*H*), 4.11-4.13 (m, 2H, *C*H*), 4.02-4.05 (m, 1H, *C*H*), 3.74-3.80 (m, 1H, *C*H*), 2.99-3.05 (m, 2H, NCH_2), 1.92-1.98 (m, 2H, *CCH_2), 1.51-1.64 (m, 2H, CH_2), 1.19-1.23 (m,

3H, C*H*$_3$), 1.02-1.06 (m, 3H, C*H*$_3$). ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm): 176.13, 172.73, 169.51, 164.85, 157.68, 131.98, 125.93, 66.20, 59.46, 52.56, 50.25, 41.39, 30.78, 24.85, 21.10, 19.22. ESI-HRMS (m/z): calcd for $C_{16}H_{28}N_6O_6$: 400.2045; found: 401.2118 $[M+H]^+$.

2.3 Modification of Au-coated quartz-crystal (QC) resonator or Au electrode with PNI-*co*-ARTA$_{0.1}$ *via* surface-initiated atom transfer radical polymerization (SI-ATRP)

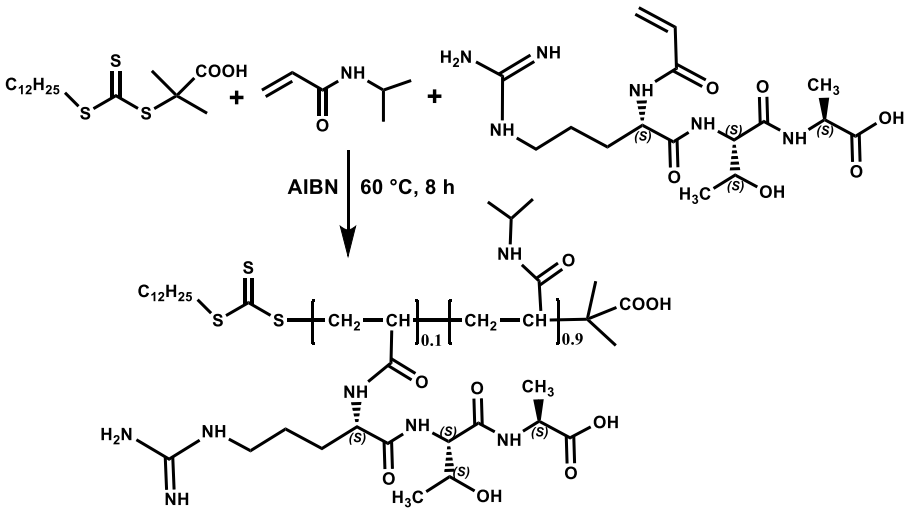


Scheme S3 Modification of QC resonator or gold electrode surfaces with PNI-*co*-ARTA$_{0.1}$.

First, Au-coated QC resonator (purchased from Q-Sense Corp.) with an intrinsic frequency (F_0) of 5 MHz was washed four times with distilled water and ethanol, respectively. After treatment with a fresh mixture solution of water, ammonia water and hydrogen peroxide at the volume ratio of 5:1:1 for 10 min at 70 ^{o}C, the resonator was rinsed with distilled water and ethanol in turn. Then the Au-coated QC resonator was treated with 2-mercaptoethylamine hydrochloride (0.01 mol L^{-1}) in ethanol (10 mL) at room temperature for 24 hours to generate Au−S bonds on the gold surface. Afterwards, the QC resonator was immersed in the mixed solution of anhydrous CH_2Cl_2 (20 mL) and pyridine (0.8 mL) at 0 ℃ for 15 min, and then the polymerization initiator bromoiso-butyryl bromide (BiBB, 0.8 mL) was added dropwise to the mixture and kept at 0 ℃ for 30 min. After that, the reaction device was removed from the ice-water bath and maintained at the ambient temperature overnight in the dark. After being rinsed with CH_2Cl_2 and dried with nitrogen gas, the bromine–modified QC resonator was soaked in a mixed solution of NIPAAm (68 mg, 0.60 mmol), ARTA (60 mg, 0.15 mmol), CuBr (1.4 mg, 0.01 mmol) and *N*,*N*,*N*',*N*'',*N*''-pentamethyldiethylenetriamine (2 μL, 0.01

mmol) in DMF (3 mL). The reaction was carried out at 60 ℃ and nitrogen atmosphere for 7 hours. Finally, the PNI-*co*-ARTA$_{0.1}$ anchored QC resonator was washed with DMF (20 mL), H_2O (10 mL) and ethanol (10 mL), respectively. Similar method was used to immobilize the copolymer on the gold electrode surface.

2.4 Preparation of PNI-*co*-ARTA$_{0.1}$ through RAFT Polymerization



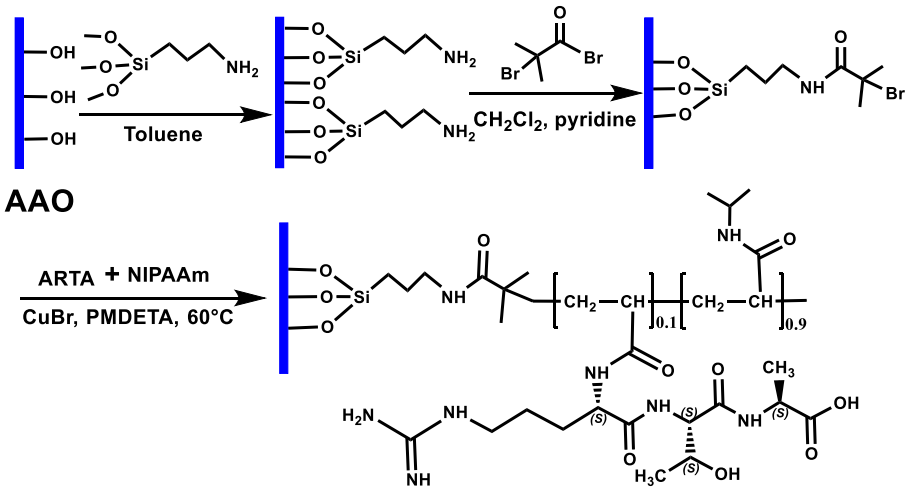
Scheme S4 Synthesis of PNI-*co*-ARTA$_{0.1}$

Chain Transfer Agent (CTA) was prepared according to literature method.[1] NIPAAm (498 mg, 4.4 mmol) and ARTA (441 mg, 1.1 mmol) were added to the solution of CTA (20 mg, 0.06 mmol) and AIBN (3 mg, 0.02 mmol) in DMF (5 mL). Then the mixture was stirred for 8 hours at 60 ℃ under nitrogen atmosphere. After cooling to room temperature, the reaction solution was purified by dialysis (molecular weight cut-off: 3500 Da) with water and ethanol in turn. After freeze-drying, white powder was obtained (378 mg). ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm): 7.07-7.36 (m, 16H, CON*H*, guanidine-N*H*), 3.79-3.88 (m, 15H, O*H*, *C*H*), 1.93-2.01 (m, 4H, CH_2), 1.33-1.56 (m, 22H, CH_2), 1.04-1.23 (m, 60H, CH_3). According to ^{1}H NMR data, the proportion of ARTA in the PNI-*co*-ARTA$_{0.1}$ copolymer was about 10%. The average molecular weight was about 10000 determined by gel permeation chromatography (GPC).

2.5 Modification of the AAO membrane with PNI-*co*-ARTA$_{0.1}$ *via* SI-ATRP

Bare AAO membrane (with average pore size of 80 nm) was immersed in distilled water

and ethanol for 10 min in turn and dried with nitrogen gas. Then the AAO membrane was soaked in the heated (100 ℃) hydrogen peroxide solution for 1 hour to generate hydroxyl groups on the inner surface of nanochannels. After that, the hydroxyl–activated membrane was washed with distilled water and ethanol successively and dried under nitrogen gas. Subsequently, the membrane was immersed in a solution of ATMS (0.5 mL) in anhydrous toluene (20 mL) and heated at 65 ℃ for 3 hours to prepare the amino–modified AAO membrane. Afterwards, the AAO membrane was rinsed with toluene for three times and dried under nitrogen gas to remove the remaining ATMS. Then the amino–modified AAO membrane was allowed to react with the polymerization initiator BiBB (0.4 mL) in dry CH_2Cl_2 (10 mL) in an ice-water bath for 30 min, then the reaction was maintained at room temperature for 12 hours in dark. After sufficient rinsing with CH_2Cl_2 to remove the BiBB residue, the Br-modified AAO membrane was soaked in a mixed solution of NIPAAm (68 mg, 0.60 mmol), ARTA (60 mg, 0.15 mmol), CuBr (1.4 mg, 0.01 mmol) and *N*,*N*,*N*',*N*'',*N*''-pentamethyldiethylenetriamine (2 μL, 0.01 mmol) in DMF (5 mL) under nitrogen for 7 hours at 60 ℃. Finally, the copolymer–modified AAO membrane was washed with DMF, ethanol and H_2O sufficiently, and dried by nitrogen gas.



Scheme S5 Modification of AAO membrane with PNI-*co*-ARTA$_{0.1}$.

2.6 Fabrication and modification of asymmetric nanochannels

Nanochannels fabrication: According to the literature method,[2] PET membrane (thickness:12 μm) irradiated by rapid heavy ions of Au with an energy of 11.4 MeV (ion track density 10^6 cm^{-2}) was firstly exposed to the UV light for 1 hour or half an hour. Then under continuous voltage of 1 V at 40 ℃, the PET membrane was etched from one side with 9

mol L^{-1} NaOH aqueous solution, the other side filled with 1 mol L^{-1} KCl and 1 mol L^{-1} formic acid aqueous solution that was able to neutralize the base solution. There was almost no ion current across the PET membrane before etching, the etching process was stopped when the ion current reached a certain expected value, generating multiple cone-shaped nanochannels with different apertures. Then, the nanochannels were washed with stopping solution to neutralize the etching solution and immersed in water to remove residual salts, respectively. Subsequently, multiple conical nanochannels with different base pore diameters of about 300 nm, 500 nm and 800 nm were obtained, respectively. The pore diameters were measured by scanning electron microscope (SEM) or Helium ion microscope (HIM) (Fig. S14). According to a classical literature,[3] the tip pore diameter could be estimated by the equation listed as below:

$$\tan\frac{\alpha}{2} = \frac{\frac{D_B}{2} - \frac{D_T}{2}}{L}$$

$$D_T = D_B - 2L\tan\frac{\alpha}{2}$$

Where D_B is the base pore diameter, D_T is the tip pore diameter, L is the thickness of the PET membrane, and α is the cone angle. According to the above formula, the tip pore diameters of the nanochannels in the PET membrane were calculated to be 25, 33 and 44 nm, respectively, corresponding to the base pore diameters of about 300 nm (α = 1.31), 500 nm (α = 2.23) and 800 nm (α = 3.63).

Modification of polymer PNI-*co*-ARTA$_{0.1}$ on the inner surface of nanochannels: A mixture solution of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI·HCl, 15 mg, 0.08 mmol) and *N*-hydroxysuccinimide (NHS, 9 mg, 0.08 mmol) in pure water (2 mL) was prepared as an activator for carboxyl acid. Then the PET membrane was soaked in the mixture aqueous solution for one hour. After being washed with distilled water, the membrane was immersed in 0.01 M ethylendiamine (EDA) aqueous solution overnight. Then the membrane was removed to a mixed solution of EDCI·HCl (15 mg, 0.08 mmol), NHS (9 mg, 0.08 mmol), and PNI-*co*-ARTA$_{0.1}$ (9 mg) in distilled water (2 mL) for 5 hours. Finally, the PET membrane was taken out and rinsed with distilled water. Evidential decrease of the transmembrane ion current of the PET conical nanochannels after being modified with PNI-*co*-ARTA$_{0.1}$ (Fig.

S15a, S16a, and S17a) illustrated that PNI-*co*-ARTA$_{0.1}$ had been successfully grafted into the nanochannels.

3. Test method

3.1 ^{1}H NMR titration experiment

Different molar ratios (0:1, 1:0, 1:1, 1:2, or 1:3) of RTA and cAMP sodium salt were mixed in DMSO-d_6 at 20 ℃, RTA concentration: 1.0 mmol L^{-1}. After equilibration for 4 hours at ambient temperature, chemical shifts of hydrogen protons in the mixture solution were recorded and analyzed by a BRUKER AVANCE III 400–MHz spectrometer.

3.2 Fluorescent titration experiment

The host FITC-labelled RTA was prepared as a stock solution (5.0×10^{-6} mol·L^{-1}) in distilled water. Guests of cAMP, ATP, ADP, AMP and adenosine were prepared into three stock solutions in distilled water (1.0×10^{-2}, 1.0×10^{-3} and 1.0×10^{-4} mol·L^{-1}), respectively. The work solutions were prepared by adding different volumes of guest solution to a series of test tubes, then the same amount of stock solution of the host of FITC-labeled RTA was added into each test tube, and they were diluted to 3.0 mL by water. After being shaken for 1 min, the work solutions were measured immediately at 20 ℃ by using a FL-6500 fluorescence spectrophotometer (excitation wavelength: 365 nm). The association constant (K_a) values between host RTA and guests (cAMP, ATP, ADP, AMP and adenosine) were obtained from the fluorescent titration experiments according to intensity changes in the emission-peak maximum through a nonlinear fitting calculation equation listed as below.

$$F = F_0 + \frac{F_{lim} - F_0}{2C_0}\left\{C_H + C_G + {}^1/_{K_a} - \left[\left(C_H + C_G + {}^1/_{K_a}\right)^2 - 4\,C_H \times C_G\right]^{1/2}\right\}$$

Where F represents the fluorescent intensity, F_0 and F_{lim} are the initial and ultimate fluorescent intensity, respectively, and C_H and C_G are the corresponding concentrations of host fluorescein–labeled-RTA and the guest, C_0 is the initial concentration of the host.

3.3 QCM-D adsorption experiment

All QCM-D measurements were performed at 20 ℃ on a Q-Sense E4 system (Sweden). Prior to binding assays between QC resonator modified with PNI-*co*-ARTA$_{0.1}$ and cAMP or ATP, QCM channels and tubes were washed carefully by using distilled water and dried by nitrogen gas. Subsequently, the copolymer–modified QC resonator was installed into a flow-cell for frequency and dissipation measurements. After stabilization of fundamental resonance frequency with the flow of pure water, cAMP, or ATP solution (10 μmol L^{-1}) was pumped into the flow-cell by a peristaltic pump at a speed of 100 μL min^{-1}. All of the time–dependent frequency and dissipation variation curves were recorded by Q-Sense software and analyzed by Q-Tools.[4]

3.4 LCST measurement

The copolymer solution was injected into a closed quartz cell and the LCST measurement could be completed within 100 min. Transmittance of copolymer solution at 500 nm was measured by PerkinElmer Lambda 365 UV–Vis spectrophotometer at different temperatures, then effect of cAMP addition on the LCST of the copolymer was investigated. According to dramatic change of transmittance near the LCST, the LCST of copolymer was measured to be approximately 40 ℃ in distilled water (pH 6.5). Upon the addition of cAMP (0.1 mg), the LCST of copolymer increased to 45 ℃. These data indicated that PNI-*co*-ARTA$_{0.1}$ was a typical thermo-responsive polymer and its LCST was strongly influenced by the addition of cAMP.

3.5 ITC experiment

The sample cell was loaded with 200 μL PNI-*co*-ARTA$_{0.1}$ solution (solvent: water, 0.1 mmol L^{-1}) while the reference cell was loaded with 200 μL of distilled water. The syringe was filled with a cAMP solution (solvent: water, 25 mmol L^{-1}, 40 μL). A run of ITC consists of 18 times successive injections of 2 μL titrant solution with 4 s duration each and an interval of 3 min between two injections. The apparent heat effect per injection, which corresponds to the change in molality of titrated solution in the sample cell, was determined by automatic peak integration of thermal power vs time curve.

Binding enthalpies, entropies and associated constant were tested at T = (298.15 ± 0.01) K

and atmospheric pressure P = (101.3 ± 5.0) kPa, which were calculated by software of MicroCal Analysis Launcher. Under the condition of constant pressure, the enthalpy change is equal to the thermal effect of the reaction system (that is $\Delta H = Q_p$, where Q_p is the constant pressure reaction heat). According to a literature,[5] a spontaneous binding interaction is defined by a negative change in the Gibbs free energy (ΔG) of the system, which is formed by the interacting partners and the surrounding solvent molecules. The more negative the ΔG, the stronger the interaction, since the ΔG value dictates the binding affinity of the process ($\Delta G = -RT \ln K$), where R is the gas constant, T is the absolute temperature, and K is the association constant.

On the basis of the relative enthalpic (ΔH) and entropic ($T\Delta S$) contributions to ΔG ($\Delta G = \Delta H - T\Delta S$) at a constant temperature, enthalpy (ΔS) can be obtained by Gibbs－Helmholtz equation:

$$\Delta S = \frac{(\Delta H - \Delta G)}{T}$$

For the data illustrated in Figure 3d, a nonlinear fitting based on a sequential binding site model (N = 2) was used to give the thermo dynamical parameters ($\Delta H1$, $\Delta H2$, $\Delta S1$, $\Delta S2$), which was calculated by software of MicroCal Analysis Launcher automatically. The detailed calculation mechanism and formula could consult an application note–"Isothermal titration calorimetry: Theory and practice", which was written by Dr. Adrian Velazquez-Campoy and Dr. Natalia Markova, as well as an ITC tutorial guide provided by Malvern Corp. The audiences interested in these documents could send an email to the corresponding authors of this manuscript. Although these calculation formula are complicated for chemists, a technician in Malvern Corp. (Dr. Peifu Han) told the authors that the MicroCal Analysis software could handle these calculation precisely and automatically.

3.6 Electrochemical impedance spectroscopy (EIS) measurement

EIS experiments were performed in 0.1 mol L^{-1} KCl solution containing 5 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$, and the experimental conditions were as follows: open-circuit potential, 0.3 V; alternative voltage, 5 mV; frequency range, 0.1-105 Hz; temperature, 20 ℃. The three electrode system consisted of Au electrode modified with the PNI-*co*-$ARTA_{0.1}$ as the working

electrode, Ag|AgCl electrode as the reference electrode and glassy carbon electrode as the auxiliary electrode.

3.7 Current-Voltage (*I-V*) measurement

The ionic transport property of the heterogeneous membrane was examined by *I–V* measurements using a commercial Keithley 6487 picoammeter (Keithley Instruments). The copolymer–modified AAO membrane was mounted between the two chambers of the conductivity cell according to the literature method.[6] Both halves of the cell were filled with NaCl solutions (0.1 $mol \cdot L^{-1}$) containing different gradient concentrations of cAMP. A scanning voltage varying from −0.2 V to +0.2 V was applied through Ag|AgCl electrodes as the transmembrane potential. The copolymer–modified PET multi-porous film was mounted between the two chambers of the conductivity cell, and both halves of the cell were filled with symmetric solutions. The main transmembrane potential applied in this work was a scanning voltage that varied from −2 V to +2 V, and the voltage was applied by Pt electrodes. For each experiment, three pieces of PET membranes were etched with the same method and grafted with the copolymers, then the *I-V* curves were calculated in parallel to guarantee the reproducibility of the data. Each test was repeated at least 5 times to obtain the average current value at different voltages.

3.8 Cycling experiment

First the copolymer-modified AAO membrane was immersed in a NaCl solution (0.1 $mol \cdot L^{-1}$) containing cAMP (10 $\mu mol \cdot L^{-1}$), and the recording current decreased from −14 to −8 μA. Then the cAMP solution was removed by pipette from the measurement apparatus, fresh NaCl solution was added and the membrane was immersed in this solution for 1 hour, allowing the copolymer chain to return back to its initial state gradually. After that, the current was measured to recover to −14 μA. Under this condition, cAMP would diffuse from the copolymer film to bulk solution, which drives the dissociation of cAMP from the copolymer, accompanied by the gradual recovery of the polymer conformation. The membrane could be alternately treated by NaCl solution with or without cAMP for several times, the current changes were recorded.

Similar procedure was adopted to evaluate the reversibility of the copolymer–grafted PET membrane. Differently, the concentrations of NaCl and cAMP were 0.01 mol·L^{-1} and 1 μmol·L^{-1}, respectively.

4. Figures

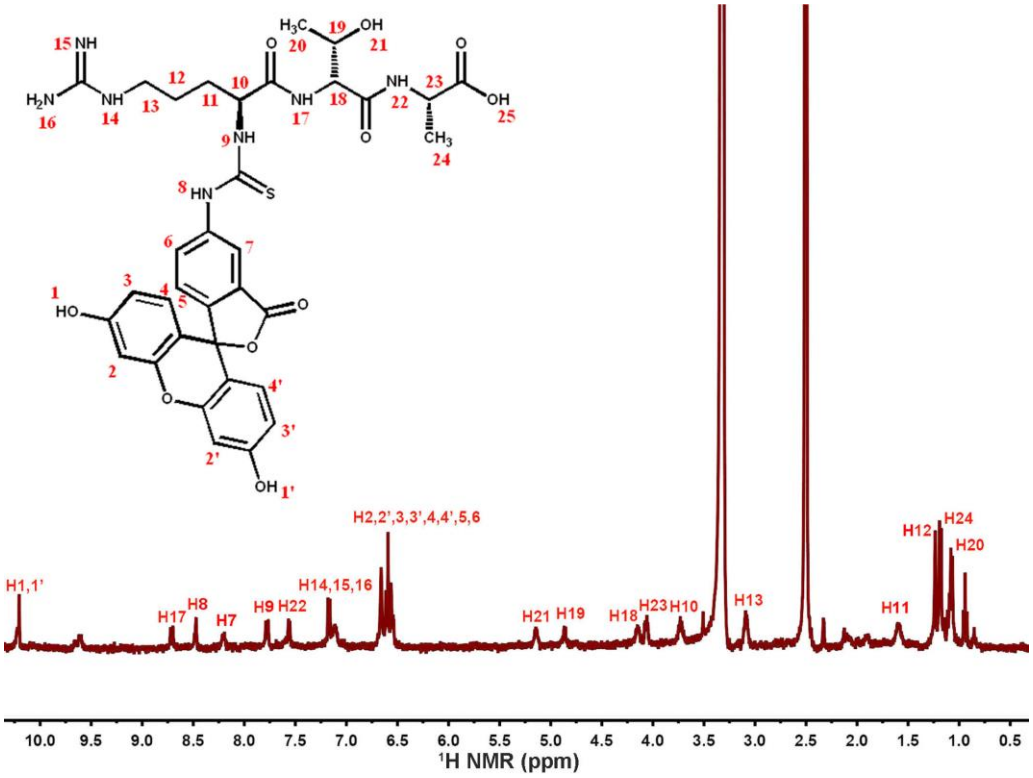


Figure S1 ^{1}H NMR spectrum of FITC-labelled RTA in DMSO-d_6.

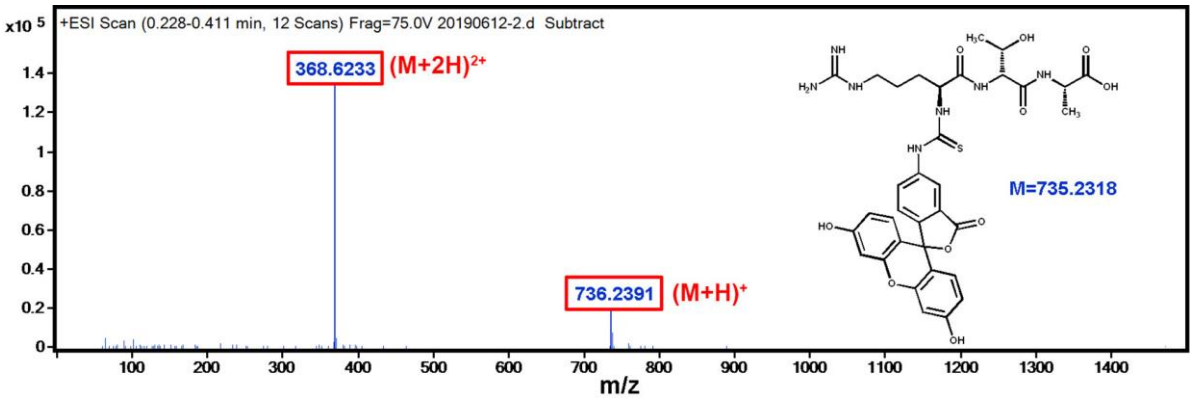


Figure S2 Mass Spectrum of FITC-labelled RTA.

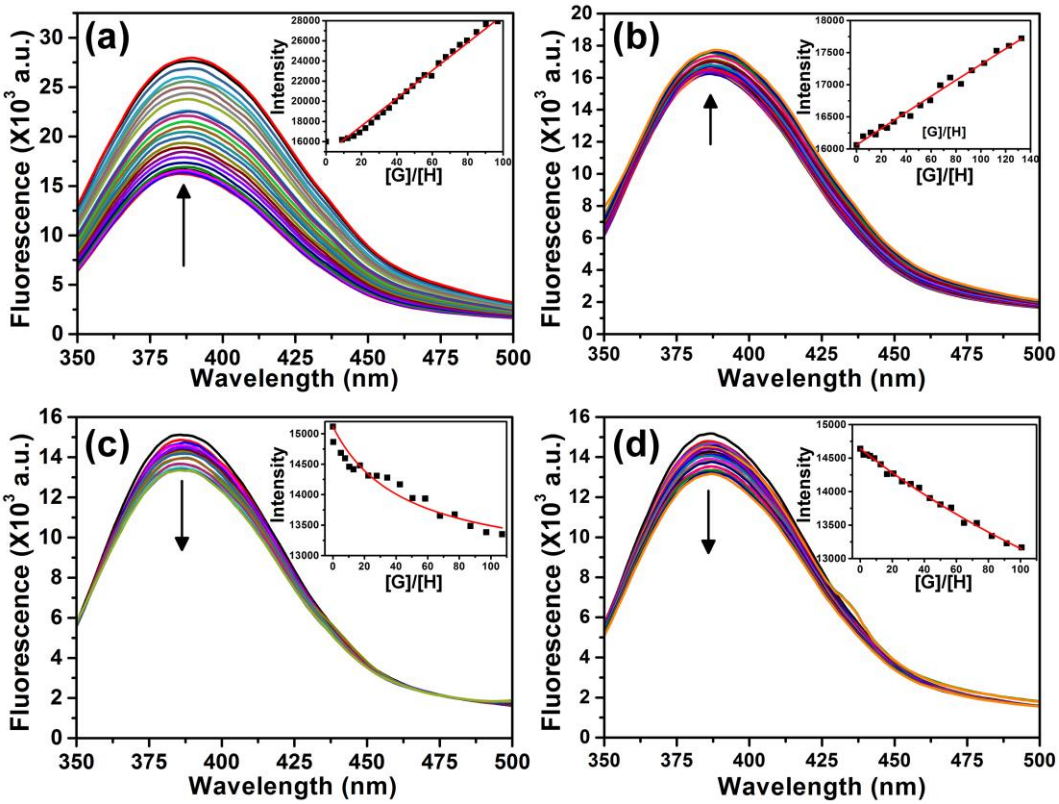


Figure S3. Fluorescence spectra of FITC-labelled RTA aqueous solution (5.0×10^{-6} mol·L^{-1}) upon additions of different equivalents of ATP (a), ADP (b), AMP (c) or adenosine (d). The insets show the fluorescent intensity changes of FITC-labelled RTA upon the additions of various guests, [G]/[H] is an abbreviation of the molar ratio of guest to host. The red lines are nonlinear–fitted curves to calculate association constants (K_a). The K_a of FITC-labelled RTA with ATP, ADP, AMP or adenosine was 32, 11, 450 and 54 L·mol^{-1}, respectively.

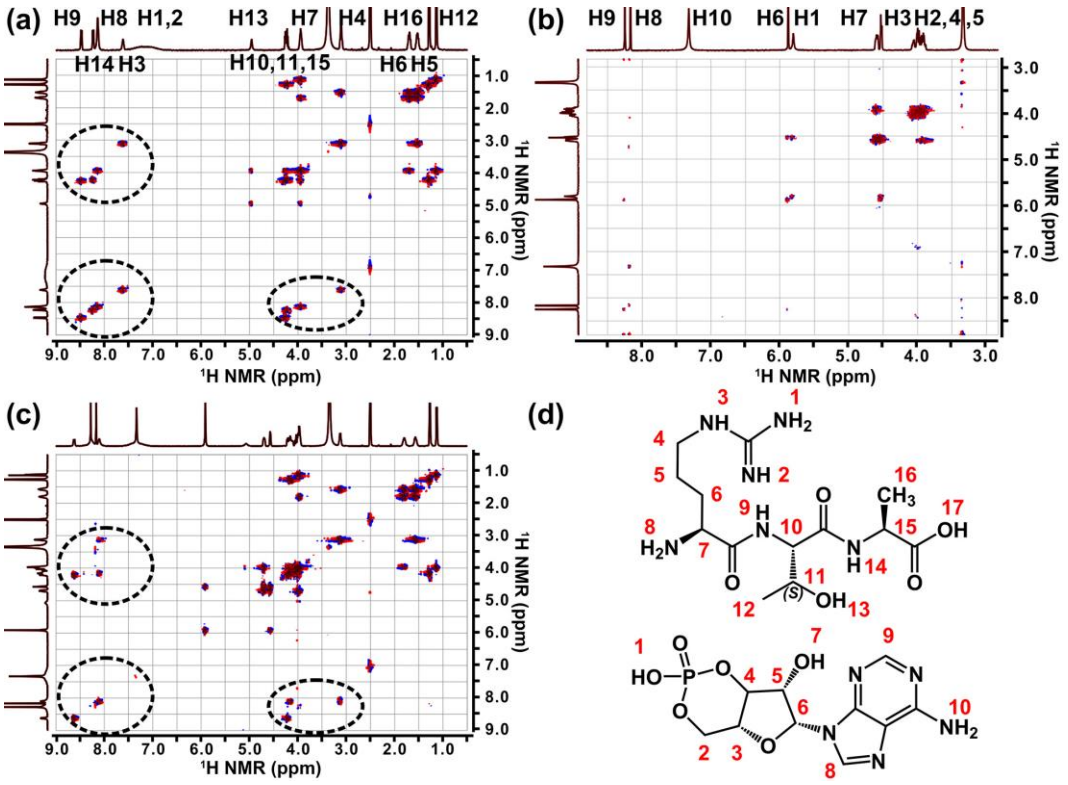


Figure S4 ^{1}H-^{1}H COSY spectra of RTA (a), cAMP (b) and their mixture at a molar ratio of 1:1 in DMSO-d_6 at 20 ℃ (c). (d) Chemical structures of RTA and cAMP and the attribution of each H proton.

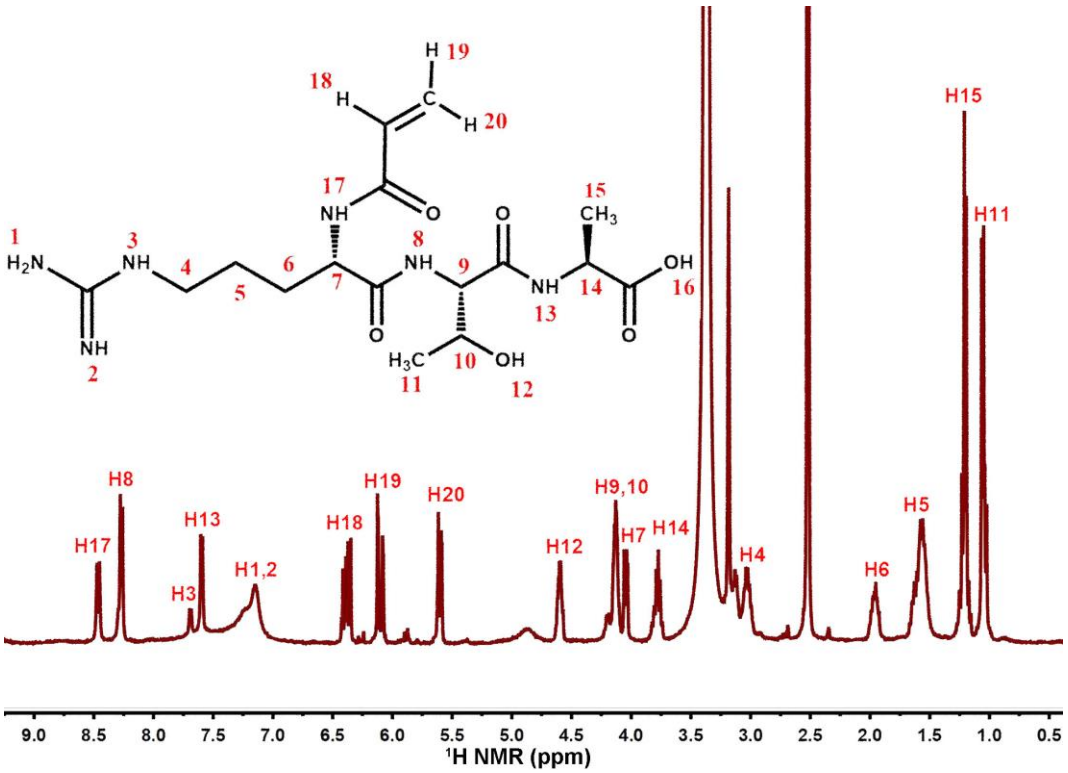


Figure S5 ^{1}H NMR spectrum of ARTA in DMSO-d_6.

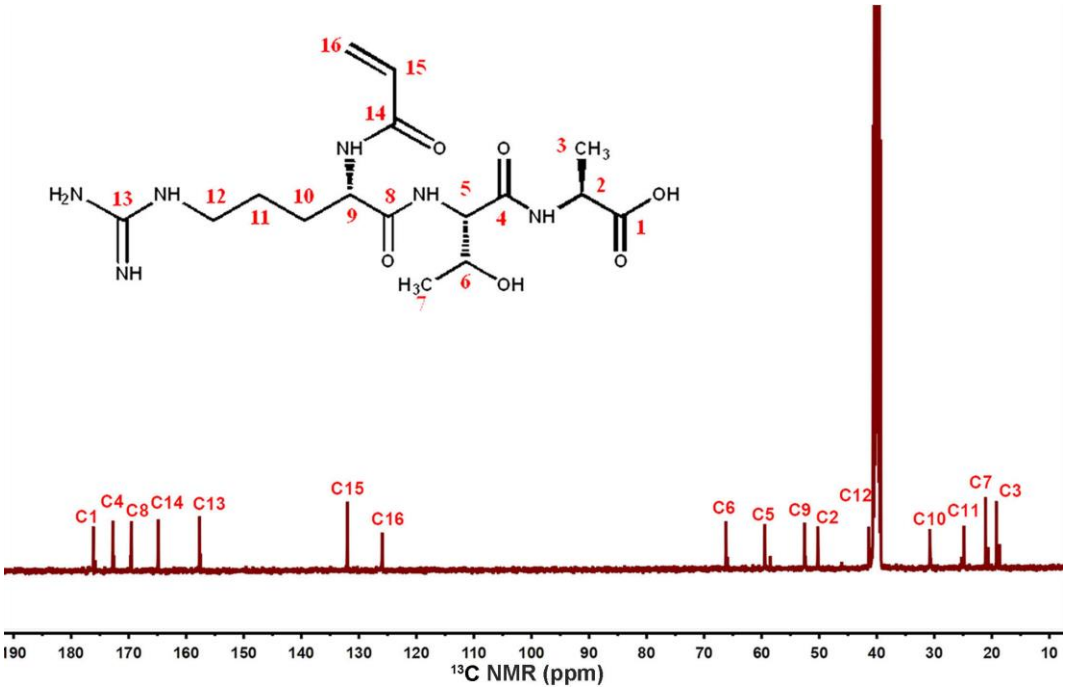


Figure S6 ^{13}C NMR spectrum of ARTA in DMSO-d_6.

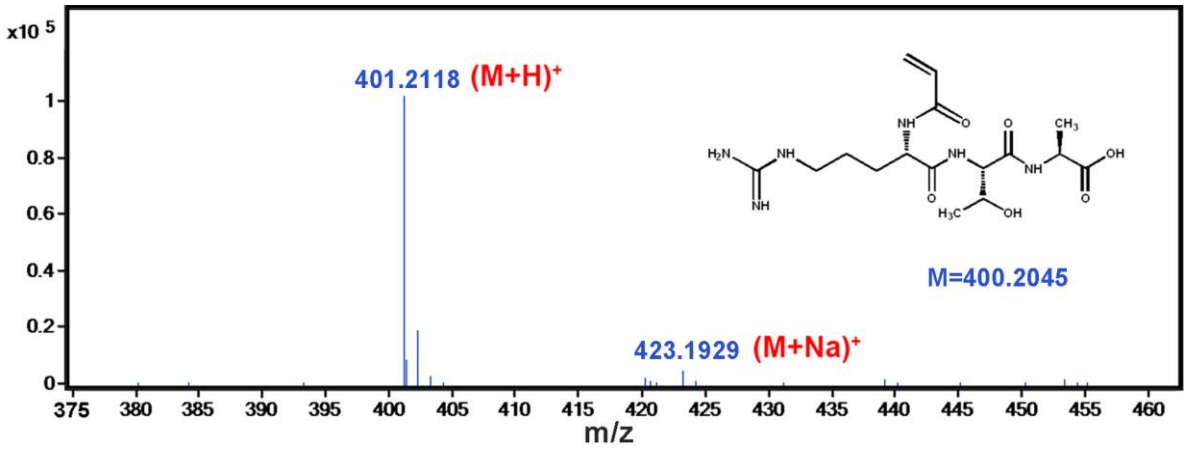


Figure S7 Mass spectrum of ARTA.

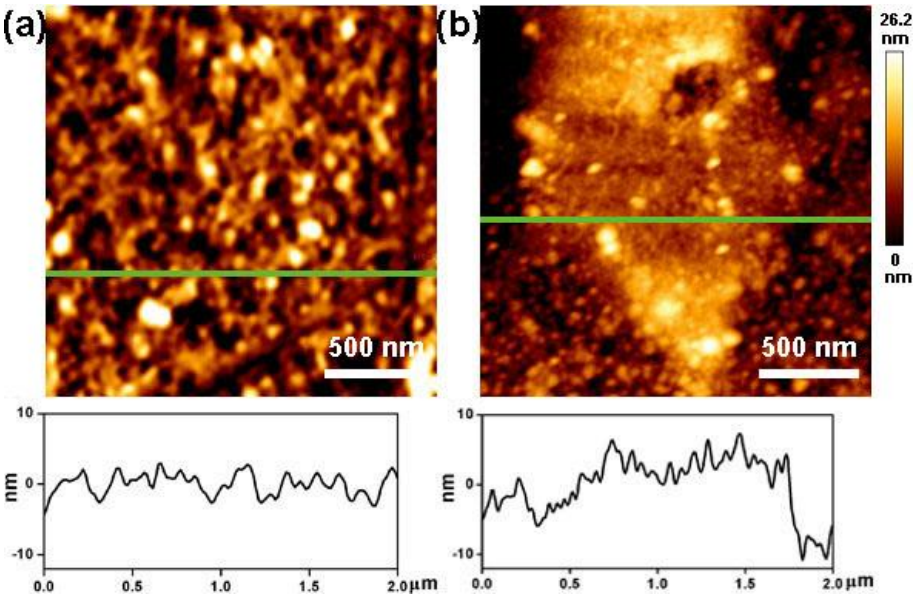


Figure S8 AFM images (2 μm × 2 μm) of the PNI-*co*-ARTA$_{0.1}$ modified QC resonator before (a) and after (b) being immersed in a cAMP solution (10 μmol·L^{-1}) for 15 min. The lower panels are section profiles of the AFM images along the green lines. The evidential increase in the fluctuation of the AFM images indicated the expansion of the copolymer film in response to the cAMP adsorption.

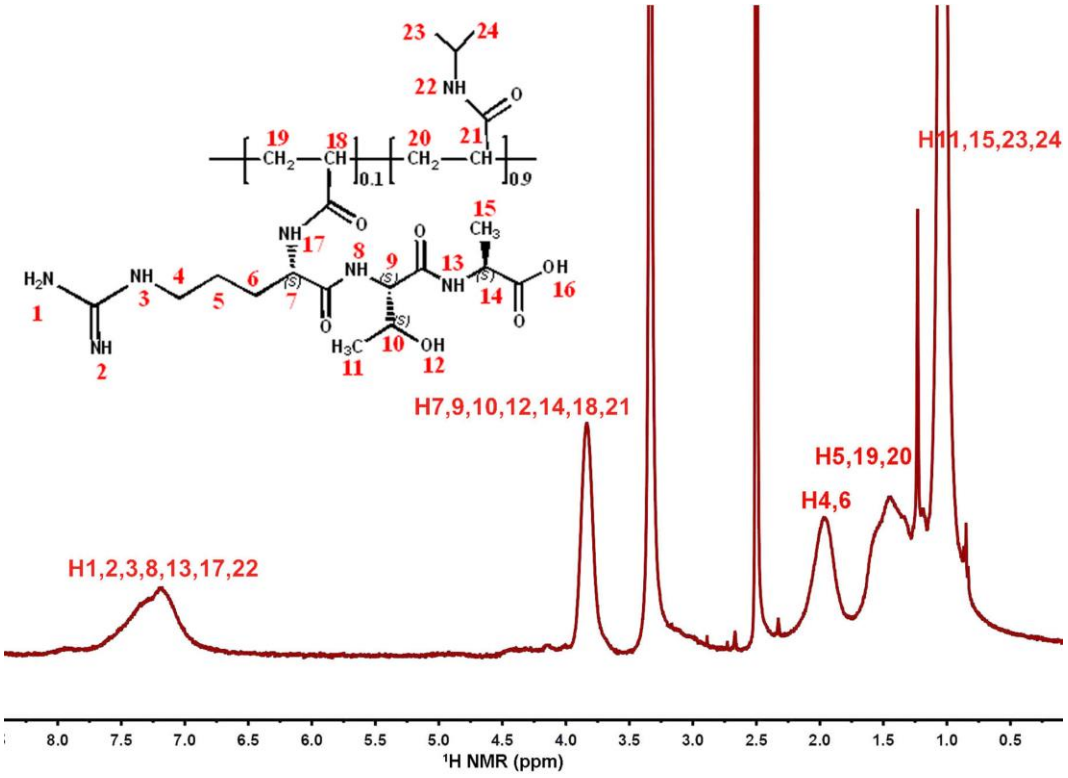


Figure S9 ^{1}H NMR spectrum of PNI-*co*-ARTA$_{0.1}$ in DMSO-d_6.

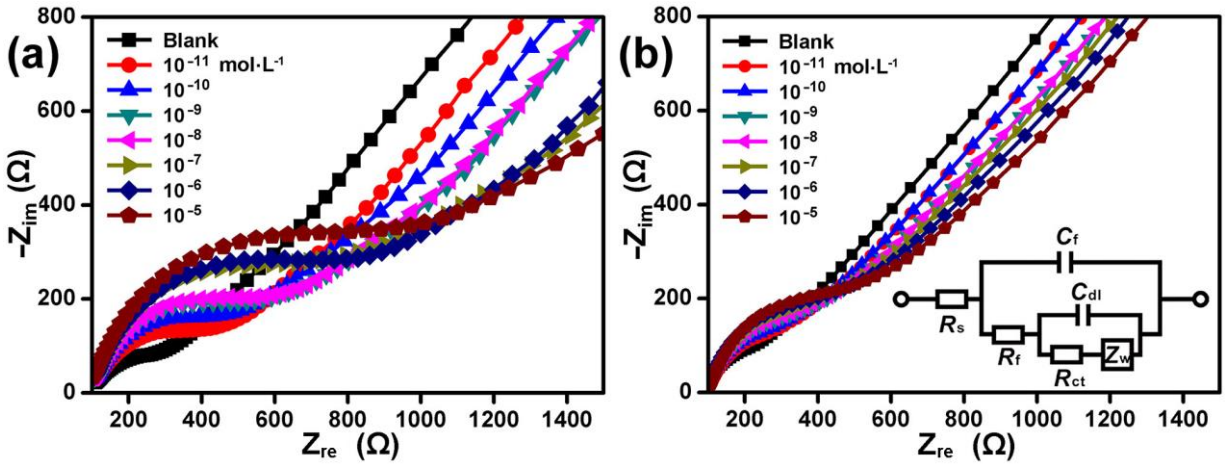


Figure S10 Nyquist plots obtained at the copolymer–modified gold electrode after being treated by different amounts of cAMP (a) or ATP (b) in 0.1 mol L^{-1} KCl solution containing 5 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$ for 10 min. Inset: equivalent circuit used for analyzing the impedance spectra for electrochemical sensor.

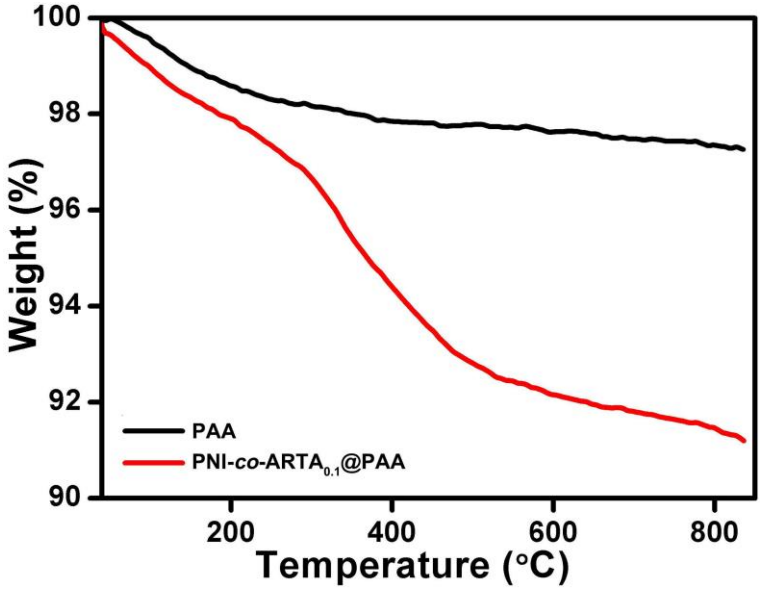


Figure S11 Thermal gravimetric analysis (TGA) of AAO membranes before (black) and after (red) PNI-*co*-$ARTA_{0.1}$ modification. Evidential weight loss of 8% in TGA spectrum indicated that PNI-*co*-$ARTA_{0.1}$ had been immobilized on the AAO membrane with high density.

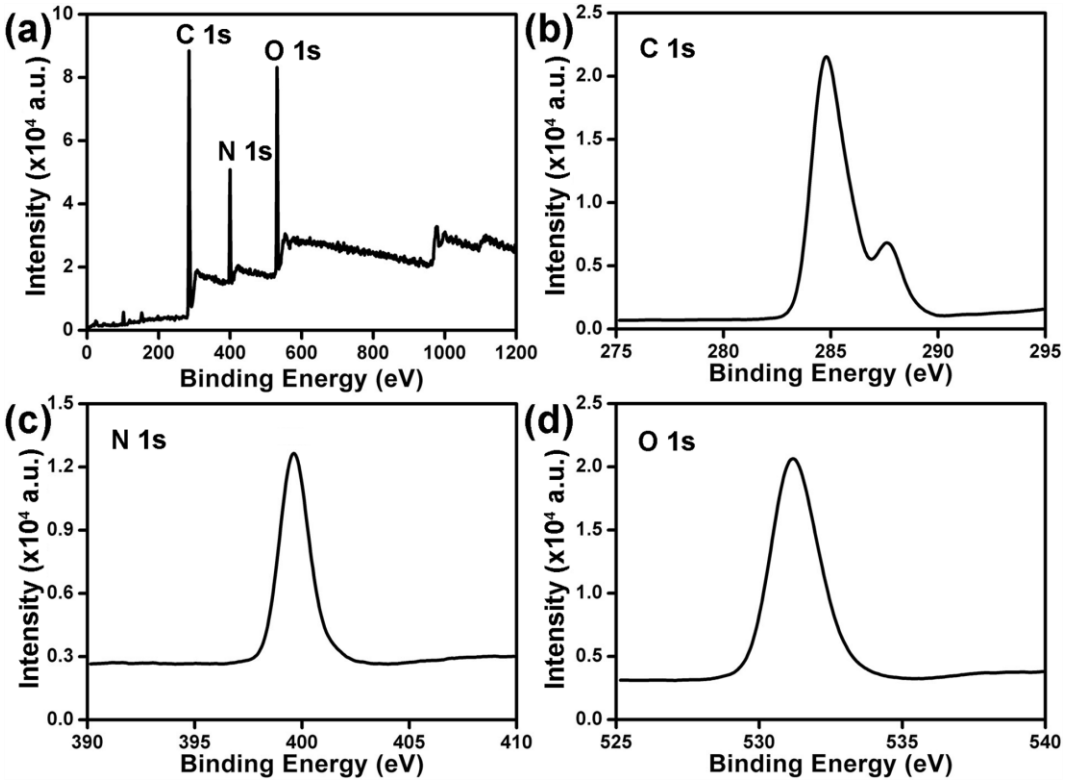


Figure S12 Survey scan (a), C 1s (b), N 1s (c) and O 1s (d) narrow scan of the copolymer modified AAO membrane, detected by X-ray photoelectron spectroscopy (XPS) spectra. The strong peaks of the C and N elements indicated that the copolymer had been successfully immobilized on the AAO membrane.

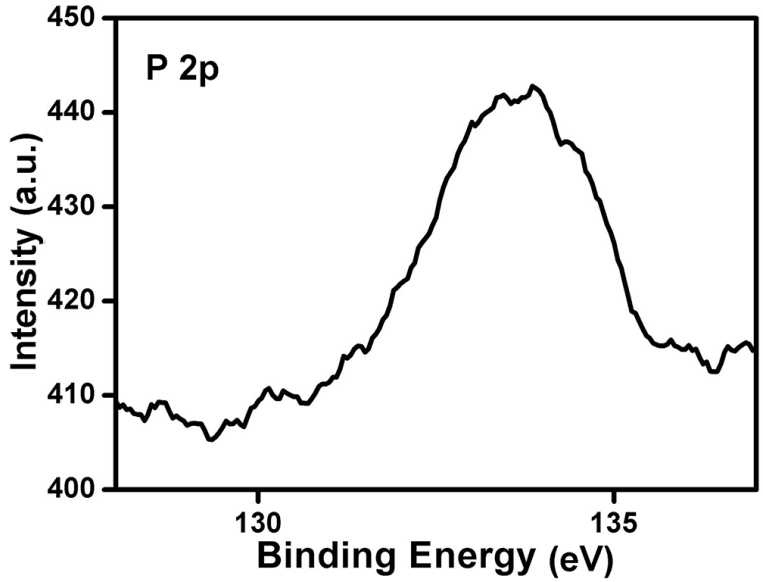


Figure S13 P 2p narrow scan of the PNI-*co*-ARTA$_{0.1}$ modified AAO membrane after being treated by a cAMP solution (10 μmol·L^{-1}). The appearance of P 2p peak indicates that cAMP has been adsorbed onto the copolymer thin film.

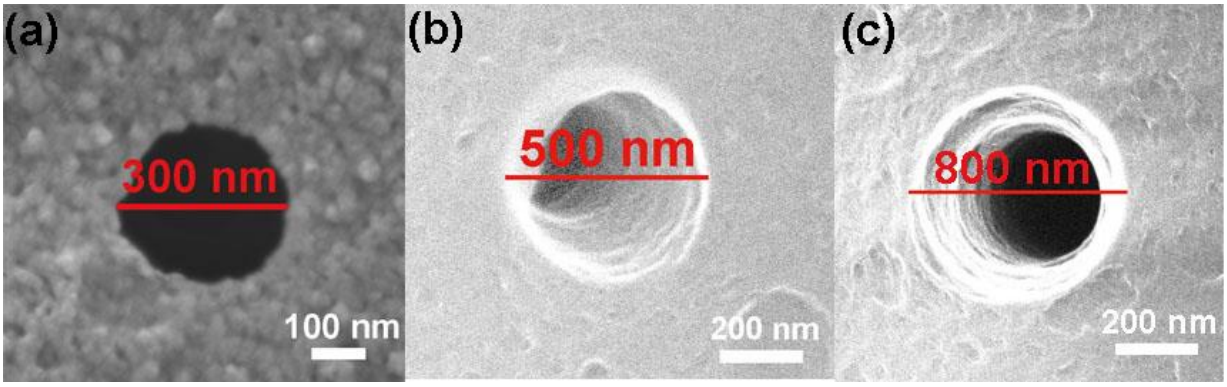


Figure S14 (a) SEM image of the PET conical nanochannel with a base pore diameter of 300 nm. Helium ion microscope (HIM) images of the PET conical nanochannels with base pore diameters of 500 (b) and 800 nm (c).

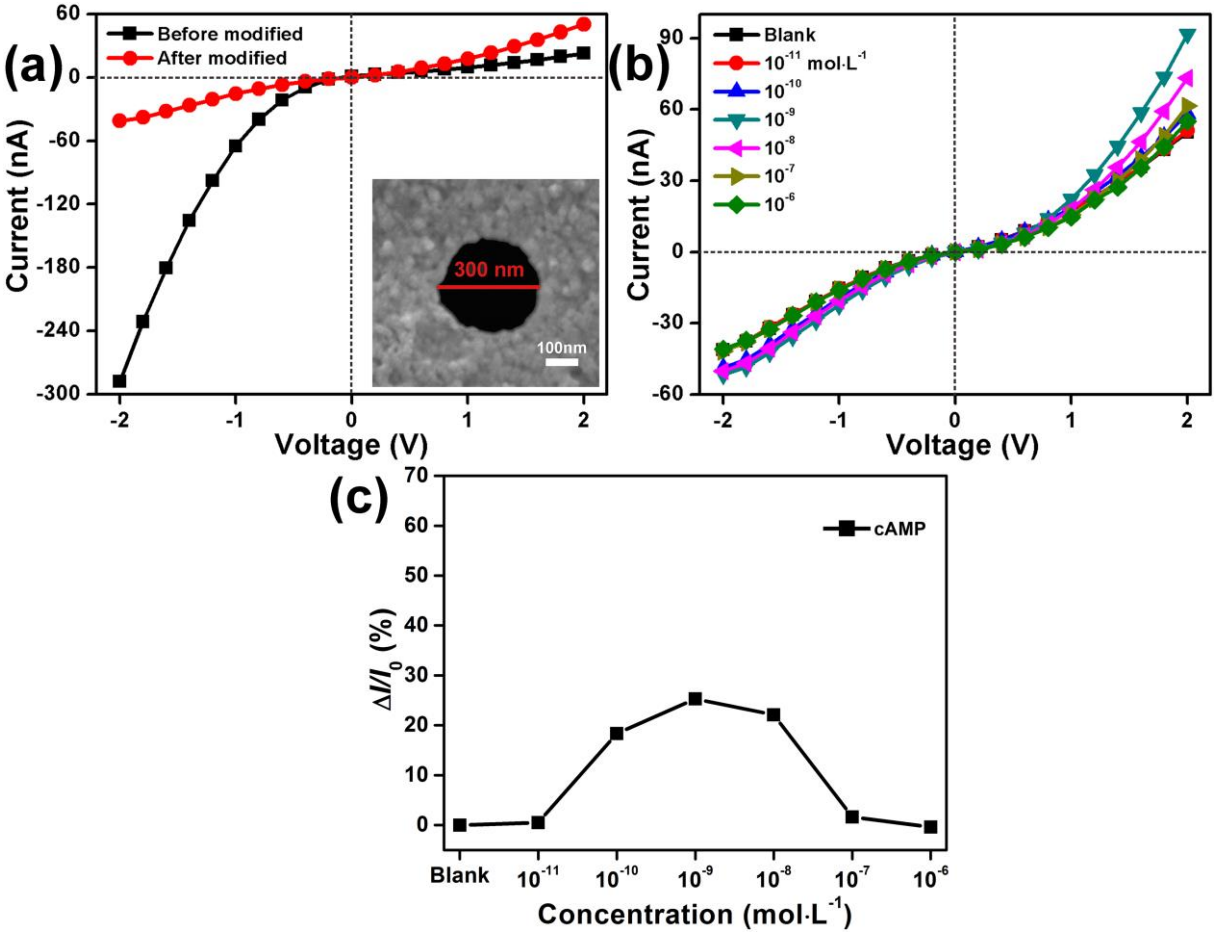


Figure S15 (a) Current–Voltage (*I*–*V*) curves of multiple conical nanochannels on PET membranes before and after the copolymer modification, the base pore diameter was about 300 nm (SEM image is shown in the inset). (b) *I*–*V* curves of the copolymer–grafted conical nanochannels treated by 0.01 mol·L^{-1} NaCl solution containing different concentrations of

cAMP. (c) Concentration−dependent ionic current changes ($\Delta I/I_0$) (at −2 V) of the copolymer-grafted conical nanochannels treated by cAMP solution. Irregular ionic current change was observed, we presumed that the grafted copolymer might obstruct the nanochannels with tip pore diameter of about 25 nm, resulting in unsatisfactory cAMP responsiveness and substantially lower ionic current as low as tens of nA.

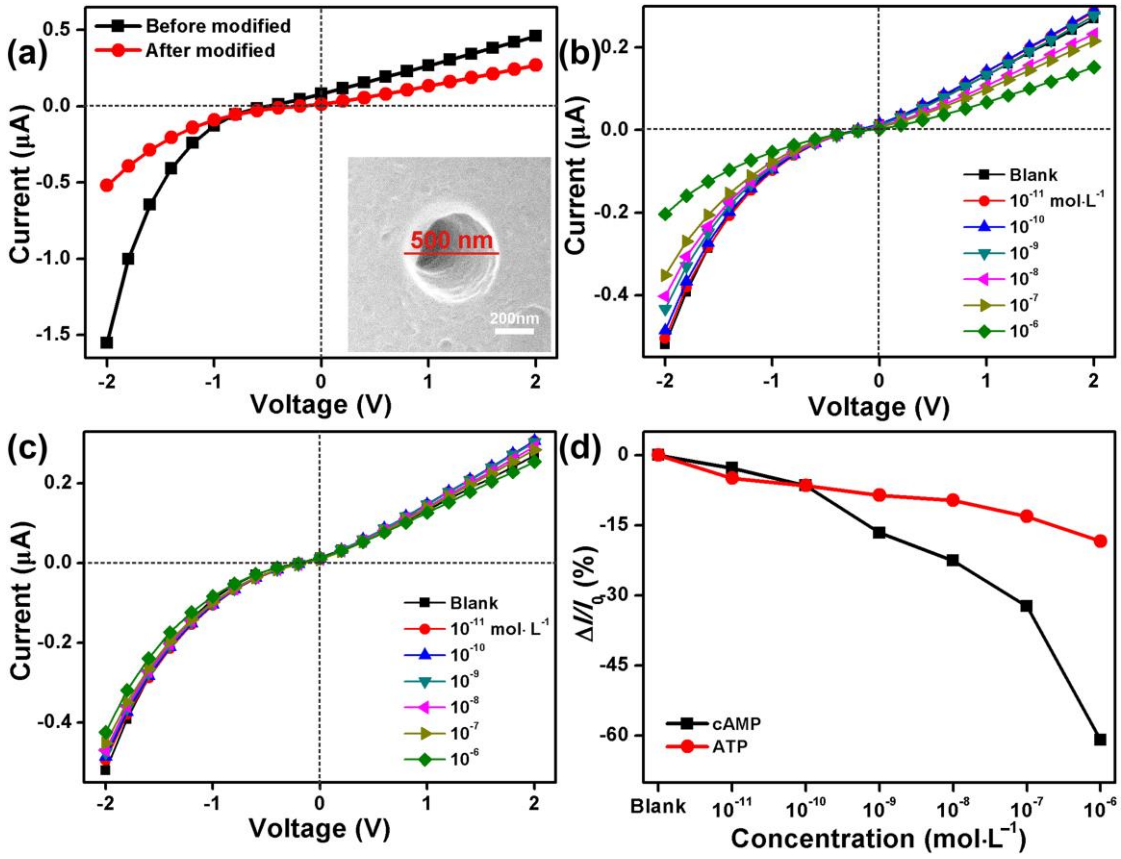


Figure S16 (a) *I–V* curves of multiple conical nanochannels on PET membranes before and after the copolymer modification, the base pore diameter was 500 nm (HIM image is shown in inset). (b, c) *I–V* curves of the copolymer–grafted conical nanochannels treated by 0.01 mol·L^{-1} NaCl solution containing different concentrations of cAMP (b) or ATP (c). (d) Concentration−dependent ionic current changes ($\Delta I/I_0$) (at −2 V) of the copolymer-grafted conical nanochannels treated by cAMP or ATP solution, illustrating evidential discrimination between cAMP and ATP.

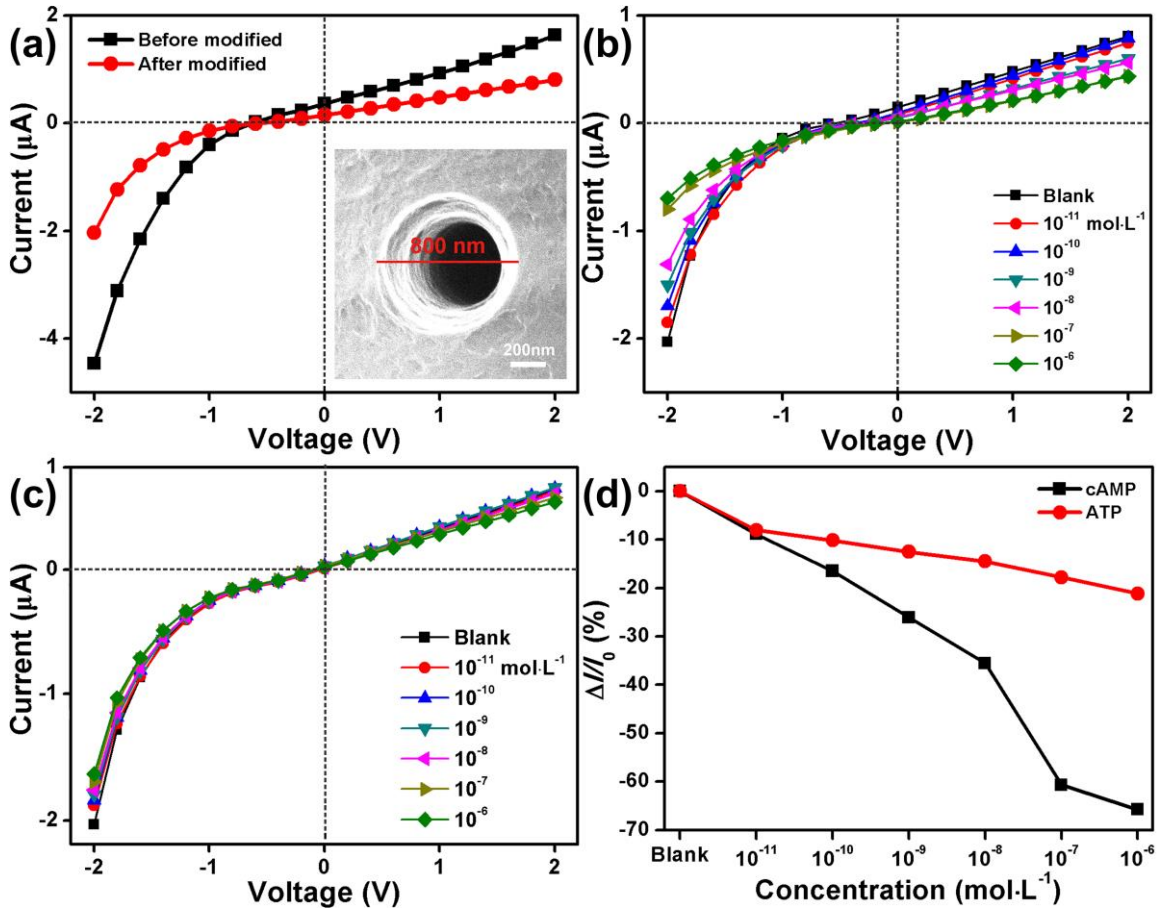


Figure S17 (a) *I–V* curves of multiple conical nanochannels on PET membranes before and after the copolymer modification, the base pore diameter was 800 nm (HIM image is shown in inset). (b, c) *I–V* curves of the copolymer–grafted conical nanochannels treated by 0.01 mol·L^{-1} NaCl solution containing different concentrations of cAMP (b) or ATP (c). (d) Concentration−dependent ionic current changes ($\Delta I/I_0$) (at −2 V) of the copolymer-grafted conical nanochannels treated by cAMP or ATP solution, illustrating evidential discrimination between cAMP and ATP.

5 References

1. Z. Chen, T. Sun and G. Qing. *J. Mater. Chem. B.*, 2019, **7**, 3710.

2. Z. Meng, C. Jiang, X. Li and J. Zhai, *ACS Appl. Mater. Interfaces*, 2014, **6**, 3794.

3. L. Gao , P. Li, Y. Zhang, K. Xiao, J. Ma, G. Xie, G. Hou, Z. Zhang, L. Wen and L. Jiang, *Small*, 2015, **11**, 543.

4. G. M. Liu, G. Z. Zhang, CM-D studies on polymer behavior at interfaces (ed. S. K. Sharma) (Springer, 2013).

5. M. Varese, S. Guardiola, J. Garc á and E. Giralt, *Chembiochem*, 2019, **20**, 2981.
6. Y. Sugawara, H. Kuroki, T. Tamaki and H. Ohashi, *Anal. Methods*, 2012, **4**, 2635.