Electronic Supplementary Information for

CAMP–modulated biomimetic ionic nanochannels based on smart polymer

Zhixiang Chen,[a] Taolei Sun[a] and Guangyan Qing*[,b]

[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.*

*Corresponding authors: qinggy@dicp.ac.cn

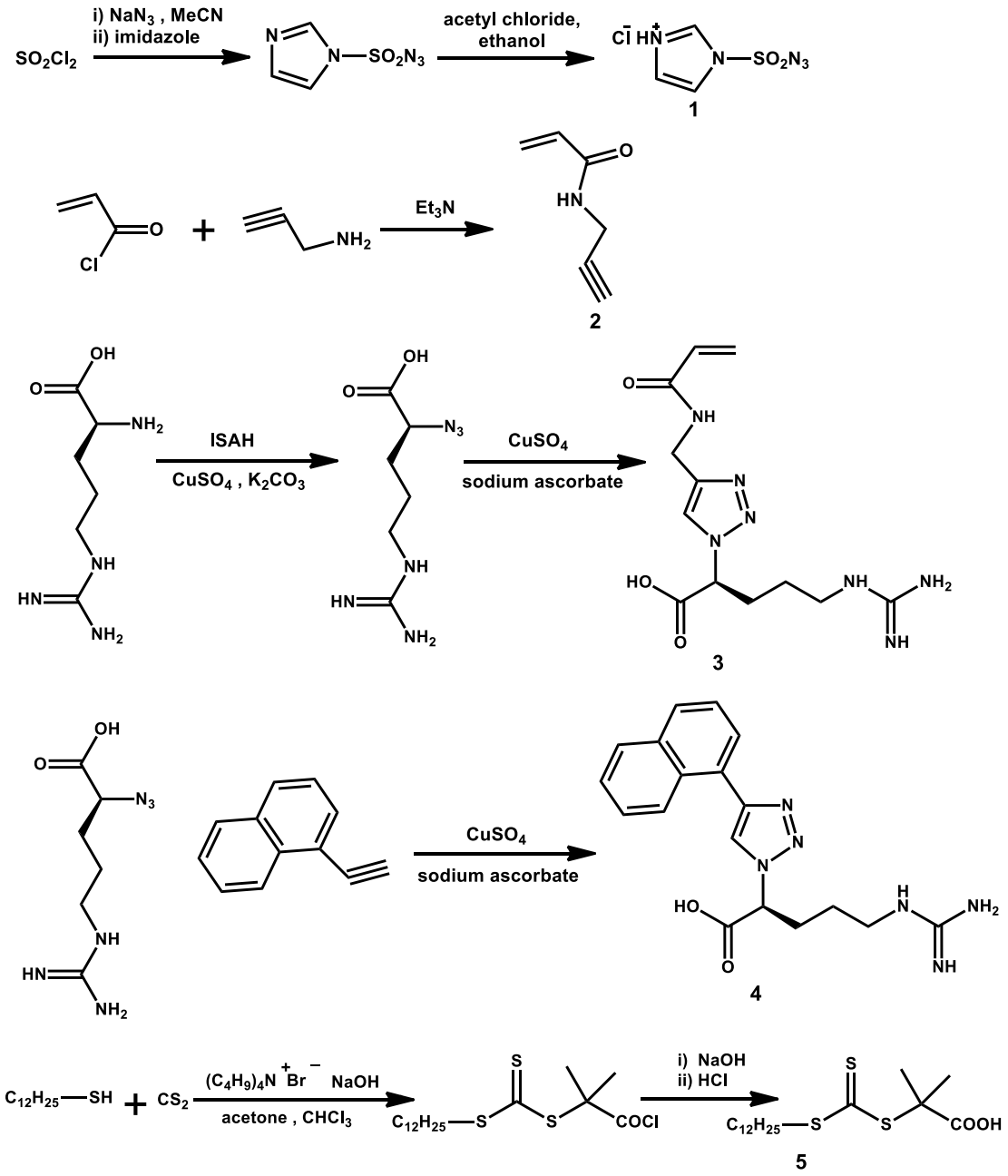
Outline

Materials and instruments

Materials: *N*–isopropyl acrylamide (99%, Sigma–Aldrich, NIPAAm) was recrystallized in n–hexane for four times before polymerization. 2–Bromoisobutyryl bromide (99%), acryloyl chloride (99%), cuprous bromide (CuBr, 99.999%), cyclic adenosine monophosphate salt (cAMP, 99%), adenosine mono-phosphate salt (AMP, 97%), adenosine 5'–diphosphate salt (ADP, 97%) and adenosine 5'–triphosphate salt (ATP, 97%) were purchased from Alfa Aesar (Germany). L–Arginine, triethylamine (Et_3N), methanol (CH_3OH), ethanol, sodium hydroxide, sulfuric acid, hydrochloric acid (HCl), hydrogen peroxide, ethyl acetate (EtOAc), acetone, toluene, dichloromethane, trichloromethane ($CHCl_3$), pyridine, *N*, *N*'–dimethylformamide (DMF), dimethyl sulfoxide (DMSO), sodium chloride (NaCl), calcium chloride ($CaCl_2$), sodium sulfate (Na_2SO_4), sodium bicarbonate ($NaHCO_3$), 2–mercapto–ethylamine(Adamas, Switzerland), 3–amino–propyl triethoxysilane (APTES, Fluka, Switzerland), deuterated methanol (CD_3OD, Aldrich, Germany), deuterated dimethyl sulfoxide (d_6–DMSO) and *N*, *N*, *N*', *N*", *N*"'–penta-methyldiethylenetriamine (Aldrich, Germany) were used as received, all chemicals used were of chromatographic pure. anodic aluminum oxide (AAO) membrane (with 80–100 nm aperture, and 60 μm thickness) were commercially obtained (Puyuan Nano, Hefei, China). Double distilled water (18.2 $M\Omega \cdot cm^{-1}$, MilliQ system, Bedford, MA, USA) was used.

Instruments: Fluorescent titration experiments were conducted by using a PerkinElmer FL–6500 fluorescence spectrophotometer. Lower critical solution temperature (LCST) was measured by using a PerkinElmer Lambda 365 UV–Vis spectrophotometer. Hydrogen and Carbon (^1H and ^{13}C) nuclear magnetic resonance (NMR) spectra were recorded on a BRUKER AVANCE III 400–MHz spectrometer, respectively. Fourier translation infrared spectra (FT–IR) were recorded on a Bruker Optics Vertex 80v FT–IR spectrometer. Quartz crystal microbalance (QCM) measurements were performed by using a Sweden Q–Sense QCM–D E4 system. Atomic force microscope (AFM) images were obtained on Multimode 8 (Bruker) and FastScan systems (Bruker). Isothermal titration calorimetry (ITC) experiment was performed on a Malvern MicroCal iTC200 system. Scanning electron microscope (SEM) images were obtained on a JSM–7500F field emission scanning electron microscope (SEM, JEOL, Japan). X–ray photoelectron spectra (XPS) were recorded on a VG MultiLab 2000 XPS system. Current–voltage was recorded with a Keithley 6487 picoammeter/voltage source. Cyclic Voltammetry (CV) and A.C. Impedance acquisition was carried out on a CHI 760E electrochemical workstation (Shanghai, China).

Synthesis and Characterization



Scheme S1. Graphic synthesis route.

General Procedures

Imidazole–1–sulfonyl azide hydrochloride 1.HCl (1)

Sulfuryl chloride (1.61 mL, 20 mol) was added drop–wise to an ice–cooled suspension of NaN_3 (1.3 g, 20 mmol) in CH_3CN (20 mL) and the mixture was stirred overnight at room temperature. Imidazole (2.59 g, 38 mmol) was added portion–wise to the ice–cooled mixture and the resulting slurry was stirred for 3 h at room temperature. The mixture was diluted with EtOAc (40 mL), washed with H_2O (2 × 40 mL) then saturated aqueous $NaHCO_3$ (2 × 40 mL), dried over $MgSO_4$ and filtered.

A solution of HCl in ethanol [obtained by the drop–wise addition of acetyl chloride (2.13 mL, 30 mmol) to ice–cooled dry ethanol (7.5 mL)] was added drop–wise to the filtrate with stirring, the mixture was chilled in an ice–bath. After filtration, the filter cake was washed with EtOAc (3 × 10 mL) to give **1**· HCl as colorless needles.[1] (2.93 g, yield: 70%). ^{1}H NMR (400 MHz, d_6–DMSO): δ (ppm): 7.70 (d, J=1.2 Hz, 2H, C–*H*), 9.15 (s, 1H, N=C–*H*); ^{13}C NMR (400 MHz, d_6–DMSO): δ (ppm): 138.0, 134.5, 119.6; IR: 2173, 1581, 1429, 1301, 1154 cm^{-1}; Matrix–assisted laser desorption ionization mass spectrometry (MALDI–MS): m/z calcd. for $C_3H_3N_5O_2S$: 173.15; found: 174.0118 $[M+H]^+$.

***N*–(prop–2–yn–1–yl) acrylamide (2)**

10 mmol propargyl amine and 15 mmol triethylamine were dissolved in 80 mL distilled $CHCl_3$. To this solution, 11 mmol acryloyl chloride diluted in $CHCl_3$ was added dropwise under cooling in ice bath. The reaction mixture was stirred at 5 ^{o}C for 30 min and then at room temperature for another 8 h. After removing most of the solvent by evaporation at reduced pressure and the precipitate salt by filtration, the residue mixture was separated on a silica column with ethyl acetate: petroleum ether (1:1 in volume) as eluent. Pure product was obtained as a yellow powder (0.96 g, yield: 88%). ^{1}H NMR (400 MHz, d_6–DMSO): δ (ppm): 8.58 (t, J=5.7 Hz, 1H, N–*H*), 6.04–6. 38 (m, 2H, C=CH_2), 5.65 (dd, J_1=J_2=2.4 Hz, 1H, C–*H*), 3.95 (dd, J_1=J_2=2.4 Hz, 2H, N–CH_2), 3.13 (t, J=2.5 Hz, 1H, C–*H*). ^{13}C NMR (400 MHz, d_6–DMSO): δ (ppm): 164.9, 131.5, 126.5, 81.4, 73.5, 28.3; IR: 3260, 3058, 1643, 1622, 1540, 1404, 1240, 1067, 980, 689 cm^{-1}. MALDI–MS: m/z calcd. for C_6H_7NO: 109.13; found: 110.0624 $[M+H]^+$.

(*S*)–2–(4–acrylamido–1H–1,2,3–triazol–1–yl)–5–guanidinopentanoic acid (3, ATGPA)

Imidazole–1–sulfonyl azide hydrochloride **1**·HCl (0.75 g, 6 mmol) was added to the L–arginine salt substrate (0.52 g, 5 mmol), K_2CO_3 (1.8 g, 13 mmol) and $CuSO_4$ (7.5 mg, 30 μmol) in CH_3OH (5 mL) and the mixture was stirred at room temperature for 9 h. The mixture was concentrated, diluted with H_2O (15 mL), acidified with conc. HCl. After removing most of the solvent by evaporation at reduced pressure, the residue was resolved in a 50mL CH_3OH:H_2O (v/v=1:1) mixture. $CuSO_4$ (75 mg, 5 mL H_2O) and sodium ascorbate (200 mg, 5mL H_2O) were added into the above mixture.[2] Then *N*–(prop–2–yn–1–yl) acrylamide was added to the solution. The reaction mixture was stirred for 24 h at room temperature and monitored by TLC. After removing most of the solvent by evaporation at reduced pressure, the residue was resolved in CH_3OH and the precipitate salt was filtrated. This procedure was repeated for three times, then the residue mixture was separated on a silica column with CH_3OH as eluent. Pure product was obtained as a white powder (0.71 g, yield: 46%). ^{1}H NMR (400 MHz, d_6–DMSO): δ (ppm): 9.16 (s, 1H, C=N*H*), 8.63 (t, J=5.6 Hz, 1H, CON*H*), 7.40–8.05 (m, 4H, N–C*H*=C, NH_2, C–N*H*), 6.28 (dd, J_1=9.6 Hz, J_2=10.4 Hz, 1H, C=C*H*), 6.12 (dd, J_1=2.4 Hz, J_2=2 Hz, 1H, C=C*H*), 5.60 (dd, J_1=J_2=2 Hz, 1H, C=C*H*), 4.87 (dd, J_1=5.2 Hz, J_2=5.6 Hz, 1H, **H*), 4.36–4.45 (m, 2H, NH–CH_2–C), 2.98–3.13 (m, 2H, CH_2), 2.20–2.28 (m, 1H, C*H*), 1.90–

1.99 (m, 1H, C*H*), 1.36–1. 43 (m, 1H, C*H*), 1.17–1. 23 (m, 1H, C*H*). ^{13}C NMR (400 MHz, d_6–DMSO): δ (ppm): 176.9, 172.6, 164.9, 157.8, 144.2, 132.0, 125.9, 122.5, 65.7, 34.7, 30.6, 26.1. Elemental analysis, calcd. (%) for $C_{12}H_{19}N_7O_3$: C, 46.60; H, 6.14; N, 31.71; found C, 46.52; H, 6.22; N, 31.64; MALDI–MS: m/z calcd. for $C_{12}H_{19}N_7O_3$: 309.15; found: 310.1642 $[M+H]^+$.

Naphthalene–TGPA (4)

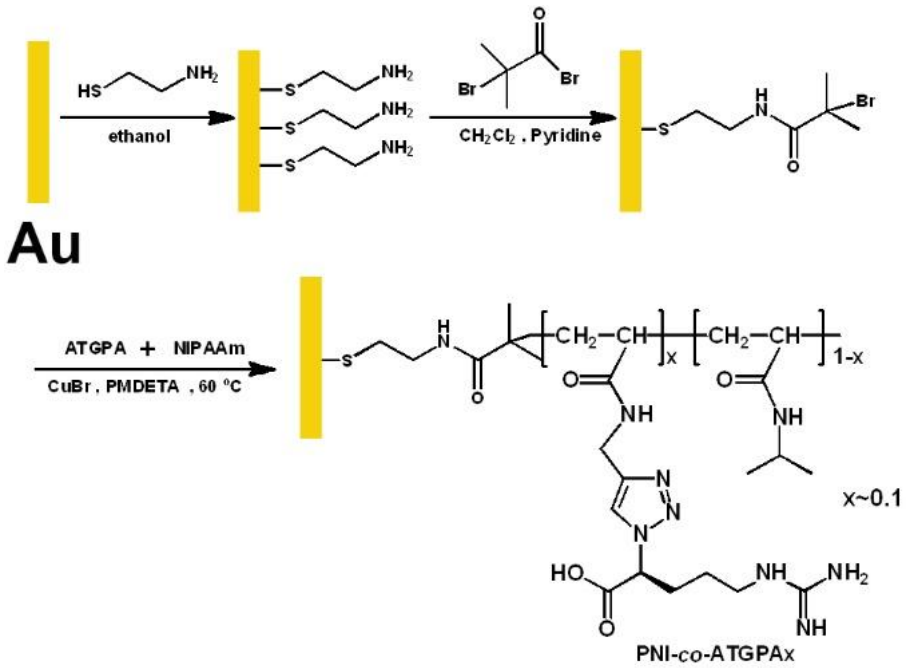
4 was prepared through a similar method described above. Imidazole–1–sulfonyl azide hydrochloride **1**·HCl (0.75 g, 6 mmol) was added to the L–arginine salt substrate (0.52 g, 5 mmol), K_2CO_3 (1.8 g, 13 mmol) and $CuSO_4$ (7.5 mg, 30 μmol) in CH_3OH (5 mL) and the mixture was stirred at room temperature for 9 h. The mixture was concentrated, diluted with H_2O (15 mL), acidified with conc. HCl. After removing most of the solvent by evaporation at reduced pressure, the residue was resolved in a 50 mL CH_3OH:H_2O (v/v=1:1) mixture. $CuSO_4$ (75 mg, 5 mL H_2O) and sodium ascorbate (200 mg, 5 mL H_2O) were added into the above mixture. Then 1–ethynylnaphthalene was added to the solution. The reaction mixture was stirred for 24 h at room temperature and monitored by TLC. After removing most of the solvent by evaporation at reduced pressure, the residue was resolved in CH_3OH and the precipitate salt was filtrated. This procedure was repeated for three times, then the residue mixture was separated on a silica column with CH_3OH as eluent. Pure product was obtained as a white powder (0.63 g, yield: 36%). ^{1}H NMR (400 MHz, d_6–DMSO): δ (ppm): 9.47–9.49 (m, 1H, C=N*H*), 8.36–8.43 (m, 4H, N–C*H*=C, N*H*$_2$, C–N*H*), 8.04–8.06 (m, 2H, Ar–*H*), 7.96–7.99 (m, 1H, Ar–*H*), 7.91–7.93 (m, 1H, Ar–*H*), 7.64–7.72 (m, 2H, Ar–*H*), 7.50–7.57 (m, 2H, Ar–*H*), 3.85–3.88 (m, 1H, **H*), 3.44–3.49 (m, 2H, *CH*$_2$), 3.03–3.07 (m, 2H, *CH*$_2$), 1.48–1.57 (m, 2H, *CH*$_2$). ^{13}C NMR (400 MHz, d_6–DMSO): δ (ppm): 175.0, 173.8, 172.1, 158.3, 146.1, 137.7, 133.7, 132.9, 128.8, 123.9, 122.2, 64.4, 33.2, 29.6, 26.4, 22.9. Elemental analysis, calcd. (%) for $C_{18}H_{20}N_6O_2$: C, 61.36; H, 5.68; N, 23.86; found C, 61.24; H, 5.73; N, 24.05; MALDI–MS: m/z calcd. for $C_{18}H_{20}N_6O_2$: 352.16; found: 353.1742 $[M+H]^+$.

CTA, S–1–dodecyl–S′–(α,α′–dimethyl–α″–acetic acid)

Trithiocarbonate CTA, *S*–1–dodecyl–S′–(α, α′–dimethyl–α″–aceticacid) trithiocarbonate bearing acid functionality was synthesized. 4.04 g of dodecanethiol (20 mmol), 10 mL of acetone, and 0.26 g of tetrabutylammonium bromide (0.8 mmol) were added to a 50 mL flask, and nitrogen gas was bubbled through the solution for 30 min at 10 °C. An aqueous solution of 1.68 g of 50 wt% sodium hydroxide (21 mmol) was subsequently slowly added at temperatures below 10 °C. After stirring for 15 min, a carbon disulfide solution in acetone was added in a dropwise manner (CS_2, 1.525 g, 20 mmol; acetone, 2.015 g, 34.5 mmol). The system was stirred for another 15 min prior to the addition of 2.4 mL of chloroform (30 mmol) and 8 g of 50 wt % sodium hydroxide at temperatures below 10 °C. The ice bath was removed 30 min later, and the reaction was allowed to proceed for 12 h before the addition of 30 mL of distilled water and 5 mL of hydrochloric acid (6.8 mol·L^{-1}). After 30 min, the system was distilled under reduced pressure to remove the volatile solvents, resulting in the appearance of a yellow precipitate which was collected by filtration. The precipitate

was dissolved in 100 mL of isopropanol under strong stirring, and the undissolved residue was removed by filtration. The filtrate was subsequently distilled under reduced pressure to remove isopropanol, and the remaining residue was recrystallized in hexane and dried in vacuum for 1 day. At the end, 4.3 g of trithiocarbonate CTA was obtained as a pale yellow solid, yield: 58%.[3] ^{1}H NMR (400 MHz, d_6–DMSO): δ (ppm): 3.29 (t, J=7.2 Hz, 2H, S–CH_2), 1.56–1.62 (m, 8H, CH_3–C–CH_3, CH_2), 1.23–1.35 (m, 18H, CH_2), 0.76–0.92 (t, J=6.4 Hz, 3H, CH$_2$–CH_3). ^{13}C NMR (400 MHz, d_6–DMSO): δ (ppm): 173.6, 56.6, 36.6, 31.8, 29.5, 29.4, 29.3, 29.2, 28.9, 28.7, 27.9, 25.5, 22.6, 14.4. MALDI–MS: m/z calcd. for $C_{17}H_{32}O_2S_3$: 364.16; found: 365.1634 $[M+H]^+$.

Polymerization

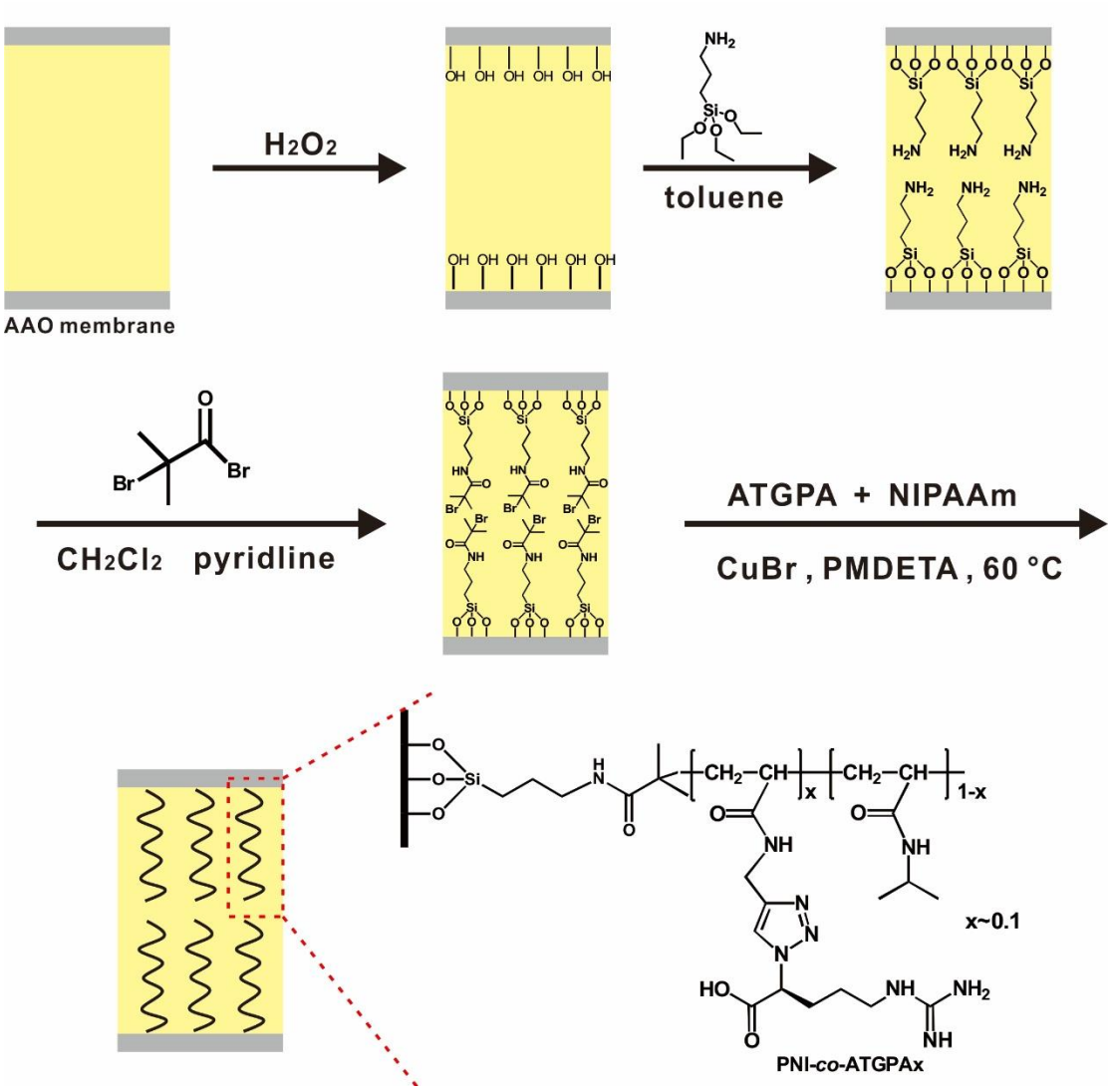


Scheme S2. Modification of Au–coated quartz–crystal (QC) resonator and Au electrode with poly[NIPAAm-*co*-(*S*)-2-(4-acrylamido-1H-1,2,3-triazol-1-yl)-5-guanidinopentanoic acid)$_{0.1}$] (denoted as PNI-*co*-ATGPA$_{0.1}$) via surface–initiated atom transfer radical polymerization (SI–ATRP).

First, Au–coated QC resonator with an intrinsic frequency (F_0) of 5 MHz (purchased from Q–Sense Corp.) was washed in order with distilled water and ethanol for three times, separately. Then, a monolayer of 2–mercaptoethylamine was grafted onto the gold surface after the Au–coated QC resonator was immersed in a solution of 2–mercaptoethylamine (0.01 mol·L^{-1}) in ethanol for 24 h at ambient temperature. For removal of the physically adsorbed 2–mercaptoethylamine, the QC resonator was rinsed with ethanol for three times and dried under a flow of nitrogen gas. The amino–modified Au–coated QC resonator were suspended in 30 mL of anhydrous CH_2Cl_2 with pyridine (0.8 mL). The polymerization initiator bromoiso–butyryl bromide (BIBB, 4.0 mL) was added

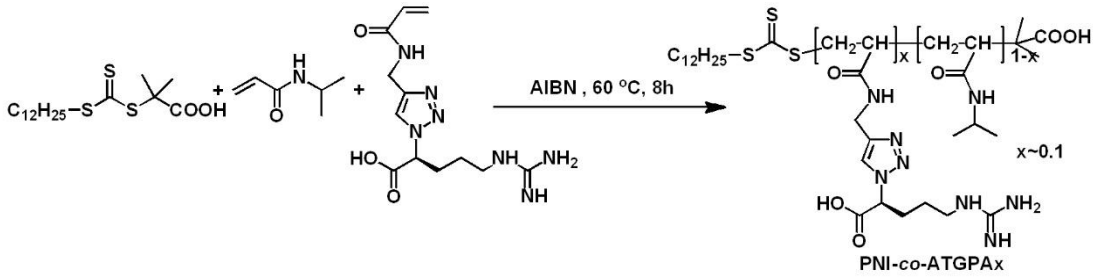
dropwise to this solution for 30 min at 0 ºC, which was continued to stir overnight at ambient temperature (This reaction should be protected from light.).

Subsequently, the QC resonator was rinsed with CH_2Cl_2, and dried under a nitrogen flow, receiving a bromine–modified QC resonator for polymerization. Then, PNI-*co*-ATGPA$_{0.1}$was grafted from the surface of the bromine–modified QC resonator through SI–ATRP. The polymeric film was achieved by immersing the bromine–modified QC resonator in a degassed solution of NIPAAm (0.452 g, 4mmol) and ATGPA (0.309 g, 1 mmol) in 10 mL of DMF containing cuprous bromide (CuBr, 0.016 g, 0.11mmol) and *N*, *N*′, *N*″, *N*‴–pentamethyl–diethylenetriamine (0.08 mL, 0.37 mmol). The reaction was carried out in nitrogen atmosphere for 7 h at 60 °C. After that, the polymerization was stopped by removing the QC resonator from the reaction bath, and then the copolymer–modified QC resonator was cleaned in order with 20 mL of DMF, 10 mL of water, and 10 mL of ethanol, separately, and was subsequently dried under a nitrogen flow. The polymer film thickness was measured to be 20 nm approximately according to AFM section profile.



Scheme S3. Modification of the AAO membrane with PNI-*co*-ATGPA$_{0.1}$ via SI–ATRP.

Bare AAO membrane (with 80–100 nm aperture, and 60 μm thickness) was immersed in order in distilled water for 10 min, in ethanol for 10 min, in a hydrochloric acid aqueous solution (5%, v/v) for 35−50 s, and in a heated hydrogen peroxide at 100 °C for 2 h to generate surface hydroxyl groups. After that, the membrane was washed in order with distilled water and ethanol, separately, followed by drying under a nitrogen flow. Then, the membrane was immersed in a solution of 40 mL of toluene containing 1.0 mL of APTES, and the mixture was left at 80 °C for 6 h. The obtained amino–modified membrane was rinsed with ethanol to remove the remaining APTES, and dried under a nitrogen flow. Then, the same method described above was adopted to get the AAO membrane modified with PNI-*co*-ATGPA$_{0.1}$.



Scheme S4. Preparation of PNI-co-ATGPA$_{0.1}$ through RAFT Polymerization.

NIPAAm (0.904 g, 8 mmol) and ATGPA (0.618 g, 2 mmol) in 10 mL of DMF containing CTA (36.4 mg, 1 mmol) and AIBN (4.92 mg, 0.3 mmol) were added in a 50 mL flask. Then the reaction was carried out in nitrogen atmosphere for 7 h at 60 °C. To this end, polymers were purified by dialysis (MWCO 3000 Da). The weight average molecular weight was 8700 determined by gel permeation chromatography. Elemental analysis, calcd. (%) for copolymer: C, 55.98; N, 15.27; H, 9.309; S, 0.901. According to the results of elemental analysis and ratio of characteristic peaks in ^{1}H NMR spectra of the prepared copolymer, the proportion of ATGPA in the PNI-*co*-ATGPA$_{0.1}$ copolymer was approximately 10%. The prepared copolymer was used in ^{1}H NMR, Bio-ATR-FT-IR, ITC titration experiments and LCST measurement.

Test method

Fluorescent Titration Experiment

For investigation of binding affinity of ATGPA with cAMP, ATP, ADP, AMP and adenosine, fluorescent titration experiments were conducted, which is a typical and widely adopted method for calculating association constant (K_a) in host–guest chemistry. In this experiment, cyclic adenosine monophosphate salt (cAMP), adenosine monophosphate salt (AMP), adenosine 5'–diphosphate salt (ADP) and adenosine 5'–triphosphate salt (ATP) were used. The host naphthalene–labeled TGPA was prepared as stock solution (1.0×10^{-6} mol·L^{-1}) in pure water. Guests (cAMP, ATP, ADP, AMP and adenosine) were prepared into three stock solutions in pure water (1.5×10^{-3} mol·L^{-1}, separately). The work solutions were prepared by adding different volumes of guest solution to a series of test tubes, and then, the same amount of stock solution of the naphthalene–labeled TGPA host was added

into each test tube, followed by dilution to 3.0 mL water solution. After being shaken for 1 min, the work solutions were measured immediately at 20 °C using a FL–6500 fluorescence spectrophotometer (excitation wavelength: 282 nm). The K_a values between TGPA and guests (cAMP, ATP, ADP, AMP, and adenosin e) were obtained through a non-linear fitting.[3]

Calculation formula of association constant (K_a)

K_a values were obtained from the fluorescent titration experiments according to intensity changes by using a nonlinear 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 naphthalene–labeled-TGPA and guest, C_0 is the initial concentration of host naphthalene–labeled TGPA.

^{1}H NMR titration experiment

To investigate the complexation between ATGPA and cAMP sodium salt, ^{1}H NMR titration experiment was also conducted in d_6–DMSO at 20 °C. For example, different molar ratios (0, 0.25, 0.5, 0.75, 1, 1.5, and 2,) of cAMP sodium salt were added to the host ATGPA solution (concentration: 1.0 mmol·L^{-1}). After equilibration for 4 h at ambient temperature, the chemical shifts of hydrogen protons were recorded and analyzed on a BRUKER AVANCE III 400–MHz spectrometer.

QCM–D Adsorption Experiment

All QCM–D measurements were performed at 25 °C on a Q–Sense E4 system. Prior to binding assays between cAMP (or ATP, ADP, AMP, separately) and the QC resonator modified with PNI-*co*-ATGPA$_{0.1}$ QCM channels and tubes were washed carefully with distilled water and dried under a flow of nitrogen gas, followed by installation of the functionalized QC resonator into a flow–cell for frequency and dissipation measurements. After stabilization of the fundamental resonance frequency with pure water, cAMP, ATP, ADP, or AMP solution (100 μmol·L^{-1}) was pumped into the flow–cell by a peristaltic pump at a constant speed of 100 μL·min^{-1}. All of the time–dependent frequency and dissipation curves were recorded by Q–Sense software and analyzed by Q–Tools.

ITC experiment

Binding enthalpies, entropies and associated constant were determined at T =(298.15 ± 0.01) K and atmospheric pressure p = (101.3 ± 5.0) kPa, and calculated by software of MicroCal Analysis Launcher. The sample cell was loaded with 200 μL PNI-*co*-ATGPA$_{0.1}$ water solution (1 mmol·L^{-1}) while the reference cell was loaded with 200 μL of pure distilled water. The 40 μL syringe was filled with a cAMP water solution (20 mmol·L^{-1}). 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. The thermal effects from the friction in the process of injection were considered to be negligible

according to the literature.

Bio–ATR–FT–IR titration experiment

The infrared spectra were recorded on a Bruker Vertex 80v FT–IR spectrometer in Bio–ATR cell II accessory [the accessory is based on dual crystal technology: the top crystal is made of silicon, and the second crystal is made of zincselenide (ZnSe) and has a hemispherical design]. All samples were dissolved in 16 μL of d_6–DMSO. For monomer sample, the concentrations (ATGPA, 40 mmol·L^{-1}; cAMP salt, 40 mmol·L^{-1}) and total volume (16 μL) were strictly controlled. For polymer sample, the concentrations (PNI-*co*-ATGPA$_{0.1}$, 5 mg; cAMP salt, 40 mmol·L^{-1}) and total volume (16 μL) were strictly controlled. For each measurement, the equipment remained in standby mode for 15 min to ensure the equilibrium of temperature (20 °C) prior to the test, and all the spectra of samples were obtained through 1200 scans subtracting the d_6–DMSO background at a 4 cm^{-1} resolution. Before each measurement, the Bio–ATR cell was cleaned in order with distilled water and ethanol, separately. Then, it was sufficiently dried under a nitrogen gas flow.

LCST measurement

The polymer solution was injected into a closed quartz cell and the LCST measurement could be completed within 1 h, under this condition, the solution pH value would not change remarkably. It is worth mentioning that various buffer solutions (e.g., phosphate buffered saline, Tris–HCl, or ammonium formate) were not used because these buffering agents might also impact on the LCST of the copolymer according to many literatures. Transmittance of copolymer solution at 320 nm was measured by PerkinElmer Lambda 365 UV–Vis spectrophotometer at different temperature, then effect of cAMP addition on the LCST of the copolymer was investigated. According to the dramatic change of transmittance near the LCST, the copolymer LCST was determined to be approximately 37 °C in pure water (pH 6.5). Upon the addition of cAMP (0.1 mg), the copolymer LCST increased to 41 °C. These data indicated that PNI-*co*-ATGPA$_{0.1}$ was a typical thermo–responsive polymer and its LCST was strongly influenced by the addition of cAMP.

Current–voltage recording

The ionic transport property of the heterogeneous membrane was examined by *I–V* measurements using a commercial Keithley 6487 picoammeter (Keithley Instruments). The AAO membrane was mounted between the two chambers of the conductivity cell. Both halves of the cell were filled with NaCl solutions (0.1 mol·L^{-1}). A scanning voltage varying from −2.0 V to +2.0 V was applied through Ag/AgCl electrodes as the transmembrane potential. The effective area for ionic conduction measurements was approximately 20 mm^2.

Fluorescence titration spectra

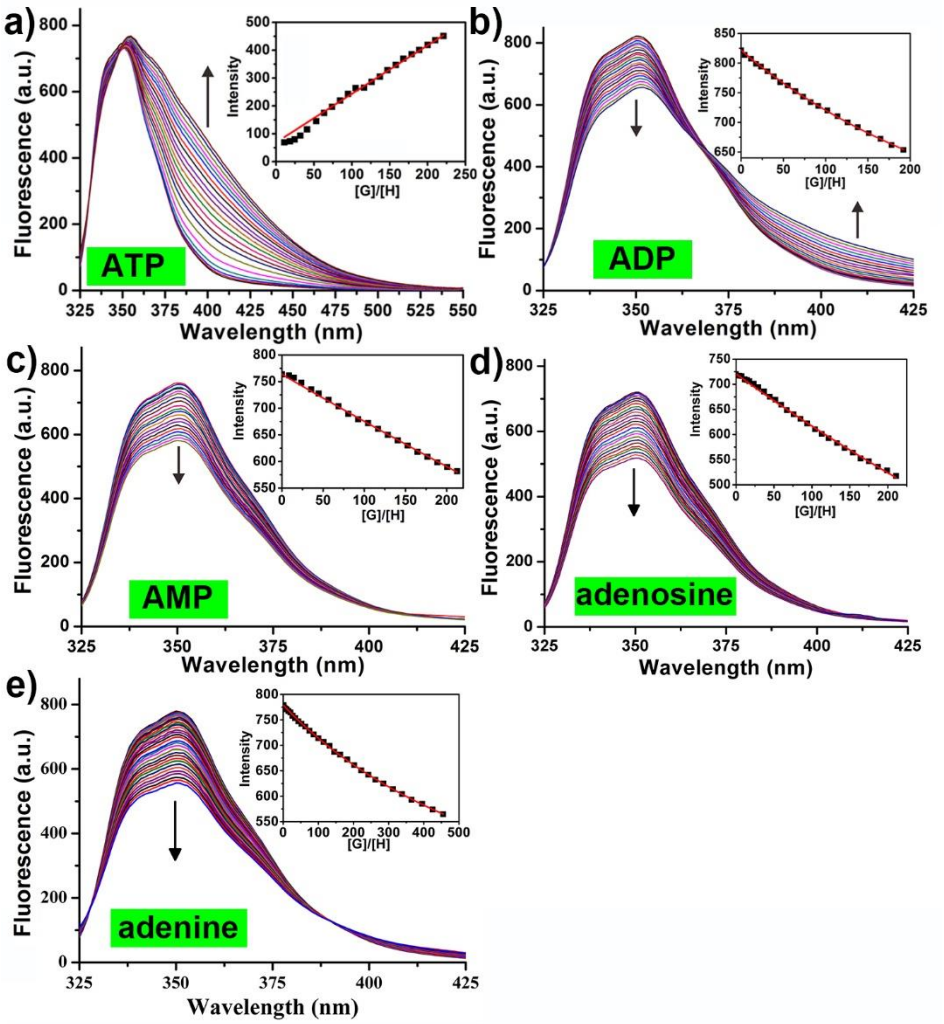


Figure S1. Fluorescence spectra of naphthalene–TGPA (1.0×10^{-6} mol·L^{-1}) upon addition of different equivalents of ATP (a), ADP (b), AMP (c), adenosine (d), adenine (e), in H_2O at 20 °C. The insets show the fluorescent intensity changes of naphthalene–TGPA host upon the additions of various guests, [G]/[H] is an abbreviation of the molar ratio of guest to host. The red lines are nonlinear–fitted curves. In H_2O solution, the K_a of naphthalene–TGPA with ATP, ADP, AMP, adenosine and adenine was 321, 245, 214, 574 and 1211 L·mol^{-1}, respectively. It is worth noting that commercially available cyclic adenosine monophosphate salt (cAMP), adenosine monophosphate salt (AMP), adenosine 5'–diphosphate salt (ADP) and adenosine 5'–tri-phosphate salt (ATP) were used in the fluorescent titration experiment.

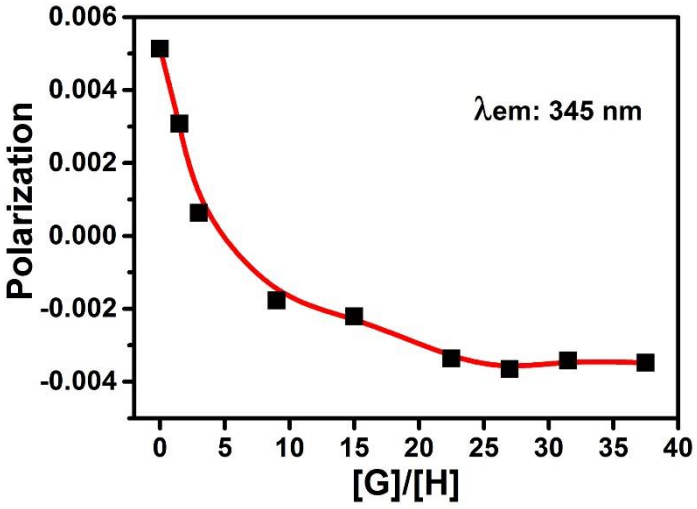


Figure S2. Fluorescent polarization changes (at 345 nm) of naphthalene–indicated TGPA (1.0×10^{-6} mol·L^{-1}) upon addition of different equivalents of adenosine cAMP. Fluorescent polarization signal is closely related to the molecular configuration. The binding of cAMP to naphthalene–indicated TGPA restricted the free rotation of the host molecule, resulting in a decrease in fluorescent polarization value.

^{1}H NMR titration spectra

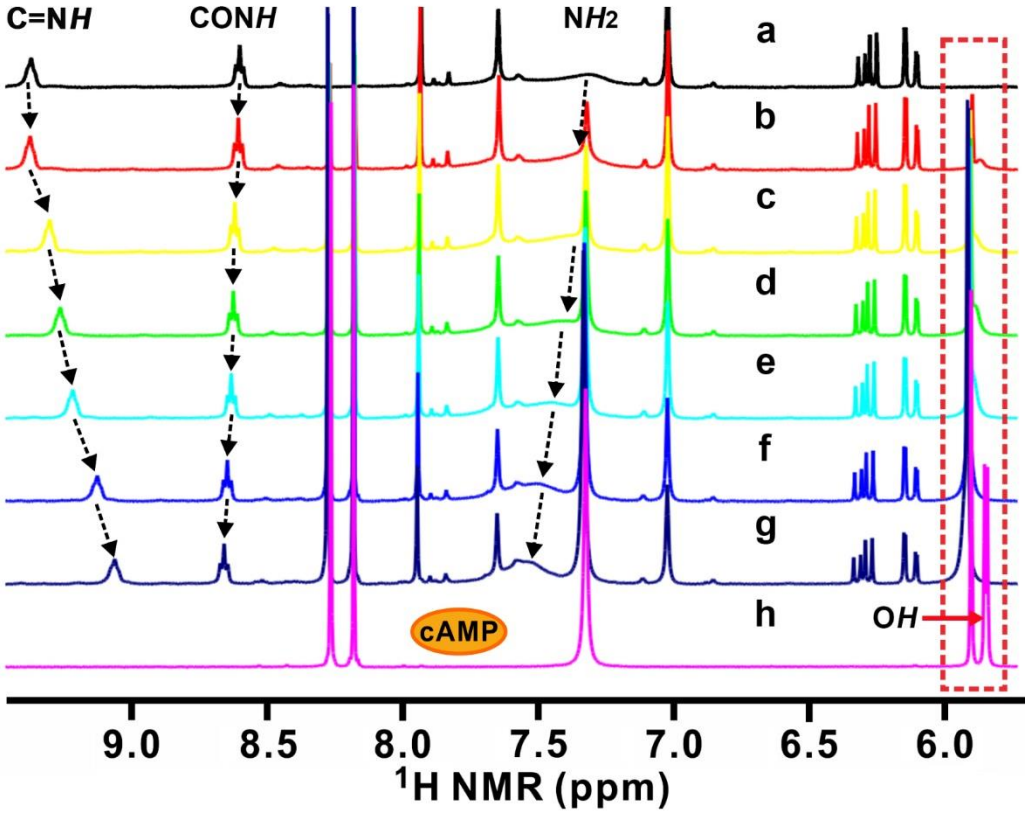


Figure S3. ^{1}H NMR spectra of cAMP (h), ATGPA before (a) and after interactions with different molar ratios (0.25 (b), 0.5 (c), 0.75 (d), 1.0 (e), 1.5 (f), 2.0 (g)) of cAMP sodium salt in d_6–DMSO

at 20 °C. Chemical shift changes of active H–protons are indicated by black dashed lines or red box, respectively. This data further validated the curial role of hydrogen bonding interaction in the complexation.

AFM images of QC resonator surface before and after modification of PNI-*co*-ATGPA$_{0.1}$

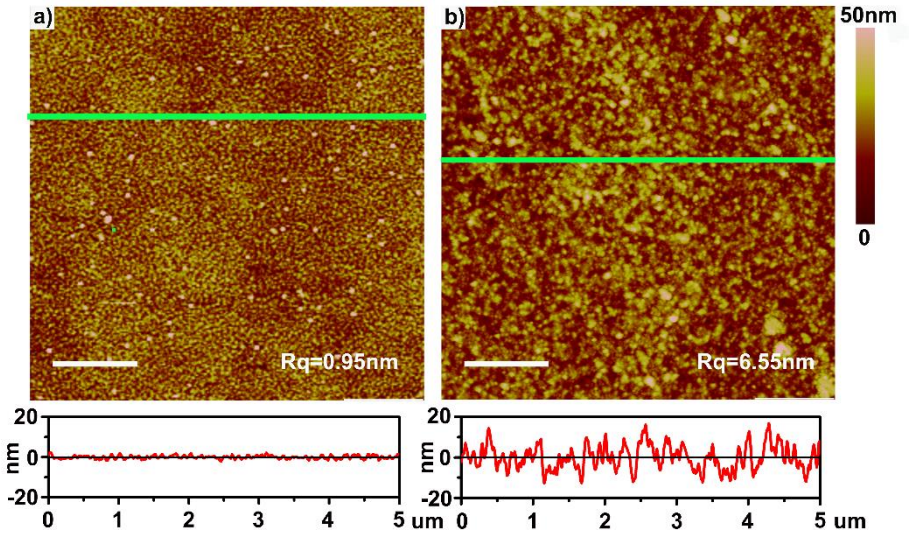


Figure S4. Typical AFM images of QC resonator surface before (a) and after (b) modification of PNI-*co*-ATGPA$_{0.1}$, and the section profiles for the corresponding AFM images along the green lines. The QC resonator is coated with a thin layer of gold. The pristine QC resonator surface is smooth with mean roughness (Rq) of 0.95 nm. After immobilizing PNI-*co*-ATGPA$_{0.1}$ on the QC resonator, many tiny polymeric aggregates with average height of approximately 20 nm could be observed on the surface, while the Rq value sharply increased to 6.55 nm. This indicates the successful modification of PNI-*co*-ATGPA$_{0.1}$ on the QC resonator surface. Scale bars: 1 μm.

EIS experiment

EIS experiments were performed in 0.1 mmol·L^{-1} KCl solution containing $[Fe(CN)_6]^{3-/4-}$ (5 mmol·L^{-1}), and the experimental conditions were as follows: open–circuit potential, 0.3 V; alternative voltage, 5 mV; frequency range, 0.1−10^5 Hz. The working electrode which is Au electrode modified with the PNI-*co*-ATGPA$_{0.1}$, an Ag|AgCl reference electrode and a graphite auxiliary electrode consisted the three electrode system. Temperature: 20 °C.

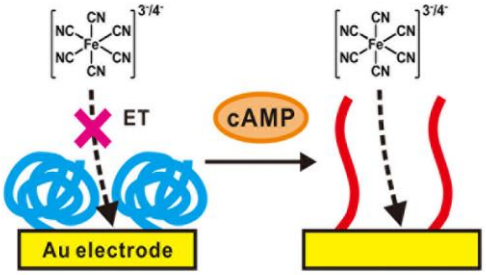


Figure S5. Graphic illustration of a potential conformational transition of the copolymer chains triggered by cAMP on the Au electrode.

Representative Bio-ATR-FT-IR spectra of copolymer interacted with cAMP

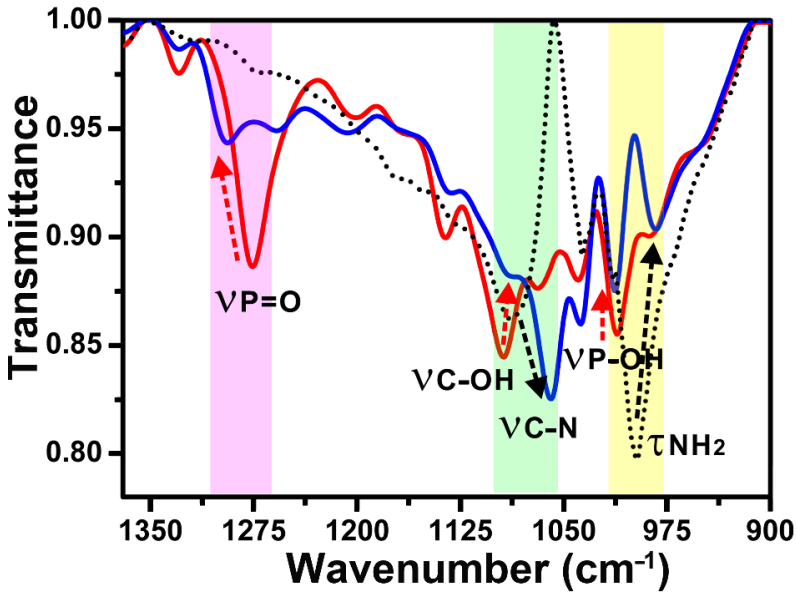


Figure S6. Representative Bio–ATR–FT–IR spectra of PNI-*co*-ATGPA$_{0.1}$ (black), cAMP (red), and their mixture (blue) in d_6–DMSO at 20 ºC. Color ribbons illustrate the remarkable changes of the characteristic peaks of PNI-*co*-ATGPA$_{0.1}$ and cAMP. Clear shifts or variation in adsorption intensity of the characteristic vibration peaks indicated that these functional groups participated in the complexation between the copolymer and cAMP.

Isothermal titration calorimetry (ITC) spectrum

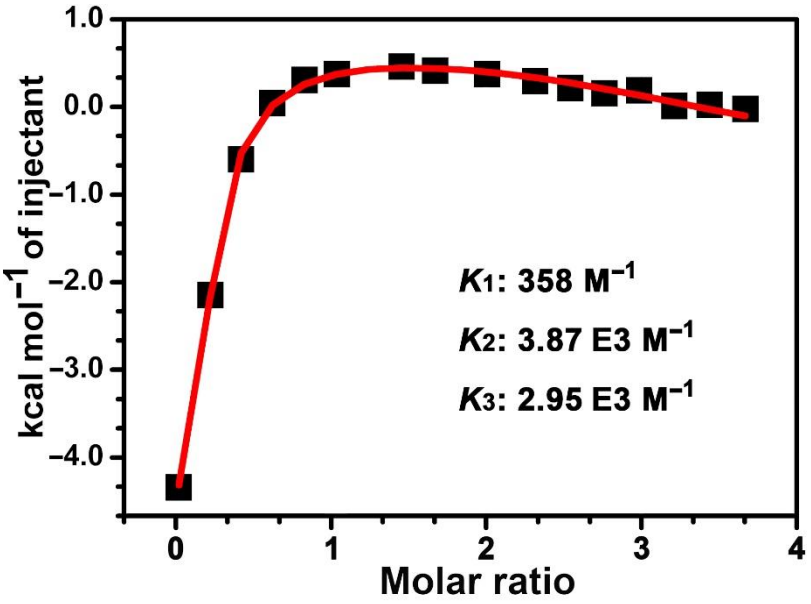


Figure S7. Isothermal titration calorimetric data for titration of PNI-*co*-ATGPA$_{0.1}$ solution (1.0 mmol·L^{-1}) with the additions of various equivalents of cAMP (20 mmol·L^{-1}) in water. The red line denotes a non-linear fitting curve using a sequential binding sites model (N = 3), resulting in an accumulative association constant (K_1×K_2×K_3) of 4.08 × 10^9 L·mol^{-1}. This data indicated an intensive complexation between the copolymer and cAMP.

FT–IR and Thermal gravimetric analysis (TGA) of AAO membrane

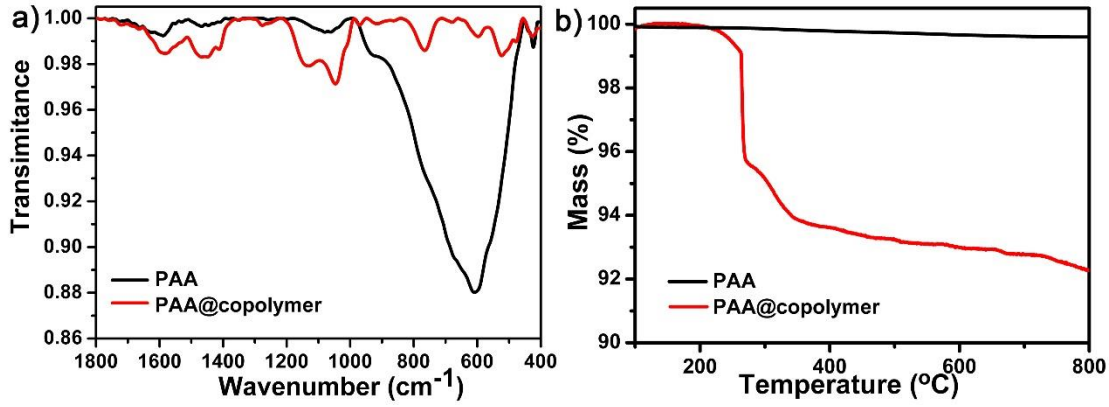


Figure S8. (a) FT–IR (in MIR–ATR mode) and (b) TGA measurement of AAO membrane before (black) and after (red) PNI-*co*-ATGPA$_{0.1}$ modification. The appearance of new characteristic peaks in IR spectra and evidential weight loss (8%) in TGA spectra indicated that PNI-*co*-ATGPA$_{0.1}$ had been modified on the AAO membrane with high density.

SEM images of AAO membrane

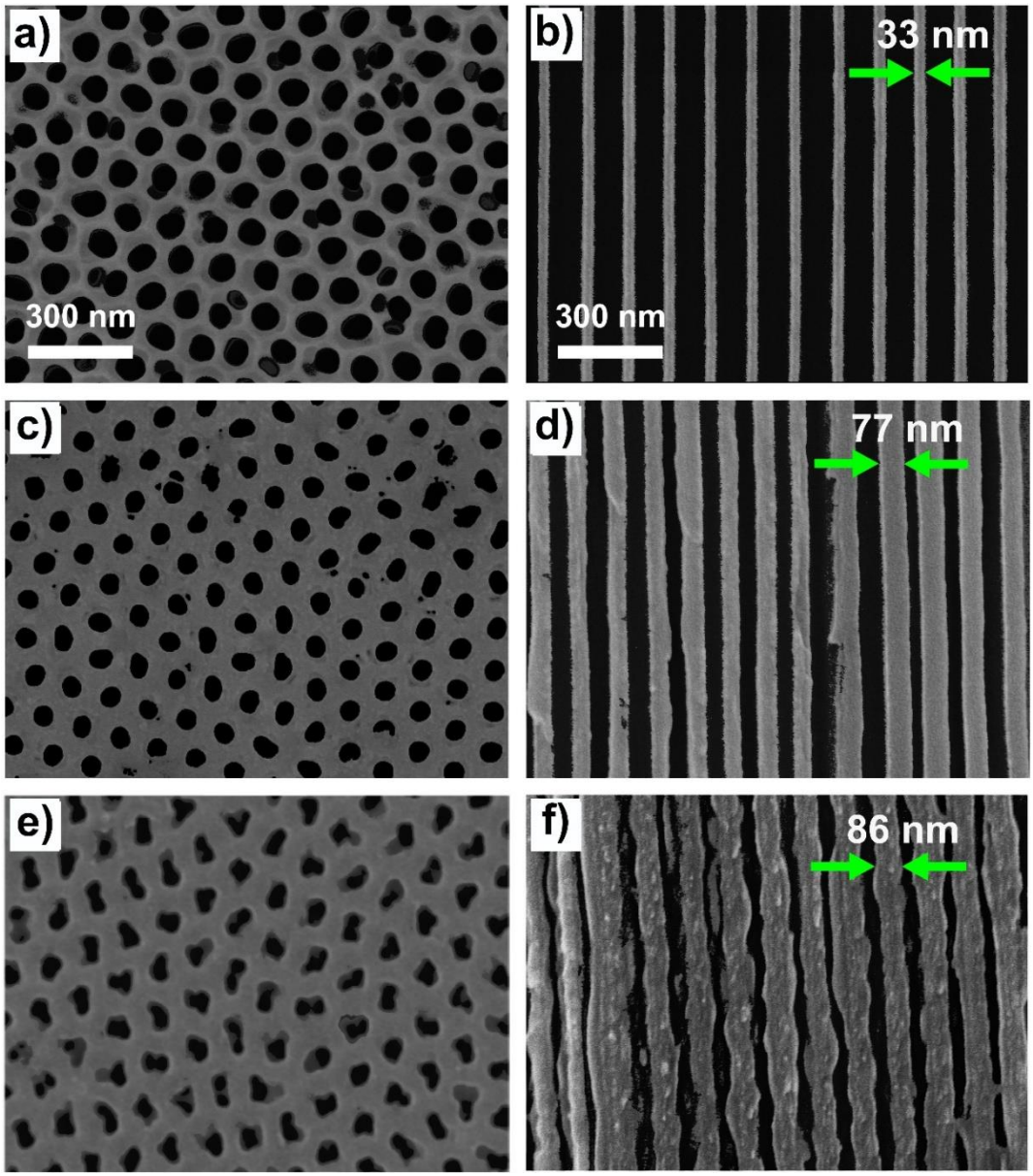


Figure S9. SEM and cross-section images of bare AAO membrane (a, b), PNI-*co*-ATGPA$_{0.1}$-modified AAO membrane before (c, d) after (e, f) being treated by cAMP salt solution (10 $\mu mol \cdot L^{-1}$) for 15 min at 20 °C. Figure a–d indicated that the copolymers had been grafted onto the AAO membrane with multiple straight nanochannels (average pore size: 80–100 nm), the porosity of the alumina nanochannels decreased considerably with average diameter of nanopores decreasing from 80 to 55 nm, approximately. From the cross–section images (b, d), the wall thickness of alumina nanochannels increased considerably with average width increasing from 33 to 77 nm. Upon cAMP treatment as shown by Figure (e, f,) the porosity of the alumina nanochannels decreased considerably and the average wall thickness of the nanochannels increased from 77 to 86 nm, resulting in a remarkable decrease of the size of the nanochannels.

XPS spectra of AAO membrane before and after copolymer modification

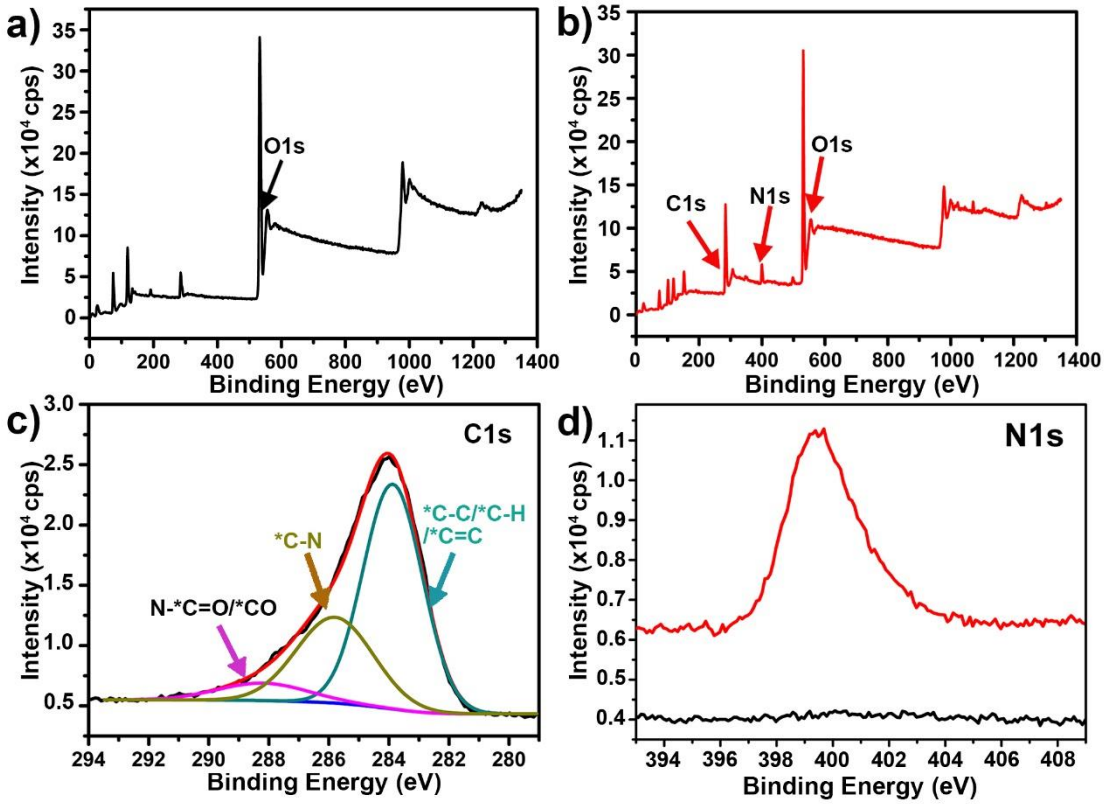


Figure S10. X-ray photoelectron spectroscopy (XPS) wide scan spectra of AAO membrane before (a) and after (b) being modified with PNI-*co*-ATGPA$_{0.1}$. C1s core–level spectrum (c) and N1s narrow scan (d) of the copolymer modified AAO membrane. The sharp increases of the C and N elemental signals indicated that the copolymer had been successfully immobilized on the AAO membrane.

Optical photograph of testing device

Figure S11. Optical photograph of testing device

Surface wettability test

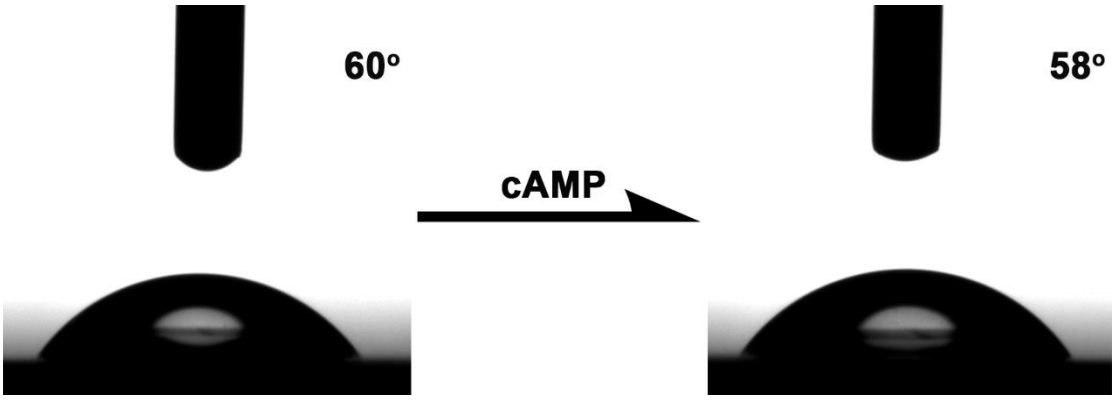


Figure S12. Water droplet profiles on the PNI-*co*-ATGPA$_{0.1}$ film before and after being treated by cAMP salt solution (10 $\mu mol \cdot L^{-1}$) for 15 min at 20 °C.

Supplementary References

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(4) G. Y. Qing, Q. Lu, X. L. Li, J. Liu, M. L. Ye, X. M. Liang and T. L. Sun, *Nat. Commun.*, 2017, **8**, 461.