Nucleotide Responsive Wettability on Smart Polymer Surface

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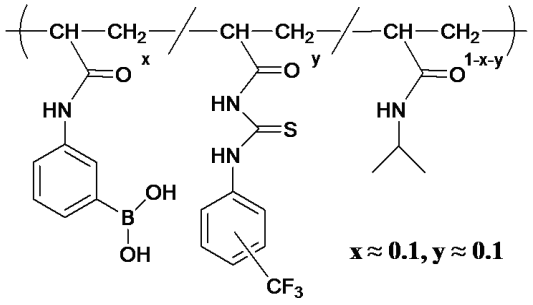
Supporting Information

1. Synthesis of the copolymers

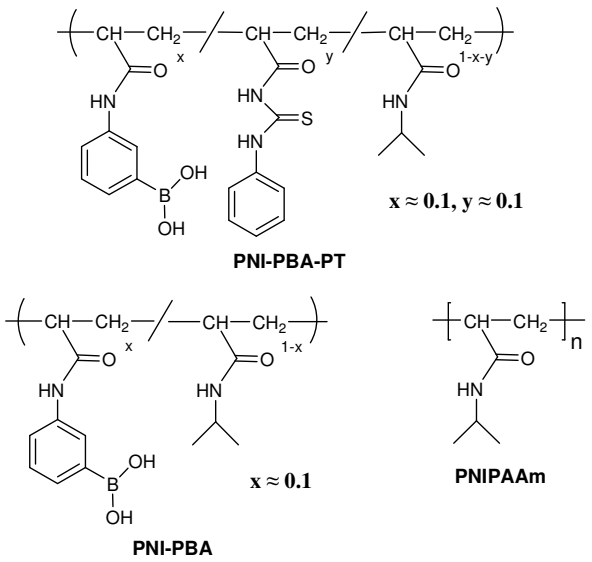
Materials: Silicon wafer was purchased from Silicon Materials Corporation (Germany). *N*-isopropyl acrylamide (Aldrich, Germany) was recrystallized in n-hexane for three times. The 3-aminophenylboronic acid hydrochloride, 2-mercaptoethylamine, acryloyl chloride (Sigma, Germany), *o*-, *m*-, *p*-(trifluoromethyl)phenylthiourea, aniline, acetone, nitric acid, methanol, DMF, sodium hydroxide, bromoisobutyryl bromide (Alfa, Germany), *N*,*N*,*N*',*N*',*N*"-pentamethyl-diethylenetriamine (Aldrich, Germany) and 3-aminopropyl trimethoxysilane (Fluka, Switzerland) were used as received. CuBr was recrystallized before being used. Toluene and dichloromethane were dried by molecular sieves for 24 hours before being used. The 3-arylamido phenylboronic acid (APBA) was synthesized according to the method reported in the literature.[1] Double distilled water (18.2 MΩ·cm, MilliQ system) was used. Adenosine 5'-diphosphate disodium salt (ADP), Adenosine 5'-monophosphate sodium salt (AMP) and adenosine used in the detection were purchased from Sigma Corporation (Germany). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker ARX300 spectrometer.

Molecular Structure of the target copolymers:



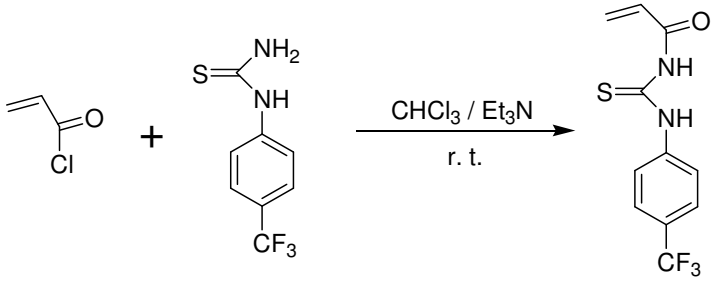
Scheme S1. Molecular structures of the target copolymers. They are named as PNI-PBA-TF(*o*), PNI-PBA-TF(*m*) and PNI-PBA-TF(*p*), respectively, according to the $-CF_3$ substitutions at *ortho*-, *meta*-, and *para*- positions on the benzene ring of the phenylthiourea unit.

Molecular Structure of the reference copolymers:



Scheme S2. The structures of reference copolymers.

*Synthesis of monomer 4-(trifluoromethyl) phenylthiourea acrylamide (**p-TF-PT**):*



Scheme S3 The synthesis of 4-(trifluoromethyl)phenylthiourea acrylamide.

Et_3N (0.505 g, 5 mmol) was added to a solution of 4-(trifluoromethyl)phenylthiourea (1.10 g, 5 mmol) in 30 mL dry $CHCl_3$, the mixture was stirred for 10 min, then acryloyl chloride (0.453 g, 5 mmol) was added dropwise to this mixture at ambient temperature, and continued to stir for 24 hours. The mixture was washed with water for three times, and the organic layer was dried over anhydrous Na_2SO_4 overnight. After filtration and evaporation of solvent, the crude product was purified by column chromatography on silica gel, with elution of CH_2Cl_2/MeOH (120:1), to obtain the pure product as yellow

semi-oil (1.07 g, yield: 78%). ^{1}H NMR (300MHz, $CDCl_3$): 5.73 (d, *J*=6 Hz, 1H, =CH), 6.31-6.36 (m, 2H, =CH), 7.01 (d, *J*=6 Hz, 2H, Ph-H), 7.28(s, 1H, CNHCS), 7.61 (d, *J*=6.5 Hz, 2H, Ph-H), 7.70 (s, 1H, CNHCS); ^{13}C NMR (300MHz, $CDCl_3$): 115.6, 117.2, 121.3, 122.1, 126.4, 127.2, 132.9, 144.4, 157.3; MADLI-MS: m/z calcd for $C_{11}H_9F_3N_2OS$: 274.04; found: 275.046.

The same method was also used to synthesize the monomers 2-(trifluoromethyl)phenylthiourea acrylamide and 3-(trifluoromethyl)phenylthiourea acrylamide.

Synthesis of PNI-PBA-TF(p) copolymer thin films on silicon substrates:

A clean silicon substrate was immersed in aqueous NaOH (0.1 mol·L^{-1}) for 5 minutes and subsequently in HNO_3 (0.1 mol·L^{-1}) for 10 minutes to generate surface hydroxyl groups. After the substrate had been washed with an excess of water and dried under a flow of nitrogen, it was heated to reflux in toluene that contained 5 wt% aminopropyl-trimethoxysilane (ATMS) for 3 hours to obtain chemically bonded $-NH_2$ groups on the surface. The surface was rinsed with toluene and dichloromethane to remove remaining ATMS, dried under a flow of nitrogen gas, and immersed in dry dichloromethane that contained pyridine (2% w/v). The polymerization initiator bromoisobutyryl bromide was added dropwise into the solvent containing the silicon substrate at 0 ^{o}C, and the mixture was left for 1 hour at this temperature then at room temperature for 12 hours. The silicon substrate was cleaned with dichloromethane, and dried under a nitrogen flow. Copolymerization of PNI-PBA-TF(*p*) was achieved by immersing the silicon substrate with the initiator grafted on the surface in a degassed solution of NIPAAm (0.814 g), APBA (0.172 g), *p*-TF-PT (0.247 g) (10 mol % APBA, 10 mol % *p*-TF-PT against NIPAAm.) in a mixture of H_2O (5 mL), MeOH (5 mL) and DMF (5 mL) containing CuBr (0.032 g, 0.23 mmol) and pentamethyl diethylene triamine (PMDETA, 0.16 mL) for 5 hours, at 60 ^{o}C. Under this condition, the thickness of film was between 15 nm and 20 nm. The same method was adopted to prepare the PNI-PBA-TF(*o*), PNI-PBA-TF(*m*) and PNI-PBA-PT copolymer thin films on silicon substrates.

Synthesis of PNI-PBA-TF(p) thin film on the Au-coated quartz-crystal resonators:

The Au-coated quartz-crystal (QC) resonator was washed with water and ethanol for three times. Then a monolayer of 2-mercaptoethylamine was covered on the gold surface after the Au-coated QC resonator was immersed for at least 24 h in a solution of 2-mercaptoethylamine (1×10^{-2} mol·L^{-1}) in ethanol. After that the QC resonator was rinsed with ethanol for three times and dried under a flow of nitrogen gas. Then the QC resonator was immersed in dry dichloromethane that contained pyridine (2% w/v). The polymerization initiator bromoisobutyryl bromide was added dropwise into the solvent containing the QC resonator at 0 ^{o}C, and the mixture was left for 1 hour at this temperature then at room temperature for 12 hours. The QC resonator was cleaned with dichloromethane, and dried under a nitrogen flow. Then same method described above was adopted to graft the PNI-PBA-TF(*p*) copolymer thin film on the Au-coated QC resonator.[2]

Static contact angle measurement:

The static CAs were measured by a Contact Angle Measurement System G2 instrument (Krüss, Germany) at ambient atmosphere and a constant temperature of 25 ^{o}C. A series of nucleotide solutions with different concentration ranging from 5×10^{-5} M to 2×10^{-2} M in water were prepared precisely, the copolymer grafted silicon substrate was immersed in the nucleotide solution for 10 minutes, then dried under a flow of N_2 gas. The static CAs were recorded for each concentration using the sessile drop method, respectively.

Dynamic contact angle measurement:

The dynamic CAs were also measured by the sessile method on the same instrument. However, in order to measure the advancing CAs or the receding CAs, the volume of the water drop would increase or decrease with a fixed flow rate of about 25 μL/min, and the data were recorded when the edge of the water drop expanded or shrank constantly, respectively. Water drop size used in measurement is in the range of 2 - 8 μL. The outer diameter for the needle used in the dynamic CA measurement is about 0.23 mm.

A water drop was firstly settled on the material surface and then the volume of the drop increased or decreased in a constant rate of about. The advancing or receding CAs were determined when the edge of the water drop expanded or shrank constantly. All the measurements were made at a constant temperature of about 25 ^{o}C.

AFM measurement:

The AFM investigation was conducted on flat silicon substrate (with polymer film on it) using a Nanoscope IIIa instrument (DI, USA) in the tapping mode.

QCM measure and the chemical characterization of the copolymer films: For details, see following.

2. QCM Measurement

The QCM measurement was carried on a QCM 200 (Stanford Research System, Inc., USA). The quartz crystals (QC) with intrinsic frequency (F_0) of 5 MHz were purchased from SI-GmbH, Germany. The Au-coated QC covered with PNI-PBA-TF(*p*) copolymer was prepared through the procedure reported in the experimental section. Initially, the polymer modified QC was washed with ethanol and water for several times, then the QC was put into a flow-cell for frequency measurement. After stabilization of fundamental resonance frequency with pure water, the nucleotide solution with different concentration was pumped into flow-cell by a peristaltic pump (DYANAMAX Co.). The frequency change was then recorded. Figure S1 shows the time dependence of the QCM frequency shift of PNI-PBA-TF(*p*) copolymer modified resonator at a flow of ADP, AMP or adenosine solution with the same concentration of 2×10^{-4} mol·L^{-1}. The polymer surface exhibits a much larger frequency shift for ADP (ΔF = 225 Hz) than that for AMP (ΔF = 117 Hz), while exhibits the smallest frequency shift for adenosine, ΔF is only 38 Hz. Similar phenomena could be observed when nucleotide solutions with higher concentration (3×10^{-4} mol·L^{-1}) were used as shown in the Figure S2. The frequency shift of polymer surface for ADP is 295 Hz at this concentration, which is still much larger than that for AMP (ΔF = 153 Hz). From these data, we could conclude that the PNI-PBA-TF(*p*) polymer modified surface has a larger absorption quantity to ADP than that to AMP, the comparatively small absorption to adenosine could be attributed to the weak combination between polymer and adenosine. Above phenomena also agree well with the data obtained from the wettability measurement and further indicate that the phosphate

group is the critical factor for the different nucleotide absorption on the PNI-PBA-TF(*p*) polymer surface.

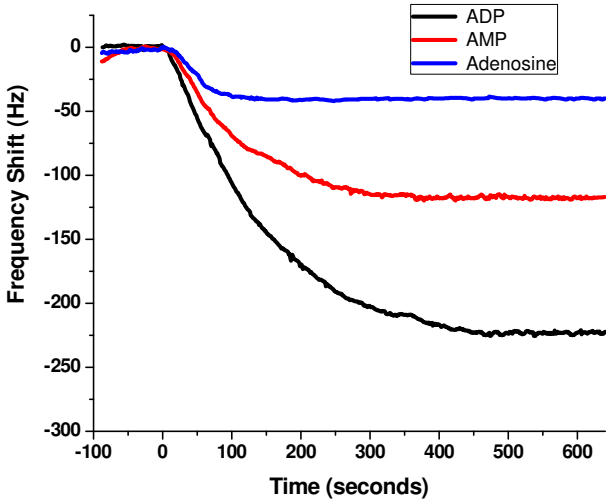


Figure S1. Time dependence of the frequency change of a QCM resonator covered with PNI-PBA-TF(*p*) polymer following injection of ADP, AMP or adenosine solution, the concentration of solution is 2×10^{-4} mol·L^{-1}. Experiment temperature: 25 ^{o}C.

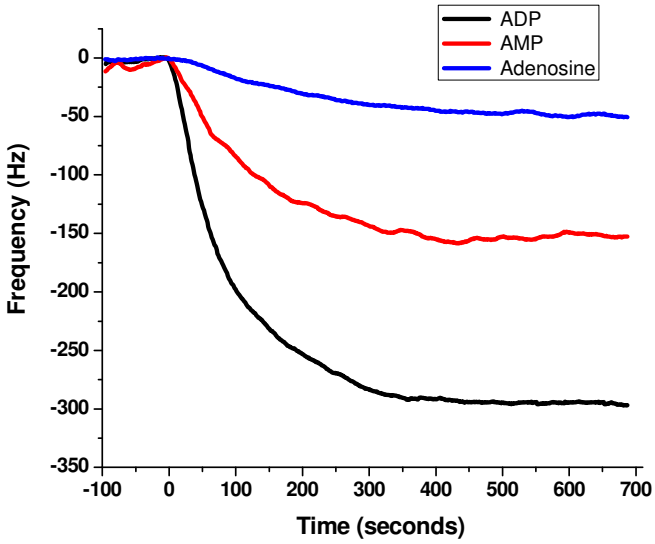


Figure S2. Time dependence of the frequency change of a QCM resonator covered with PNI-PBA-TF(*p*) polymer following injection of ADP, AMP or adenosine solution, the concentration of solution is 3×10^{-4} mol·L^{-1}. Experiment temperature: 25 ^{o}C.

3. ^{1}H NMR Titration Experiment for the Interaction between PBA Unit and Adenosine.

Adenosine is an important component of nucleotide, In order to prove that the phenylboronic acid unit could combine adenosine. ^{1}H NMR titration experiments were

undertaken to investigate the interaction details. Although both the monomer APBA and adenosine could be soluble in water, the NMR experiment could not be done in the D_2O solution due to its suppressive effect for the active hydrogen proton signals. We chose d_6-DMSO as the solvent because it has similar polarity to D_2O, while it is also a common solvent in the NMR detection. As shown in the Figure S3, the addition of 1.0 equiv. of adenosine to the solution of monomer PBA in d_6-DMSO could induce the amide occurs split, one peak exhibits downfield shift from 10.08 ppm to 10.22 ppm, while the original peak become much weakened. The characteristic peak (8.04 ppm) of boronic acid splits into two peaks, which downfield shift to 8.35 or 8.13 ppm, respectively. When 3.0 equiv.

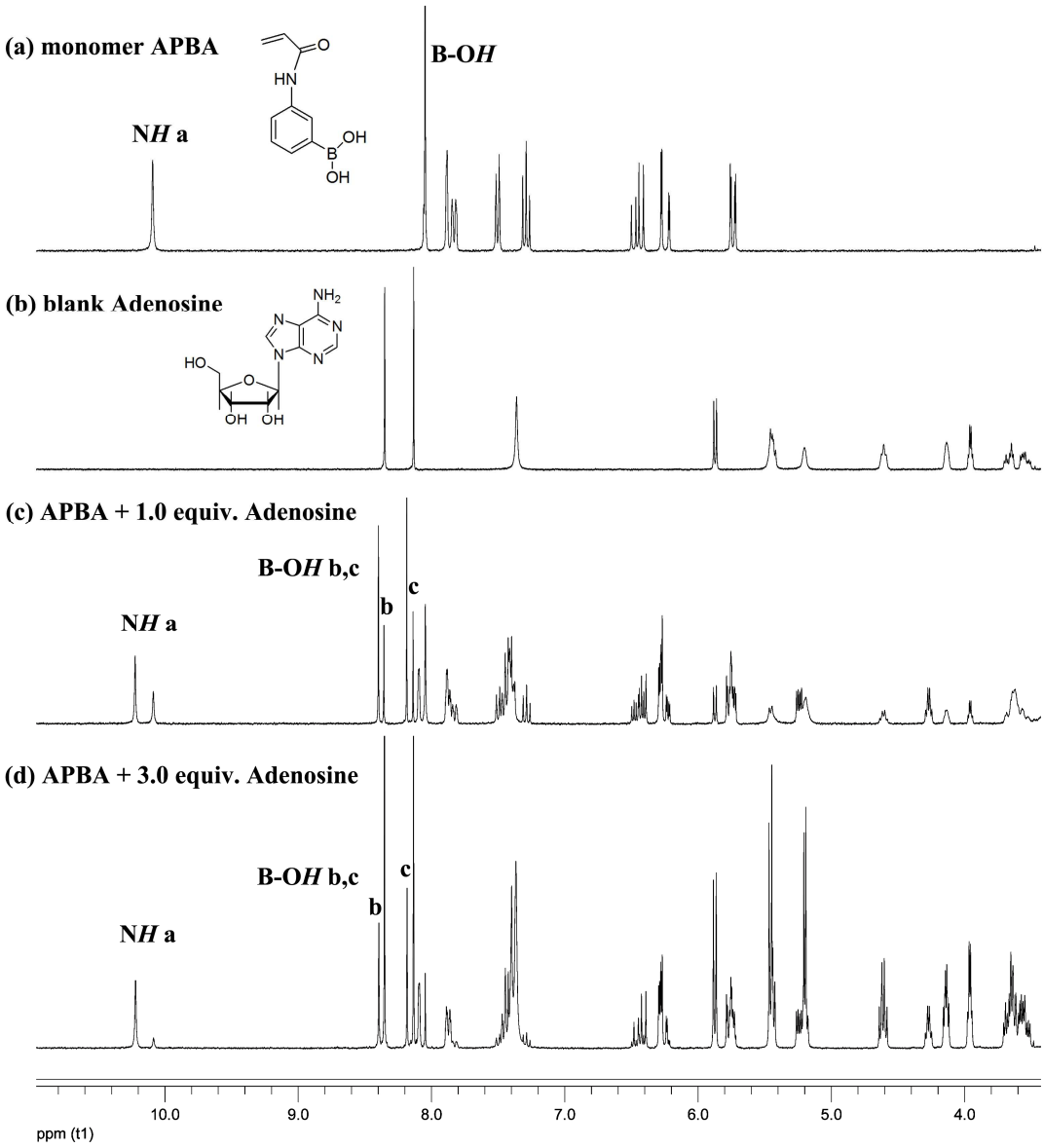


Figure S3 ^{1}H NMR spectra of monomer APBA interacts with Adenosine in d_6-DMSO at 25 ^{o}C. (The concentration of APBA solution is 2×10^{-3} mol·L^{-1}). "NH a" represents the signal of amide, while "B-OH b, c" represents the splitted peaks of boronic acid.

of adenosine is added to the monomer solution, these peaks exhibit more obvious changes: the peak of amide at 10.08 ppm nearly disappears, while the peaks of boronic acid further shift to 8.39 or 8.18 ppm, respectively. These data clearly prove that the phenyl boronic acid unit could combine the adenosine group through both the hydrogen bond and covalent bond interaction.

4. UV/Vis Titration Experiments of Interaction between Different Phenythiourea Units Phosphate Species.

The UV/Vis spectroscopic titration experiment was adopted to study the binding properties between the phenylthiourea units and the pyrophosphate groups in nucleotides, using different phenylthiourea monomers (*o*-, *m*-, *p*-CF_3 substituted, being denoted as *o*-TF-PT, *m*-TF-PT, *p*-TF-PT, respectively) and PO_4^{3-}, HPO_4^{2-} or $H_2PO_4^{2-}$ as the model system.

Because monomer *p*-TF-PT was insoluble in water, DMSO was chosen as the solvent to process the UV/Vis titration experiment to investigate the interaction between monomers and phosphate anion species, the counter cations are tetrabutylammonium. Due to the huge volume of tetrabutylammonium, it has no influence on the recognition research. Figure S4 shows the UV/Vis spectra change of monomer *p*-TF-PT interacts with PO_4^{3-}, the absorbance intensity increases gradually with the continuous addition of the phosphates. Because these phosphate species have no absorption in the UV/Vis spectra, so the change in the spectra could be attributed to the interaction between thiourea monomer *p*-TF-PT and phosphate. For the complex of 1:1 stoichiometry, an association constant K_{ass} can be calculated by using the following equation 1 in Origin 7.0,[3] the K_{ass} between *p*-TF-PT and PO_4^{3-} is 6852.6 mol^{-1}·L. While the interaction between *p*-TF-PT and HPO_4^{2-} or $H_2PO_4^-$ have also been investigated through the same method, the values of K_{ass} are listed in Table S1. These data prove that thiourea monomer could combine phosphate units through hydrogen bonding interaction,[4] which further disclose the possible chemical change inside the polymer film and verify the above mechanism analysis from at least one aspect.

$$A = A_0 + \frac{A_{lim} - A_0}{2C_0}\{C_H + C_G + 1/K_{ass} - [(C_H + C_G + 1/K_{ass})^2 - 4\,C_H\,C_G]^{1/2}\} \quad \text{eq 1.}$$

Where A represents the absorption intensity, and C_H and C_G are the corresponding concentrations of host and anion guest.

Table S1. Association constants (K_{ass}) of interaction between phenylthiourea monomers and PO_4^{3-}, HPO_4^{2-}, $H_2PO_4^-$ anions in DMSO at 25 °C using the UV-vis titration method.

monomer	K_{ass} ($mol^{-1}\cdot L$) [a]		
	PO_4^{3-}	HPO_4^{2-}	$H_2PO_4^-$
o-TF-PT	717.2 ± 83.4	641.8 ± 74.5	[b]
m-TF-PT	3517.1 ± 419.5	754.1 ± 48.7	[b]
p-TF-PT	6852.6 ± 507.5	1090.2 ± 93.4	55.73 ± 21.1

[a] K_{ass} values were calculated from the absorbance intensity change at 350 nm, all error values were obtained by the results of nonlinear curve fitting.

[b] Reliable value could not be obtained due to the small change in the UV-vis spectra.

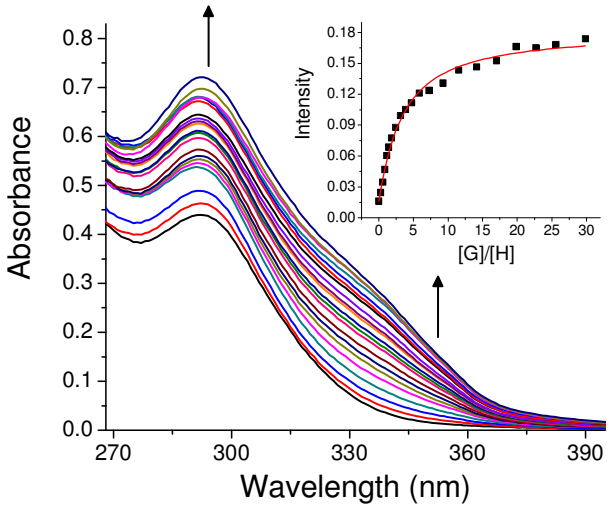


Figure S4. UV/Vis absorption spectra of monomer *p*-TF-PT (5.0×10^{-5} $mol\cdot L^{-1}$) upon the addition of various amounts of PO_4^{3-} in DMSO at 25 °C. Equivalent of PO_4^{3-}: 0, 0.3, 0.6, 0.9, 1.2, 1.5, 1.8, 2.4, 3.0, 4.0, 5.0, 6.0, 7.0, 9.0, 11.5, 14, 17, 20, 22.5, 25 and 30. The curve-fitting line through data points at 350 nm is shown in the inset, where G is the guest and H is the host. The correlation coefficient (R) of non-linear curve fitting is 0.991. K_{ass} = 6852.6 ± 507.5 $mol^{-1}\cdot L$.

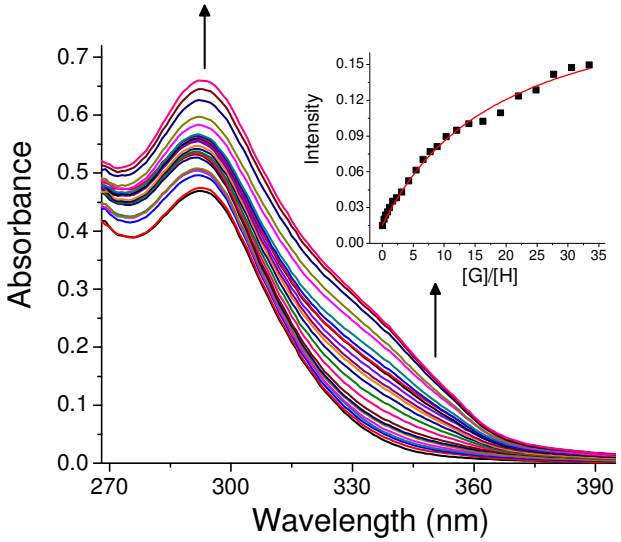


Figure S5. UV/Vis absorption spectra of monomer *p*-TF-PT (5.0×10^{-5} mol·L^{-1}) upon the addition of various amounts of HPO_4^{2-} in DMSO. Equivalent of HPO_4^{2-}: 0, 0.3, 0.6, 0.9, 1.2, 1.7, 2.3, 3.0, 4.5, 5.5, 6.5, 7.5, 9.0, 10, 12, 14, 16, 19, 22, 25, 27.5, 30.5 and 33.5. The curve-fitting line through data points at 350 nm is shown in the inset. The correlation coefficient (*R*) of non-linear curve fitting is 0.994. K_{ass} = 1090.2 ± 93.4 mol^{-1}·L.

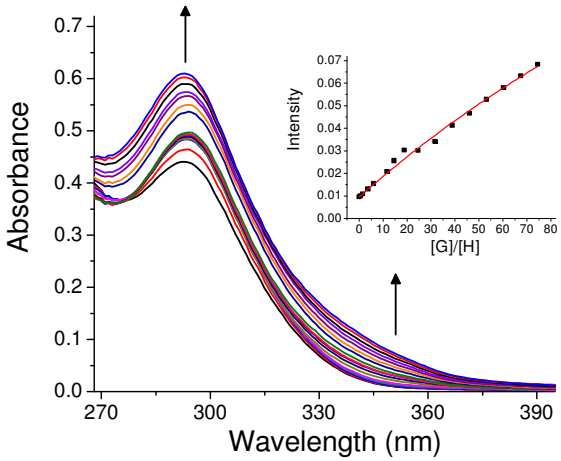


Figure S6. UV/Vis absorption spectra of monomer *p*-TF-PT interacts (5.0×10^{-5} mol·L^{-1}) upon the addition of various amounts of $H_2PO_4^-$ in DMSO. Equivalent of $H_2PO_4^-$: 0, 0.6, 1.5, 3.5, 6.0, 11.5, 15, 19, 25, 30, 40, 45, 55, 60, 67.5 and 75. The curve-fitting line through data points at 350 nm is shown in the inset. The correlation coefficient (*R*) of non-linear curve fitting is 0.993. K_{ass} = 55.73 ± 21.1 mol^{-1}·L.

5. Dynamic wettability evaluation of the responsive wettability of the PNI-PBA-TF(*p*) film.

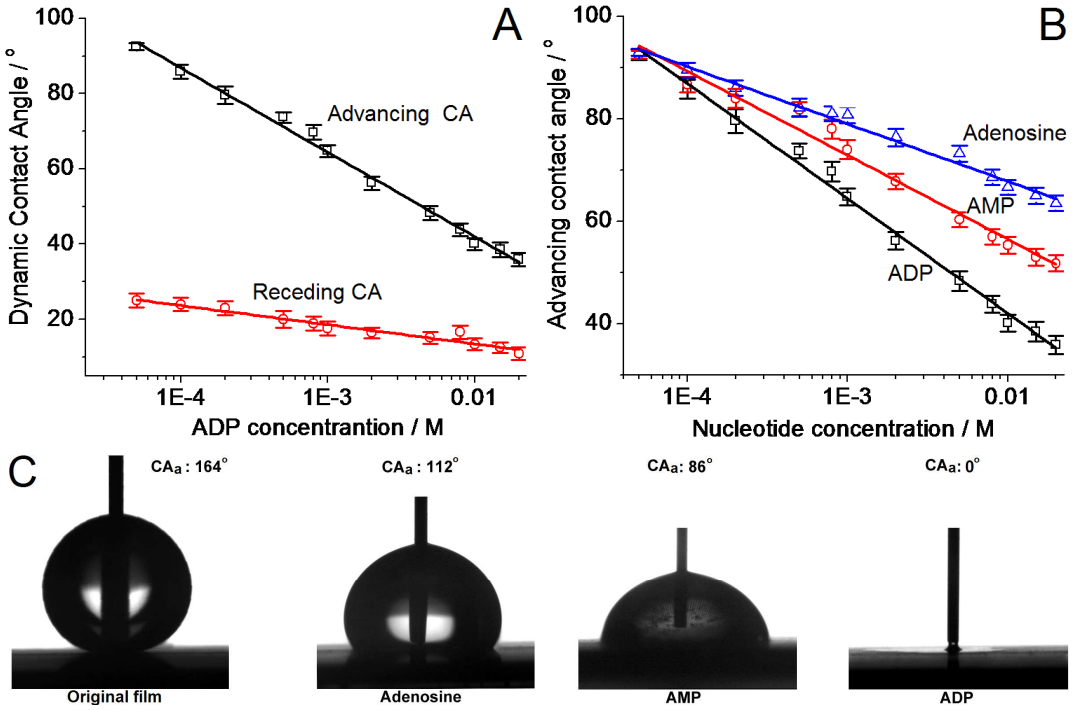


Figure S7. Dynamic CA results for the responsive wettability on the PNI-PBA-TF(*p*) film. (A) Concentration dependence of advancing and receding CAs on flat PNI-PBA-TF(*p*) film upon ADP treatment. It clearly shows that the result of the advancing CAs is similar to the static CA result. However, for the receding CAs, both the CAs before and after ADP treatments are much smaller than the static CAs, which can be explained by the hysteresis due to the relatively high adhesiveness of the film to water. (B) Concentration dependence of advancing CAs upon different nucleotide treatment, which shows a distinct selectivity to adenosine, AMP and ADP. This result is in excellent consistence with the static CA result presented in the main text. (C) Water drop profiles for the advancing CA measurement of the structured PNI-PBA-TF(*p*) film on etched silicon substrate. The pictures were caught during the procedure of water drop volume increase. Nucleotide concentration: 1 x 10^{-2} M. The result is also very similar to that of the static CA measurement.

6. Potential Molecular Mechanism for the Nucleotide Responsive Wettability

In order to investigate the possible mechanism of the PNI-PBA-TF(*p*) copolymer film for the nucleotide responsive wettability, pure PNIPAAm polymer film was introduced to proceed the control experiment, but the change of contact angle was less than 2 ^{o} under the treatment of an ADP solution of 2×10^{-2} mol·L^{-1}. Another control experiment was also conducted using reference copolymer with only the PNIPAAm blocks and the PBA units (PNI-PBA). However, a CA decrease of only about 6 ^{o} was observed towards the treatment of ADP solution. It reveals that the phenylthiourea unit is crucial for the nucleotide responsive wettability switching.

Considering above results and those described in the text, we proposed a potential molecular mechanism for the nucleotide responsive wettability switching of the PNI-PBA-TF(*p*) polymer film (as schematically shown in Figure S7): Initially, the thiourea and PBA units form complicated intramolecular hydrogen bonding network with the carboxyl and imine groups on PNIPAAm chains or with each other at different chains, resulting in a crimped and collapsed configuration of copolymer chains and a relatively hydrophobic property of the film. When being exposed to the nucleotide solutions, the nucleotide molecules can combine chemically with the PBA units through the covantenly bond between boronic acid and dihydroxyl of ribose unit; and at the same time, thiourea unit could combine phosphate functionalities through the hydrogen bonding interaction. The combination of nucleotide leads to the breakdown of the intramolecular H-bonds system, the copolymer chains thus become stretched and the hydrophilic groups can be fully exposed, leading to a much hydrophilic property of the film. The cooperation among these effect results in the excellent nucleotide responsive wettability of the film.

The electron-withdrawing $–CF_3$ group could induce the electron-deficient condition of benzene ring, thus further enhance the hydrogen bond donating ability of thiourea group, which differs very much according to its *ortho*-, *meta*-, *para*-positions on the benzene ring. In this way, the responsive wettability can be conveniently tailored by the existence and the substitute position of the $–CF_3$ group in the phenylthiourea unit.

Furthermore, the subsequent water treatment will cause the decomposition of the PBA-nucleotide-thiourea complex because the complex formation is dynamic and reversible, and the intramolecular H-bonds will come into being again.

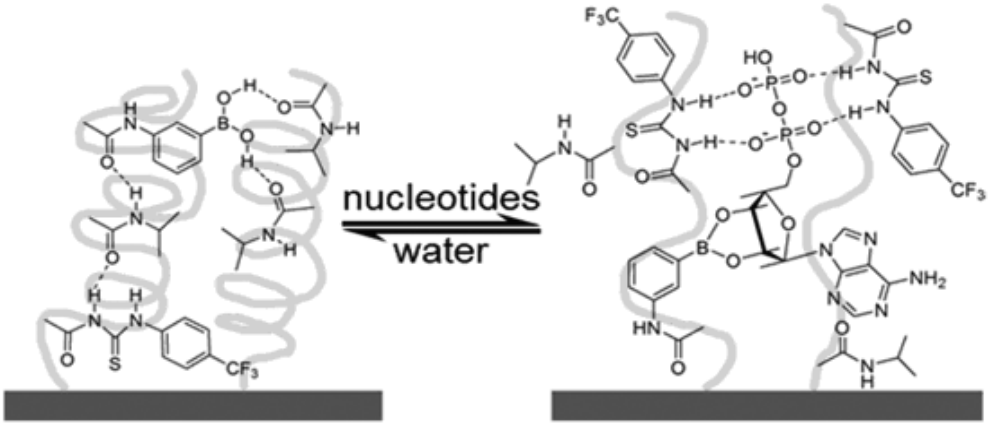


Figure S7. Potential molecular mechanism for the nucleotide responsive wettability.

In order to prove above mechanism, ^{1}H NMR titration experiment was introduced to investigate the combination of phenylboronic acid unit with adenosine, while UV/Vis titration experiments were undertaken to explore the hydrogen bonding interaction between thiourea unit and phosphate group.

7. Fabrication of the structured silicon substrate

The silicon wafer was used directly as the smooth substrate. The structured silicon substrate was fabricated by the combination of the photolithography and inductively coupling plasma (ICP) deep etching technique, and a chemical etching process. The photolithography and ICP technique were used to obtain the patterned silicon micropillar structure[5] on silicon wafer, and the chemical etching process, as described in detail in the literature,[6] was used to create further aligned nanofibrous structure on each micropillar. Figure S8 shows a typical SEM image of a rough surface introducing geometrical structures with patterned square pillars on a flat silicon wafer, 20 μm high, 9 μm long and with spacing of 12 μm between the silicon pillars. The SEM images were recorded by the Field Emission SEM apparatus (JSM 6700F, Hapan; and LEO VP 1530, Germany).

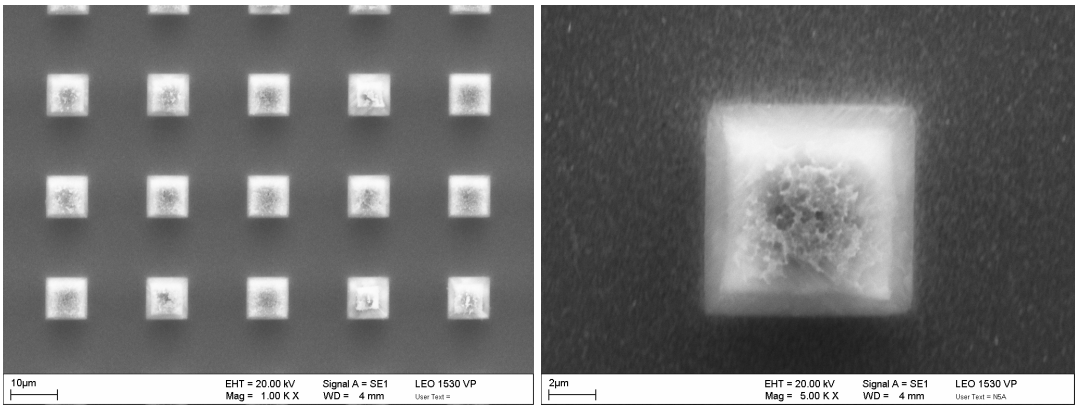


Figure S8. Typical SEM image of the rough silicon substrate.

8. The measurement of thickness of PNI-PBA-TF(*p*) film on flat silicon wafer.

The AFM investigation was conducted on flat silicon substrate (with copolymer film on it) using a Nanoscope IIIa instrument (DI, USA) in the tapping mode at room temperature (~ 25 ^{o}C).

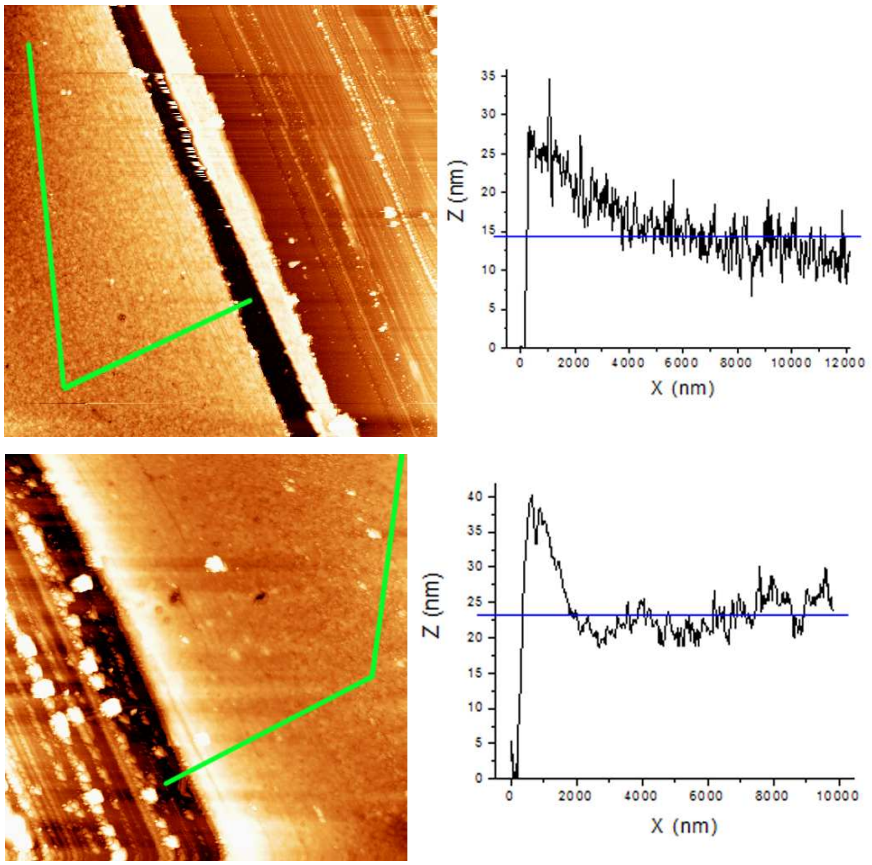


Figure S9. Typical AFM images of PNI-PBA-TF(*p*) film before (upper) and after (lower) immersing in the solution of ADP (2×10^{-2} mol·L^{-1}), 10×10 μm^2 size images are shown at Z-axis maximum height of 100 nm. The right sides are section profiles for the corresponding AFM images along the green lines at the left sides. The average thickness of original polymer film is 14.5 ± 2.0 nm, this value increases to 23.0 ± 1.5 nm after immersing in ADP solution.

References

1. Kanekiyo, Y.; Sano, M.; Iguchi, R.; Shinkai, S. *J. Polymer Science: Part A: Polymer Chemistry*, **2000**, *38*, 1302-1310.

2. Zayats, M.; Katz, E.; Willner, I. *J. Am. Chem. Soc.*, **2002**, *124*, 14724-14735.

3. (a) Valeur, B.; Pouget, J.; Bourson, J. *J. Phys. Chem.* **1992**, *96*, 6545-6549; (b) Bernard, V. *Molecular Fluorescence: Principles and Applications*; Wiley-VCH: Weinheim, Germany, **2002**; (c) Birks, J. B. *Photophysics of Aromatic Molecules*; Wiley: New York, **1970**, p 313.

4. Martnez-Mez, R.; Sancenn, F. *Chem. Rev.* **2003**, *103*, 4419-4476.

5. Williams, S. J.; Davies, G. J. *Trends Biotechnol.* **2001**, *19*, 356-362.

6. Roseman, S. *J. Biol. Chem.* **2001**, *276*, 41527-41542.