Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supplementary information

Nucleotide Sensing with a Perylene-based Molecular Receptor *via* Amplified Fluorescence Quenching

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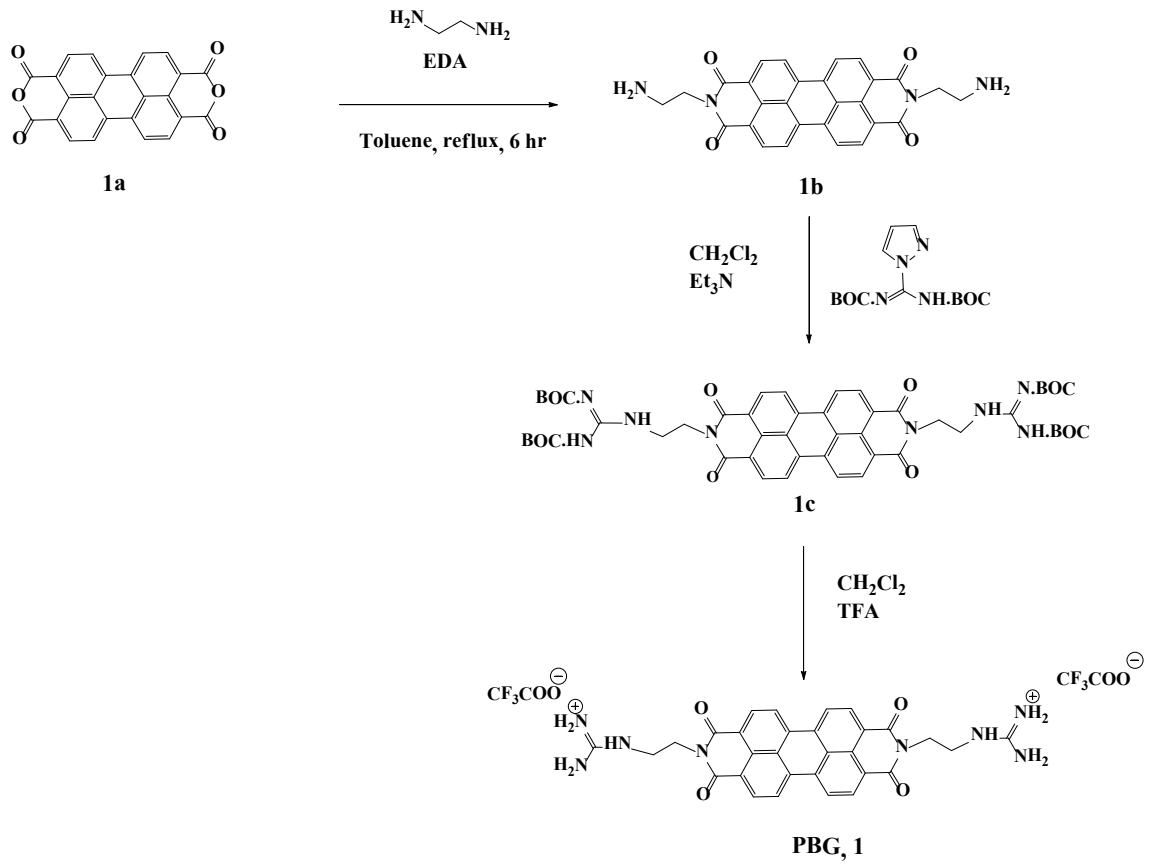
Materials:

All commercially available chemicals were of reagent grade and used as received. 3,4,9,10-perylenetetracarboxylic dianhydride, Ethylene diamine, *N,N*'-dimetlylethylenediamine, Et_3N, Methyl Iodide, Formic acid, Isopropanol, Trifluoroacetic acid (TFA), Toluene (super dehydrated, stabilizer free), CH_2Cl_2 (dehydrated), DMF (dehydrated) and MeOH were purchased from Wako Pure Chemical, Ltd. 1-H-pyrazole-1-(*N,N*'-bis(tert-butyloxycarbonyl))-carboxamidine and HEPES were obtained from Sigma-Aldrich Chem. Co., respectively. $CDCl_3$ and DMSO-d^6 for NMR were purchased from ACROS ORGANICS containing 0.03 (*v/v*)% TMS. TFA-d was purchased from Sigma-Aldrich and residual solvent (δ 11.5) was used as the internal referenced. Phosphates and nucleotides were purchased from Wako Pure Chemical, Ltd. Water was purified with a Direct-Q system (Millipore, Co.). The 1mM buffer solution of pH 7.4 (25 ^oC) was prepared by using 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES).

Methods:

^1H NMR (300 MHz) and ^{13}C NMR (75 MHz) were recorded on a Bruker Avance 300 spectrometer. Chemical shifts were reported in ppm with the signals of TMS or residual solvent as an internal standard for ^1H NMR and residual solvent for ^{13}C NMR measurements. FTIR spectra were performed on a JASCO FTIR-4200 using KBr palate. Mass spectra were obtained by a Waters 3100 MS. UV-vis absorption spectra were recorded on a JASCO V-670 quipped with a peltier-type thermostatic cell holder using a quartz cell with 1 cm path length. Fluorescence spectra were recorded by Perkin-Elmer LS55 luminescence spectrophotometer at room temperature (25 °C) using quartz cells with 1 mm path lengths and excitation wavelength is of 480nm. DLS measurements were conducted on the Malvern Zeta sizer Nano-ZS. Atomic force microscopy (AFM) was experimented using a JEOL JEM-2010 (acceleration voltage 120 kV) and Veeco Nanoscope IIIa (Tapping mode). The fluorescence images, irradiated under the UV light of 365nm, of the glass vials containing solutions were snapped with a digital camera.

(A) Synthesis of bis(2-(guanidinium)ethylene)perylene-3,4,9,10-tetracarboxyldiimide (PBG, 1):



Synthesis of **1b:**

To a suspension of 3,4,9,10-perylenetetracarboxylic dianhydride (**1a**) (1 g, 2.55 mmol) in toluene (40ml) was added ethylene diamine (**EDA**) (1.7 ml, 25.6 mmol). The mixture was refluxed for 6 h, and then filtered and washed with toluene. The solid was added to 5M KOH (40 ml) and stirred at room temperature for 6 h. The reaction mixture was filtered and washed with water. The resulting solid was dissolved in formic acid (~100 ml) and filtered. The solidification was achieved in isopropanol (400 ml) and the product was appeared as dark brown solid (1.06 g, 87%).

^{1}H NMR (300 MHz, TFA-d, ppm): δ 11.49 (TFA), 8.93 (d, 6.0 Hz, 4H), 8.86 (d, 6.0Hz, 4H), 8.15 (s), 4.85 (br s, 4H), 3.86 (br s, 4H).

MS (AP^+) m/z 498.3, $(M + Na)^+$

Synthesis of **1c:**

To a suspension of **1b** (600 mg, 1.26 mmol) in CH_2Cl_2 (20 ml), Et_3N (0.5 ml, 3.6 mmol) and 1-*H*-pyrazole-1-(*N*,*N*'-bis(*tert*-butyloxycarbonyl))carboxamidine (1.2 g, 3.6 mmol) were added. The resulting solution was stirred at room temperature for 72 h, and the solvent was evaporated to dryness. The crude product was purified by column chromatography on silica (CH_2Cl_2/MeOH 99:1, R_f =0.24) to afford **1c** as reddish brown solid (580 mg, 48%).

^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 8.70 (d, 6.0 Hz, 4H), 8.63 (d, 6.0 Hz, 4H), 8.59 (t, 4.1 Hz, 2H) 7.25 (s, $CHCl_3$), 4.49 (t, 4.3 Hz, 4H), 3.86 (q, 4.2 Hz, 4H), 1.54 (s, H_2O), 1.46 (s, 18H), 1.32 (s, 18H)

Synthesis of **PBG, 1:**

TFA (5 ml, 65 mmol) was added to a solution of **1c** (500 mg, 0.52 mmol) in CH_2Cl_2 (20 ml), and the resulting solution was stirred at room temperature for 12 h. The reaction mixture was then poured into Et_2O, and the resulting precipitate was collected by filtration, washed with Et_2O, and dried in vacuum. Reprecipitation was performed in tert-butyl alcohol and DCM, separately, to get **PBG, 1** as reddish brown solid (172mg, 42%).

^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ 8.78 (d, 6.0 Hz, 4H), 8.46 (d, 6.0 Hz, 4H), 7.57 (t, 4.6 Hz, 2H), 7.60-6.90 (br, 8H), 4.24 (t, 3.8 Hz, 4H), 3.53 (q, 4.2 Hz 4H), 3.35 (s, H_2O), 2.50 (s, DMSO).

^{13}C NMR (75 MHz, DMSO-d_6, ppm): δ 161, 157 (q, 31 Hz, $CF_3\underline{\boldsymbol{C}}OO^-$), 155.5, 131.8, 129, 126.3, 123.2, 122.3, 120.5, 117 ($\underline{\boldsymbol{C}}F_3COO^-$), 114, 38 (DMSO). (The expected alkyl carbon in the lower δ value (*ref. 5b*) was masked by strong solvent residual peak of DMSO-d_6; in fact the hidden peak appeared at 37.27 ppm in MeOH-d_4)

MS (AP^+) m/z 280.1, M/2

Elemental Analysis: Calc. for $C_{34}H_{26}F_6N_8O_8$ (**PBG, 1**): C, 51.78; H, 3.32; N, 14.21%. Found: C, 51.16; H, 3.37; N, 14.15%.

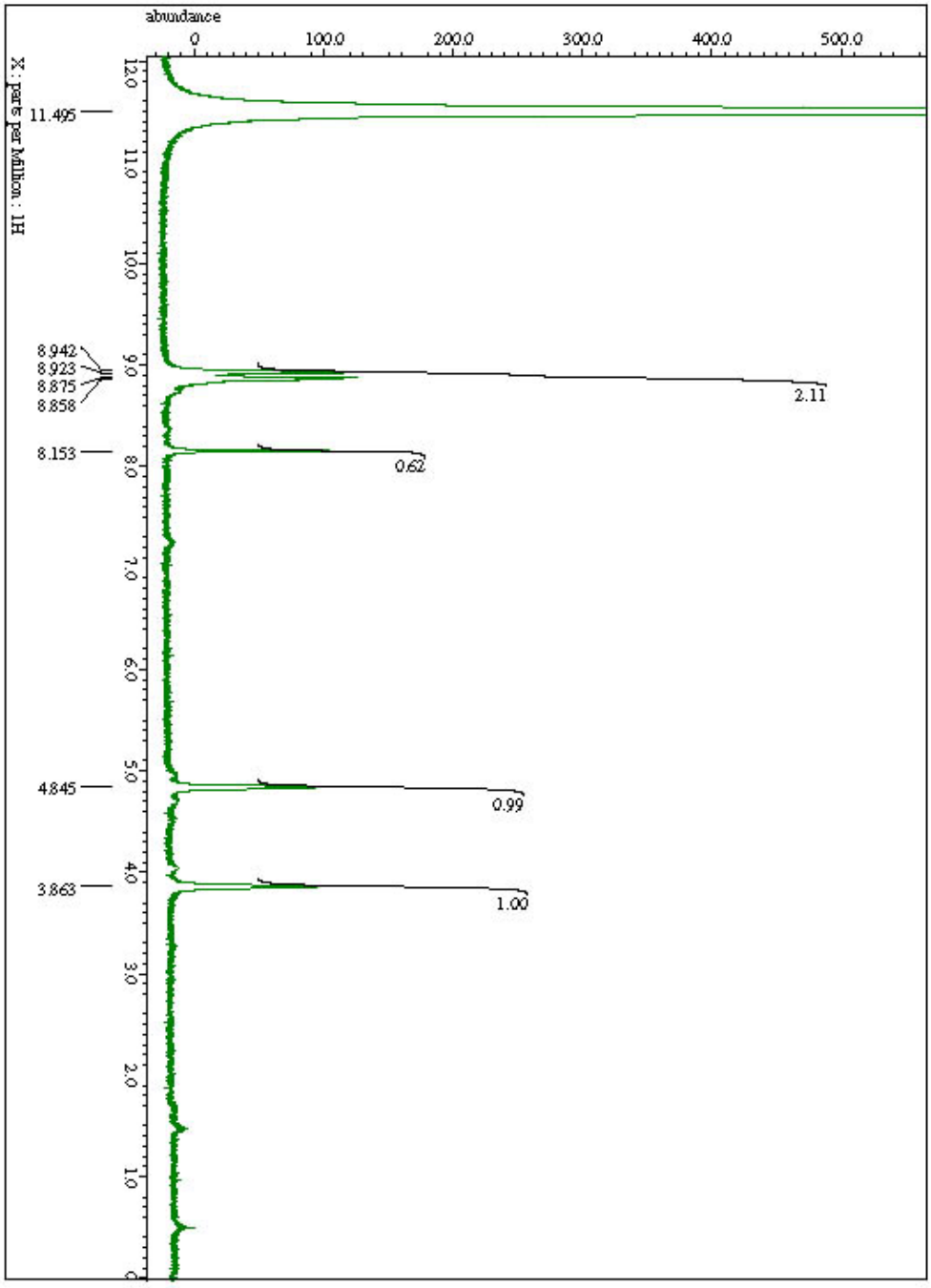
(B) Synthesis of *N,N′*-bis(2-(trimethylammonium)ethylene) perylene-3,4,9,10-tetracarboxyldiimide (PBTA, 2):

PBTA, 2 was synthesized by following the method described in *reference 9a*, using *N,N*'-dimetlylethylenediamine.

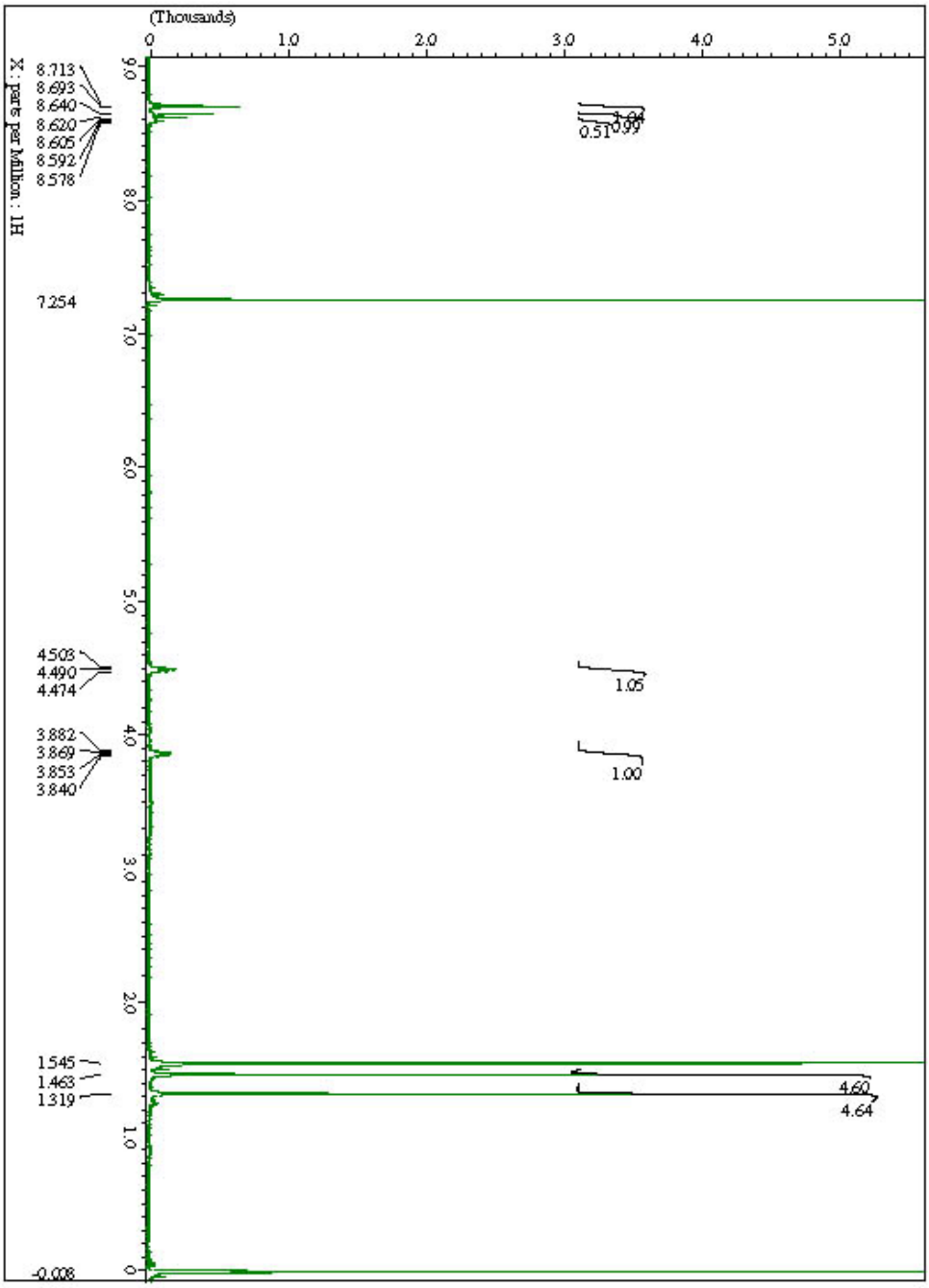
^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ 9.04 (d, 6.0Hz, 4H), 8.66 (d, 6.0 Hz, 4H), 4.51 (t, 5.5 Hz, 4H), 3.66 (t, 5.4 Hz, 4H), 3.34 (s, H_2O), 3.25 (s, 18H), 2.50 (s, DMSO).

NMR Spectra:

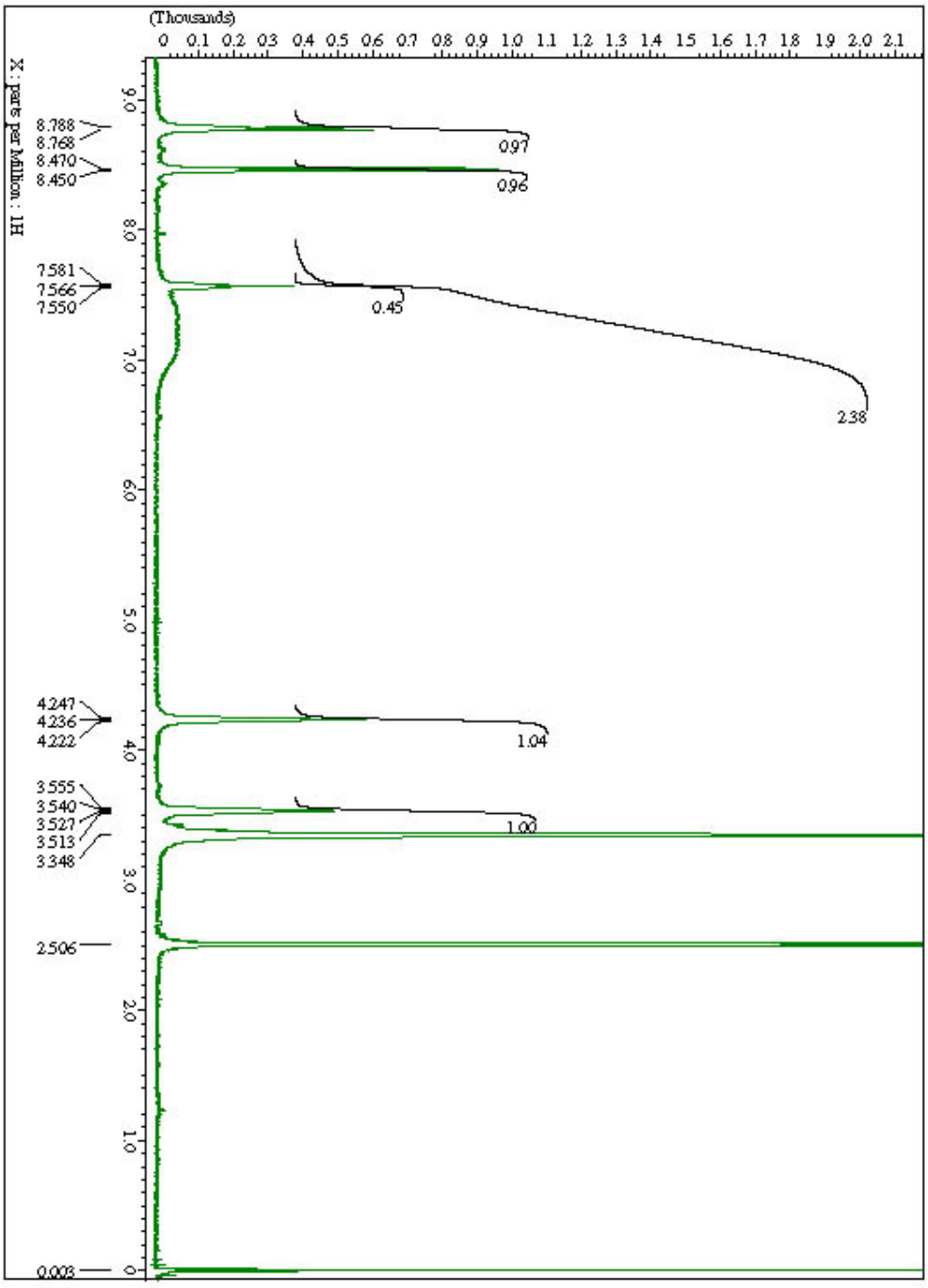
(i) ^{1}H NMR of **1b**:



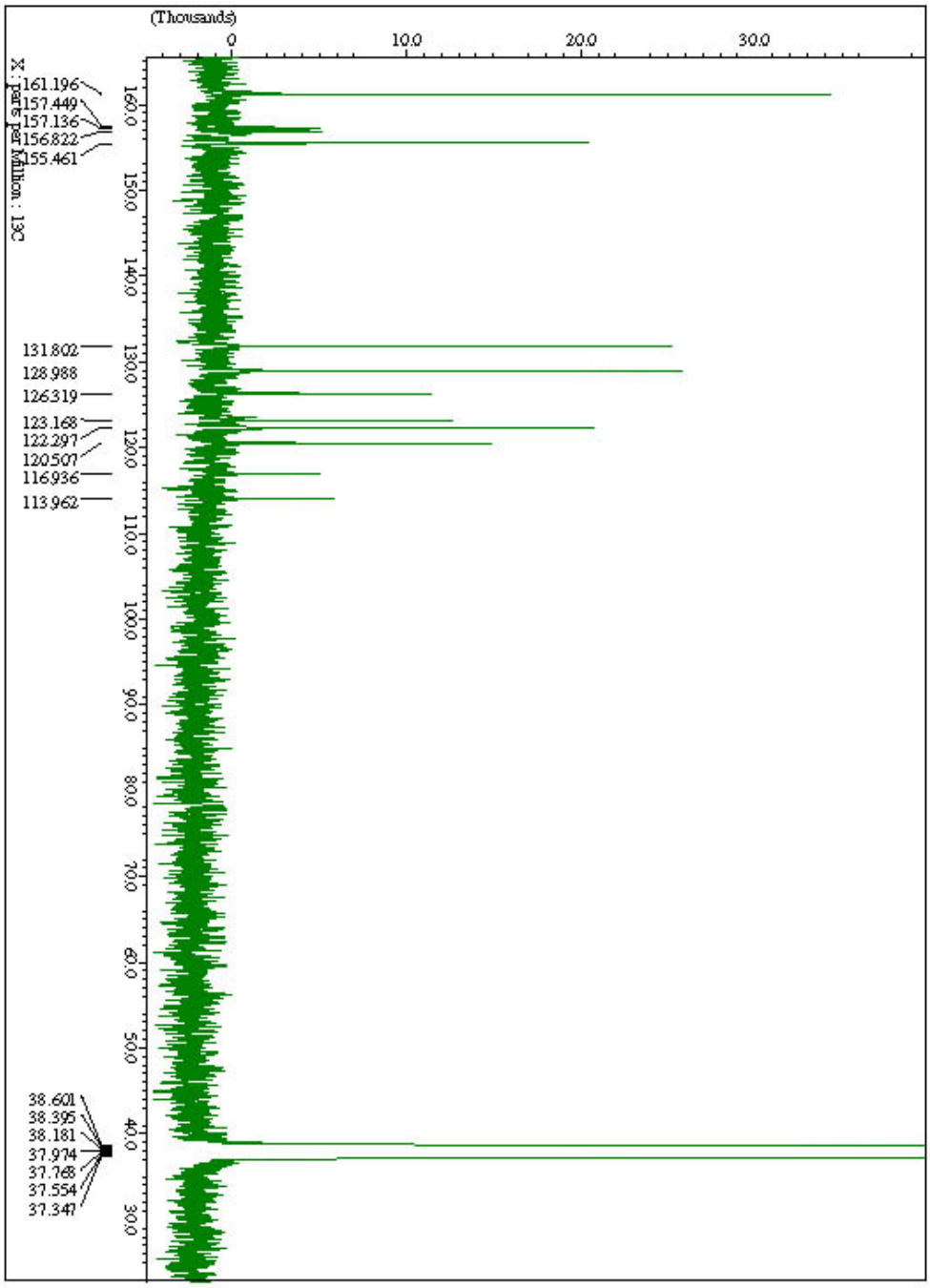
(ii) ^{1}H NMR of **1c**:



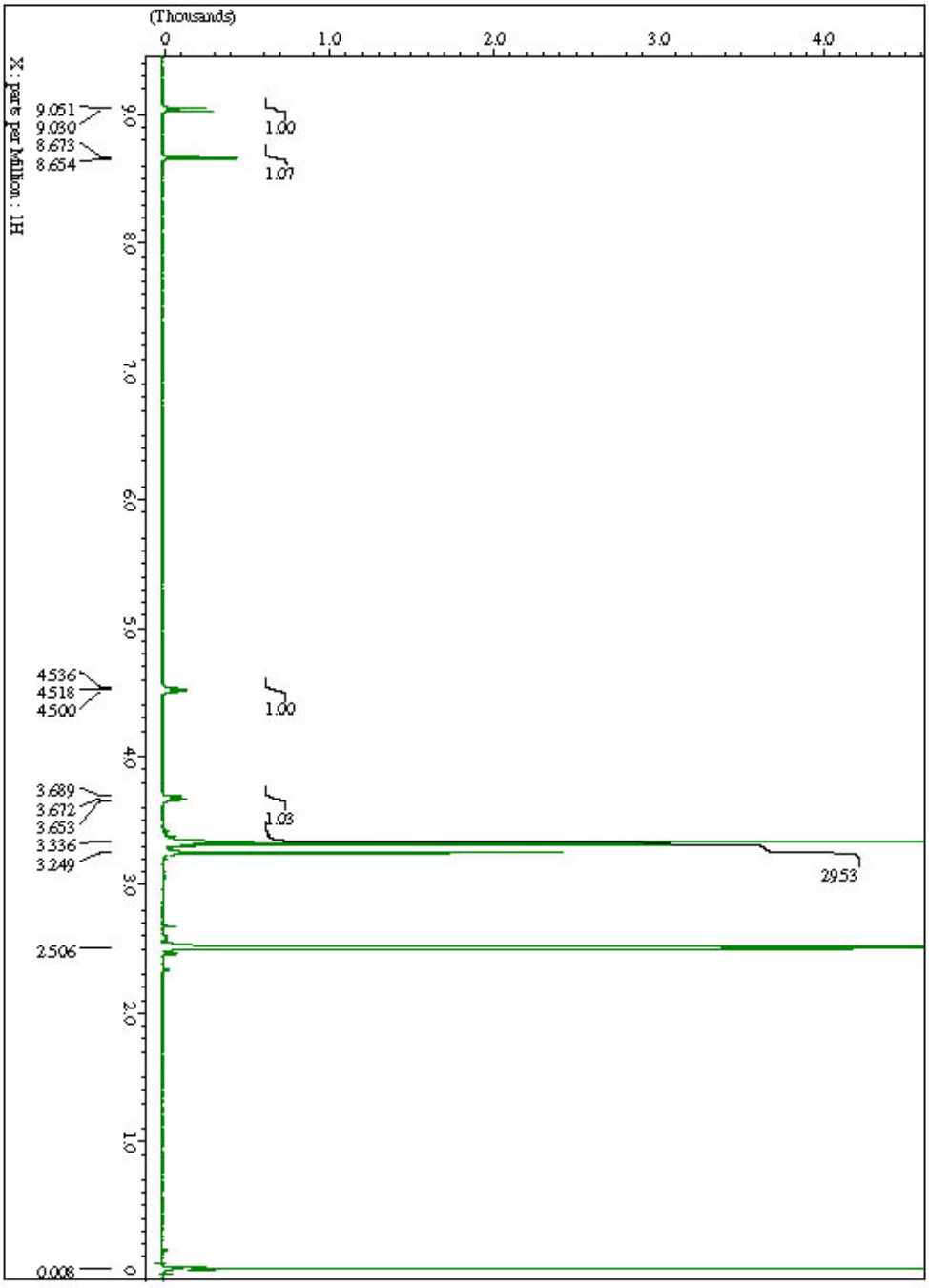
(iii) ^{1}H NMR of **PBG, 1**:



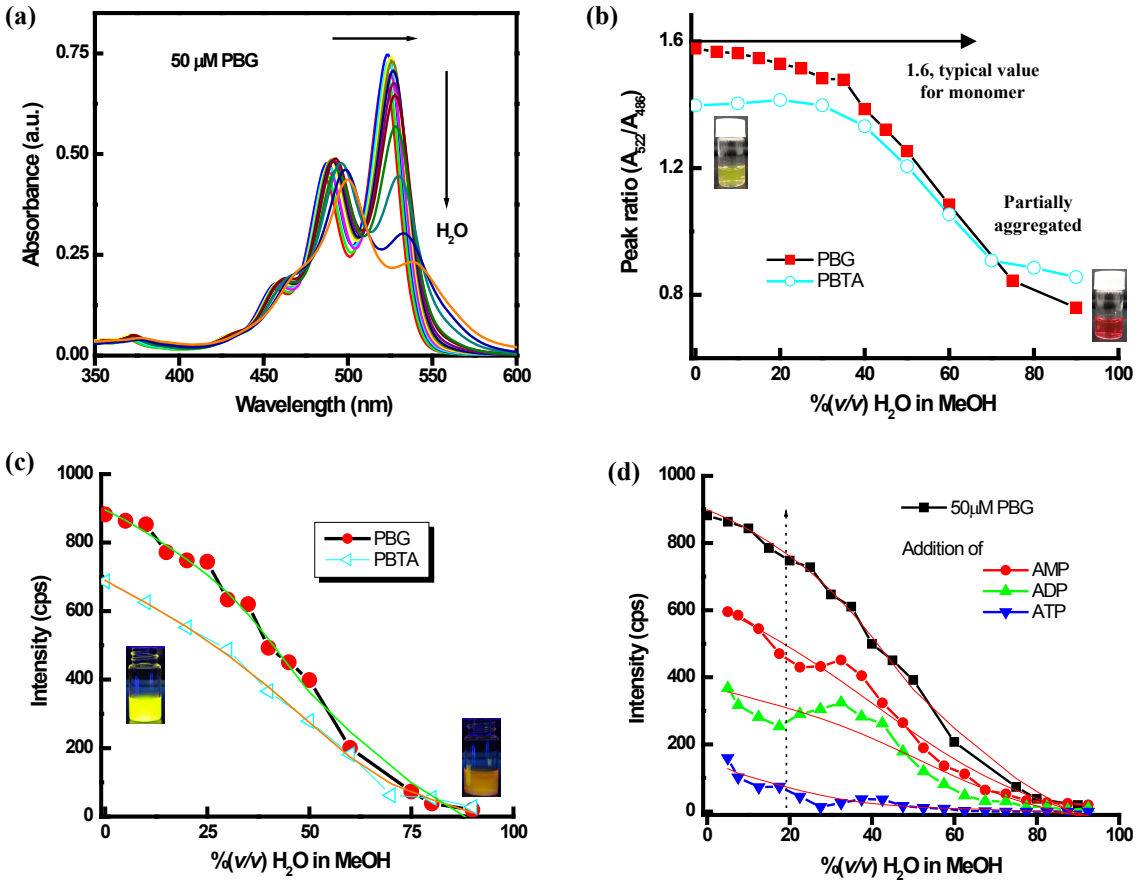
(iv) ^{13}C NMR of **PBG, 1**:



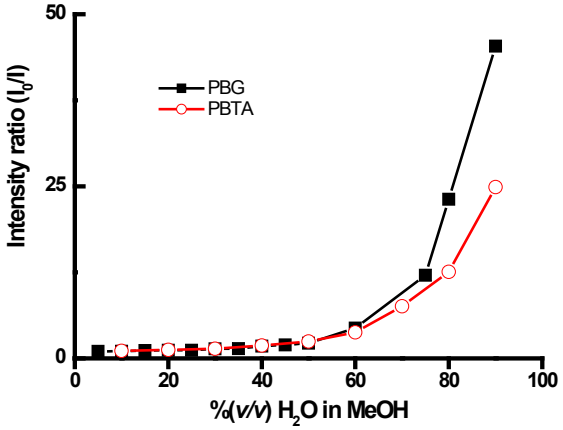
(v) ^{1}H NMR of **PBTA, 2**:



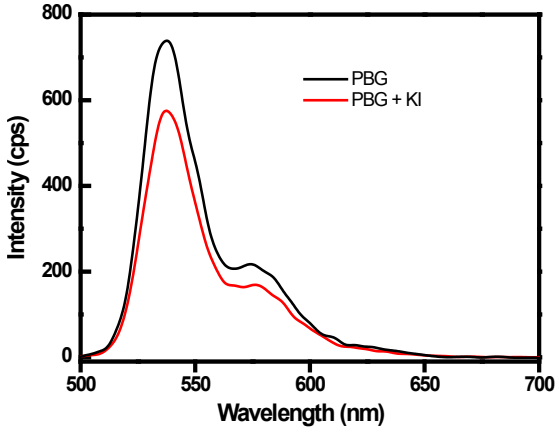
Standardization and application of the sensing systems:



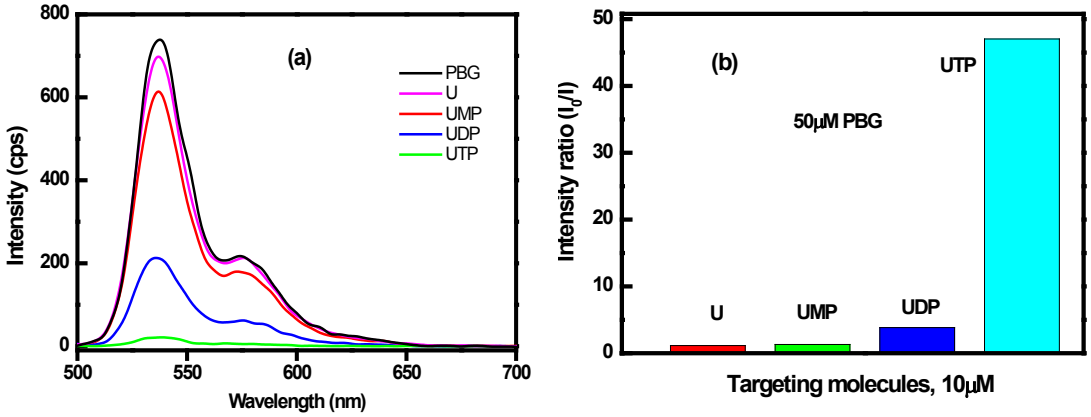
SI Figure 1. (a) and (b) Changes in the absorbance values of UV-vis spectra with increasing water (HEPES buffer, 1mM, pH 7.4) content(*v/v*) in 50μM methanolic solution of PBG and PBTA probes at 25 ^{o}C [inset Fig. 1b: visual appearance of the 50μM PBG in 100% water and methanol], (c) Corresponding fluorescence intensity change [inset Fig. 1c: appearance of the 50μM PBG in 100% water and methanol under UV light at 365nm], and (d) fluorescence intensity change with water content(*v/v*) of only 50 μM PBG and PBG in presence of different adenosine nucleotides (10 μM) at 25 ^{o}C.



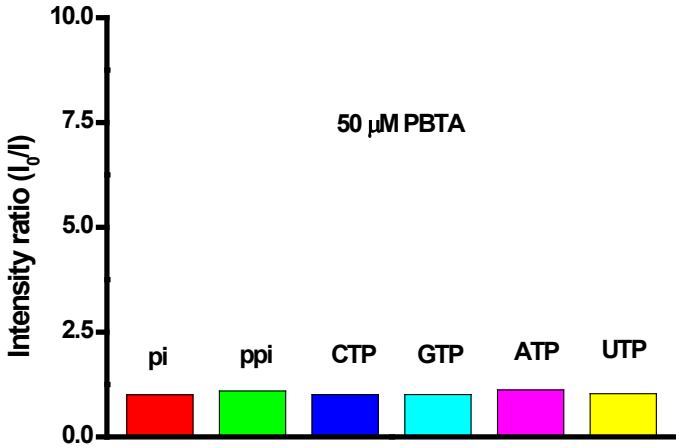
SI Figure 2. Investigation of SV quenching efficiencies (I_0/I) with increase of water content (*v/v*) in 50μM methanolic solutions of PBG and PBTA probes (in absence of anionic guest molecules) at 25 ^oC.



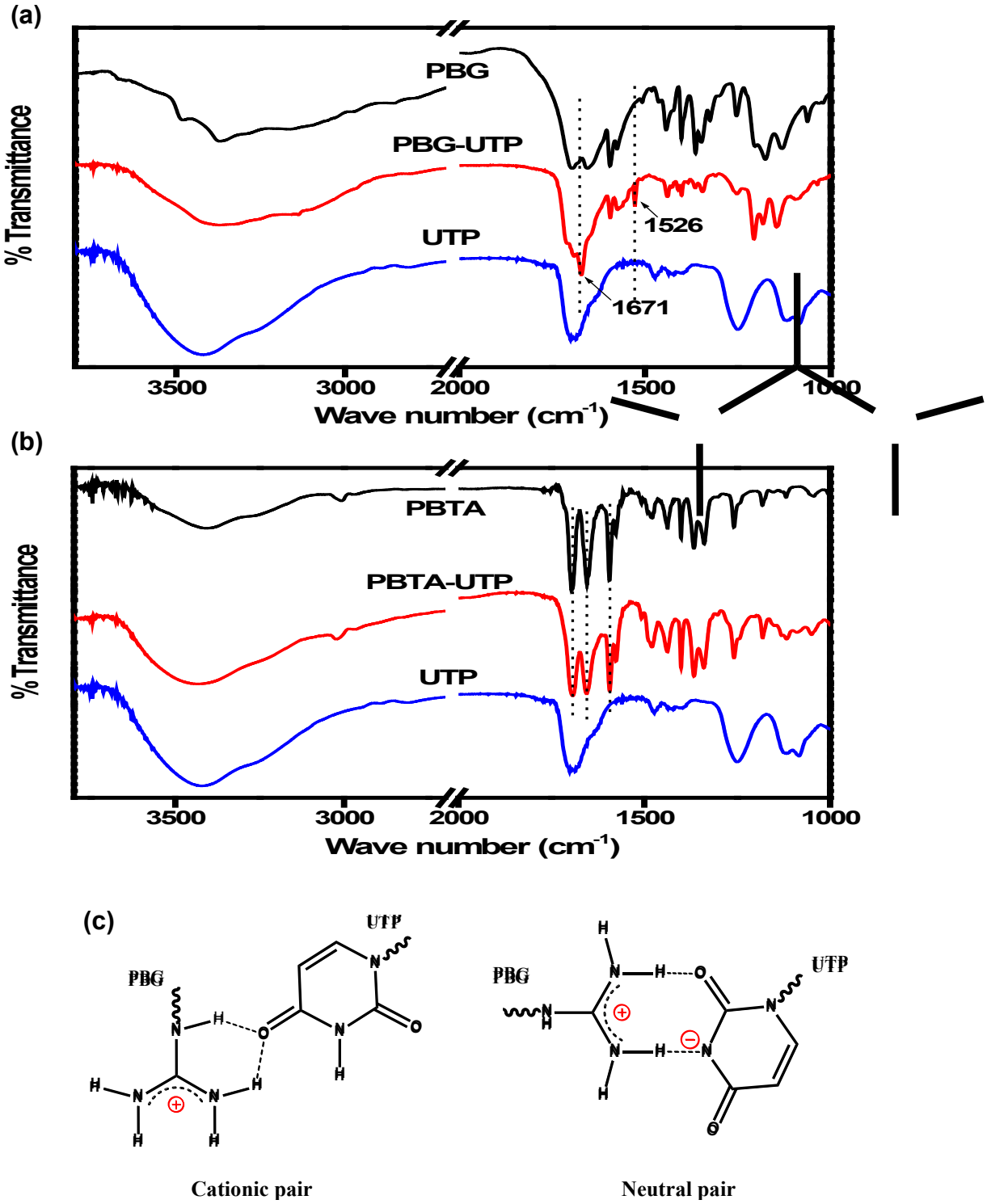
SI Figure 3. Effect in fluorescence intensity change of 50μM PBG with the addition of Potassium Iodide, KI (100μM) at 1:2 ratio, in 20%(*v/v*) H_2O/MeOH at 25 ^oC.



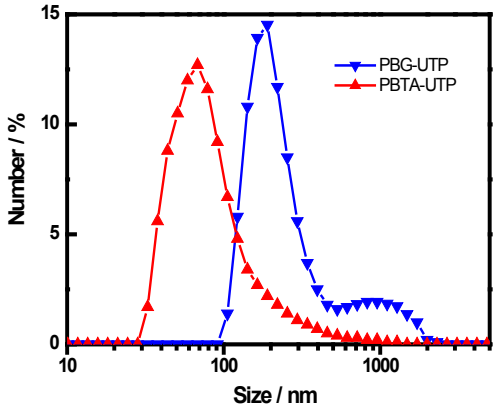
SI Figure 4. Assessments of quenching effect in addition of Uridine (U) and Uridine nucleotides (10μM) into 50μM, 20%(*v/v*) H_2O/MeOH solution of PBG at 25 °C.



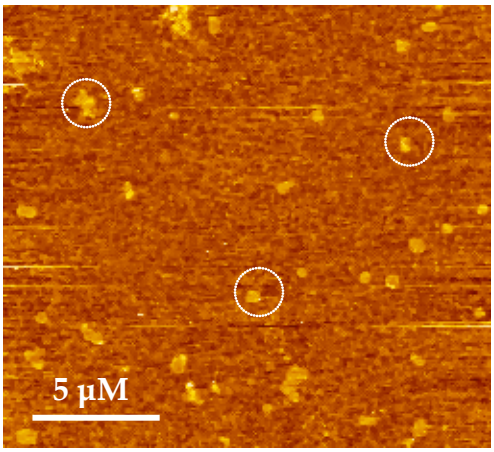
SI Figure 5. Investigation of quenching effect in presence of anionic guest molecules (10μM) into 50μM, 20%(*v/v*) H_2O/MeOH solution of tert-ammonium (PBTA) system at 25 °C.



SI Figure 6. (a),(b) Comparisons of the vibrational peaks in PBG-UTP and PBTA-UTP complexes with corresponding individual components, and (c) Schematic Presentations of possible binding modes between PBG and uridine head group.



SI Figure 7. Size distribution measurements in DLS experiment for PBG & PBTA systems (50μM) containing 10μM UTP in 20%(*v*/*v*) H_2O/MeOH at 25 ^oC.



SI Figure 8. The AFM Image obtained from the UTP recognized PBG system, aged for 6hr.