Supporting Information

Ethynylpyrene induces pH-dependent, fluorescence-detectable, reversible DNA condensation and decondensation

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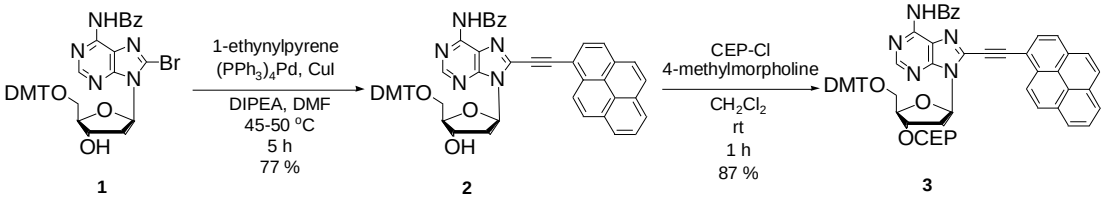
Synthesis of A^{PY}



Scheme 1 Synthesis of pyrene-labeled deoxyadenosine derivatives.

***N*6-Benzoyl-5´-*O*-[bis(4-methoxyphenyl)phenylmethyl]-8-(1-ethynylpyrenyl)-2´-deoxyadenosine (2).** $(PPh_3)_4Pd$ (18 mg, 0.026 mmol) and CuI (10 mg, 0.053 mmol) were added to a solution of **1** (190 mg, 0.26 mmol) in DMF (3 mL) under Ar and then the mixture was stirred for 5 min at room temperature. 1-Ethynylpyrene (60 mg, 0.26 mmol) and *N*,*N*-diisopropylethylamine (68 µL, 0.39 mmol) were added to this solution. After degassing, the reaction mixture was stirred at 45–50 °C and monitored by TLC. After 5 h, water was added to the solution and the product was extracted with excess EtOAc. The organic phase was washed twice with water. After evaporation of the solvent *in vacuo*, the residue was subjected to chromatography (SiO_2; hexane/EtOAc, 1:1) to yield **2** (188 mg, 85%). M.p. 140–141 °C. $[\alpha]^{23}_D$ = +33° (*c* = 0.525, CH_2Cl_2). IR (film): 3366, 2923, 2203, 1684, 1582, 1508, 1464, 1339, 1174, 846, 702 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 9.15 (s, 1H; NH), 8.64 (s, 1H; H-2), 8.25–7.15 (m, 23H; ArH), 6.89 (t, *J* = 6.9 Hz, 1H; H-1´), 6.68–6.73 (m, 4H; ArH), 5.10 (m, 1H; H-3´), 4.22 (dd, *J* = 10.1, 6.0 Hz, 1H; H-4´), 3.70 (s, 6H; OCH_3), 3.71–3.65 (m, 1H; H-2´), 3.47–3.44 (m, 2H; H-5´), 2.45–2.34 (m, 1H; H-2´), 2.34 (br s, 1H; OH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 163.9, 158.0, 157.9, 152.5, 148.7, 144.2, 137.4, 135.5, 135.3,133.3, 132.5, 132.3, 130.5, 130.3, 129.6, 129.5, 129.4, 129.0, 128.4, 127.7, 127.4, 127.3, 127.1, 26.7,126.6, 126.3, 126.2, 125.9, 124.4, 124.0, 123.7, 123.4, 113.5, 112.6, 96.8, 85.9, 85.0, 82.5, 72.7, 63.6, 54.7, 36.9. HRMS–FAB (*m*/*z*): $[M + H]^+$ calcd for $C_{56}H_{44}N_5O_6$, 882.3294; found, 882.3288.

***N*6-Benzoyl-5´-*O*-[bis(4-methoxyphenyl)phenylmethyl]-8-(1-ethynylpyrenyl)-3´-{bis[1-methylethyl)phosphoramidyl]cyanoethyl}-2´-deoxyadenosine (3).** 4-Methylmorpholine (55 µL, 0.50 mmol) was added to a solution of **2** (150 mg, 0.16 mmol) in CH_2Cl_2 (2 mL) under N_2 and then the mixture was stirred at room temperature for 5 min. 2-Cyanoethyldiisopropylaminochlorophosphoramidite (48 µL, 0.22 mmol) was added, after which the mixture was stirred and monitored by TLC. After 1 h, the solvent was evaporated *in vacuo* and the residue purified chromatographically through a short column (SiO_2; hexane/EtOAc/Et_3N, 97:97:6) to yield **3** (160 mg, 87%). M.p. 110–112 °C. $[\alpha]^{23}_D$ = +81° (*c* = 1.06, $CHCl_3$). IR (film): δ 3447, 2968, 2932, 1702, 1608, 1580, 1508, 1463, 1250, 1178, 1117, 1087, 979, 849, 829, 706 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 9.10 (s, 1H; NH), 8.65–8.05 (m, 9H; Ar PyH), 7.56–7.14 (m,14H; ArH), 6.68 (dd, *J* = 11.9, 6.3 Hz, 1H; H-1´), 6.71–6.65 (m, 4H; ArH), 5.27–4.98 (m, 1H; H-3´), 4.43–4.40 (m, 1H; H-4´), 3.74–3.41 (m, 13H; OCH_3, H-2´, NCH, OCH_2, H-5´), 2.67–2.54 (m, 1H; H-2´), 2.52–2.36 (m, 2H; CH_2CN), 1.15–1.06 (m, 12H; NCHCH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 163.9, 157.9, 152.5, 150.4, 148.7, 144.3, 137.7, 135.5, 135.4, 133.4, 132.6, 132.4, 132.3, 130.7, 130.4, 129.9, 129.6, 129.5, 129.0, 128.4,

127.7, 127.4, 127.2, 126.7, 126.2, 125.9, 124.5, 124.1, 123.9, 123.5, 122.8, 117.0, 113.6, 112.5, 96.8, 85.7, 85.4, 85.3, 82.7, 73.5, 63.4, 63.2, 59.9, 58.2, 57.9, 54.7, 42.9, 24.0, 19.7. ^{31}P NMR (121 MHz, $CDCl_3$): δ 151.6, 151.3. MS (FAB) *m*/*z*: 1082.3 $[M + H]^+$.

Solid-phase oligonucleotide synthesis

The phosphoramidite $\mathbf{A}^{\mathbf{PY}}$ was introduced as a building block to produce fluorescent oligodeoxynucleotides (ODNs) on a controlled-pore glass (CPG) solid support, using the phosphoramidite approach and an automated DNA synthesizer (POLYGEN DNA-Synthesizer). For comparison, unmodified ODNs were also prepared. The synthesized ODNs were cleaved from the solid support upon treatment with 30% aqueous NH_4OH (1.0 mL) for 10 h at 55 °C. The crude products from the automated ODN synthesis were lyophilized and diluted with distilled water (1 mL). The ODNs were purified through HPLC (Merck LichoCART C18 column; 10 × 250 mm; 10 µm; pore size: 100 Å). The HPLC mobile phase was held isocratically for 10 min with 5% MeCN/0.1 M triethylammonium acetate (TEAA) (pH 7.0) at a flow rate of 2.5 mL/min. The gradient was then increased linearly over 10 min, from 5 to 50% MeCN/0.1 M TEAA, at the same flow rate. The fractions containing the purified ODN were cooled and lyophilized. 80% Aqueous AcOH was added to the ODN. After 30 min at ambient temperature, the AcOH was evaporated under reduced pressure. The residue was diluted with water (1 mL); this solution was then purified through HPLC using the same conditions as those described above. The ODNs were analyzed through HPLC (Hewlett-Packard, ODS Hypersil; 4.6 × 200 mm; 5 m; 79916OD-574) using almost the same eluent system.

(a)

S1 5'- GTG CCG AAA $\mathbf{A^{PY}}$AA $\mathbf{A^{PY}}$AA $\mathbf{A^{PY}}$AA $\mathbf{A^{PY}}$AAA CTG CGC
S2 3'- CAC GGC AAA $\mathbf{A^{PY}}$AA $\mathbf{A^{PY}}$AA $\mathbf{A^{PY}}$AA $\mathbf{A^{PY}}$AAA GAC GCG
S3 5'- GTG CCG AAA AAA AAA AAA AAA A CTG CGC
S4 5'- GTG CCG TTT TTT TTT TTT TTT T CTG CGC
S5 5'- AAA $\mathbf{A^{PY}}$AA $\mathbf{A^{PY}}$AA AAA AAA AAA TT
S6 5'- AAA AAA AAA AAA AAA AAA TT
S7 3'- TTT TTT TTT TTT TTT TTT AA

(b)

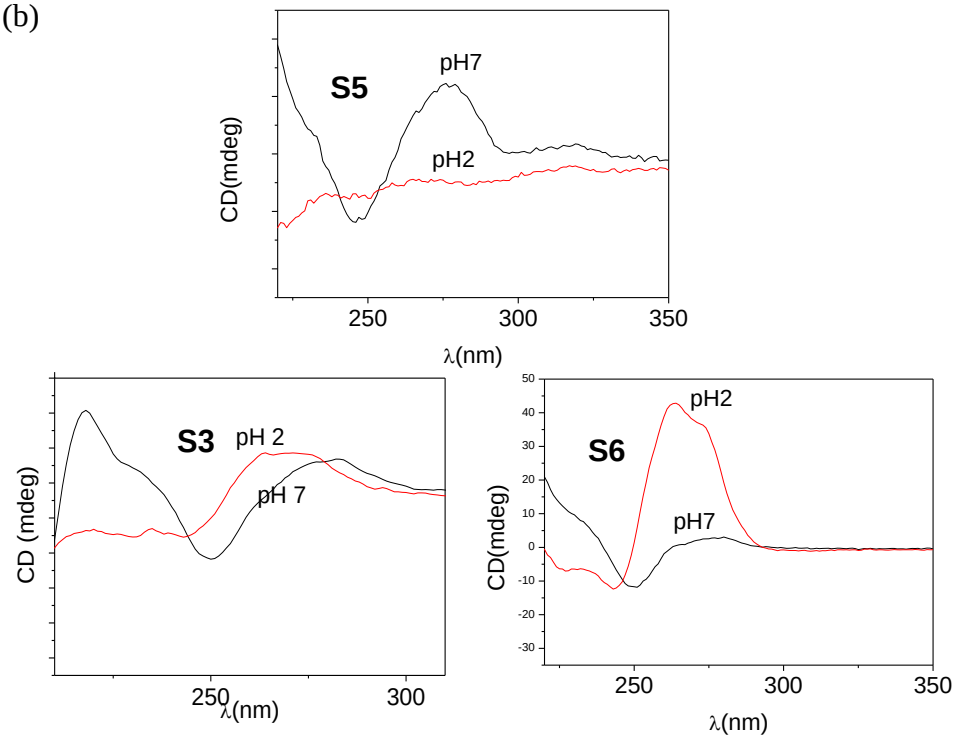


Figure S1. (a) Oligonucleotide sequences of **S1**–**S7**. (b) Circular dichroism (CD) spectra of ODNs **S3**, **S5**, and **S6** at pH 7 and 2, recorded at 1.5 μM in buffer (pH 7.2, 100 mM trizma·HCl, 10 mM $MgCl_2$, 100 mM NaCl) at 20 °C.

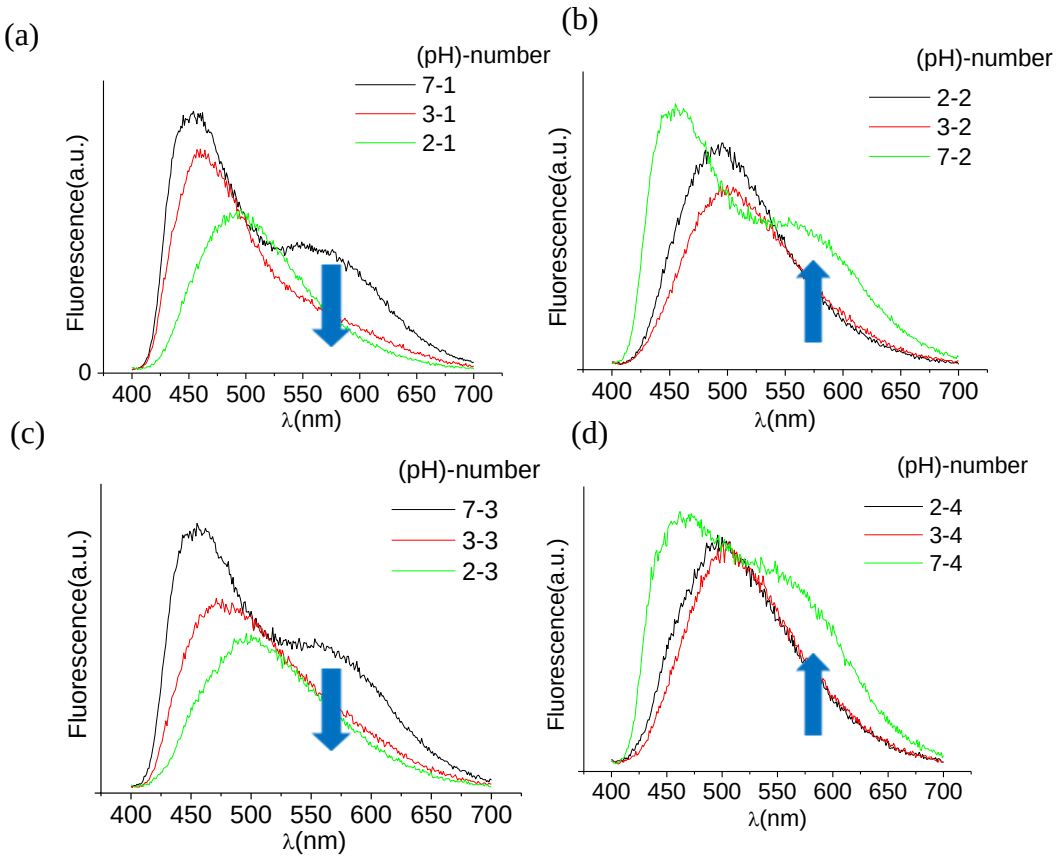


Figure S2. Reversible condensation and decondensation detected in the fluorescence spectra of the duplex **S1**·**S2** at pH 7, 3, and 2. (a) First, (b) second, (c) third, and (d) fourth cycles, recorded at 1.5 μM in buffer (pH 7.2, 100 mM trizma·HCl, 10 mM $MgCl_2$, 100 mM NaCl) at 20 °C.

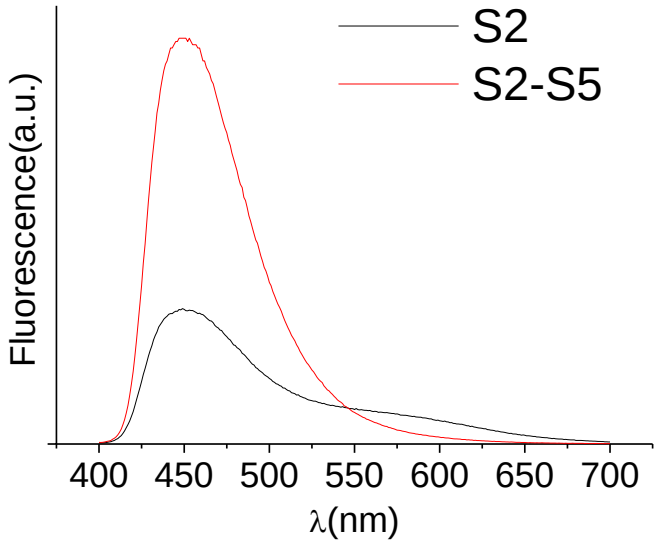
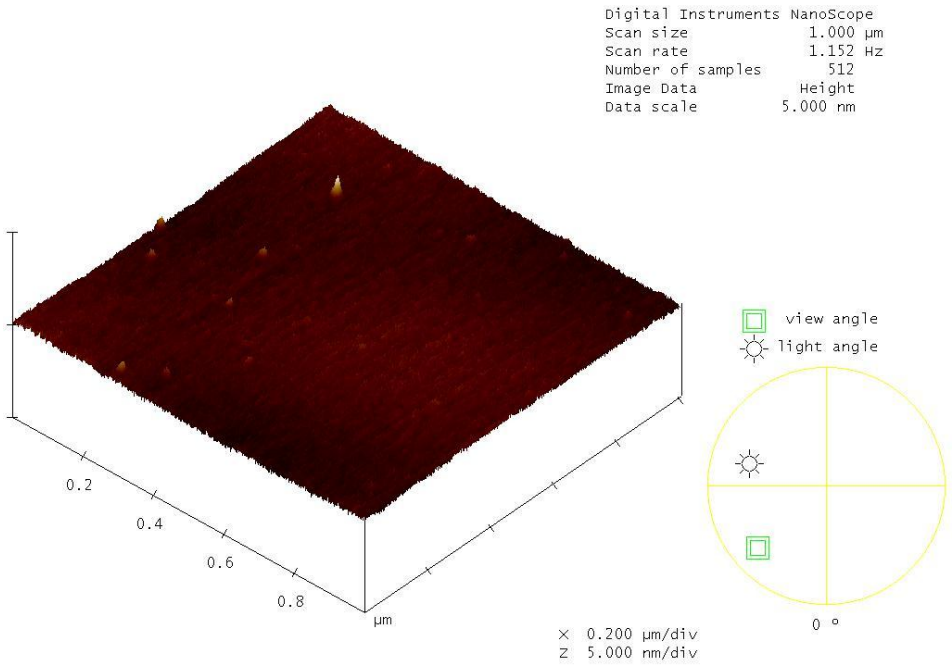


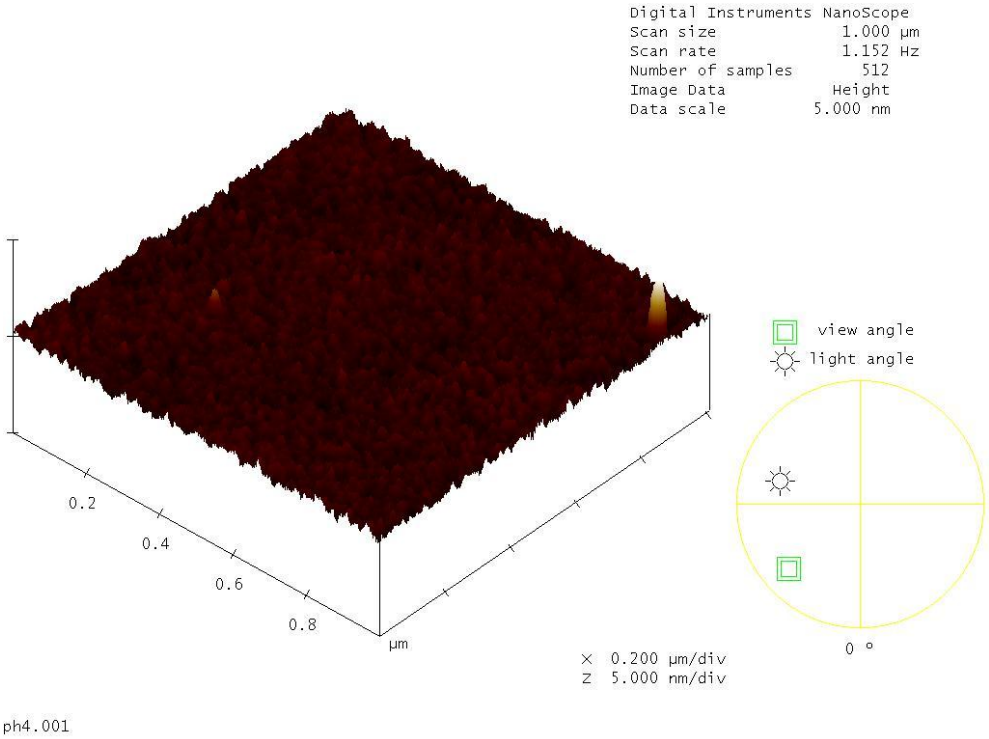
Figure S3. Fluorescence spectra of the ODN **S2** and the duplex **S2**·**S5** at pH 7, recorded at 1.5 μM in buffer (pH 7.2, 100 mM trizma·HCl, 10 mM $MgCl_2$, 100 mM NaCl) at 20 °C.

Detailed AFM images of DNA condensation

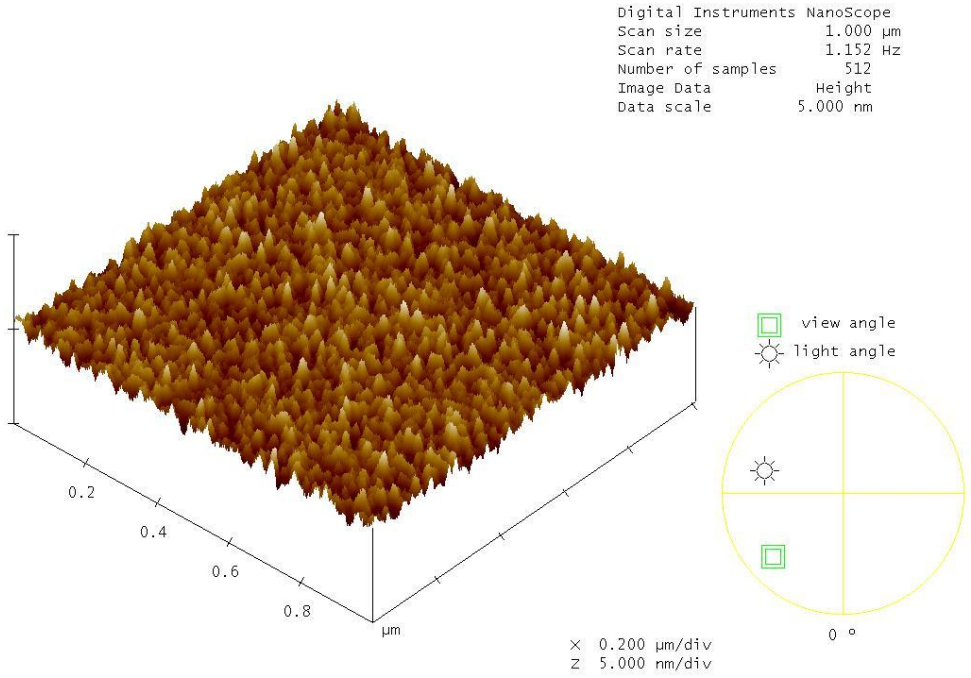
AFM image (pH 7)



AFM image (pH 4)

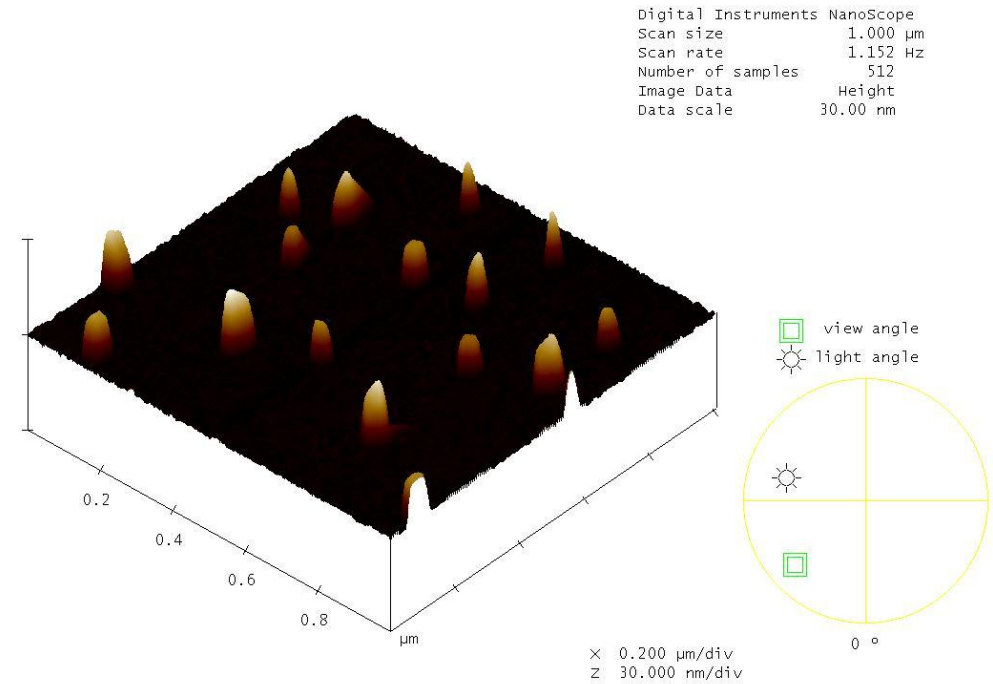


AFM image (pH 3)



ph3.001

AFM image (pH 2)



ph2.002