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Electronic Supplementary Information

"Nano-catalyst" for DNA transformation[†]

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1. Materials

N-isopropyl acrylamide (NIPAAm, Acros, 99%) was recrystallized from a toluene/hexane solution (50%, v/v) and ¹⁰ dried under vacuum prior to use. Copper (I) bromide (CuBr, Fluka, 98%) was purified by stirring in acetic acid, washing with methanol, and then dried in vacuum. 3-Aminopropyltriethoxysilane (APTES, Aldrich), bromoisobutyryl bromide (BIBB, Fluka) and 1,1,4,7,7-pentamethyldiethylenetriamine (PMDETA, Aldrich, 99%) were used as received. Triethylamine (TEA) and all other solvents were purchased from Shanghai Chemical Reagent Co. and purified according to standard methods before use. Silicon wafers (n-doped, (100)-oriented, 0.56 mm thick, 15 and 100 mm in diameter) were purchased from Guangzhou Semiconductor Materials (Guangzhou, China). The asreceived silicon wafers were cut into 0.5 cm×0.5 cm square chips. Deionized water purified by a Millipore water purification system to give a minimum resistivity of $18.2M\Omega$ cm was used in all experiments.

2. Preparation of PNIPAAm-grafted silicon nanowire arrays (SiNWAs)

- ²⁰ The silicon nanowire arrays (SiNWAs) investigated in this study were prepared by chemical etching of silicon wafer in AgNO₃/HF aqueous solution.¹ Briefly, silicon wafers were cleaned in a freshly prepared piranha solution $(H_2SO_4:H_2O_2=7:3(v/v))$ at 90°C for 2 h and were then rinsed with distilled water and dried in a stream of argon. The cleaned silicon wafers were immersed in the etching solution containing 5.0 mol·L⁻¹ HF and 0.015 mol·L⁻¹ AgNO₃ at 50°C for 30 min. The resulting surfaces were immersed in 20% nitric acid for 1 min and then rinsed extensively with 25 deionized water.

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The initiator was immobilized on the SiNWAs and SI-ATRP of NIPAAm was carried out following the procedures reported previously.² Briefly, NIPAAm (6.25 g, 55.23 mmol), PMDETA (0.7 mL, 3.35 mmol) and CuBr (0.16 g,1.12 mmol) were dissolved in a 1:1 mixture of methanol and water (25 mL) to prepare the reaction solution. The polymerization was carried out at room temperature under a nitrogen atmosphere for 10 min, 2 h and 8h,

³⁰ respectively. After polymerization, the arrays were rinsed with deionized water to remove unreacted NIPAAm monomer and ungrafted PNIPAAm, and then dried under a nitrogen flow. For comparison, PNIPAAm grafted silicon wafer surfaces (Si-PNIPAAm) were prepared following the same procedures as mentioned above. SNP1-SNP3 refer to PNIPAAm modified silicon nanowire arrays (10 min, 2 h, 8 h) and SiP1- SiP3 refer to PNIPAAm modified silicon wafer surfaces (10 min, 2 h, 8 h).

3. Surface characterization

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3.1 Electron microscopy

The surface morphology of as-prepared SiNWAs and SiNWAs-PNIPAAm were observed using a field-emission scanning electron microscope (FESEM, S-4800, Japan) and a transmission electron microscope (TEM, Tecnai G2 F20 S-Twin, USA).

3.2 Ellipsometry

The thickness of the PNIPAAm grafted layer on the smooth silicon substrate was measured by ellipsometry (M-88 spectroscopic ellipsometer, J.A. Woollam Co). Results are summarized in Table S1.

¹⁰ Table S1. Thickness of PNIPAAm layers on Si-PNIPAAm surfaces.

Surface	Polymer thickness (nm)	
SiP1	13.12 ± 1.04	
SiP2	37.71 ± 2.01	
SiP3	52.35 ± 2.35	

3.3 Water contact angle

Static water contact angles on the pristine and functionalized silicon surfaces were measured using the sessile drop method (SL200C optical contact angle meter, Solon Information Technology Co consisting of a sample stage, a light ¹⁵ source, a computer-controlled CCD camera with a macrolens, and a heating element on the sample stage for

temperature control). Results are shown in Fig. S1.



Fig. S1 Water contact angles vs. temperature for Si-PNIPAAm and SiNWAs-PNIPAAm surfaces. Each symbol represents the mean of three independent experiments with bar as SE.

4. Bacterial Strain and Plasmid

Bacterial cells of *Escherichia coli* DH5 α were used for bacterial adhesion and release experiments. The cells were cultured in Luria-Bertani (LB) broth medium and LB agar plates. Plasmid pUC18 was used for DNA transformation into *E. coli* DH5 α . The plasmid carries *bla* gene, coding for β -lactamase that confers resistance to ampicillin.

5. Bacterial adhesion

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Bacterial adhesion was performed as follows. *E. coli* DH5 α cells were grown at 37°C in LB broth medium. Cells were collected in the exponential phase of growth (OD₆₀₀=0.5) by centrifugation, washed in PBS buffer (pH 7.4) and finally resuspended in PBS buffer (pH 7.4) at a density of ~2.5×10⁹ mL⁻¹.

The materials were maintained at 4°C or 42°C on a thermal cycler (Mastercycler ep gradient S, Eppendorf). Cells were layered on the wafers, and after 2 min at 4°C or 90 s at 42°C, the materials were placed in PBS (at 4°C or 42°C). The OD₆₀₀ was measured and bacterial numbers were counted by colony forming test.

This journal is © The Royal Society of Chemistry 2011 For bacterial release assessment, bacterial suspension was layered on the wafers and maintained at 42°C for 90 s. The number of attached cells was determined by colony forming test, and was calculated from the difference in cell number in the suspension before and after exposure to the surface. The temperature was then quickly decreased to 4°C, and after 2 min the OD₆₀₀ was measured and the bacterial number determined by colony forming test at varying 5 dilution.³

Detachment (%) = Released cell number / Attached cell number \times 100



Fig. S2 Adhesion of E. coli cells on different materials (silicon and silicon nanowire arrays, unmodified and PNIPAAm modified) at 42°C. Each column represents the mean of three independent experiments with bar as SE.



Fig. S3 Adhesion and release of E. coli cells on SNP3 from 42°C to 4°C for different incubation times. (1'>1' indicates E. coli cells were incubated with SNP3 for 1 min at 42°C, then 1 min at 4°C; 2'>2' indicates E. coli cells were incubated with SNP3 for 2 min at 42°C, then 2 min at 4°C, and so on.) Each column represents the mean of three independent experiments with bar as SE.

6. Fluorescence microscopic observation of bacterial cells

To differentiate the live and dead cells on the materials in cell adhesion test, the mixture of SYTO 9 and PI in LIVE/DEAD® BacLight bacterial viability kit (Invitrogen, USA) were used to stain the healthy cells and dead cells, ²⁰ respectively. And for the transformation examination, cells were stained with SYTO 9, while plasmids were stained with PI. All the samples were observed by fluorescence microscopy (IX71, Olympus, Japan).

7. Plasmid purification and concentration determination

E. coli DH5 α strain that had been transformed with pUC18 was used to propagate plasmids (pUC18) for ²⁵ transformation experiments. In order to isolate the single colony with pUC18 including in the cell, all the cells were streaked on LB agar plate supplement with ampicillin (100 µg mL⁻¹) and cultured at 37°C for 14 h. The plate was preserved at 4°C in order to prevent the formation of secondary colonies around the primary colony. Before overnight

This journal is © The Royal Society of Chemistry 2011 culture, the single colony on the agar plate was inoculated into 2 ml LB medium supplement with ampicillin (100 μ g mL⁻¹) and cultured at 37°C for 2 h with vigorous shaking (~200 rpm) to disperse and recover the cells. 2 ml LB medium supplement with ampicillin (100 μ g mL⁻¹) in a flask or other vessel were inoculated with a volume of at least 4 times the volume of the short time culture (depending on cell density in the culture). The cells were grown at 5 37°C for 14 h with vigorous shaking (~200 rpm) to reach a cell density of approximately 3–4×10⁹ cells mL⁻¹. The bacterial cells were harvested by centrifugation (12000 rpm, 1 min at 4°C) and the plasmid was purified using a Qiaprep Purification Kit (Qiagen). The concentration of plasmid DNA was estimated from the absorbance at 260 nm measured by Varioskan Flash (Thermo Scientific, USA). The purified plasmid was preserved at -20°C. Before DNA

transformation the plasmids were kept at 4°C and diluted to a concentration of 100 ng μ L⁻¹. The plasmid DNA ¹⁰ provided to chemical transformation, SiP3 and SNP3 was from the same batch of purified DNA.

8. DNA transformation

DNA chemical transformation to competent *E. coli* DH5 α cells was performed by the CaCl₂ method. Briefly, competent cells suspended in 50 mM CaCl₂ were mixed with 0.1 ng pUC18 plasmid. The cells were then heat ¹⁵ shocked at 42°C for 90s. All the cells were diluted and spread on LB agar plate supplement with ampicillin (100 µg mL⁻¹). After overnight culture the transformants (transformed bacterial cells) formed colonies. Colony numbers were counted and the transformation efficiency (TE, transformant cfu µg⁻¹ DNA) was calculated.

To introduce DNA into *E. coli* DH5 α cells adhered on the PNIPAAm modified materials, approximately 1×10^7 fresh cells were suspended in 4 µL 50 mM CaCl₂, and loaded on the wafers at 4°C. 0.1 ng pUC18 plasmid was then ²⁰ added. After 2 min, the temperature was increased to 42 °C for 90s. The temperature was then decreased to 4°C. The bacterial cells were transferred to and diluted in LB medium. After overnight culture of the cells on LB agar plate supplement with ampicillin (100 µg mL⁻¹), transformation efficiency was calculated as described for chemical

25 **References**

transformation.

- 1 K. Peng, Y. J. Yan, S. P. Gao and J. Zhu, Adv. Mater., 2002, 14, 1164.
- 2 Q. Yu, Y. X. Zhang, H. Chen, Z. Q. Wu, H. Huang and C. Cheng, Colloid. Surface. B, 2010, 76, 468.
- 3 J. Sambrook and D. W. Russell, *Molecular Cloning: A Laboratory Manual. (3rd ed.)* Cold Spring Harbor Laboratory Press, New York, 2001, Chapter 1.

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