Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supporting Information for

Zn^{2+}, small addition in Ca^{2+}@DNA, achieves large elevation in gene transfection by aminated PGMA modified silicon nanowire arrays

Xuejin Huang,[a] Zhenhua Li,[a] Jingxian Wu,[a] Yingjie Hang,[a] Hongwei Wang,*[a] Lin Yuan*[a] and Hong Chen[a]

State and Local Joint Engineering Laboratory for Novel Functional Polymeric Materials, College of Chemistry, Chemical Engineering and Materials Science, Soochow University, 199 Ren'ai Road, Suzhou 215123, P. R. China.

Corresponding Authors

*Tel: +86-512-65880567. Fax: +86-512-65880583. E-mail: wanghw@suda.edu.cn; yuanl@suda.edu.cn.

Characterization of Material Surfaces:

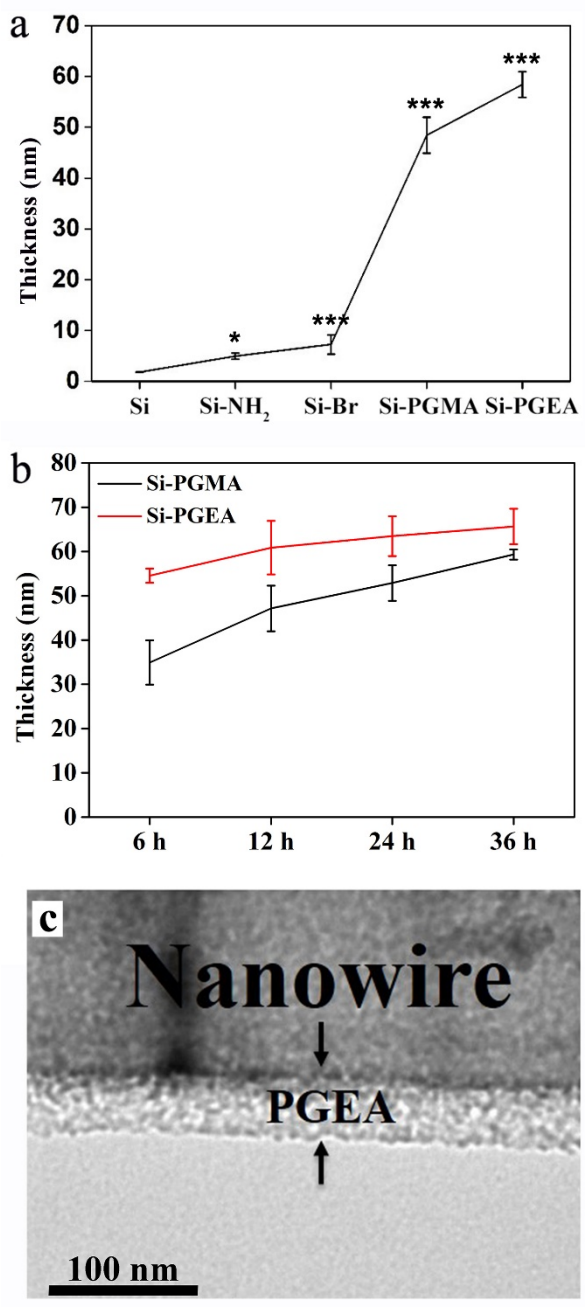

Figure S1. (a) Thickness of PGEA grafted on Si by SI-ATRP measured with an Ellipsometer. Significant increasing of the thickness of each samples at each step of the grafting process indicated the successful grafting of PGEA on the surface; (b)Thickness of polymer grafted on Si at different polymerization time (6 h, 12 h, 24 h, 36 h). The

thickness increased as the polymerization time prolonged; (c) TEM image of a single silicon nanowire surface modified with PGEA. Data are presented as the mean ± SD (n = 3); *, $p < 0.05$; ***, $p < 0.001$ (Si was the control group for analysis of significant differences).

Surface element analysis：

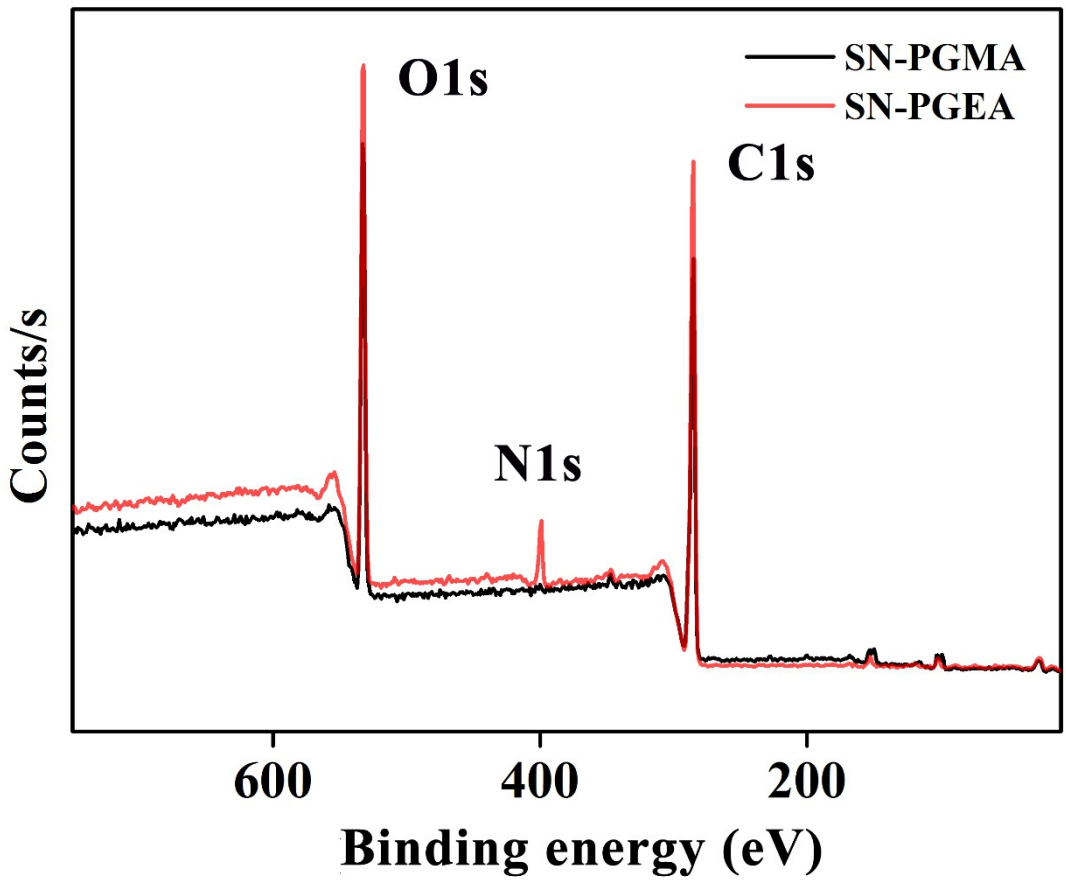

Figure S2. XPS spectra of SN-PGMA and SN-PGEA. The results showed that compared with SN-PGMA, the surface of SN-PGEA appears a peak of N element near 399 eV in addition to the peak of C and O elements, indicating the successful grafting of PGEA on the surface.

The stability of Zn^{2+}/Ca^{2+}@DNA nanoparticles and SN-PGEA:

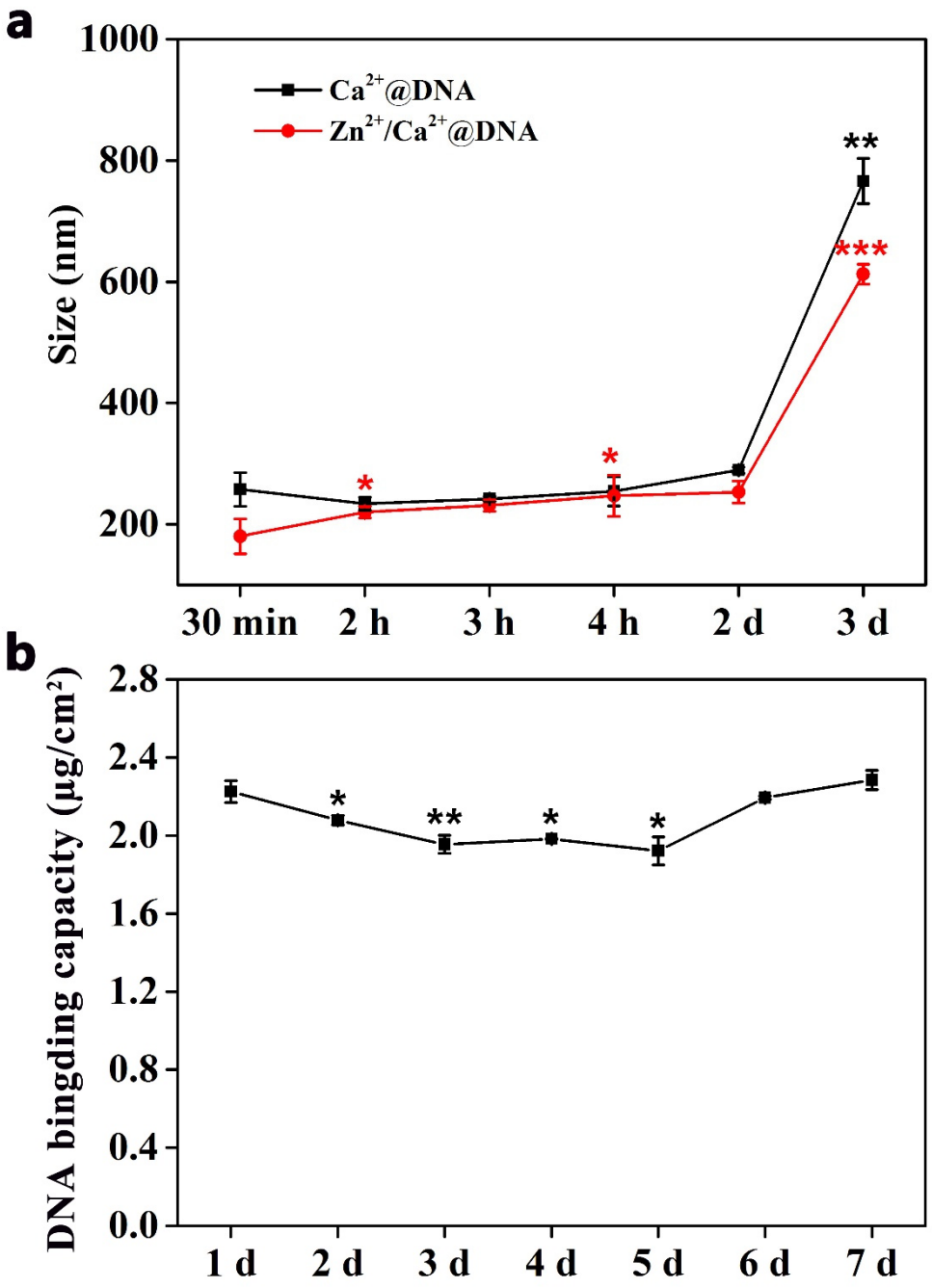

Figure S3. (a) The sizes of Ca^{2+}@DNA and Zn^{2+}/Ca^{2+}@DNA nanoparticles over time. The results demonstrate that during two days, the sizes of these particles have no obvious change. (b) DNA binding capacity of SN-PGEA over time. It was found that the DNA binding capacity maintained at the same level at least for a week. Data are

presented as the mean ± SD (n = 3); *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ (30 min and 1 d were the control group for analysis of significant differences respectively).

The transfection efficiency of Zn^{2+}@DNA:

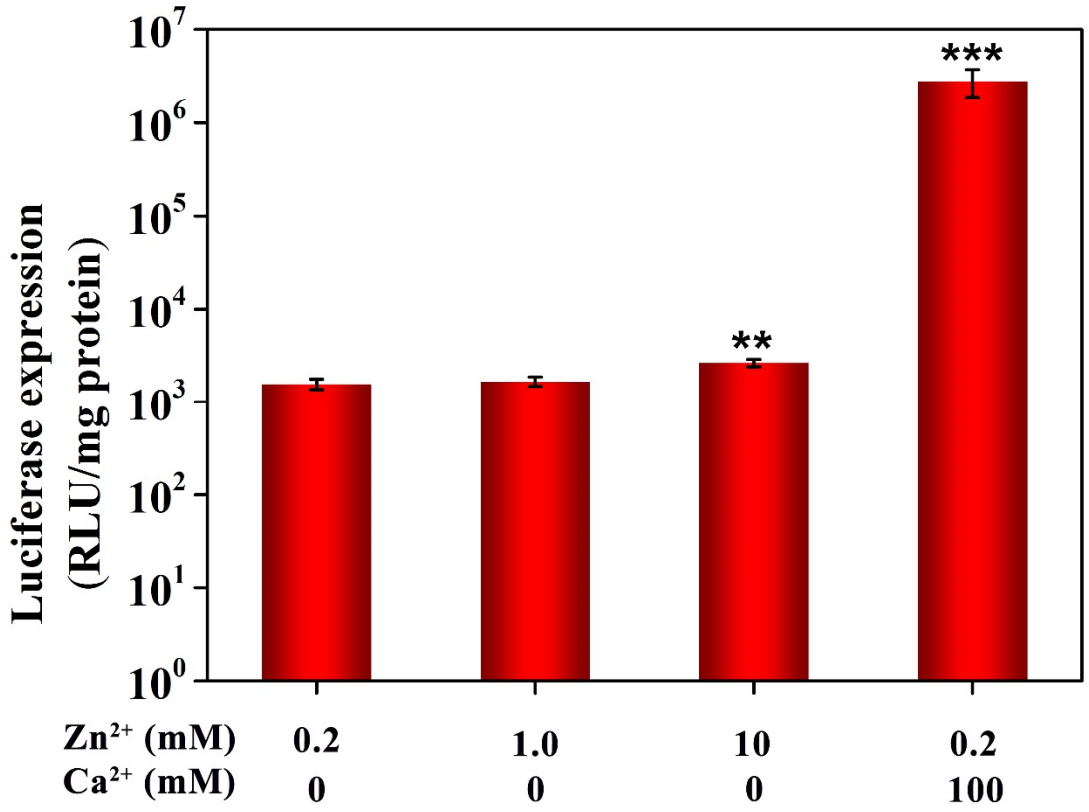

Figure S4. The transfection efficiency of Zn^{2+}@DNA and Zn^{2+}/Ca^{2+}@DNA. The results showed that the transfection efficiency of Zn^{2+}@DNA was lower than Zn^{2+}/Ca^{2+}@DNA. Since Zn^{2+} is a trace element in cells, and high concentrations of Zn^{2+} will cause harm to the cells, the addition of Ca^{2+} can achieve high transfection efficiency without obvious cytotoxicity. Data are presented as the mean ± SD (n = 3); **, $p < 0.01$; ***, $p < 0.001$ (the sample of 0.2 mM Zn^{2+} and 0 mM Ca^{2+} was the control group for analysis of significant differences).

DNA Loading Assay:

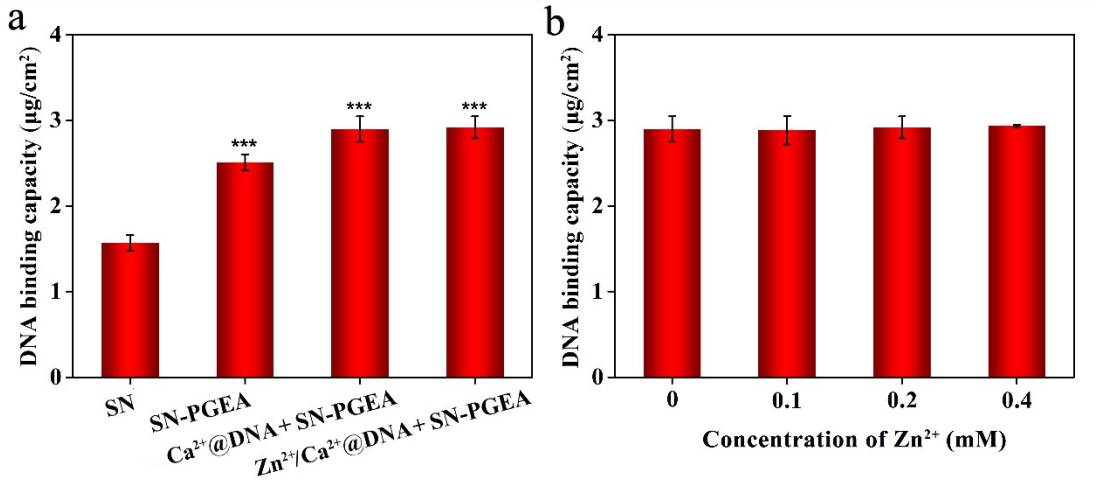

Figure S5. (a) DNA loading capacity of different surfaces. The results demonstrated that after being modified with PEGA, the DNA loading capacity of SN increased significantly compared to the unmodified SN. Ca^{2+} is widely accepted as an effective DNA condensing ion, which can dramatically increase the DNA loading capacity. However, no obvious change in the loading ability of DNA on SN-PGEA was observed after Zn^{2+} was added; (b) Loading capacity of Zn^{2+}/Ca^{2+}@DNA on SN-PGEA with different concentrations of Zn^{2+} (0, 0.1, 0.2, 0.4 mM). Results showed that the loading capacity of DNA complexes on SN-PGEA remained almost unchanged disregarding of the concentration of Zn^{2+}. Data are presented as the mean ± SD (n = 3); ***, $p < 0.001$ (SN was the control group for analysis of significant differences).

The Influence of Free Zn ions on Transfection Efficiency:

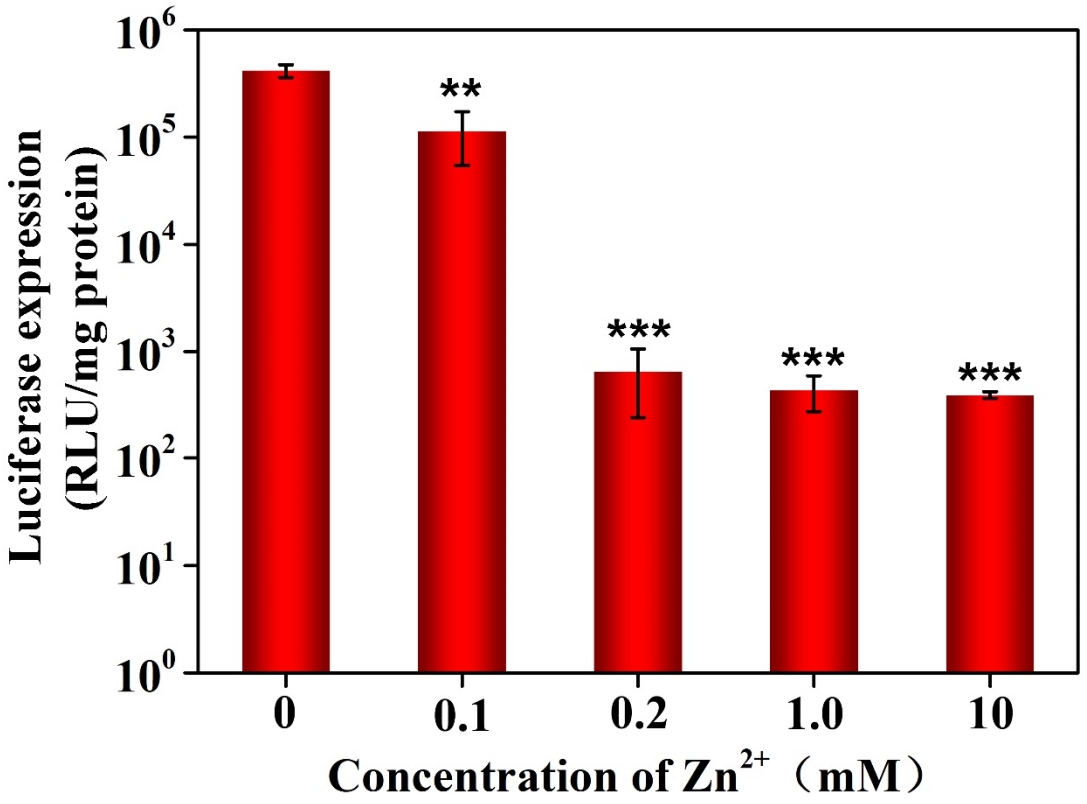

Figure S6. The transfection efficiency of Ca^{2+}@DNA with additional Zn^{2+}. Ca^{2+}@DNA was incubated with SN-PGEA, followed by the addition of different concentrations of free Zn^{2+} (0, 0.1, 0.2, 1.0, 10 mM). Transfection efficiency was detected 48 h after transfection. The results showed that the addition of free Zn^{2+} does not improve the transfection efficiency of the system while an inhibitory effect was observed. With the increase of the concentration of free Zn^{2+}, the transfection efficiency decreased due to the low viability of cells. Data are presented as the mean ± SD (n = 3); **, $p < 0.01$; ***, $p < 0.001$ (0 mM was the control group for analysis of significant differences).

The Influence of Free Zn ions on Cell Viability:

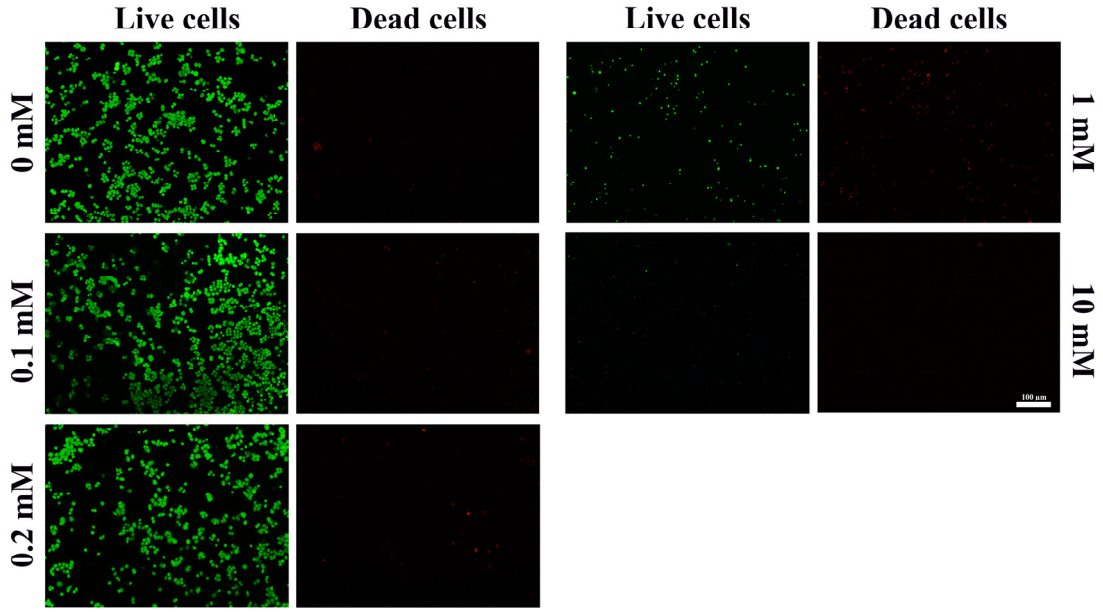

Figure S7. The observation of Live/Dead cells by fluorescence microscopy. Green and red fluorescence showed the live and dead cells, respectively. When investigating the effect of free Zn ions on transfection efficiency, it was found that when the concentration of Zn^{2+} exceeds 1 mM, the transfection efficiency is very low, presumably related to the lower cell viability. To testify the hypothesis, the effect of different concentrations of free Zn^{2+} on cell viability was studied. The experimental results showed that the system maintains good cell compatibility when the added concentration of free Zn^{2+} is low. However, the cytotoxicity of the system began to change significantly after the concentration of free Zn^{2+} increased to 0.2 mM, as the number of dead cells increased. When the concentration of free Zn^{2+} was further

increased to 1 mM, the cytotoxicity was further increased. In addition to the increased number of dead cells, the viable cell activity was also decreased as the cells were smaller and rounder than normal ones. When the concentration of free Zn^{2+} reached 10 mM, the cells died in large numbers and almost no adherent cells were seen on the substrate.