Supporting Information

pH-Operated Mechanized Porous Silicon Nanoparticles

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Materials and Methods

Synthesis of PSiNPs. Silicon wafers with a resistivity of 0.008-0.02 Ω·cm was pre-cleaned with water, acetone and isopropanol. The native oxide layer on the silicon was removed by immersing the wafer into a buffered oxide etchant (BOE), leading to a hydrogen (H)-terminated surface. The porous silicon nanowires were then prepared by Ag coating followed by the etching in an etchant solution containing HF and H_2O_2 for 60 minutes. The Ag nanoparticles were removed by soaking the samples into concentrated nitric acid for one hour. The porous silicon nanowires were then separated and suspended in methanol via sonication. The porous silicon nanowires were fragmented into PSiNPs by strong sonication (70 W, 42 kHz) for 2 hours. After the sonication, PSiNPs with different size were collected by filtering the suspension with 800 nm, 450 nm and 220 nm membrane filter. In this study, PSiNPs with a size range of < 450 nm were chosen for subsequent studies.

Grafting of Nanovalves. 20 mg of PSiNPs were suspended in 1 ml of absolute ethanol, followed by the addition of 5 μl of 3-Iodopropyltrimethoxysilane (IPTMS, 90%, Gelest). The solution was stirred under inert atmosphere at 60 ^oC for 10 hrs. The IPTMS-modified PSiNPs were then collected via centrifugation and washed with ethanol. Then, the PSiNPs were re-suspended in 2 ml of anhydrous DMF, in which 20 mg of benzimidzole (98%, Fluka) and 20 μl of *N*,*N*-diisopropylethylamine (99.5%, Sigma) were added. The solution was stirred at 70 ^oC under inert atmosphere for 24 hrs. The PSiNP-PBI were collected via centrifugation and washed with DMF and H_2O.

Fluorescent Labeling of PSiNP-PBI. 0.5 mg of fluorescein isothiocyanate (FITC, 90%, Sigma) was mixed with 1μl of *N*-(2-aminoethyl)-3-aminopropyltrimethoxysilane (90%, Gelest) in 1 ml of absolute ethanol. The solution was stirred under N_2 for 2 hrs. 20 mg of PSiNP-PBI were suspended in 1 ml of absolute ethanol, and mixed with the FITC ethanol solution. The mixture was stirred at 60 ^oC for 10 hrs. The resulted nanoparticles were centrifuged and washed with ethanol and H_2O.

Loading of Hoechst 33342. 20 mg of PSiNPs-PBI were mixed with 1 ml of Hoechst 33342 (Sigma) in aqueous solution (1 mM). The suspension was stirred for 12 hrs before β-cyclodextrin (40 mg) was added. The mixture was stirred for another 12 hrs, and the nanoparticles were centrifuged and washed with H_2O. The content of Hoechst in the washing solution was monitored by UV-vis spectrometer and fluorescence spectrometer to ensure the maximal removal of surface associated dye molecules. The pH value of the loading and washing solution was also closely monitored by a pH meter so that the nanovalve should not open during these processes.

Release Studies. The release profiles were obtained by time-resolved fluorescence spectroscopy, as previously described (Reference 9c-9f from the manuscript). Briefly, a probe beam (377 nm, 20 mW) was directed into the water or DMEM cell culture medium to excite the dissolved Hoechst molecules. The luminescence spectrum of the dissolved cargo was collected in 1 sec intervals over the course of the experiment. Acetic acid was added to decrease the pH to desired value. The luminescence intensity at the emission maximum of the dye was plotted as a function of time to generate a release profile.

Characterization of PSiNPs. The as-prepared silicon nanowires were characterized by scanning electron microscopy (SEM JEOL 6700) and transmission electron microscopy (TEM, CM120). The nanoparticle sizes were measured by ZetaSizer Nano (Malvern Instruments Ltd., Worcestershire, UK). N_2 adsorption-desorption experiment was performed on QuadraSorb SI (Quantachrome Instruments). The C-13 CPMAS NMR spectrum was collected on a DSX300 NMR spectrometer (Bruker, 300 MHz), using a 4 mm CPMAS probe at room temperature.

Cell Culture. Human pancreatic cancer PANC-1 cells grown in DMEM supplemented with 10% FBS were seeded overnight into 8 well chamber slides (Nalge Nunc International). FITC-Hoest containing PSiN and unlabeled PSiN dissolved in water were sonicated briefly prior to being added to cells for five hours at 37 ^{o}C or 4 ^{o}C. To demonstrate that PSiNP uptake occurs via an energy dependant process such as endocytosis or phagocytosis, one group of cells was pre-incubated at 4 ^{o}C for 30 minutes prior to PSiNP addition and subsequent incubation for another 5 hours at that temperature.

Cells were washed three times in PBS and fixed in 1% PFA for 10 minutes prior to mounting in 50% glycerol and imaging by confocal microscopy.

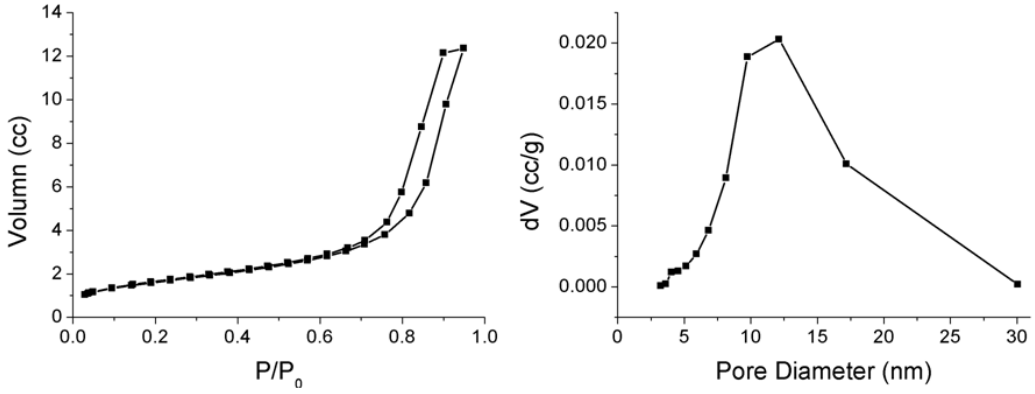

Figure S1. (Left) N2 adsorption-desorption isotherms of the PSiN. The hysteresis indicates the existence of the pore. **(Right)** Pore diameter calculated from the desorption isotherm using BJH method. The relatively wide pore size distribution is consistent with the pore forming mechanism, where re-nucleated Ag nanoparticles vary in size.

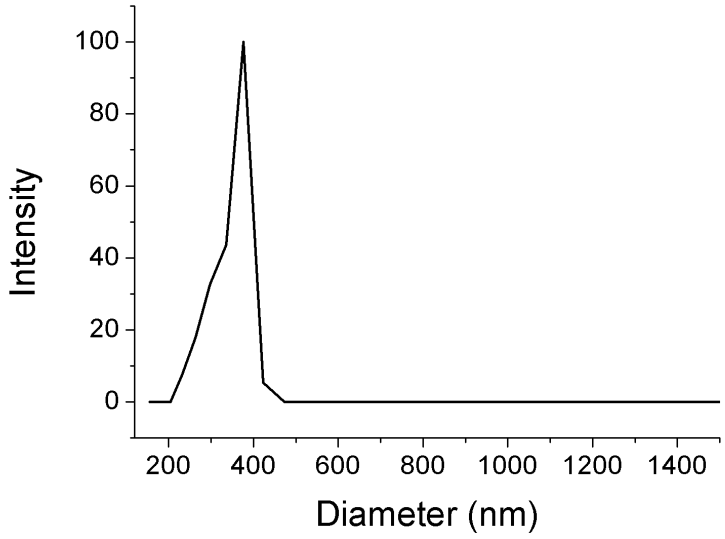

Figure S2. Dynamic light scattering result of PSiN in aqueous suspension. The non-symmetric distribution is due to the rod-like shape of the PSiN nanoparticles. The effective hydrodynamic size of the PSiN is calculated to be 320 nm,

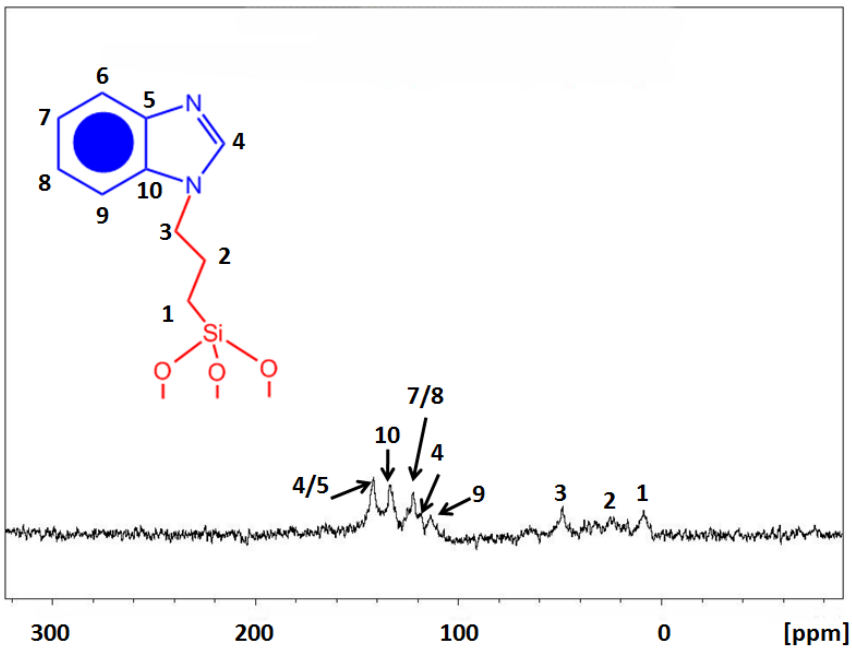

Figure S3. C-13 CPMAS NMR spectrum of nanovalve-modified PSiNPs. The peaks are assigned as indicated in the figure. During the synthesis, any absorbed or unreacted compounds will be removed from the PSiNPs' surface. This result proved the success of the modification.

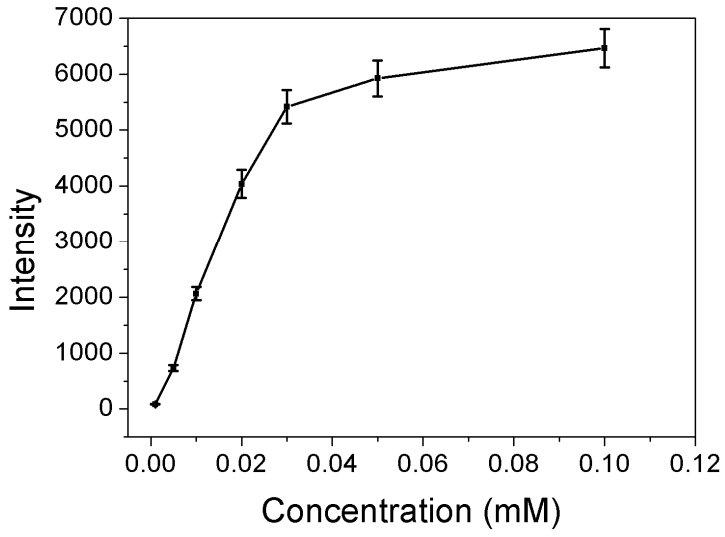

Figure S4. Fluorescence intensity as a function of Hoechst 33342 concentration. At low concentration, the relation between intensity and concentration is almost linear. In the controlled release experiment, the concentration of Hoechst dye released into the solution is lower than 0.01 mM.

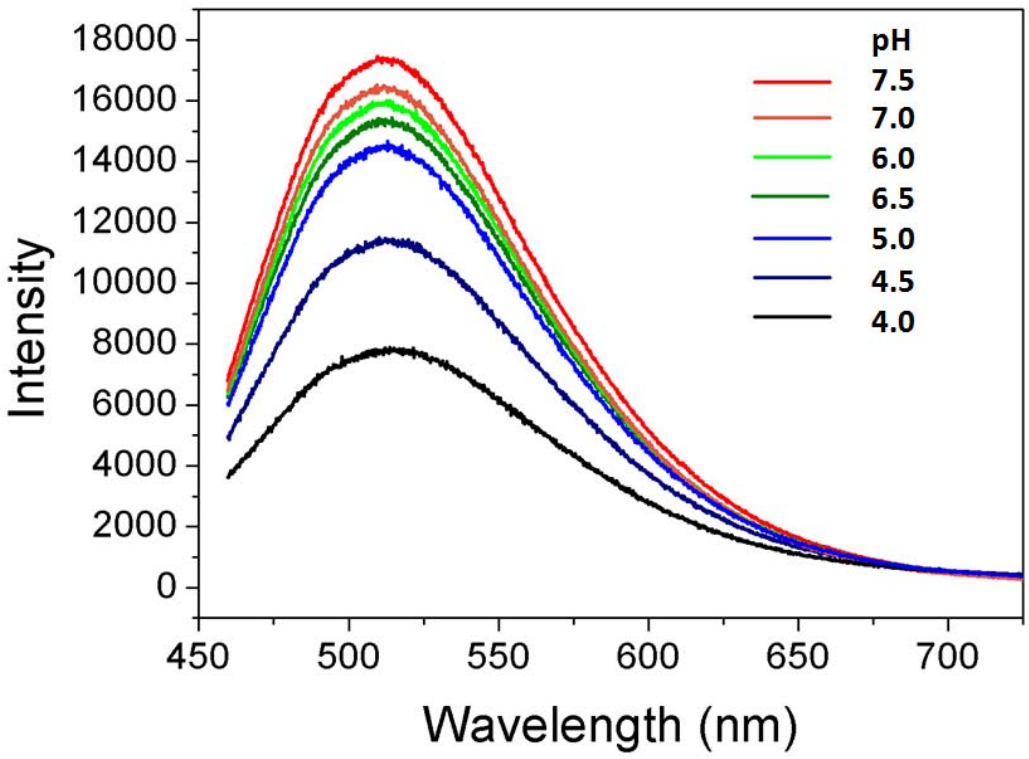

Figure S5. Fluorescence Spectra of a 0.01 mM Hoechst 33342 water solution under different pH. The pH value was adjusted by NaOH or HCl. The fluorescent intensity slightly drops from pH 7.5 - pH 5.0. In the controlled release experiments, the fluorescence intensity increased upon opening the nanovalve, which proved that the concentration of the Hoechst dye in the solution increased.

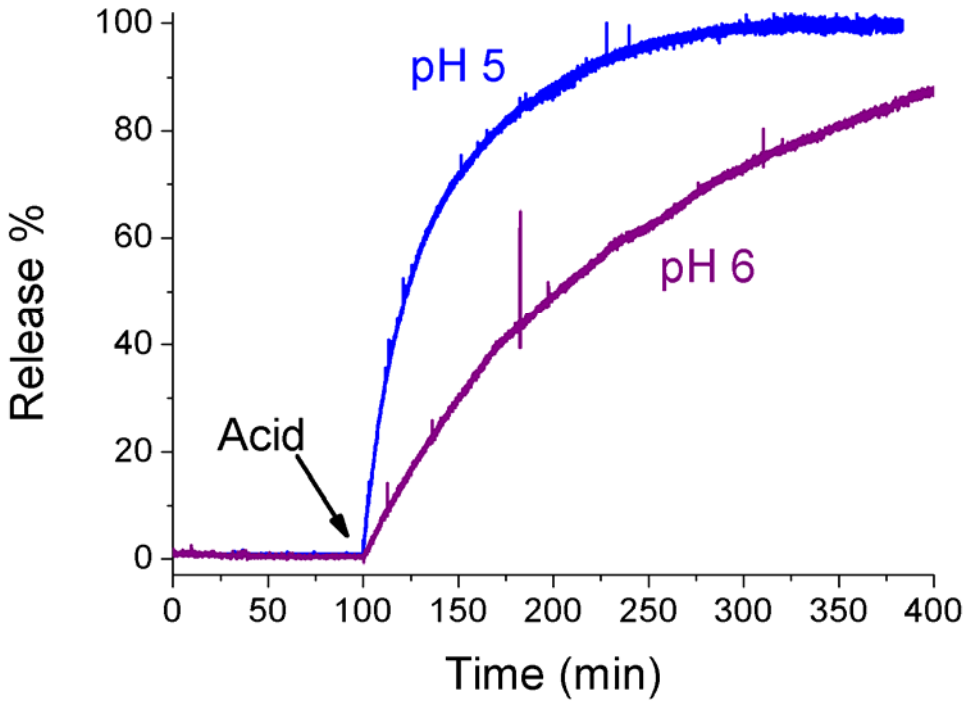

Figure S6. Plot of the release of Hoechst 33342 from PSiNPs in aqueous solution at different pH. We have observed that less acidity will lead to prolonged release course. This is, however, believed to be due to the interaction between the positively charged cargo and the negatively charged PSiNPs surface.