SUPPORTING INFORMATION

For

Hybrid Polymer/Porous Silicon Nanofibers for Loading and Sustained Release of Synthetic DNA-based responsive devices

Jonathan M. Zuidema,[#] Alessandro Bertucci,[#] Jinyoung Kang, Michael J. Sailor[*], and Francesco Ricci[*]

Experimental Details

Preparation of porous silicon nanoparticles (pSiNPs). The pSiNPs were prepared following the published "perforation etching" procedure. [1] Highly boron-doped p-type silicon wafers (≈1 mΩ cm resistivity, 100 mm diameter, Virginia Semiconductor, Inc.) were anodized in an electrolyte composed of 3:1 (*v:v*) 48% aqueous HF:ethanol. Etching waveforms were generated using Labview software (National Instruments, Inc.), and the electric current was driven by a Keithley 2651A Sourcemeter power supply interfaced to the LabView program. The etching waveform consisted of a square wave in which a lower current density of 46 mA cm^{-2} was applied for 1.818s, followed by a higher current density pulse of 365 mA cm^{-2} applied for 0.363 s.[1] This waveform was repeated for 150 cycles, generating a stratified pSi film with thin, high porosity "perforations" repeating approximately every 170 nm through the porous layer. The film was removed from the silicon substrate by application of a current density of 3.4 mA cm^{-2} for 150 s in a solution containing 1:20 (*v:v*) of 48% aqueous HF:ethanol. The freestanding film was fractured into nanoparticles of mean diameter (z-average, intensity based) 166 nm by immersion in DI H_2O (1 film/1 mL of DI water) and ultrasonication for ≈ 18 h. A 50T ultrasonic bath (VWR International) was used for ultrasonic fracture of the pSi films. The pSiNPs were centrifuged for 10 minutes at 15,000 RPM (Eppendorf 5424R) and washed 3 times with 100% ethanol. This protocol gives pSiNPs with pore sizes between 10-20 nm,[2] verified by electron microscopy.

Preparation of calcium-silicate coated DNA-loaded pSiNPs (DNA-pSiNPS) and DNA beacon-loaded pSiNPs (DNA beacon-pSiNPs). A stock solution of 4 M calcium chloride ($CaCl_2$) (M_W = 110.98, anhydrous, Spectrum Chemicals) was prepared in DNase-free water. The solution was centrifuged to remove any precipitates. For DNA loading, a 22-nt DNA sequence (5'-TCAACATCAGTCTGATAAGCTA-3') (Biosearch Technologies) was used to determine DNA release from pSiNPs and hybrid nanofibers, while FAM labeled DNA (5'-FAM-TCAACATCAGTCTGATAAGCTA-3') (Biosearch Technologies) was used for confocal microscope imaging. Lyophilized DNA was re-suspended in DNase-free water at a concentration of 100 μM. 100 μL of the 100 μM DNA solution was added to a pSiNP solution (1mg pSiNPs, 400 μL DNAse-free water). This solution was then mixed with 500 μL of 4 M $CaCl_2$, giving a final concentration of 10 μM DNA, 1 mg pSiNPs, and 2 M $CaCl_2$ in 1 mL of DNAse-free water. The solution was agitated for 60 min at room temperature, and then centrifuged for 10 minutes at 15,000 RPM. pSiNPs were washed 1x in DIH_2O, 1x in 50% EtOH: DIH_2O, and 1x in absolute ethanol. DNA-loading efficiency was determined by measuring the supernatants of each centrifugation step using a NanoDrop 2000 spectrometer (Thermo Science, ND-2000), and the drug loading[3] was determined to be 4.7 ± 0.4% of DNA by mass, calculated as the mass of DNA loaded divided by the total mass of the DNA-

loaded pSiNPs. For DNA beacon-loading, the DNA beacon (5'-Quasar 570-GCGCGTCAACATCAGTCTGATAAGCTACGCGC-Black Hole Quencher 2-3') (Biosearch Technologies) was re-suspended in DNAse-free water at a concentration of 100 μM. The loading procedure was then carried out using the protocol described above.

Fabrication of PLGA, PLA, and PCL Nanofibers containing pSiNPs. Polycaprolactone (PCL) (Sigma, 80,000 MN), poly-$_L$-lactic acid (PLA) (NatureWorks, Grade 6201D), and 75:25 poly($_{DL}$-lactide-*co*-glycolide) (PLGA) (Lactel, Inherent Viscosity Range: 0.55-0.75 dL/g in $CHCl_3$) were used to prepare hybrid polymer nanofibers. The three different polymers were dissolved in chloroform so they could be sprayed out of the airbrush. Because of differences in individual properties of PCL, PLA, and PLGA such as solution viscosity, the optimal percent composition of polymer needed to prepare nanofibers was different for each of these polymers. Thus we first optimized the percent composition of polymer in solution such that the spray nebulization process generated consistent nanofibers of similar diameters (in the range of 423-495 nm) for each of the polymer types used in this study. The percent compositions in chloroform were 3.8% by mass PCL, 7.4% by mass PLA, and 9.1% by mass PLGA. These solutions were prepared by dissolving 30 mg PCL in 750 mg $CHCl_3$, 30 mg PLA in 375 mg $CHCl_3$, and 30 mg PLGA in 300 mg $CHCl_3$. Three different ratios of polymer mass to nanoparticle mass were prepared for each polymer (total of 9 formulations). The DNA-loaded pSiNPs to be incorporated into the nanofiber scaffolds were first washed 1 time with absolute ethanol, then pelleted by centrifugation for 10 minutes at 15,000 RPM and then re-suspended in chloroform. The relevant mass of pSiNPs was then added to the polymer solution such that the nominal mass percent of pSiNPs in the final polymer composite would be the same across all the fiber groups. The percent composition of DNA-loaded pSiNPs in the polymers reported in the text was determined as follows: the 2.4% (*w/w*) pSiNP nanofiber formulations were prepared from solutions containing 30 mg of the indicated polymer (PCL, PLA, or PLGA) and 0.75 mg of the DNA-loaded pSiNPs, the 4.7% (*w/w*) pSiNP nanofiber formulation solutions contained 30 mg of the indicated polymer (PCL, PLA, or PLGA) and 1.5 mg of the DNA-loaded pSiNPs, and the 9.1% (*w/w*) pSiNP nanofiber formulation solutions contained 30 mg of the indicated polymer (PCL, PLA, or PLGA) and 3 mg of the DNA-loaded pSiNPs. The solutions were added to the hopper of an airbrush (Model G222, Master Airbrush), and nebulized under nitrogen gas at 25 psi. The nanofiber collection surface of interest was placed 20 cm from the airbrush nozzle.

Materials Characterization: Porosity of etched silicon was verified using the Spectroscopic Liquid Infiltration Method (SLIM) measurement.[4] TEM images were obtained with a JEOL-1200 EX II 120 kV instrument. Scanning electron microscope (SEM) images were obtained using a FEI XL30 field-emission SEM. The SEM images of the hybrid nanofibers were collected at a beam accelerating voltage of 2 kV. SEM images were used to determine the nanofiber diameter. For this measurement, 20 nanofibers in 4 different images (80 fibers total) were quantified using NIH ImageJ software (NIH). Hydrodynamic size and zeta potential of pSiNPs were measured by dynamic light scattering (DLS) using a Zetasizer ZS90 from Malvern Instruments. An Ocean Optics QE-Pro spectrometer was used to obtain steady-state photoluminescence spectra with a λ_{ex} = 365 nm LED excitation source (LLS-365, Ocean optics, USA) connected to a band pass filter (370 ±36 nm) and a 500 nm long-pass emission filter. For time-gated imaging of silicon nanoparticles,[5] the same LED source (λ_{ex}: 365 nm) was used but it was pulsed at a repetition rate of 10 Hz, externally synchronized and triggered by a function generator (Keithley3390 50 MHz arbitrary waveform generator). Time-resolved images were obtained with an intensified CCD camera (iSTAR 334T,

Andor Technology Ltd.) fitted with a Nikon AF micro lens (Nikko 105 mm). Andor Solis software was used to program delays and timing pulses, and to analyze images including signal-to-noise ratio (SNR). For time-gated imaging, a time delay of 5 μs was used and the signal was collected for 250 μs. This signal was corrected for background and then accumulated 50 times for 0.3 sec. SNR was calculated from the relationship:

$$SNR = \frac{Mean_{sig} - Mean_{bg}}{\sigma_{bg}}$$

where $Mean_{sig}$ is the mean integrated value of intensity measured in the ROI defined for the signal; $Mean_{bg}$ is the mean integrated value of intensity measured in the ROI defined for the background; and σ_{bg} is the standard deviation of the mean of the background signal. For confocal microscope images, a conventional confocal microscope (Zeiss LSM 710 NLO, 40x oil immersion) was used to visualize the luminescence signals from the DNA-pSiNPs in the hybrid nanofibers. PCL hybrid nanofibers containing 2.5% DNA-pSiNPs were fixed between a glass slide and cover glass using transparent mounting medium. The FAM signal from FAM-labeled DNA and the photoluminescence signal from pSiNPs were obtained at the same position and merged into a single frame with the bright field image.

DNA Release: DNA-loaded pSiNPs were suspended in PBS (pH 7.4) at a starting concentration of 0.5 mg mL^{-1} (0.1 mg pSiNPs in 0.2 mL PBS) and incubated at 37 °C. PBS solutions were collected following centrifugation, and the remaining pSiNPs were re-suspended in 0.2 mL of PBS at 1, 2, 4, 8, and 24 h. DNA release was determined by measuring DNA concentration in the supernatants at each centrifugation step using a NanoDrop 2000 spectrometer (Thermo Science, ND-2000). Following 24 h of release, 4.46 ± 0.303 μg was released from 0.1 mg of pSiNPs. DNA release from DNA-pSiNP containing hybrid nanofibers was measured as follows: 5 mg hybrid nanofibers (which contained 6.125 μg total loaded DNA, 12.25 μg total loaded DNA, and 24.5 μg total loaded DNA, for the 2.4%, 5% and 9% pSiNPs, respectively, calculated from amount of DNA loaded into the DNA-pSiNPs) were suspended in 0.5 mL PBS at 37 °C and PBS solutions were collected and replaced every 48 h for the first 6 days (days 2, 4, 6). In order to keep DNA release in the detection range of the NanoDrop 2000, the PBS volume was then changed to 0.1 mL PBS for the remainder of the release study. To calculate the rate of DNA release, the total amount of DNA released from each hybrid nanofiber scaffold was set to a value of 1. Prior release data points were then normalized accordingly, and this was plotted over time.

DNA Beacon Fluorescence Measurements:

Fluorescence emission spectra were acquired on a Horiba Fluoromax-4 spectrophotometer equipped with a 450 W Xenon arc lamp as excitation source. The excitation wavelength, λ_{ex}, was set to 545 nm ($slit_{ex}$ = 5 nm), the acquisition window was set to a value between λ = 555 nm and λ = 650 nm ($slit_{em}$ = 5 nm), and a quartz cuvette of reduced volume (100 μL) was used to contain the samples. All measurements were carried out at 37 °C. Binding calibration curves of the fluorescence emission spectra were obtained by dilution of the anti-miR-21 DNA beacon (10 nM) into increasing concentrations of stock microRNA-21 in the range of 0.1–5000 nM. The maximum fluorescence intensity at λ = 562 nm was used to plot the binding curve.

DNA Beacon-loaded pSiNPs were suspended in PBS (pH 7.4) at a starting concentration of 0.1 mg mL^{-1} (0.1 mg pSiNPs in 1 mL PBS) and incubated at 37 °C for 1, 2, 4, and 8 h. At each indicated time point, the supernatant was collected following centrifugation and assayed, and the remaining pSiNPs were re-suspended in 1 mL of PBS. Fluorescence emission of the DNA beacon released

from the DNA-loaded pSiNPs was first acquired and used as reference background. Next, miR-140 (5'-CAGUGGUUUUACCCUAUGGUAG-3') or miR-21 (5'- UAGCUUAUCAGACUGAUGUUGA-3') (Biosearch Technologies) were added to the supernatant at a concentration ≈10 times higher than the calculated concentration of the DNA beacon in the supernatant to ensure full hybridization. Fluorescence emission was then recorded and reported as % signal gain. DNA release from hybrid nanofibers was similarly calculated, using concentrations of released DNA beacon at days 2, 10, and 20. PLGA, PLA, and PCL nanofiber scaffolds all contained the DNA beacon-loaded pSiNPs at a mass percentage of 2.4%. For the PLGA formulation, 0.5 mg of the PLGA nanofibers containing 2.4% by mass of the DNA beacon-pSiNPs were suspended in 3 mL of PBS and the supernatant was removed after every 2 days of incubation at 37 °C. Fluorescence emission from the supernatant was first acquired, then measured again in the presence of either 5 μM miR-140 or 5 μM miR-21 to ensure quantitative hybridization with miR-21. For the PLA formulation, 2 mg of the PLA nanofibers containing 2.4% by mass of the DNA beacon-pSiNPs were suspended in 800 μL of PBS and the supernatant was removed and replaced after every 2 days of incubation at 37 °C. At day 10, fluorescence from the supernatant was first acquired, and then measured again in the presence of either 5 μM miR-140 or 5 μM miR-21. For the PCL formulation, 5 mg of PCL nanofibers containing 2.4% by mass of the DNA beacon-pSiNPs were suspended in 600 μL of PBS and the supernatant was removed and replaced after every 2 days of incubation at 37 °C. At day 20, fluorescence from the supernatant was first measured, and then measured again in the presence of either 5 μM miR-140 or 5 μM miR-21.

Supporting Figures

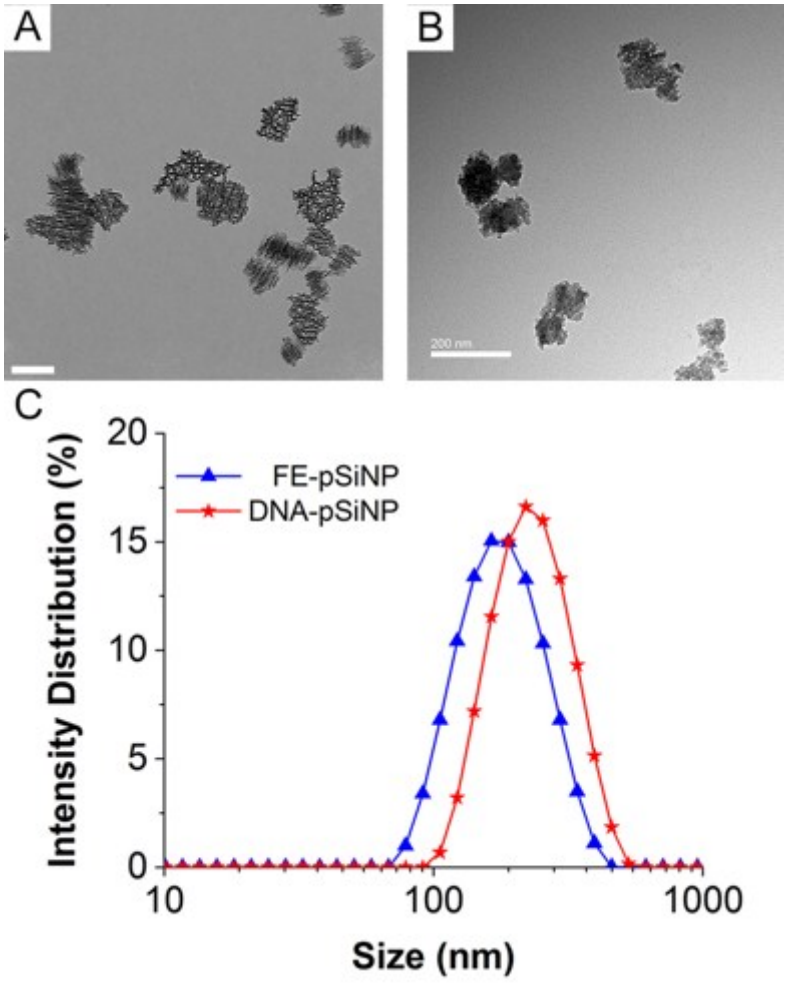

Figure S1. TEM images of freshly etched (FE) pSiNPs (A) and DNA-loaded pSiNPs (B). Intensity-weighted size distribution (from DLS) of FE-pSiNPs and DNA-loaded pSiNPS (C). Scale = 200 nm for (A) and (B).

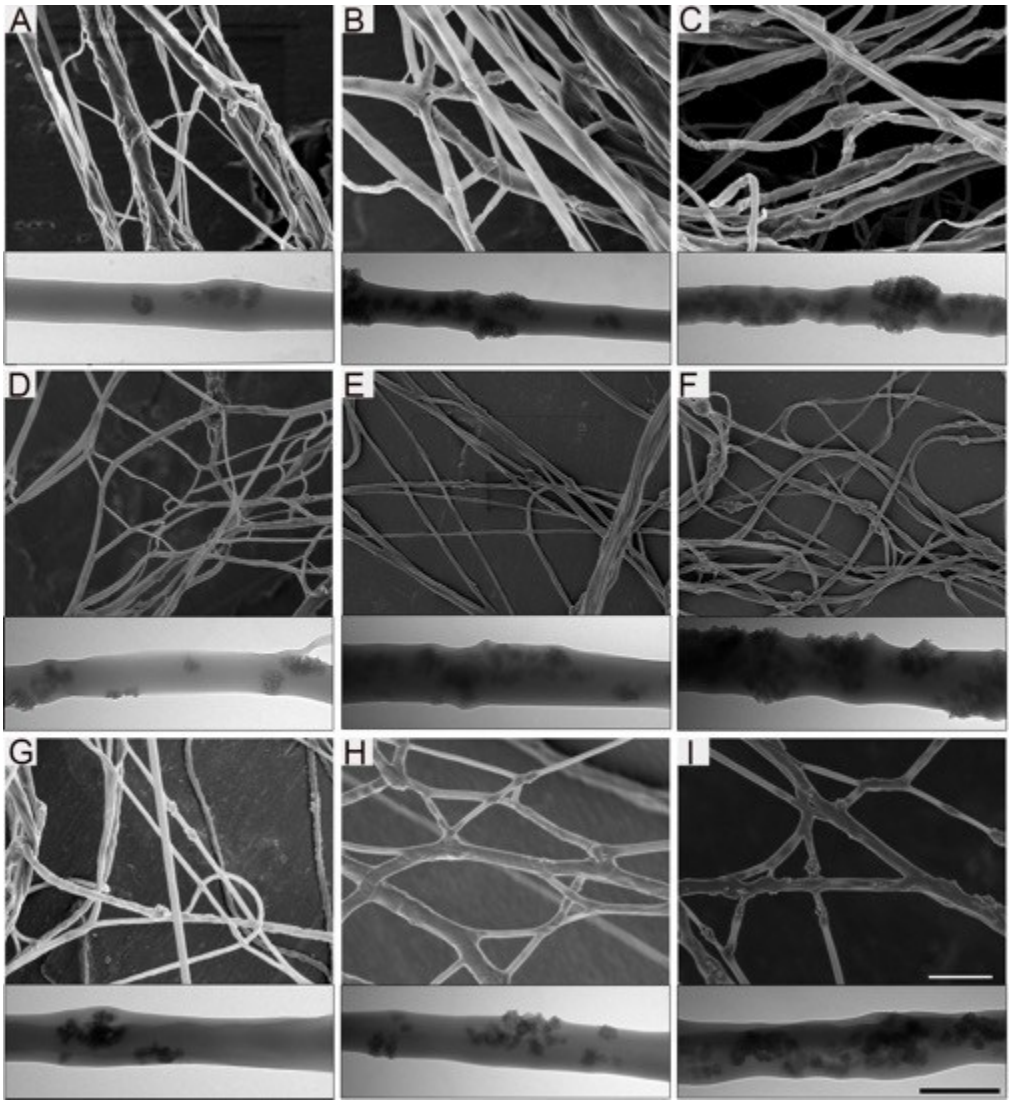

Figure S2. SEM images of several PLGA nanofibers showing the overall fiber morphology (top) and TEM images of a single PLGA nanofiber showing the pSiNPs embedded within the fiber (bottom): PLGA nanofibers prepared containing 2.4% (A), 4.7% (B) and 9.1% (C) by mass of the DNA-loaded pSiNPs; PLA nanofibers prepared containing 2.4% (D), 4.7% (E) and 9.1% (F) by mass of the DNA-pSiNPs; PCL nanofibers prepared containing 2.4% (G), 4.7% (H) and 9.1% (I) by mass of the DNA-pSiNPs. SEM Scale = 2 μm and TEM Scale = 500 nm.

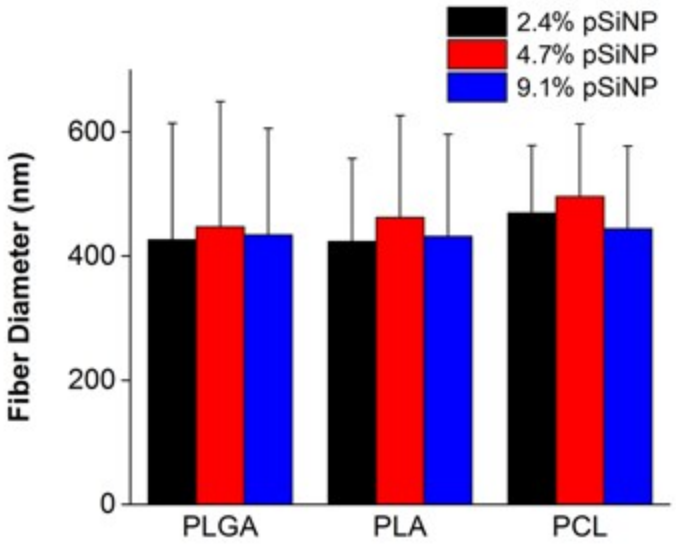

Figure S3. Mean and standard deviation of the nine different hybrid nanofiber scaffolds studied in this work. The designations 2.4% pSiNP, 4.7% pSiNP, and 9.1% pSiNP indicate polymer that contained 2.4%, 4.7%, and 9.1% by mass of the DNA-loaded pSiNPs, respectively. PLGA nanofiber mean and standard deviation were: 2.4% pSiNP = 426 ± 188 nm; 4.7% pSiNP = 447 ± 201 nm; and 9.1% pSiNP = 434 ± 171 nm. PLA nanofiber mean and stadard deviation were: 2.4% pSiNP = 423 ± 133 nm; 4.7% pSiNP = 462 ± 163 nm; and 9.1% pSiNP = 431 ± 164 nm. PCL nanofiber mean and stadard deviation were: 2.4% pSiNP = 469 ± 108 nm; 4.7% pSiNP = 495 ± 117 nm; and 9.1% pSiNP = 444 ± 133 nm.

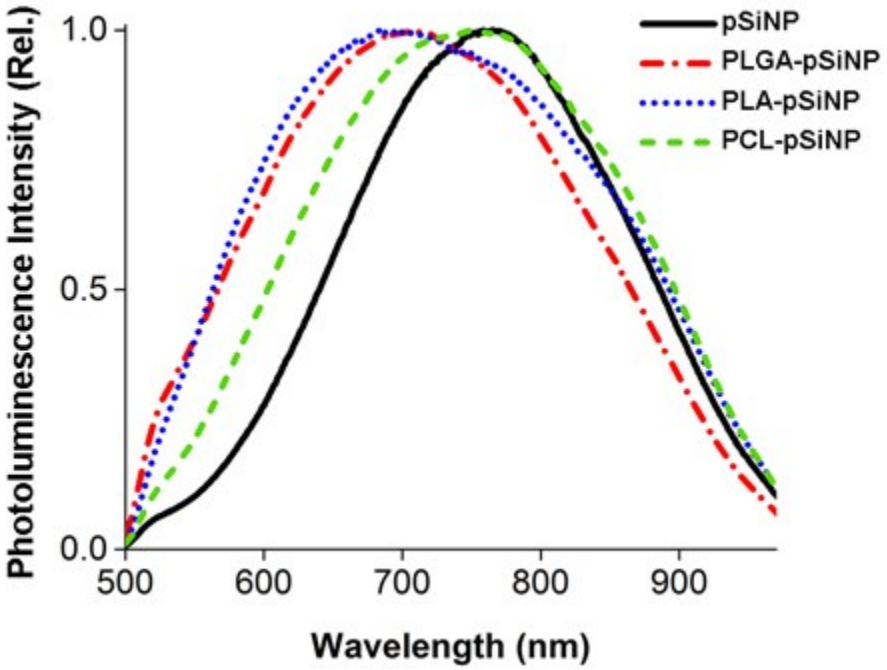

Figure S4. Photoluminescence emission spectra (λ_{ex} = 365 nm) of dry DNA-loaded pSiNPs (designated pSiNP) and PLGA, PLA, or PCL polymers that contained 2.4% by mass of the DNA-loaded pSiNPs (designated PLGA-pSiNP, PLA-pSiNP, or PCL-pSiNP). Maximum photoluminescence intensities were normalized to 1 for each polymer-pSiNP formulation. A blue shift is observed in the wavelength of maximum intensity when DNA-pSiNPs are embedded into PLA and PLGA. Additionally, the emission spectra show substantial broadening of the band when DNA-loaded pSiNPs are emedded in the nanofibers. Photoluminescence spectra of the pristine polymers, obtained under the same conditions, are shown in Fig. S5, and they have a negligible contribution to the emission spectra shown here. The DNA used in these experiments contained no fluorescent dye labels. Thus the observed photoluminescence in these samples is attributed to the intrinsic photoluminescence from the pSiNPs.

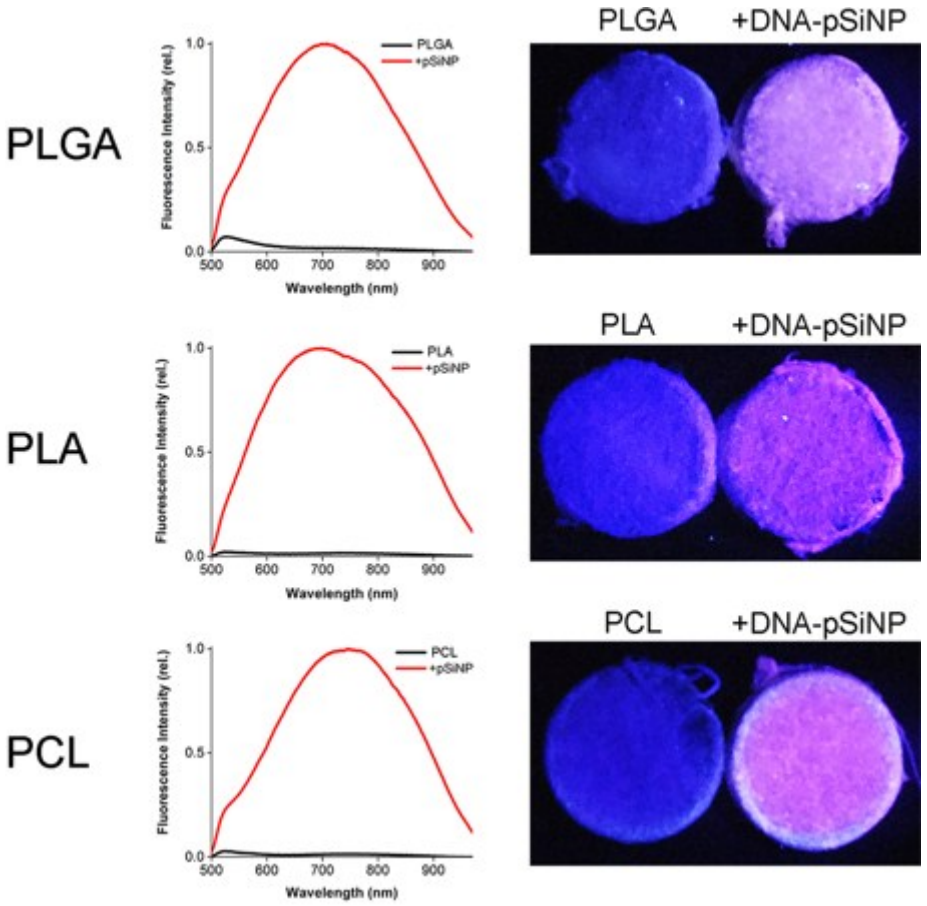

Figure S5. Photoluminescence emission spectra (λ_{ex} = 365 nm) of pristine PLGA, PLA, and PCL nanofibers (containing no incorporated pSiNPs), compared to nanofibers embedded with 2.4% by mass of the DNA-loaded pSiNPs (designated +pSiNP in each plot). The photoluminescence spectrum of each of the pure polymers is presented with a y-axis scaling equivalent to its respective polymer-pSiNP formulation**.** Photographs corresponding to the relevant samples are shown to the right of each plot. These images were obtained under 365 nm LED illumination, and they show the characteristic red emission of the pSiNPs in the pSiNP-doped nanofibers (sample on the right of each image).

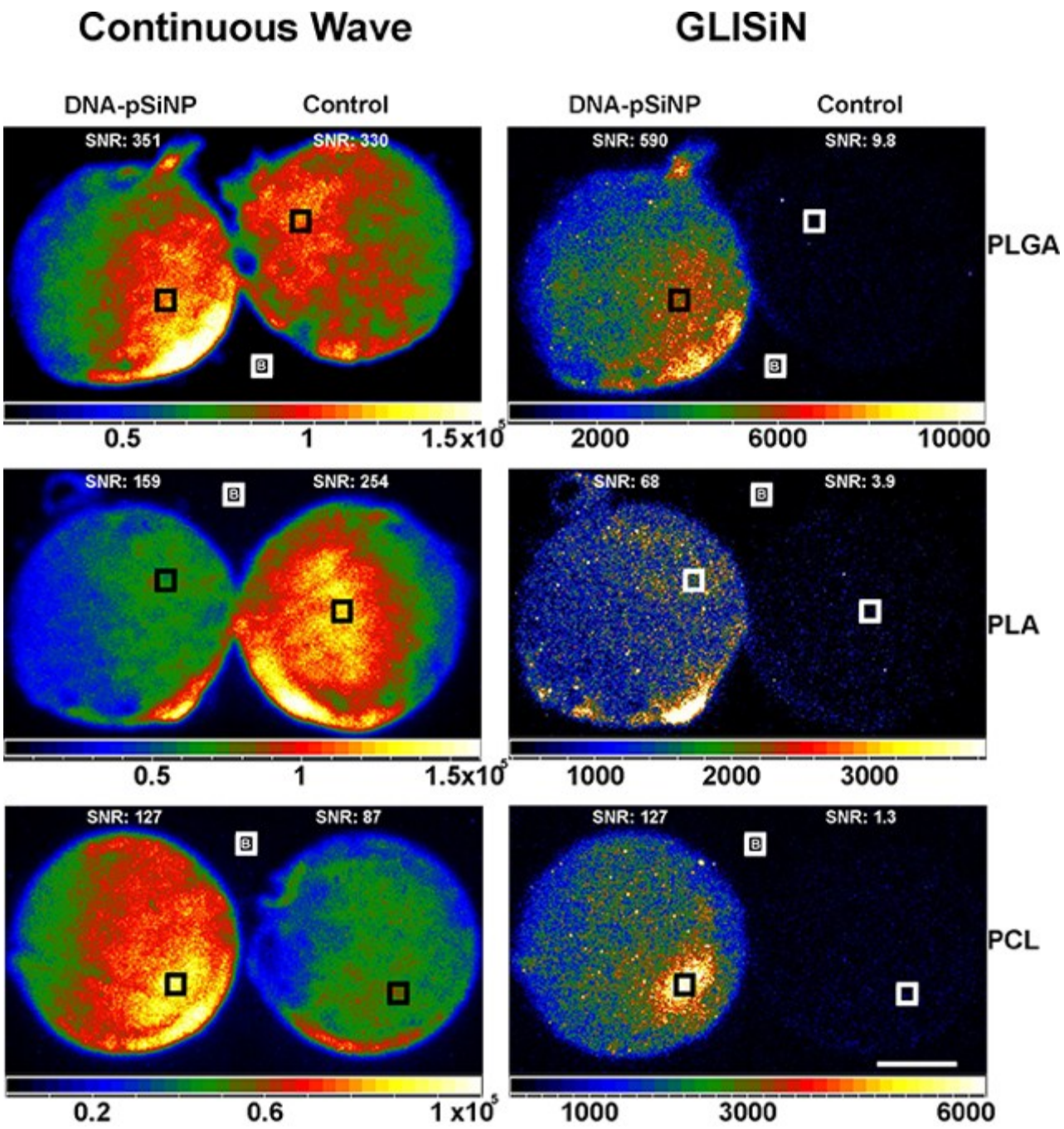

Figure S6. Gated luminescence imaging of silicon nanoparticles (GLISiN) compared with continuous wave images of both 2.4% DNA-pSiNP nanofibers and control nanofibers. Boxes show the regions of interest (ROI) that were used for data analysis. The background ROI is depicted with a B. Average signal to noise ratios (SNR) under continuous-wave imaging (no time gate) for control PLGA, PLA, and PCL nanofibers were 376 ± 58, 270 ± 14, and 78 ± 8 respectively. Polymer nanofibers containing 2.4% DNA-pSiNPs had SNRs of 365 ± 55, 161 ± 5, and 138 ± 20 for PLGA, PLA, and PCL, respectively. Using a gate time of 5 μs, control PLGA, PLA, and PCL nanofibers had SNRs of 10 ± 2, 5 ± 2.5, and 1.5 ± 0.3, respectively. Polymer nanofibers containing 2.4% DNA-pSiNPs had SNRs of 641 ± 44, 66 ± 3, and 186 ± 108 for PLGA, PLA, and PCL, respectively.

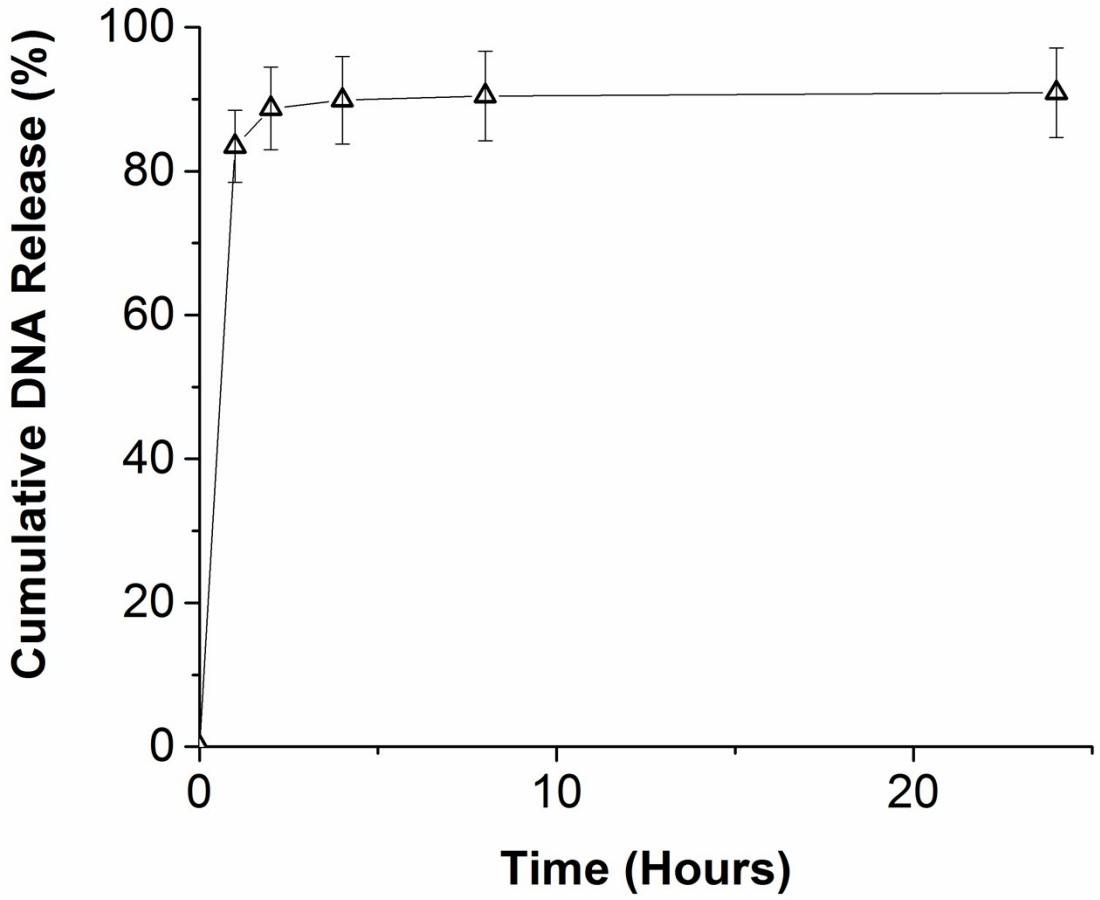

Figure S7. Percent of DNA released from DNA-loaded pSiNPs (PBS, 37° C). Eluent buffer (PBS) was sampled at 1, 2, 4, 8, and 24 hours.

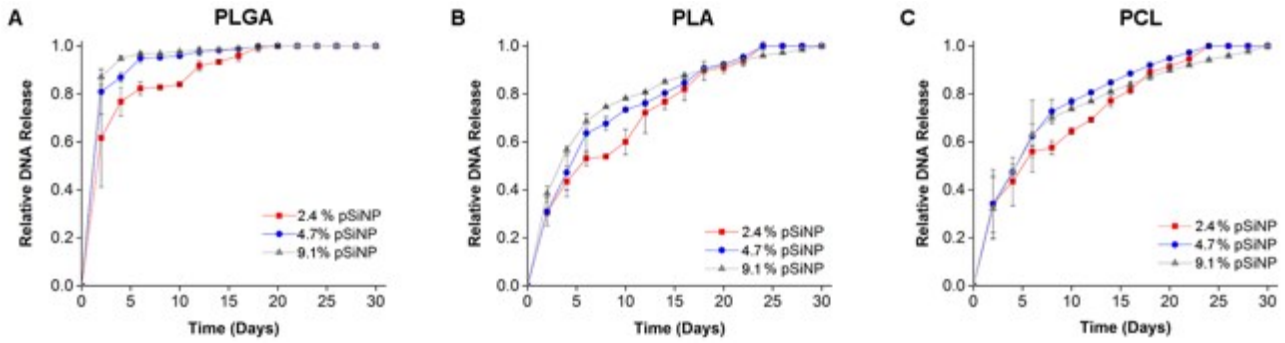

Figure S8. Comparison of the effect of the quantity of nanoparticles (percentage by mass) loaded into a given polymer formulation on the temporal release of DNA. Percentages given in the legends correspond to the percent by mass of DNA-loaded pSiNPs contained in the polymer nanofibers fabricated from PLGA (A), PLA (B), and PCL (C). The total amount of DNA released from a given formulation in 30 days was set to a value of 1, and data for each polymer are normalized accordingly. The data show that for a given polymer, the mass percent of pSiNPs (loaded with DNA) in that polymer did not exert a substantial influence on the temporal release of DNA. DNA used in these eperiments was 22 nucleotides, single-stranded. Error bars on each trace represent independent measurements from three polymer nanofiber preparations.

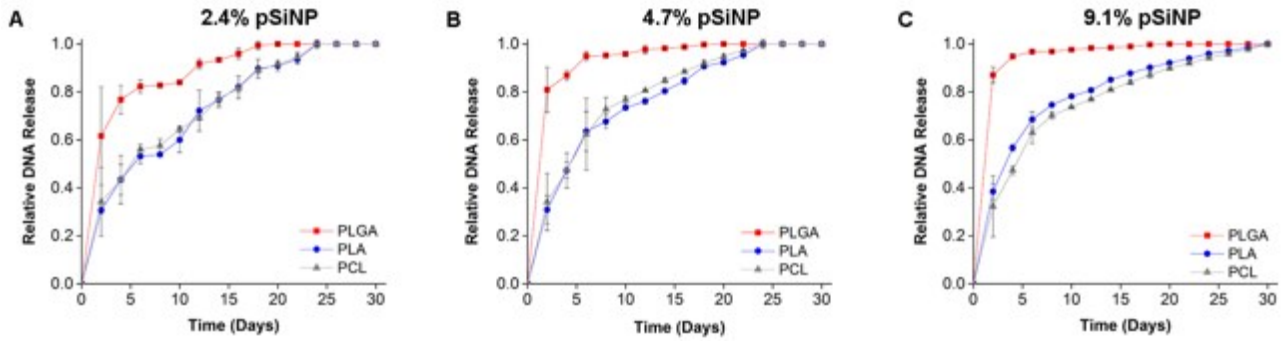

Figure S9. Comparison of the effect of the polymer type on the temporal release profile of DNA from the polymer formulations. Each plot compares the three polymers, with the mass percentage of pSiNPs (loaded with equivalent quantities of DNA) held constant at 2.4% (A), 4.7% (B), and 9.1% (C). The total amount of DNA released from a given formulation in 30 days was set to a value of 1, and data for each mass percentage of pSiNPs are normalized accordingly. PLGA shows a faster rate of DNA release compared with either PLA or PCL, regardless of the conentration of DNA-pSiNPS incorporated. PCL and PLA show similar DNA release rates for all three concentrations.

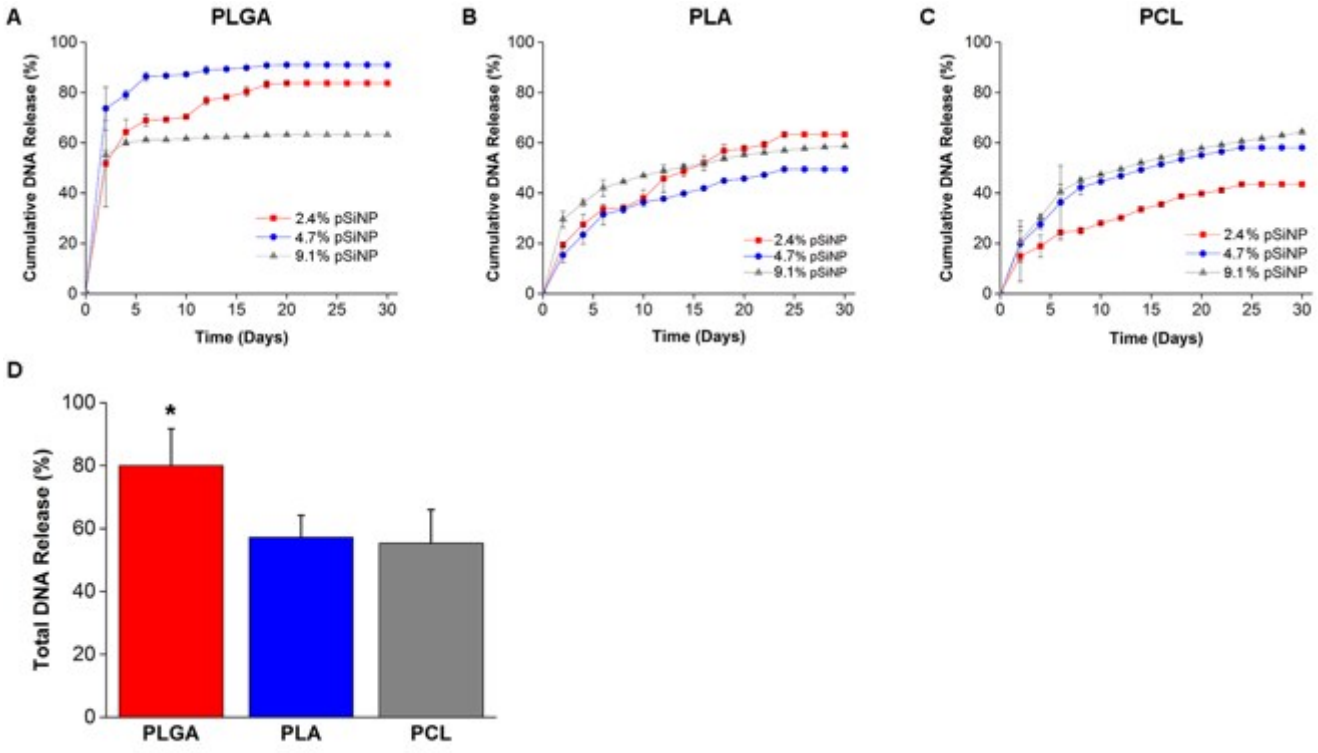

Figure S10. Cumulative percent DNA-release from DNA-pSiNP loaded PLGA (A), PLA (B) and PCL (C) nanofibers. The average cumulative percent of DNA released from all PLGA scaffolds is higher than that of both PLA and PCL nanofibers (D). * = $p<0.05$ compared to both PLA and PCL DNA-pSiNP nanofibers.

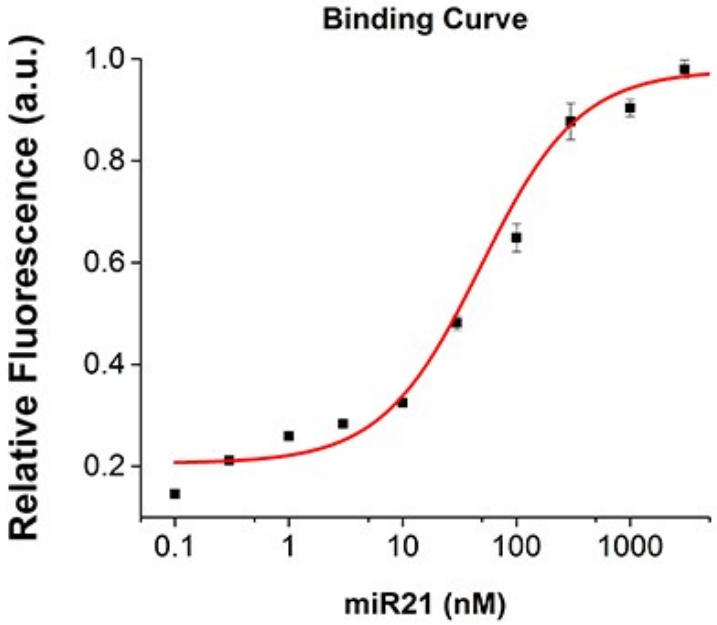

Figure S11. Binding curve of the DNA beacon for its target miR-21. Relative fluorescence emission (λ=562 nm) of the DNA beacon (10 nM) is plotted as a function of increasing concentration in solution of miR-21 (0.1 – 5000 nM). A Langmuir fit was applied to generate the final curve.

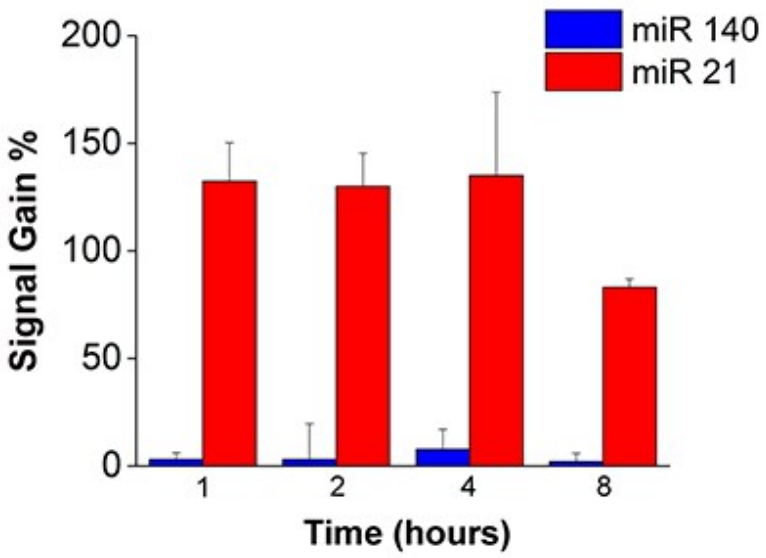

Figure S12. Hybridization-induced DNA beacon fluorescence signal increase in the presence of target miR-21. PBS was sampled and replaced at 1, 2, 4, and 8 hours. The fluorescence signal from the supernatant was first aquired as background, and then a concentration of either miR-140 (blue) or miR-21 (red) that was 10x greater than the calculated amount of DNA beacon in solution was added to the supernatant. The fluorescence signal was analyzed and the signal gain % was determined.

Supporting References

1. Qin, Z.; Joo, J.; Gu, L.; Sailor, M. J. *Part. Part. Syst. Charact.* **2014,** 31, (2), 252–256.
2. Zuidema, J. M.; Kumeria, T.; Kim, D.; Kang, J.; Wang, J.; Hollett, G.; Zhang, X.; Roberts, D. S.; Chan, N.; Dowling, C.; Blanco-Suarez, E.; Allen, N. J.; Tuszynski, M. H.; Sailor, M. J. *Advanced Materials* **2018,** 30, (12).
3. Judefeind, A.; de Villiers, M. M., *Drug Loading into and in vitro release from nanosized drug delivery systems*. Springer Science & Business Media: New York, NY, 2008.
4. Segal, E.; Perelman, L. A.; Cunin, F.; Di Renzo, F.; Devoisselle, J. M.; Li, Y. Y.; Sailor, M. J. *Adv Funct Mater* **2007,** 17, (7), 1153-1162.
5. Joo, J.; Liu, X.; Kotamraju, V. R.; Ruoslahti, E.; Nam, Y.; Sailor, M. J. *ACS Nano* **2015,** 9, (6), 6233–6241.