Supporting Information

A Mild Two-Step Method to Construct DNA-Conjugated Silicon Nanoparticles: Scaffolds for the Detection of MicroRNA-21

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1. General experimental

All chemicals, unless specifically mentioned, were obtained from Sigma-Aldrich or Acros Organics. Solvents were purchased from Fischer Scientific. Silicon wafers (undoped, mirror finished, 111 orientation) were purchased from Silrec Corp. (Lexington, KY). Tetramethylsilane (TMS; 99.9%) and chloroform-d_1 ($CDCl_3$; 99.96%) were purchased from Cambridge Isotope Laboratories, Inc. 30k Amicon centrifugal filter was purchased from Millipore Corporation. ^{1}H NMR spectra of SiNPs in $CDCl_3$ were recorded using Bruker 300MHz spectrometer. MALDI-TOF spectra were recorded on a Bruker Autoflex 3 matrix assisted laser desorption ionization time-of-flight mass spectrometer (MALDI-TOF MS). The matrices used were 2',4',6'-trihydroxyacetophenone monohydrate (THAP) for oligodeoxyribonucleotide (ODNs). UV−Vis spectra were obtained using a Varian Cary 50 Bio UV-Visible spectrophotometer. Fluorescence spectra were recorded on a Varian Cary Eclipse Fluorescence spectrophotometer. FTIR spectra of SiNPs were recorded using a Thermo Nicolet NEXUS 870 FTIR E.S.P spectrometer. Quantification of ODN concentration was carried out in a quartz cuvette using H_2O as a solvent. UV absorption at 260 nm was used to calculate ODN concentration based on Beer-Lambert law ($\boldsymbol{A = \varepsilon \cdot c \cdot L}$), in which the molar absorption coefficient was obtained from a Oligonucleotide Properties Calculator tool by Northwestern

University based on nearest neighbor coefficient for a pair of bases.

PAGE Studies.

Non-denaturing polyacrylamide gel (15%) electrophoresis (PAGE) studies were performed using a BioRad mini protean tetra cell that is equipped with BioRad PowerPac HC. For the gel shown in Figure 1b (main text), in Lane 1, 2×10^{-4} μmole of free ODN was introduced, and in Lane 2, 2×10^{-4} μmole of conjugated ODN (i.e., ODN linked to the SiNP) was used. The loading buffer was 1× TBE. The running buffer was 1× TBE buffer at 110 V, and the gel was run for 50 min. The gel was subsequently imaged on a Sony DSC-WX1 digital camera upon excitation with a hand held UV-lamp (λ_{exc} = 254 nm).

Microscopy Protocols.

(a). TEM experiments were performed on a FEI Tecnai G2 F30 Twin Transmission Electron Microscope Instrument (accelerating voltage = 120 kV). Sample was prepared on 200 mesh copper grids with lacey carbon film (purchased from Electron Microscopy Science). A 10 μL aqueous solution of 1μM SiNP ODN conjugate (in terms of ODN concentration) or a 10 μL CH_2Cl_2 solution of 50 nM SiNP was introduced to the copper grid followed by evaporation in air.

(b). AFM Experiments were carried out on a Veeco Bioscope AFM (Digital Instruments) under tapping mode in air. Bruker OTESPA AFM probes with nominal frequency, tip diameter, and spring constants of 300 kHz, 7 nm, and 42 N/m, respectively, were used. Mica (highest grade V1 Mica disc, 10 mm diameter, was purchased from TED PELLA, Inc.) was used as the substrate and the mica plate was freshly exfoliated using Scotch tape to achieve a flat surface before use. The samples (20 μL aqueous solution of 1 μM SiNP ODN conjugate (in terms of ODN concentration) or 20 μL CH_2Cl_2 solution of 50 nM SiNP) were applied on the mica substrate for 10 min, then dried with nitrogen gas flow before imaging.

HPLC Conditions.

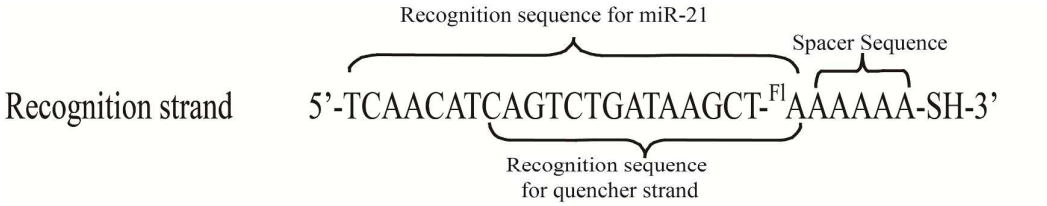
RP-HPLC purification was carried out using a Varian Prostar HPLC system, equipped with an Agilent 100 Å 5 μm PLRP-S reverse phase column. The column was maintained at 65 °C for all runs. The flow rate was set at 1 mL/min. A gradient composed of two solvents (Solvent A is 0.1 M TEAA in 5% acetonitrile and solvent B is 100% acetonitrile) was used.

Time (min)	Flow (mL/min)	%A	%B
0.00	1.00	100	0
2.50	1.00	100	0
7.50	1.00	88	12
12.50	1.00	80	20
32.50	1.00	50	50
40.00	1.00	0	100

Table SI-1. HPLC eluent gradient used for the purification of ODNs.

MicroRNA-21 Sensing Experiments.

Recognition sequence (i.e., the ODN conjugated to the SiNP), quencher strand, and miR-155 were synthesized by the Keck Foundation Biotechnology Resource Laboratory at Yale University using standard automated solid phase synthesis. miR-21 was obtained from Integrated DNA Technologies, Inc. The sequences of the ODNs used in this study are listed below:



Quencher strand 3'-GTCAGACTATTCGAT-Dabcyl-5'

Target miR-21 3'-AGUUGUAGUCAGACUAUUCGAU-5'

Control miR-155 3'-UGGGGAUAGUGCUAAUCGUAAUU-5'

Stage 1 (Preparation of OFF State). For the titration experiment, the quencher sequence was added to a 500 nM (concentration in terms of conjugated ODN) SiNP ODN conjugate solution in 500 μL phosphate buffered saline (PBS; 137 mM NaCl, 2.7 mM KCl, pH 7.4) at 25 °C. Specifically, 0.5 μL of a stock solution containing the quencher strand (in PBS) was titrated sequentially into the SiNP ODN solution such that each aliquot addition increased the final concentration of the quencher strand (in the SiNP ODN solution) by 50 nM increments. After each addition, the solution was allowed to incubate for 30 min before measuring the fluorescence. Upon completion of thirteen additions, the final quencher strand concentration reached was 650 nM.

Stage 2 (Detection of miR-21 via an ON State). All solutions contained the same PBS buffer. First, 1 equiv. of the quencher strand was added to the SiNP ODN conjugate solution and let to incubate for 30 min (total volume 500 μL). Next, a stock solution of miR-21 (or miR-155) was titrated into the SiNP ODN conjugate/quencher strand solution. A total of 15 μL miR-21 solution was added and the final concentration was 4.45 μM (that is 8.9 equiv. of SiNP ODN conjugate). Note: 5 equivalents of miR-21 were required to completely displace the quencher strand from the SiNP ODN conjugate.

Control Experiment

All solutions contained the same PBS buffer. To confirm that the above mentioned

experiments were operating under equilibrium conditions and that the quencher strand was fully displaced by miR-21 (after 5 equiv. of miR-21 was added in the Stage 2 titration, above), a control experiment was conducted. Here, 5 equiv. of miR-21 was first incubated with the SiNP ODN conjugate (total volume was 500 μL), followed by addition of 1 equiv. (0.5 μL) quencher strand. As can be observed in Figure SI-1, the maximum emission intensity is close to what is observed when the Stage 2 titration shown in Figure 3C (of the main text) reaches completion.

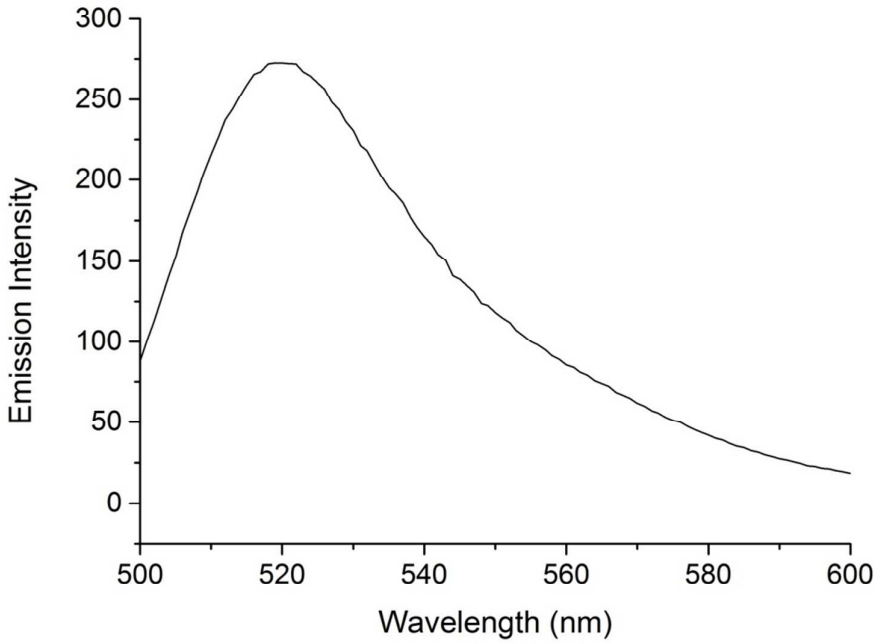


Figure SI-1. Emission spectrum after adding 5 equiv. miR-21 to the SiNP ODN conjugate followed by the addition of 1 the equiv. quencher strand.

2. Synthesis and characterization of alkene terminated SiNPs

Synthesis of Alkene Terminated SiNPs

Silicon wafers (1.0 g), 1,7-octadiene (12.1 mL; 0.08 mole) and 1-hexene (15.4 mL; 0.12 mole) were added into a stainless steel ball milling canister with 2 stainless steel

milling balls (1.2 cm in diameter, 8.1g/each) in a nitrogen filled glove box. The mixture was subjected to reactive high energy ball milling (RHEBM) using a SPEX CertiPrep 8000D Mixer/Mill for 24 hours in a cold room at 13°C. After RHEBM was complete, the SiNPs were purified by centrifugation at 3800 RPM for 30 min to remove insoluble sediments. The solution was then rotary-evaporated and pumped for 24 hours to completely remove unreacted 1,7-octadiene and 1-hexene.

TEM Image and Model of SiNP

The size of the SiNP core was analyzed by TEM. Figure SI-2 shows a typical TEM image of the SiNPs. From the size distribution (see, SI-2 inset) obtained from Image-J software (http://imagej.nih.gov/ij/), mean particle size was estimated to be 3.0 ± 1.4 nm. Thus, we chose to use 3.0 nm as the average diameter to further characterize the alkene grafted SiNPs (*vide infra*).

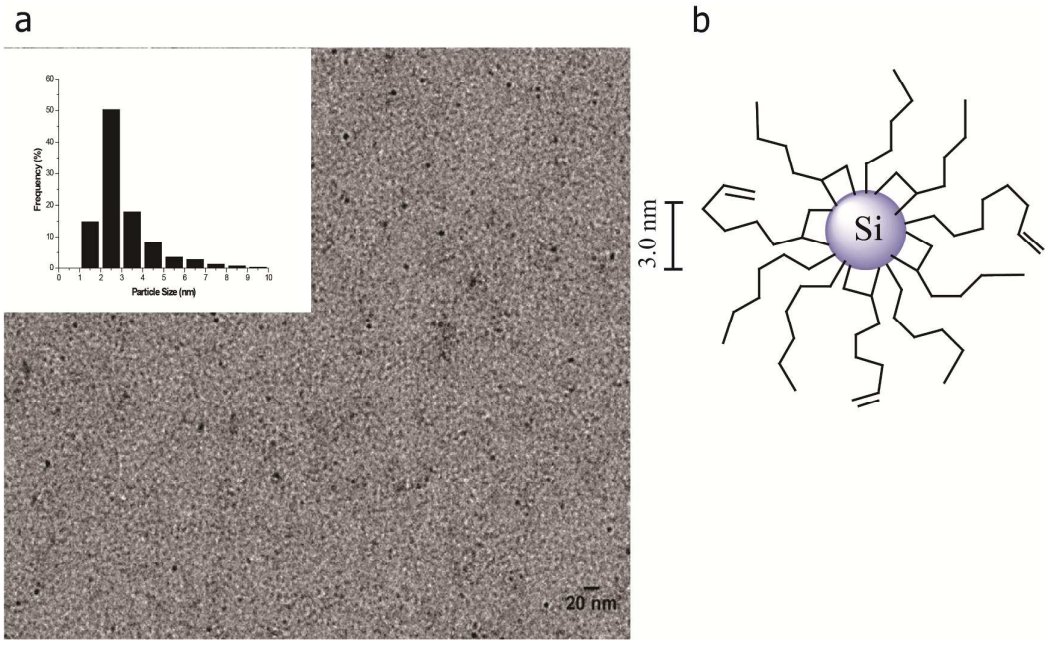
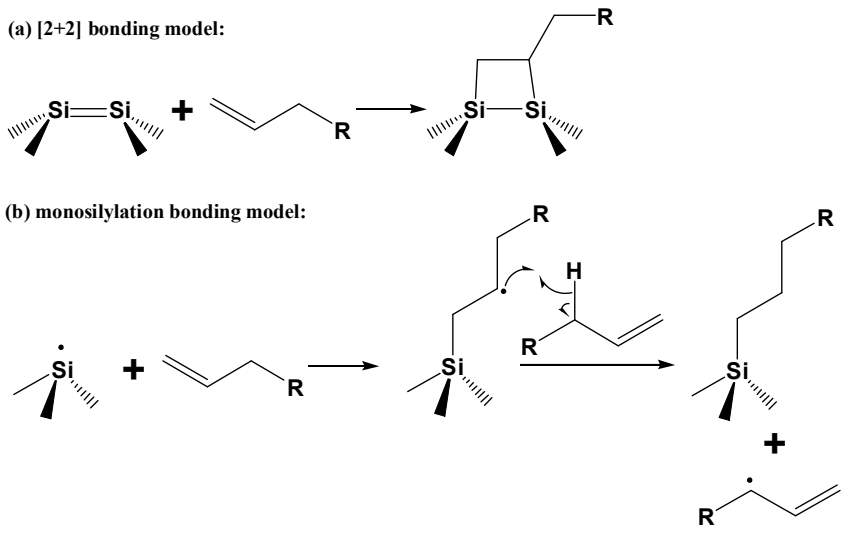


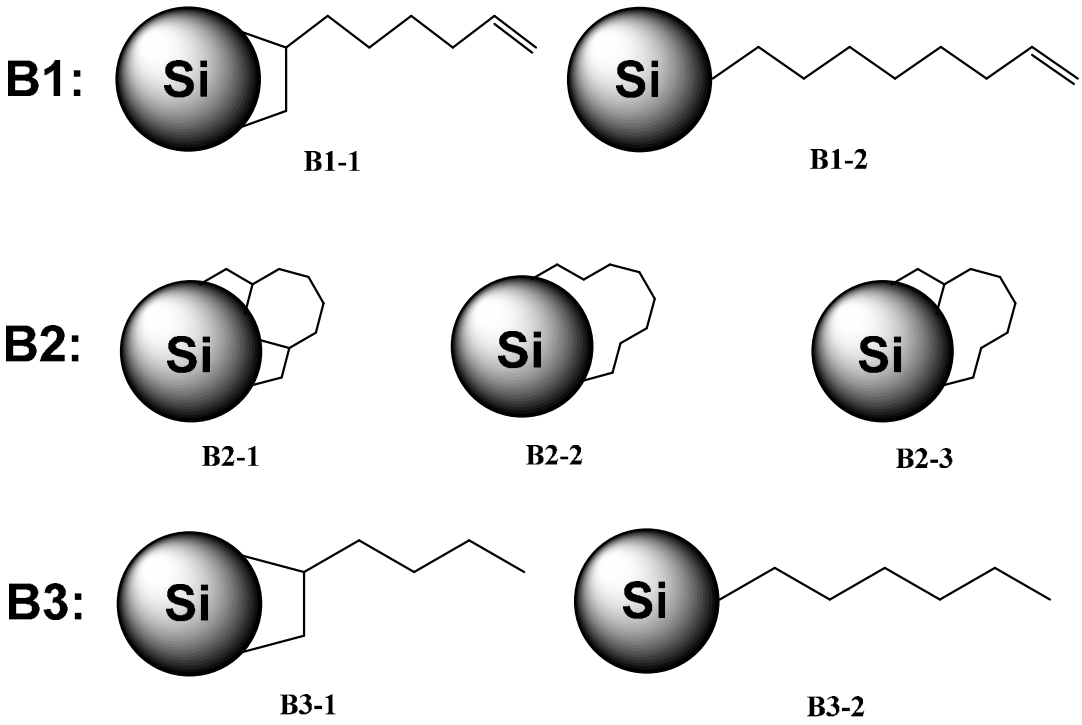
Figure SI-2. (a) TEM image of alkene terminated SiNPs and a size distribution histogram based on 2577 particles (inset). (b) Schematic illustration of alkene terminated SiNP.

Ball milling of silicon wafers create highly active surface species, including Si=Si bonds and silicon dangling bonds (radicals). The mechanochemical functionalization of silicon surfaces with an alkene can yield two basic bonding models on the surfaces (see Scheme SI-1). [1] First, Si=Si bonds can react with alkenes through cycloaddition between a Si=Si bond and a C=C bond (Scheme SI-1a). In addition, silicon dangling bonds can react with alkenes through radical silylation followed by abstraction of allylic protons from surrounding alkenes (Scheme SI-1b).



Scheme SI-1. Basic bonding models on silicon surfaces when silicon wafer is ground with a monoene: **(a)** [2+2] bonding model; **(b)** monosilylation bonding model

Scheme SI-2 show all potential surface products derived from 1.7-octadiene (B1 and B2) and 1-hexene (B3). Only products derived from 1,7-octadiene (B1-1 and B1-2) have terminal vinyl groups. Products containing only alkyl protons are B2 (from hairpinning of the 1,7-octadiene) and B3 (from 1-hexene). All of the products considered derive from the reaction pathways shown in SI-1.



Scheme SI-2. Major bonding models on SiNP surfaces by 1-hexene (B3) and 1,7-octadiene (B1 and B2) during RHEBM.

NMR Spectroscopy of SiNPs

The ^{1}H NMR spectrum of SiNPs (7.0 mg) and tetramethylsilane (TMS) (6.2 mg, 0.070 mmole) in $CDCl_3$ (0.5 mL) were recorded using a Bruker Avance 300 MHz spectrometer. Since the grafted SiNPs should consist of three major species of surface structures ($\boldsymbol{X_1}$ mmole of B1, $\boldsymbol{X_2}$ mmole of B2, and $\boldsymbol{X_3}$ mmole of B3) in the NMR sample, the mass fraction of the organic layer can be estimated by the peak areas from ^{1}H NMR using TMS as an internal standard. As shown in Figure SI-3, we assigned the integrated area of vinyl protons (from 4.5 ppm to 6.0 ppm) as 1. The integrated area of TMS standard (from -0.5 ppm to 0.25 ppm) is 26 and the integrated area of

alkyl protons (from 0.25 ppm to 3 ppm) is 13. Since X_1 mmole of B1 structure can contribute 3 X_1 mmole of vinyl protons, while 0.07 mmole TMS has 0.84 mmole protons (0.07 mmole × 12 = 0.84 mmole), we can obtain X_1 equals to 0.010 mmole ($X_1 = (0.84\ \text{mmole} \times \frac{1}{26})/3$). Furthermore, based on the molar fraction of initial reactants added in RHEBM (40% 1,7-octadiene and 60 % 1-hexene), and assuming that the reactivity of both reactants are identical with the SiNPs, we can obtain Equation 1 as follows (Since 1,7-octadiene can generate X_1 mmole B1 and X_2 mmole B2 species and 1-hexene can only generate X_3 species):

$$\textbf{Equation 1}: \frac{X1 + X2}{X3} = \frac{40\%}{60\%}$$

As illustrated in Scheme SI-2 each sub-structure of B1, B2 and B3 major species has very similar alkyl proton numbers (e.g. 11 alkyl protons for B1-1 and 12 alkyl protons for B1-2). We used 11, 14 and 12 alkyl protons for B1, B2 and B3 respectively for calculation (there is no significant difference by using other proton numbers). We can obtain Equation 2 based on integrated area of alkyl protons.

$$\textbf{Equation 2}: 11 \times X1 + 14 \times X2 + 12 \times X3 = 0.84\ \text{mmole} \times \frac{13}{26}$$

Since X_1= 0.010 mmole, based on Equation 1 and Equation 2, we can obtain X_2 = 0.004 mmole and X_3= 0.021 mmole. Furthermore, since the *MW* of 1,7-octadiene is 110 g/ mole and 1-hexene is 84 g/mole, we can calculate the mass of surface organic

layer as follows: (0.010 mmole + 0.004 mmole) × 110 g/mole + 0.021 mmole × 84 g/mole = 3.3 mg. Since the surface organic layer weighs 3.3 mg, and the sample of the SiNP is 7.0 mg, we obtain the mass fraction of the surface organic groups as 47% (wt %). Thus the mass fraction (f_{core}) of silicon cores of the SiNPs is 53%. We can further determine the amount of surface alkene functional groups per gram of the SiNPs to be C=1.42 × 10^{-3} mole/g ($\frac{0.010 \text{ mmole}}{7 \text{ mg}}$).

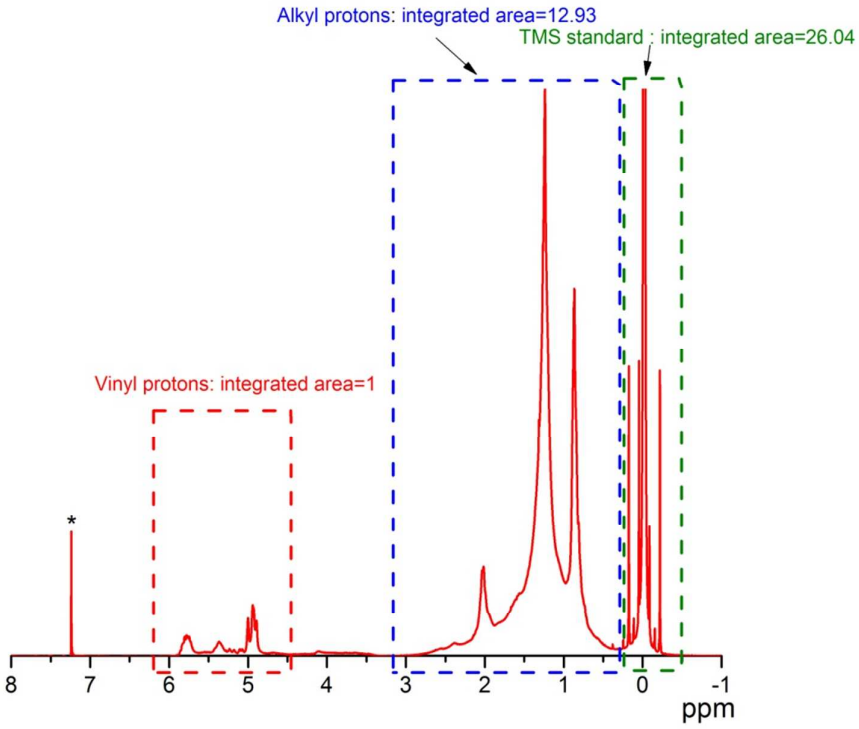


Figure SI-3. ^{1}H NMR spectrum of alkene grafted SiNPs in $CDCl_3$.

Calculation of the average molecular weight of the SiNPs

The average molecular weight of the silicon core ($\overline{MW_{core}}$) of the SiNPs can be calculated from Equation **3**, in which ***r*** is the average radius of silicon cores (half of the average diameter from TEM), **D** is the density of silicon (2.329 g/cm^3) and N_A is

the Avogadro constant (6.02×10^{23}*/mole*). $\overline{MW_{core}}$ is calculated to be 20000 *g/mole* using 3 nm as the average diameter for a SiNP.

Equation 3:

$$MW_{core} = \frac{4}{3} \times \pi \times r^3 \times D \times N_A$$

Based on mass fraction (f_{core}) of silicon core (53%) from ^{1}H NMR analysis, the molecular weight of the SiNPs ($\overline{MW_{SiNP}}$) is 38000 g/mole (based on Equation 4).

Equation 4:

$$\overline{MW_{SiNP}} = \frac{\overline{MW_{core}}}{f_{core}}$$

The number of alkene functional groups per SiNP (N_{vinyl}) can then be obtained from Equation **5**, in which $\overline{MW_{SiNP}}$ = 38000 g/mole and C= 1.42×10^{-3} mole/g based on the ^{1}H NMR spectrum. The resultant $\overline{N_{vinyl}}$ = 54, meaning that each SiNP contains an average of 54 surface alkene groups.

Equation 5:

$$\overline{N_{vinyl}} = C \times \overline{MW_{SiNP}}$$

Optical Properties of the Silicon NP core

The UV-Vis absorption spectrum of the alkene terminated SiNPs in CH_2Cl_2 is shown in Figure 1a (main text, inset). The concentration of the sample is 0.15 g/L and the

molarity of the SiNPs is estimated to be 4.0×10^{-6} mole/L based on an average molecular weight of 38000 g/mole. The absorption ***A*** of SiNP at 260 nm is 0.24 and the pathlength (***L***) is 1 cm. Therefore, based on Beer-Lambert law ($\boldsymbol{A=\varepsilon\cdot c\cdot L}$), the extinction coefficient of the SiNP at 260 nm is 6.1×10^{4} L·mole^{-1}·cm^{-1}.

The photoluminescence (PL) spectrum of the SiNPs excited at 340 nm is shown in Figure SI-4.

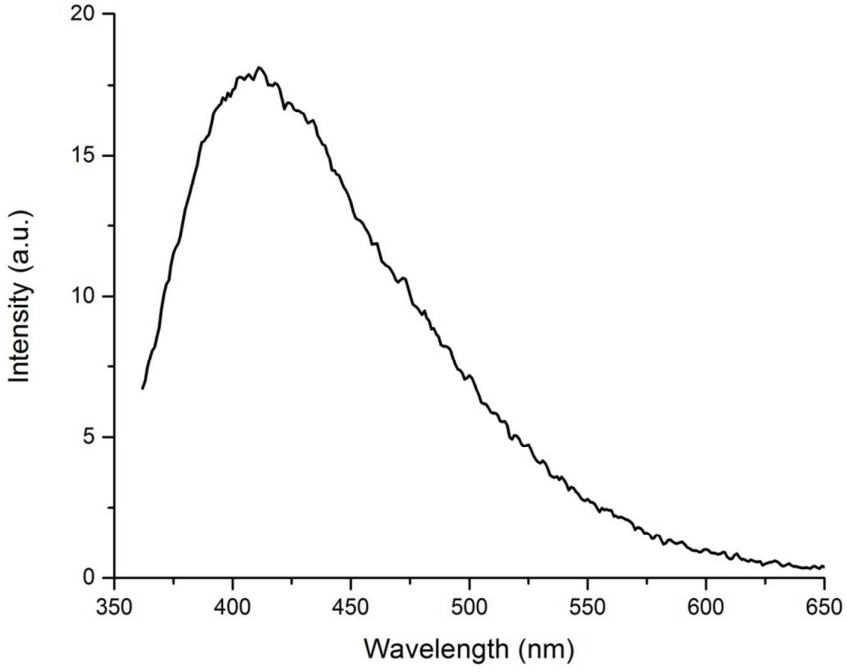
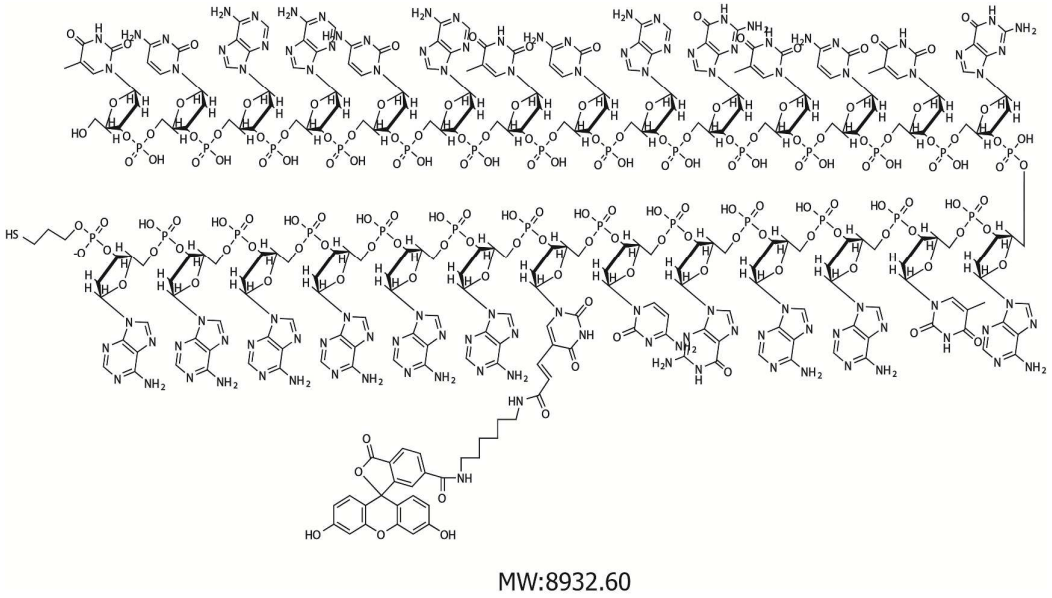


Figure SI-4. Emission spectrum of alkene terminated SiNPs (λ_{ex} = 340 nm).

3. ODN purification and characterization

The recognition sequence was synthesized using 3'-Thiol-Modifier C3 S-S CPG and

Fluorescein-dT (Glen Research) respectively. After cleavage from bead, the recognition sequence was first treated with 100 mM DTT (pH 8.5) at room temperature for 30 min to break the S-S bond and generate the free terminal thiol group. After cleavage, the reaction was desalted using a sephadex resin Microspin G-25 column (GE Healthcare) followed by chromatography on a Varian Prostar reverse-phase HPLC equipped with a MetaTherm column heater. The purified ODNs were characterized by MALDI-TOF in linear negative mode (see below). Other ODNs were purified and used as is.



Scheme SI-3. Structure of the recognition sequence. Note: Calculated mass $[M-H]^-$ is included.

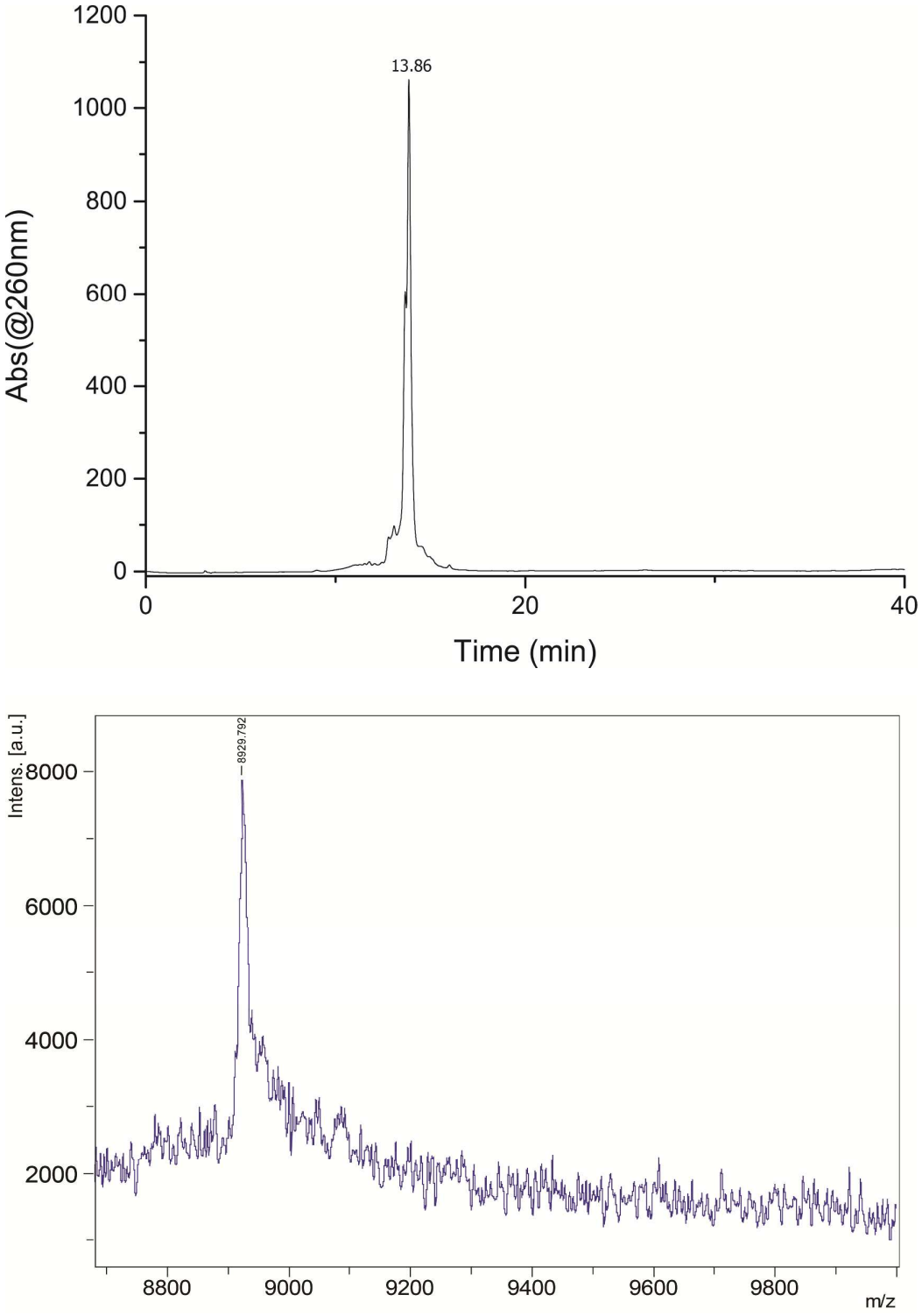


Figure SI-5. top: A representative RP-HPLC trace of the recognition sequence ODN. **Bottom**: MALDI-TOF mass spectra of the recognition sequence. (Observed mass: 8929.792 Da using linear negative mode).

4. SiNP ODN thiol-ene click reaction

An aqueous solution of thiol-modified ODNs (10uL, 250 nmole) and a catalytic amount (5 nmole) of 2,2-dimethoxy-2-phenylacetophenone (DMPA: used as the radical initiator) was added to a 190 μL DMSO solution of alkene functionalized SiNP (82.6 μg, 2.18 nmole , 118 nmole surface alkene functional groups based on $\overline{N_{vinyl}}$ =54) in a 0.5mL glass vial. The thiol-ene click reaction was initiated by placing the glass vial on top of a hand held UV-lamp (facing up) and irradiating for 30 min (365 nm UV light). After 30 min, the reaction solvents were removed using a Savant SPD IIIV speed rotorvap and the reaction residue was re-dissolved in aqueous solution (no precipitate was observed during this process suggesting the complete conversion of the H_2O insoluble SiNP to H_2O soluble SiNP ODN conjugate). The crude product was then purified via a 30k Amicon centrifugal filter to remove unreacted ODNs. For each washing cycle, 400 μL aqueous solution was used and the UV-vis absorption of the filtrate was recorded to monitor the purification process. After the 6^{th} wash, no significant amount of unreacted ODNs remained (see Figure below).

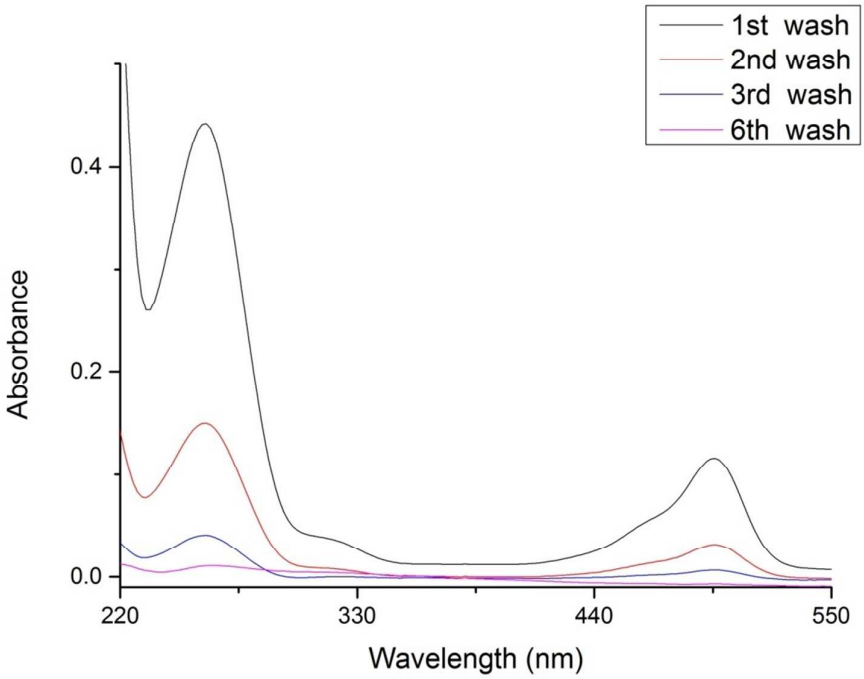


Figure SI-6. UV-vis absorption spectra of filtrate after different number of washing steps .

5. TEM image of SiNP ODN conjugates

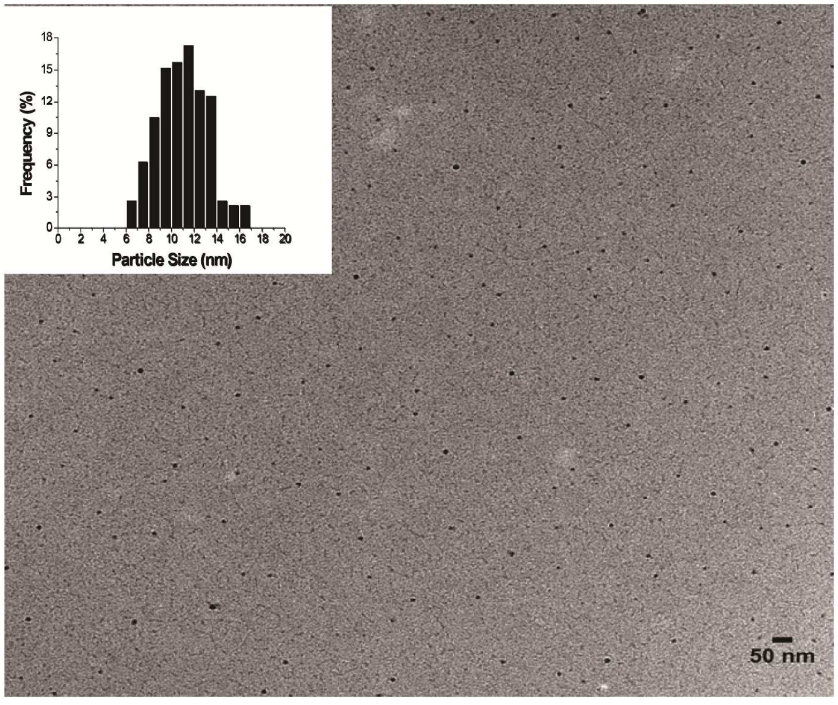


Figure SI-7. TEM image of SiNP ODN conjugates and a size distribution histogram based on 191 particles (inset).

6. Calculation of the surface coverage of the ODNs for each SiNP

Based on UV-Vis absorption spectrum of the SiNP ODN conjugates after purification (see Figure 1a main text), the concentration of the ODNs (i.e., the recognition sequence) on the SiNP ODN conjugate was calculated from the UV absorption of ODN region at 260 nm. (Note: the extinction coefficient of the ODN at 260 nm is 5.5 fold higher than the extinction coefficient of the SiNP and, moreover, the overall DNA concentration is multiple fold higher than the SiNP. Therefore, the absorption from the SiNP core at 260 nm is assumed to be negligible). The concentration of this ODN was also verified using the absorption of the fluorescein moiety at 490 nm (the molar absorption coefficient for fluorescein is 7.5×10^4 $L\cdot mole^{-1}\cdot cm^{-1}$ at 490 nm, at neutral pH). The amount of conjugated ODN was determined to be 10 nmole. Since 118 nmole of surface alkene functional groups (2.18 nmole SiNPs) were initially present in the thiol-ene click reaction assuming complete conversion of SiNP to SiNP ODN conjugate. (see SI section **4**), we can reasonably estimate that 8.4% of the surface alkenes on each SiNP reacted with the ODN, giving an average of 4-5 ODNs for each SiNP.

[1] Buriak, J. M. (2002) Organometallic chemistry on silicon and germanium surfaces. *Chem. Rev.* 102, 1271-1308.