Supplementary Information

Dye-Doped Silica Nanoparticles as Efficient Labels for Glycans

Xin Wang,[†] *Olof Ramström,*[‡]* *Mingdi Yan*[†‡]*

[†]Department of Chemistry, Portland State University, P.O. Box 751, Portland, Oregon, 97207-0751; [‡]Department of Chemistry, KTH - Royal Institute of Technology, Teknikringen 30, S-10044 Stockholm, Sweden

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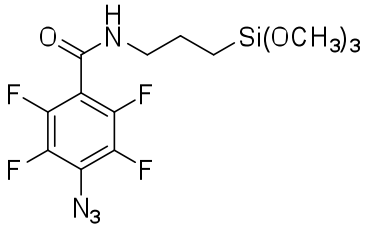
1. Experimental section

Scheme 1



PFPA-silane

Materials. 3-aminopropyltriethoxysilane (APTMS), D-(+)-mannose (Man), D-(+)-glucose (Glc), D-(+)-galactose (Gal), maltopentaose (Glc5) were obtained from TCI America. Anthrone (97%), FITC (Fluorescein isothiocyanate isomer I, 90%), tetraethyl orthosilicate (TEOS), Con A (lectin from *Canavalia ensiformis* (Jack bean), Type IV), SBA (from *Glycine max*), bovine serum albumin (BSA) were purchased from Sigma-Aldrich. 2-*O*-α-D-Mannopyranosyl-D-mannopyranose (Man2) and 3,6-di-*O*-(α-D-mannopyranosyl)-D-mannopyranose (Man3) were obtained from V-Labs Inc (Covington, Louisiana). Absolute ethanol (200-proof) was purchased from PHARMCO-AAPER. 3-(Trimethoxysilyl)butyl aldehyde (90%) was obtained from United Chemical Technologies, Inc. All chemicals were used as received without purification. Water used was from a Milli-Q ultrapure water purification system. Dialysis tubes (G-Biosciences Tube-O-dialyzer, 15K, medium) were purchased from VWR International. *E. coli* ORN178 and ORN208 were grown in the Luria-Bertani Broth medium at 37 °C to an optical density of 0.9 at 600 nm (approximately 109 cells per mL) and precipitated by centrifugation at 3000 rpm for 4 min.[1]

Fluorescence measurements were conducted on a PTI spectrofluorometer (Photon Technology International). TEM images were obtained on a JEOL 100CX transmission electron microscope operating at an accelerating bias voltage of 100 kV. The specimens were prepared by dropping nanoparticles suspensions (10 μL) onto a 200 mesh copper grid (coated with carbon supporting film, Electron Microscopy Sciences). Dynamic light scattering (DLS) experiments were carried out on Horiba LB-550 Dynamic Light Scattering Nano-Analyzer.

Synthesis of FITC-doped silica nanoparticles FSNPs. Fluorescein isothiocyanate (39 mg, 0.10 mmol) was mixed with APTMS (23 μL, 0.10 mmol) in 100 mL of absolute ethanol, and was stirred at 42 ºC for 24 h to yield the FITC-silane precursor as a bright yellow solution. The fluorescent nanoparticles were synthesized following a modified protocol from the classic Stöber protocol,[2] similar to what was previously described.[3] The dye precursor solution (5 mL) was mixed with TEOS (2.8 mL), and the mixture was added to 200 proof absolute ethanol (34 mL) followed by NH_4OH (25%, 2.8 mL). The reaction was allowed to proceed at room temperature for at least 8 h with vigorous stirring to yield a bright yellow colloidal solution. The particle diameters were determined by TEM and DLS.

Functionalization of silica nanoparticles with PFPA. PFPA-silane (80 mg, Scheme 1), synthesized following a previously reported procedure,[4] was added directly to the Stöber solution prepared above, and the mixture was stirred at room temperature overnight. The next day the mixture was brought to reflux while continuing stirring for 1 h at ~78 ºC to

facilitate the covalent bond formation between PFPA-silane and the silica nanoparticles.[3] The mixture was centrifuged at 8,000 rpm for 10 min, and the precipitate was redispersed in the fresh solvent by sonication. This centrifugation/redispersion procedure was repeated three times with ethanol and twice with acetone.

Conjugation of carbohydrates onto FSNPs. Our previous reported procedure of coupling carbohydrates on gold nanoparticles was followed.[5] The solution of PFPA-functionalized FSNPs in acetone (20 mg/mL, 5 mL) was placed in a flat-bottom dish, and an aqueous solution of carbohydrate (10 mg/mL, 1 mL) was added. The mixture was covered with a 280-nm long-path optical filter (WG-280, Schott Glass) and was irradiated with a 450-W medium pressure Hg lamp (Hanovia) for 10 min under vigorous stirring. Centrifugation of the solution at 8,000 rpm for 10 min separated the carbohydrate-attached FSNPs as precipitates. Excess carbohydrate was removed by membrane dialysis in water for 24 hours. The concentration of FSNP, ~17.2 mg/mL, was determined by drying the solution under reduced pressure for 3 hours and weighing.

Determination of carbohydrate density on glyco-FSNPs. A previously developed colorimetric method was followed to determine the density of carbohydrates immobilized on FSNPs.[5] Calibration curves were first obtained for each carbohydrate where carbohydrate solutions of various concentrations were incubated with anthrone/sulfuric acid and the absorbances at 620 nm were measured.[6] A freshly-prepared anthrone solution in concentrated H_2SO_4 (0.5 wt%, 1 mL) was added to a carbohydrate solution in water (0.5 mL) in an ice bath under stirring. The solution was then heated to 100 $^{\circ}$C and

stirred for 10 min. After cooled to room temperature, the UV-vis spectra of the resulting solutions were recorded on a Perkin-Elmer Lambda 45 UV-vis spectrometer.

Carbohydrates coupled on nanoparticles were subjected to the same assay where solutions of the glyco-FSNPs in Milli-Q water (30–50 μg/0.5 mL) were treated with anthrone/H_2SO_4. Background absorption due to FSNPs themselves was accounted for by treating FSNPs solution of the same concentration with anthrone/H_2SO_4, and the absorbance at 620 nm was subtracted from that of the glyco-FSNPs. The amount of surface-bound carbohydrate was then computed from the corresponding calibration curve.

Binding with Con A and *E. coli*. The binding affinity of FSNP-labeled Man, FSNP-Man, was evaluated using Con A and *E. coli* strain ORN178 and ORN208 according to the following procedure. FSNP-Man (2.5 mg) were incubated in a pH 7.2 HEPES buffer solution (1 mL, 10 mM) containing 3% BSA for 30 min, centrifuged, and the particles were incubated in the pH 7.2 HEPES solution without BSA for another 20 min. The nanoparticles were subsequently treated with a solution of Con A in HEPES buffer (1 mL, 10 μg/mL) containing $MnCl_2$ (1 mM) and $CaCl_2$ (1 mM), or *E. coli* solution for 1 hour while shaking. In cases where aggregation was induced after binding with Con A, the suspension was transferred to a centrifuge tube and was centrifuged at 8,000 rpm for 10 min.

Fabrication of lectin microarrays. Aldehyde-coated glass slides were prepared following a reported procedure.[7] Piranha-cleaned glass slides were treated with a solution

of 3-(trimethoxysilyl)butyl aldehyde in toluene (2 mM) for 4 hours, rinsed with toluene and dried with N_2. Solutions of lectins were prepared in pH 7.4 phosphate-buffered saline (PBS) at varying concentrations of 0.1-1 mg/mL with 40% glycerol added to prevent evaporation of the liquid droplets after printing. Con A and SBA were then printed onto the aldehyde-functionalized glass slide using a robotic printer (BioOdyssey Calligrapher miniarrayer; Bio-Rad Laboratories, Inc.). The glass slides were then incubated in a humid chamber (80% humidity) at 25 ºC for 3 h to facilitate the immobilization of the lectins. After incubation, the blocking solution of BSA in pH 7.4 PBS buffer (1%) was added and the slides were incubated for 1 h, rinsed with the PBS buffer and was dried with N_2.

Microarray assay and fluorescence imaging. The lectin microarrays were incubated in the solution of glyco FSNPs in HEPES (0.5 mg/mL) for 2 h, and were then gently rinsed with the HEPES buffer 3 times and dried. The slides were scanned under a microarray scanner (GenePix 4000B, Molecular Devices, Inc) at excitation of 532 nm. The fluorescent images were recorded and the data were analyzed using the supplied software (Axon GenePix Pro 5.1).

2. TEM and DLS characterization of FITC-doped SNPs

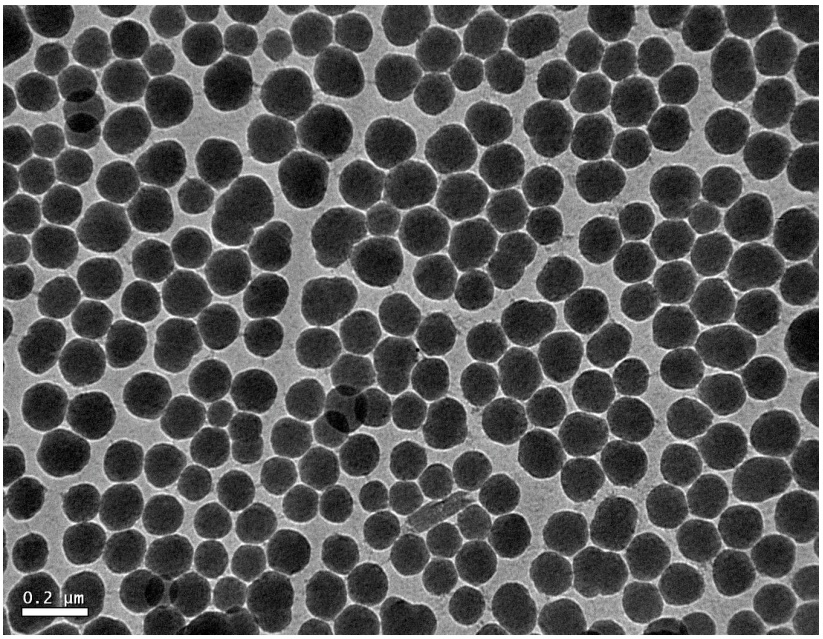


Fig. 1S TEM image of FITC-doped SNPs. (Scale bar: 0.2 µm)

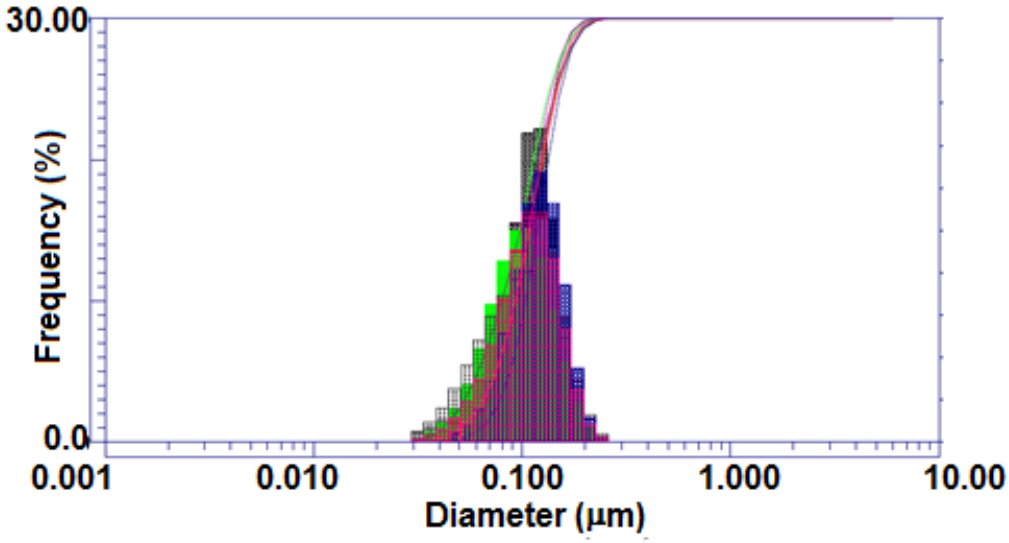


Fig. 2S Size distribution of FITC-doped SNPs measured by DLS.

3. Photo-stability of FITC-doped silica nanoparticles and FITC

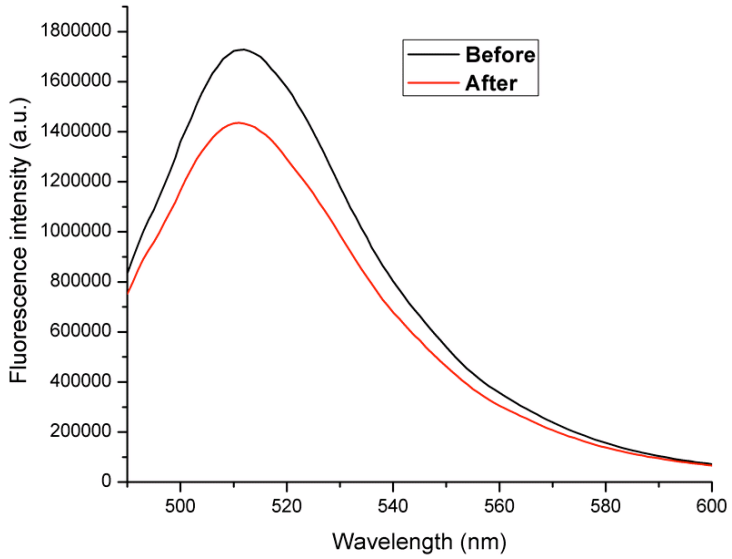


Fig. 3S Fluorescence spectra of FITC-doped silica nanoparticles before and after UV irradiation for 10 min.

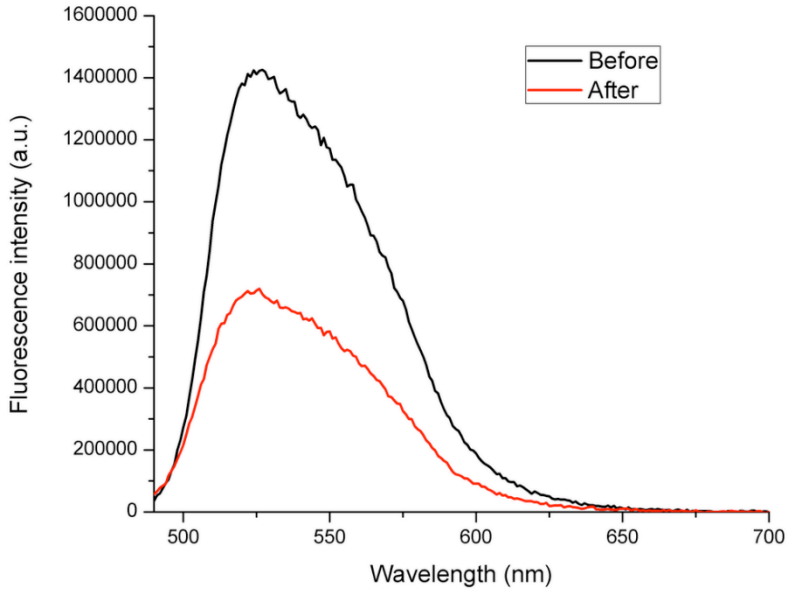
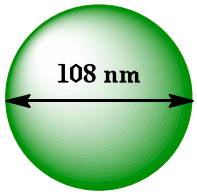


Fig. 4S Fluorescence spectra of FITC before and after UV irradiation for 10 min.

4. Determination of immobilized carbohydrate density and coupling yield



Surface area of each FSNP: $S=\pi*d^2= 36625\ nm^2$
Volume of each FSNP: $V=\pi*d^3/6=6.59x10^5\ nm^3=6.59x10^{-16}\ cm^3$
Density of FSNP=2.3 g/cm^3
Concentration of FSNP = 20.61 mg/mL
So in 1 mL , No. of FSNP= $(0.02061/2.3)/(6.59x10^{-16})= 1.36x10^{13}$

By assuming the projection area of carbohydrate molecules, such as D-mannose 0.24 nm^2 (see Ref 5 for information of other carbohydrates), the number of maximal ligands occupied on each FSNP is: $N_{Max}=36625/0.24= 1.53x10^5$

The experimental number of ligands (N_{exp}) was determined by anthrone-H_2SO_4 colorimetric measurements (using FSNP as background). The coupling yield was calculated as $N_{exp}/N_{Max}x100\%$

Table 1S. Ligand density and coupling yield of glyco FSNPs

Carbohydrate ligand	**No. of ligand per FSNP ($x10^4$, experimental)**	**No. of ligand per FSNP ($x10^4$, calculated)**	**Coupling yield (%)**
D-Mannose (Man)	5.52	15.3	36
α-1,2-Mannobiose (Man2)	2.72	5.56	49
α-1,3-1,6-Mannotriose (Man3)	2.48	4.76	52
D -Glucose (Glc)	5.64	15.3	37
Maltopentaose (Glc5)	1.72	3.16	54

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