SUPPLEMENTARY INFORMATION

Antifouling Surfaces for Proteins Labeled with Dye-doped Silica Nanoparticles

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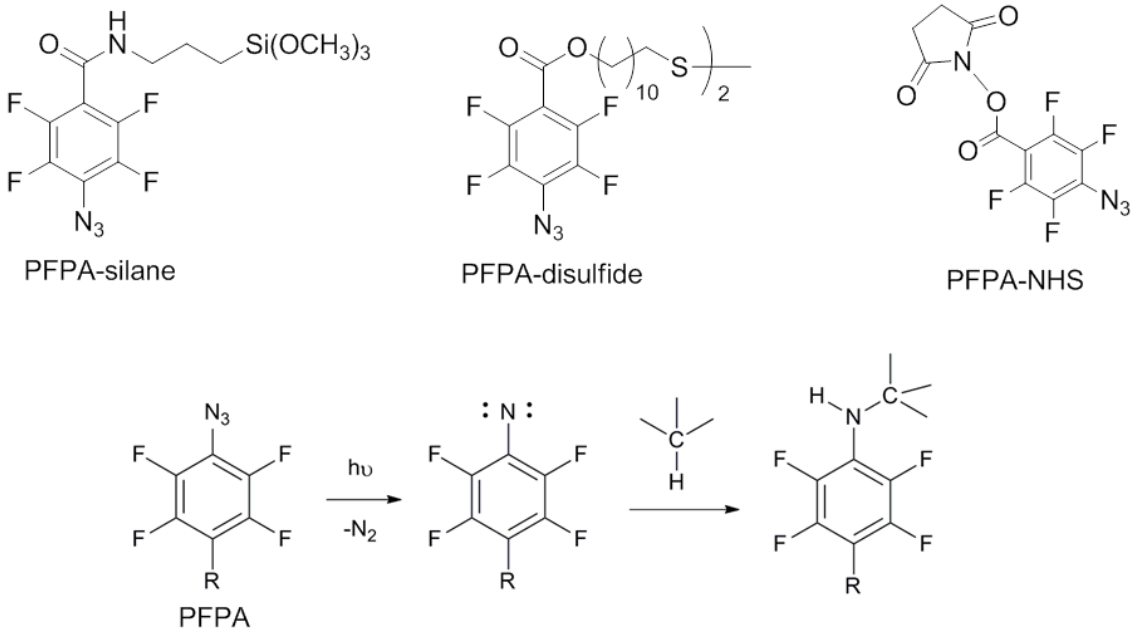
Contents

Part A: Experimental Procedures

Materials

Milli-Q water for contact angle measurements as well as for cleaning silicon wafer was obtained from a Millipore Milli-Q system with at least 18.2 MΩ resistivity. Concentrated H_2SO_4, H_2O_2 (35%), toluene, dichloromethane, and chloroform were purchased from Fisher. Dichloromethane was dried by refluxing in CaH_2 for 3 h and was distilled before use. Other solvents were used as received. Polystyrene (PS, $\overline{M_w}$ *ca* 280,000), poly(ethylene oxide) (PEO, $\overline{M_v}$ 1,000,000), poly(vinylpyrrolidone) (PVP, $\overline{M_w}$ *ca* 1,300,000), poly(methyl methacrylate) (PMMA, $\overline{M_w}$ *ca* 97,000), poly(vinyl acetate) (PVA, $\overline{M_w}$ *ca* 100,000), poly(allylamine hydrochloride) (PAAm, $\overline{M_w}$ *ca* 15,000) and tetraethyl orthosilicate (TEOS, 98%) were received from Aldrich. Poly(2-ethyl-2-oxazoline) (PEOX, $\overline{M_w}$ *ca* 500,000) was obtained from Alfa Aesar. 3-Aminopropyltriethoxysilane (APTMS), 3-glycidyloxytrimethoxysilane (GOPTS), D-(+)-mannose (Man), D-(+)-glucose (Glc) and D-(+)-galactose (Gal) were purchased from TCI. Fluorescein isothiocyanate isomer I (FITC), concanavalin A (Con A, Type IV), FITC conjugated concanavalin A (FITC-Con A, Type IV, 102 kD) and bovine serum albumin (BSA, 96%, ~66 kDa), phosphate buffered saline (PBS, pH 7.4) and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer (HEPES, pH 7.5) were purchased from Sigma. Ethanol (absolute, anhydrous) was purchased from Pharmco-AAPER Inc. (Brookfield, CT). 2-*O*-α-D-Mannopyranosyl-D-mannopyranose (Man2, 96.3%) and 3,6-di-*O*-(α-D-mannopyranosyl)-D-mannopyranose (Man3, >99%) were obtained from V-labs Inc. (Covington, LA). PFPA-silane,[1,2] PFPA-disulfide[3] and PFPA-NHS[4,5] were synthesized according to previously published procedures. They were freshly prepared and purified using a silica-gel column before being used to treat the wafers. Silicon wafers with a 35 Å native oxide layer were purchased from WaferNet, Inc. (San Jose, CA). The long-pass optical filter (280-nm) was purchased from Schott Glass Technologies, Inc. (Fullerton, CA).



Scheme S-1. Structures of PFPA-silane, PFPA-disulfide, PFPA-NHS, and CH insertion reaction of PFPA.

Preparation of FSNPs and FSNP-Con A

Silica nanoparticles and FSNPs were prepared following the Stöber method with slight modifications.[6-9] Silica nanoparticles were synthesized by mixing TEOS (2.8 mL), absolute ethanol (34 mL) and NH_4OH (2.8 mL) followed by stirring at room temperature for 8 h. The resulting mixture was centrifuged and the silica nanoparticles were washed 3 times with PBS (pH 7.4).

FSNPs were prepared using a previously developed procedure.[9] Briefly, fluorescein isothiocyanate (39 mg, 0.10 mmol) was mixed with APTMS (23 μL, 0.10 mmol) in absolute ethanol (100 mL), and the mixture was stirred at 42 ºC for 24 h to yield FITC-silane. FITC-silane (5 mL) was then mixed with TEOS (2.8 mL), and absolute ethanol (34 mL) was added followed by NH_4OH (2.8 mL). The reaction was allowed to proceed at room temperature for at least 8 h with vigorous stirring. The resulting FSNPs were centrifuged and washed 3 times with PBS (pH 7.4) to give nanoparticles of 116 nm in average diameter as determined by dynamic light scattering (DLS) using a Horiba SZ100 DLS nanoparticle analyzer (see Part C,

Figure S-2). The polydispersity index (PDI) of FSNPs was 0.064 by DLS. The zeta potential was -94 mV measured by DLS in PBS buffer (pH 7.4) containing $CaCl_2$ (1 mM) and $MnCl_2$ (1 mM).

Con A was covalently immobilized on FSNPs following a previously developed procedure.[10] Briefly, GOPTS (18 mg) was added directly to a solution of FSNPs (20 mL) prepared above, and the mixture was stirred at room temperature overnight and then at 78 °C for 1 h to give epoxy-functionalized FSNPs. The product was isolated by centrifugation and then re-dispersion in the fresh solvent by sonication. This centrifugation/re-dispersion procedure was repeated three times with ethanol and twice with HEPES (pH 7.5), and the particles were finally dispersed in HEPES buffer at a concentration of 9.9 mg/mL. A solution of Con A in pH 7.5 HEPES buffer (2 mL, 3.3 mg/mL) was added to epoxy-FSNPs (2 mL). The amount of added Con A was 3.3 times of that of theoretical maximal surface coverage of Con A on FSNP (see Part D for detailed calculation). The mixture was stirred for 3 h, which was then carefully centrifuged and washed 10 times with HEPES buffer (pH 7.5) containing $CaCl_2$ (1 mM) and $MnCl_2$ (1 mM). The average diameter and PDI of FSNP-Con A measured by DLS were 117.5 nm and 0.094, respectively. The zeta potential was -89.3 mV measured by DLS in PBS buffer (pH 7.4) containing $CaCl_2$ (1 mM) and $MnCl_2$ (1 mM).

Additional FSNP-Con A samples were prepared where a solution of Con A in pH 7.5 HEPES buffer (1 mL, 20 μg/mL or 200 μg/mL or 1 mg/mL) was added to epoxy-FSNPs (20 mL, 9.9 mg/mL). Therefore, the Con A loading concentration vs. epoxy-FSNPs was 1 μg/mL, 10 μg/mL, and 50 μg/mL, respectively, and the amount of added Con A was 0.1%, 1%, 5% of that of theoretical maximal surface coverage of Con A on FSNP (see Part D for detailed calculation). The epoxy-functionalized SNPs and FITC-Con A-immobilized SNPs were prepared following the same procedures described above except that SNPs instead of FSNPs were used.

Determination of Surface Coverage of FITC-Con A on SNP

A solution of epoxy-SNPs in HEPES (pH 7.5) (1 mL, 9.9 mg/mL) containing $CaCl_2$ (1 mM) and $MnCl_2$ (1 mM) was added to a quartz curvet and the sample was used as the blank. Varying amount of FITC-Con A (5 – 400 μg) was added to the above solution, and the fluorescence intensity at 520 nm was measured by a Cary Eclipse Fluorescence Spectrophotometer (Agilent Technologies, Inc. Santa Clara, CA). The fluorescence intensity of epoxy-SNPs was subtracted, and the difference was plotted against the concentration of FITC-Con A to construct the calibration curve (Figure S-1). To determine the amount of FITC-Con A immobilized on SNPs, the fluorescence intensity of FITC-Con A-immobilized SNPs was measured. After subtracting the fluorescence intensity of epoxy-SNPs, the amount of immobilized FITC-Con A was calculated using Figure S-1. For 1 μg/mL and 10 μg/mL FITC-Con A loading concentrations, the fluorescence intensities of the resulting SNP conjugates were very low and were out of the linear range of the calibration curve, and therefore, only results for 50 μg/mL and 3.3 mg/mL loading concentrations were determined and reported (Table S-1).

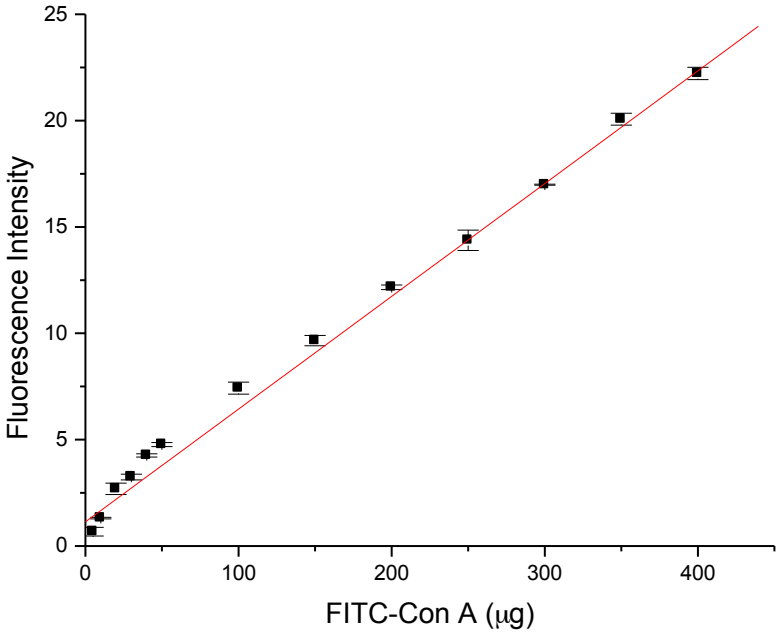


Figure S-1. Calibration curve: fluorescence intensity vs. amount of FITC-Con A. $R^2 = 0.99$.

Table S-1. The surface coverage of immobilized FITC-Con A on SNP.

Loading concentration (FITC-Con A/SNPs)	Amount of FITC-Con A immobilized (μg/mL SNPs) [a]	Surface Coverage (%)[a]
3.3 mg/mL	192 ± 44	20 ± 5
50 μg/mL	24 ± 6	3 ± 1

[a] Each data was the average of 3 samples.

Preparation of Polymer Thin Films

Silicon wafers were cut into 1 × 1 inch pieces, cleaned in the piranha solution (3:1 v/v conc. H_2SO_4/H_2O_2) at 80-90 °C for 1 hour, washed in boiling water three times for 60 minutes each, and dried carefully under a stream of nitrogen. Caution: the piranha solution reacts violently with organic solvents and should be handled in a designated fume hood. The cleaned wafers were soaked in a solution of PFPA-silane in toluene (12.6 mM) for 4 hours, rinsed with toluene, and dried under nitrogen. The wafers were allowed to cure at room temperature for 24 hours. The cured wafers were spin-coated at 2000 rpm for 60 seconds with a solution of polymer in chloroform (10 mg/mL) using a P6204 spin-coater (Specialty Coating Systems, Inc., Indianapolis, IN). The films were irradiated with the medium-pressure Hg lamp (450 W, Hanovia Ltd.) for 9 min. The lamp reached its full power after ~2.5 min warm-up to an intensity of 3.5 mW/cm^2 at 18 cm from the source as measured by an OAI 306 UV power meter (Optical Associates Inc. Milpitas, CA) with a 260-nm sensor. A 280-nm optical filter was placed on the film surface during irradiation to remove the deep-UV light that can cause crosslinking and degradation of the polymers.[11] The films were then sonicated in chloroform followed by Milli-Q water for 5 min each using a Branson 1510 sonicator (Fisher), and dried under nitrogen.

Contact angles were measured on a Ramé-Hart model 250 standard goniometer (Ramé-Hart Instrument Co., Netcong, NJ). Data were recorded using the DROPimage Advanced v2.2 software.

Fabrication of Polymer Arrays

Polymer arrays were generated by manually spotting solutions of polymers (10 mg/mL) onto the PFPA-functionalized silicon wafers using a pipette tip (Scheme 1). The printed wafers were dried under the ambient condition at room temperature for 30 min, and then UV-irradiated for 9 min. A 280-nm long-path optical filter was placed on the film surface during irradiation to avoid crosslinking of the polymer films. The wafers were then sonicated in chloroform followed by Milli-Q water for 5 min each, and finally dried under nitrogen.

Evaluation of FSNPs Adsorption on Polymer Arrays by Fluorescence Imaging

The wafers containing the polymer arrays were incubated in a solution of FSNPs in pH 7.4 PBS buffer (0.01 M) (1.6 mg/mL) for 1 hour, and were washed with PBS buffer twice for 10 min each followed by Milli-Q water for 10 min. The wafers were finally dried with nitrogen, and imaged immediately with a fluorescence array scanner (GenePix 4100A, Axon Instruments Inc., Foster City, CA) at 635 nm excitation and 532 nm emission.

Fabrication of Polymer Arrays on SPR Chips

SPR chips were prepared as follows. High refractive index N-SF10 glass slides (Schott Glass Technologies, Inc., Fullerton, CA) were cleaned in the piranha solution at room temperature for 60 min and washed thoroughly in boiling water three times for 60 minutes each. The slides were then dried with nitrogen and coated with a 2 nm thick Ti followed by a 45 nm Au film in an electron beam evaporator (SEC-600, CHA Industries, Fremont, CA) at the Microfabrication Lab, Washington Technology Center (University of Washington).

The SPR chips were cleaned with the piranha solution at room temperature for 60 s, washed in boiling water 3 times for 60 minutes each, and dried under a stream of nitrogen. The cleaned chips were immediately soaked in a solution of PFPA-disulfide in chloroform (10 mM) for 24 hours. The slides were then rinsed with chloroform and dried under nitrogen. Polymer arrays were generated by manually spotting solutions of polymers in chloroform (10 mg/mL) onto the PFPA-functionalized SPR chip using a micropipettor tip. The polymer-

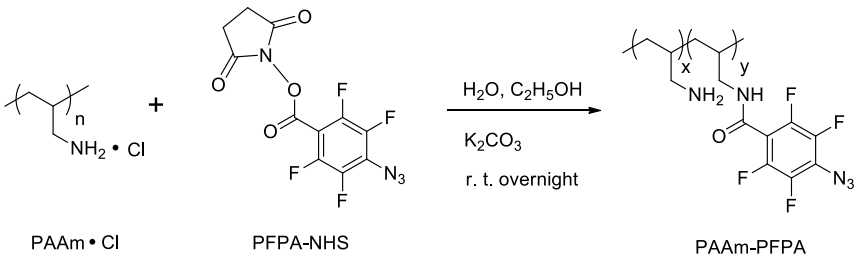
printed SPR chip was dried under the ambient condition at room temperature for 30 min followed by vacuum drying for 4 hours. The chip, covered with 280-nm optical filter, was then irradiated for 9 min with the medium-pressure Hg lamp. The resulting sample was washed thoroughly in chloroform for 4 hours and dried under nitrogen.

Evaluation of FSNP Adsorption on Polymer Arrays by SPR Imaging

SPRi experiments were conducted at room temperature using a SPRimager® II system (GWC Technologies, Inc.). The angle of incident light on the prism was optimized and remained unchanged in all experiments. The polymer microarray was primed in pH 7.4 PBS buffer until a stable baseline was reached. The solution of FSNP in PBS (~10 μg/mL) was then injected, and SPR responses from each polymer spot were recorded simultaneously. The flow rate was kept at 100 μL/min. Data were acquired by selecting the area within printed spots on a microarray image, i.e., region of interest (ROI). An average of 30 images/frames was utilized and SPR signals converted to normalized percentage in reflectivity (%ΔR) following the protocol provided by GWC. All SPR images were collected and analyzed using the Digital Optics V++ Version 4 image analysis software provided by the vendor.

Fabrication of Carbohydrate Microarrays

PAAm-PFPA was prepared following a previously reported procedure (Scheme S-2).[12] Briefly, PAAm hydrochloride (10.0 mg, 0.107 mmol) and K_2CO_3 (25 mg) were dissolved in Milli-Q water (2 mL). A solution of PFPA-NHS in absolute ethanol (4.44 mg/mL, 2 mL) was added, and the mixture was stirred vigorously overnight to yield a stock solution of PAAm-PFPA.



PAAm • Cl PFPA-NHS PAAm-PFPA

Scheme S-2. Synthesis of PAAm-PFPA.

Piranha-cleaned silicon wafers were treated with a solution of GOPTS in toluene (12.6 mM) for 4 hours, rinsed with toluene, and dried under nitrogen. The wafers were immersed in the PAAm-PFPA solution prepared above at 50 °C for 5 h, sonicated in Milli-Q water twice for 5 min each, and dried under nitrogen to give PAAm-PFPA-functionalized wafers.

Aqueous solutions of carbohydrate (10 mg/mL) were printed on PAAm-PFPA-functionalized wafers using a robotic printer (BioOdyssey Calligrapher miniarrayer, Bio-Rad Laboratories, Inc.). The wafers were dried under vacuum for 1 h and were spin-coated with a solution of PS 280,000 or PEO 1,000,000 in chloroform (5 mg/mL). The wafers were then UV-irradiated for 9 min in the presence of a 280-nm optical filter, sonicated in chloroform followed by Milli-Q water for 5 min each, and dried under nitrogen.

The control sample was prepared following a similar procedure without the PS or PEO coating. The wafer was incubated in a 3% BSA solution in pH 7.4 PBS buffer at room temperature for 2 h before treating with FSNP-Con A.

Fluorescence Imaging of Carbohydrate Microarrays Treated with FSNP-Con A

A FSNP-Con A solution prepared using 3.3 mg/mL Con A loading concentration was diluted 5 times in pH 7.5 HEPES buffer containing $CaCl_2$ (1 mM) and $MnCl_2$ (1 mM), and the carbohydrate microarrays prepared above were incubated at room temperature for 1 h. The wafer was then rinsed with HEPES buffer 3 times and dried under nitrogen. The wafer was imaged using a GenePix 4100A fluorescence array scanner at 635 nm excitation and 532 nm emission.

Part B: Static Water Contact Angles of Immobilized Polymer Films

Table S-2. The static water contact angle of immobilized polymer films.

Polymers	Contact Angle (°)[a]
PS 44,000	94.7 ± 0.6
PS 111,000	96.7 ± 0.8
PS 280,000	94.9 ± 0.1
PS 570,000	93.2 ± 0.9
PS 1,045,000	94.9 ± 2.1
PMMA 97,000	64.9 ± 0.6
PVA 100,000	65.4 ± 1.2
PEOX 5,000	43.3 ± 1.0
PEOX 50,000	39.6 ± 0.7
PEOX 200,000	39.4 ± 0.5
PEOX 500,000	41.5 ± 0.5
PEO 1,000,000	42.7 ± 1.6
PVP 1,300,000	25.7 ± 1.2

[a] Each data was the average of 3-5 samples. Milli-Q water (2 μL) was dropped onto the sample from a syringe dispenser attached to the instrument. The contact angle was measured immediately after dispensing (~2 s).

Part C: Dynamic Light Scattering (DLS) Characterization of FSNPs

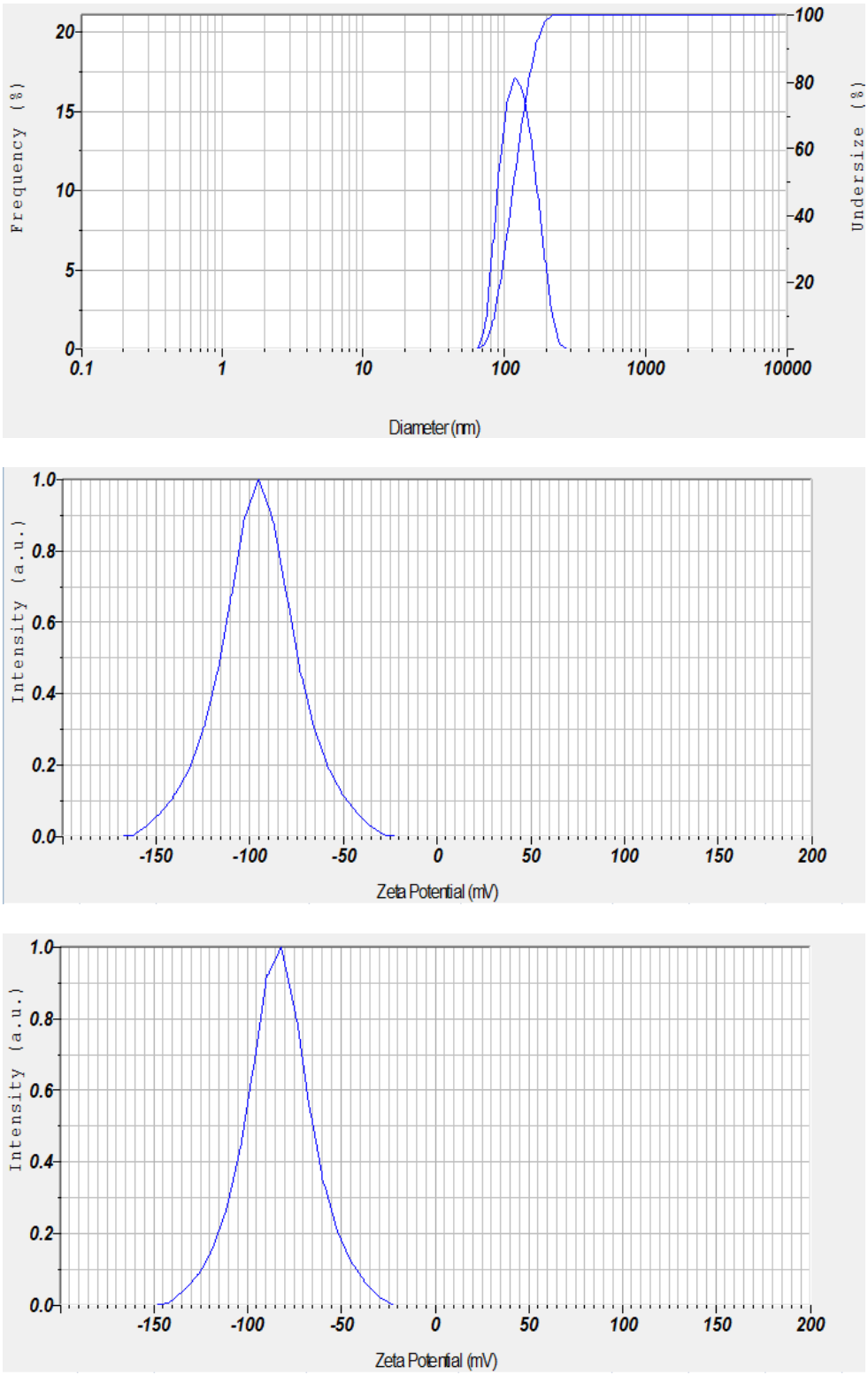


Figure S-2. Particle size distribution of FSNPs (top), zeta potential of FSNPs (middle) and zeta potential of FSNP-Con A (bottom) in PBS buffer (pH = 7.4).

Part D. Determination of surface coverage of immobilized FITC-Con A on SNP

Theoretical maximal amount of FITC-Con A on a SNP

Average diameter of SNP (from DLS): $d = 116\ nm$

Surface area of each SNP: $S = \pi d^2 = 4.23 \times 10^4\ nm^2$

Volume of each SNP: $V = \pi d^3/6 = 8.17 \times 10^5\ nm^3 = 8.17 \times 10^{-16}\ cm^3$

Density of SNPs[13]: $\rho = 2.3\ g/cm^3$

The mass of each SNP: $M_{SNP} = V\rho = 1.88 \times 10^{-15}\ g$

Considering the FITC-Con A as an equilateral triangle with 9 *nm* edges and a 35.1 nm^2 footprint,[14] the maximal number of FITC-Con A that a SNP surface can accommodate is 1,205 ($4.23 \times 10^4\ nm^2/35.1\ nm^2$), i.e., 1.73×10^{-16} g (the mass of the tetrameric Con A is 102 kDa), corresponding to 0.1 *mg* FITC-Con A/*mg* SNPs (M_{max}).

Therefore, the surface coverage is $M_{exp}/M_{max} \times 100\%$,

where (M_{exp}) is the amount of FITC-Con A immobilized on SNPs obtained experimentally from Figure S-1 (Table S-1).

Part E. Evaluation of FSNP-Con A Adsorption on Polymer Arrays by Fluorescence Imaging

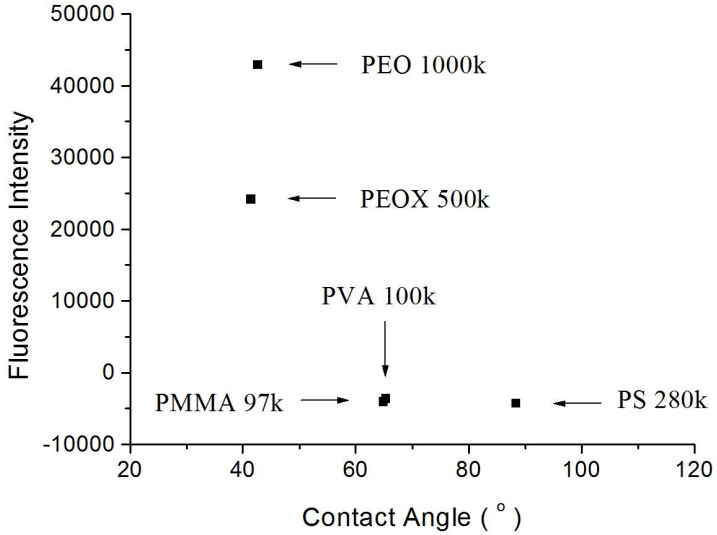


Figure S-3. The fluorescence intensity vs. static water contact angle of polymer. The contact angles were measured on polymer films prepared by spin-coating each polymer on PFPA-functionalized silicon wafer followed by UV immobilization and solvent extraction (see ESI for experimental details. The contact angle values are presented in Table S-2).

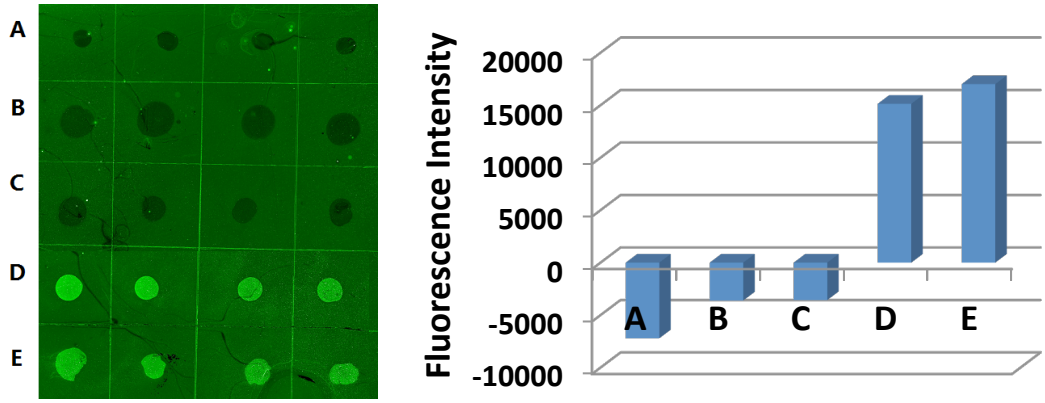


Figure S-4. Fluorescence image (left) and intensities (right) of polymer array after treating with FSNP-Con A. The array contains immobilized PS 280,000 (A), PMMA 97,000 (B), PVA 100,000 (C), PEOX 500,000 (D) and PEO 1,000,000 (E). On the fluorescence intensity plot, each data was the average of the 4 spots on the array, and the error bars were omitted for clarity. FSNP-Con A was prepared from the loading concentration of 50 μg Con A/mL epoxy-FSNPs.

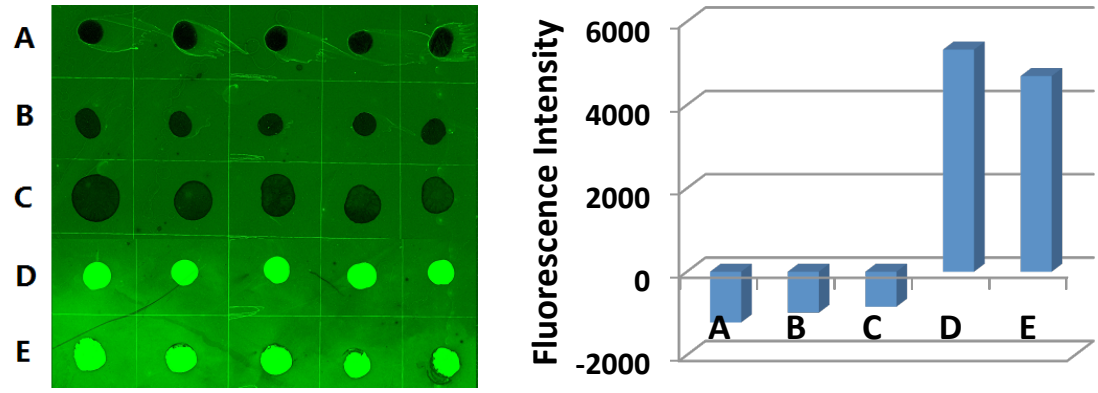


Figure S-5. Fluorescence image (left) and intensities (right) of polymer array after treating with FSNP-Con A. The array contains immobilized PS 280,000 (A), PMMA 97,000 (B), PVA 100,000 (C), PEOX 500,000 (D) and PEO 1,000,000 (E). On the fluorescence intensity plot, each data was the average of the 5 spots on the array, and the error bars were omitted for clarity. FSNP-Con A was prepared from the loading concentration of 10 μg Con A/mL epoxy-FSNPs.

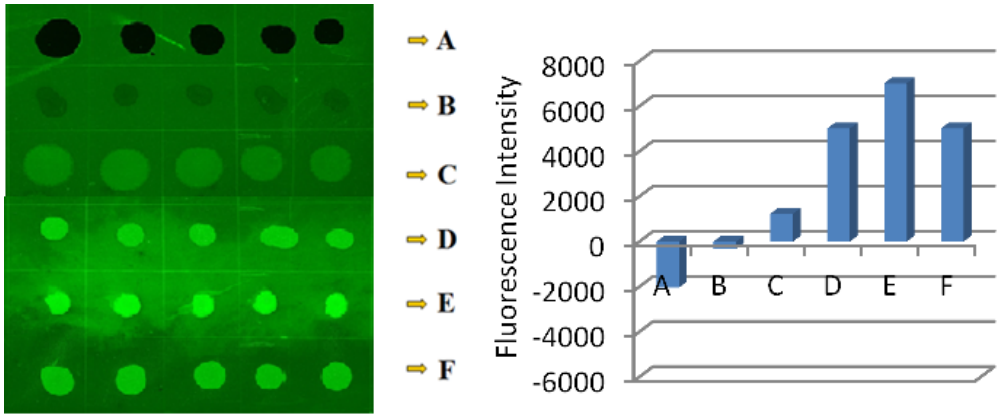


Figure S-6. Fluorescence image (left) and intensities (right) of polymer array after treating with FSNP-Con A. The array contains immobilized PS 280,000 (A), PMMA 97,000 (B), PVA 100,000 (C), PEOX 500,000 (D), PEO 1,000,000 (E), and PVP 1,300,000 (F). On the fluorescence intensity plot, each data was the average of the 5 spots on the array, and the error bars were omitted for clarity. FSNP-Con A was prepared from the loading concentration of 1 μg Con A/mL epoxy-FSNPs.

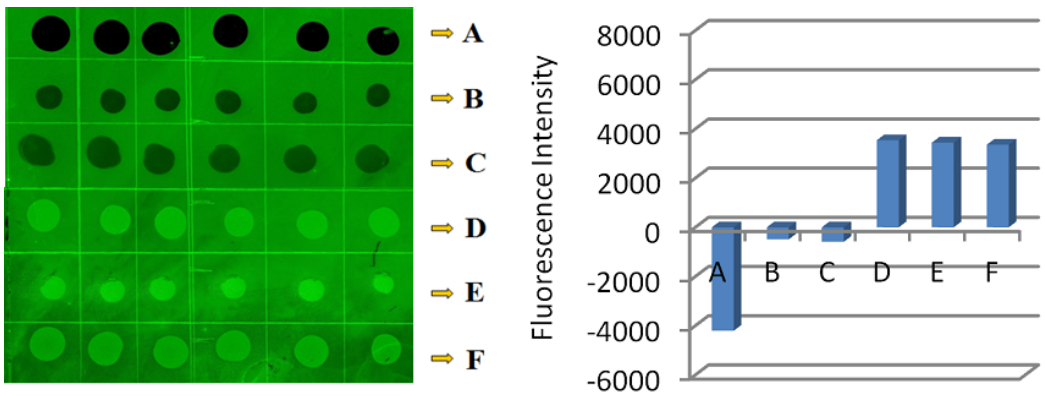


Figure S-7. Fluorescence image (left) and intensities (right) of polymer array after treating with FSNPs. The array contains immobilized PS 280,000 (A), PMMA 97,000 (B), PVA

100,000 (C), PEOX 500,000 (D), PEO 1,000,000 (E), and PVP 1,300,000 (F). On the fluorescence intensity plot, each data was the average of the 6 spots on the array, and the error bars were omitted for clarity.

Part F. Evaluation of FSNP Adsorption on Polymer Arrays by SPR

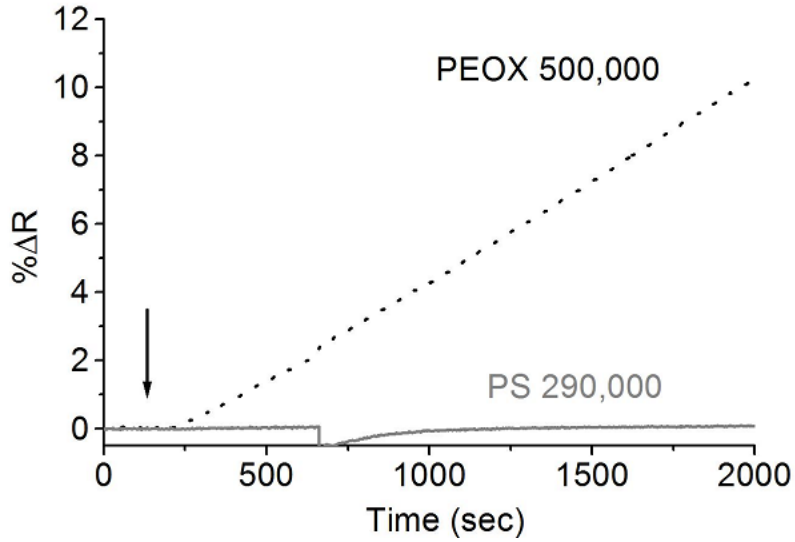


Figure S-8. SPR sensorgram monitoring the adsorption of FSNPs (in pH 7.4 PBS buffer) on a polymer array of PEOX 500,000 and PS 290,000. The array was equilibrated with PBS buffer before the FSNPs solution was injected at 120 s (arrow). For clarity, only one sensorgram is shown for each polymer.

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