Supplementary Information

Sensing lectin-glycan interactions using lectin super-microarrays and glycans labeled with dye-doped silica nanoparticles

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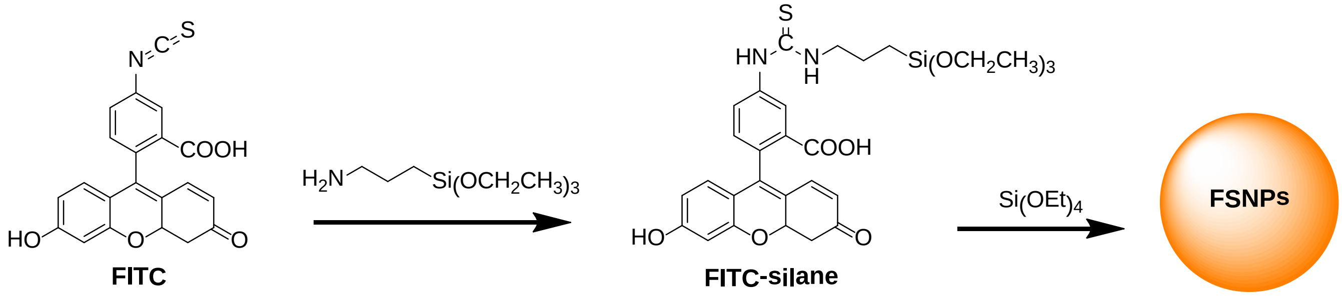
1. Synthesis and characterization of FITC-doped silica nanoparticles (FSNPs)

The FITC-doped silica nanoparticles were synthesized following a modified Stöber protocol (**Scheme S1).**[1,2] The FITC-silane precursor was synthesized by mixing fluorescein isothiocyanate (39 mg, 0.10 mmol, Sigma-Aldrich) with APTMS (23 μL, 0.10 mmol) in absolute ethanol (100 mL), and the solution was stirred at 42 ^{o}C for 24 h. The resulting FITC-silane solution (5 mL) was then mixed with TEOS (2.8 mL), and the mixture was added to a solution of NH_4OH (25%, 2.8 mL) in absolute ethanol (34 mL). A bright yellow colloidal solution was obtained after the reaction mixture was stirred vigorously overnight at room temperature. The resulting FSNPs were characterized by transmission electron microscopy (TEM) and dynamic light scattering (DLS) (**Fig. S1**).



Scheme S1. Synthesis of FSNPs.

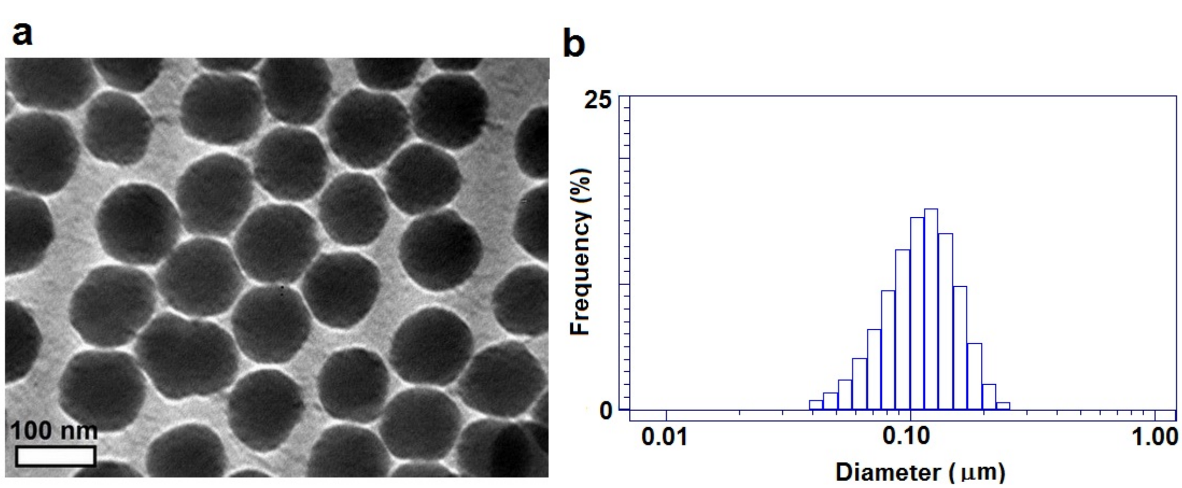


Figure S1. (**a**) TEM image and (**b**) DLS particle size distribution of FSNPs.

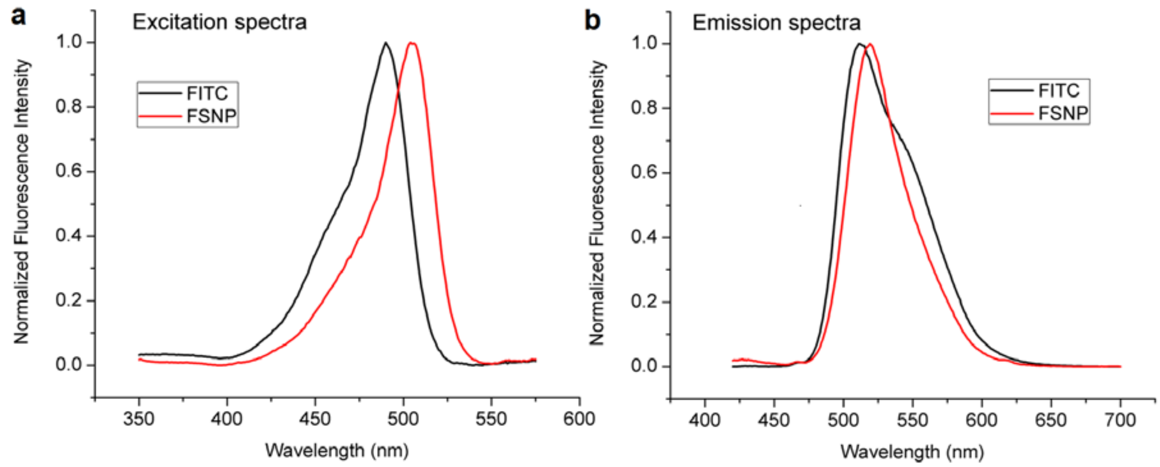


Figure S2. Fluorescence spectra of FITC (black) and FSNPs (red): (**a**) excitation and (**b**) emission.

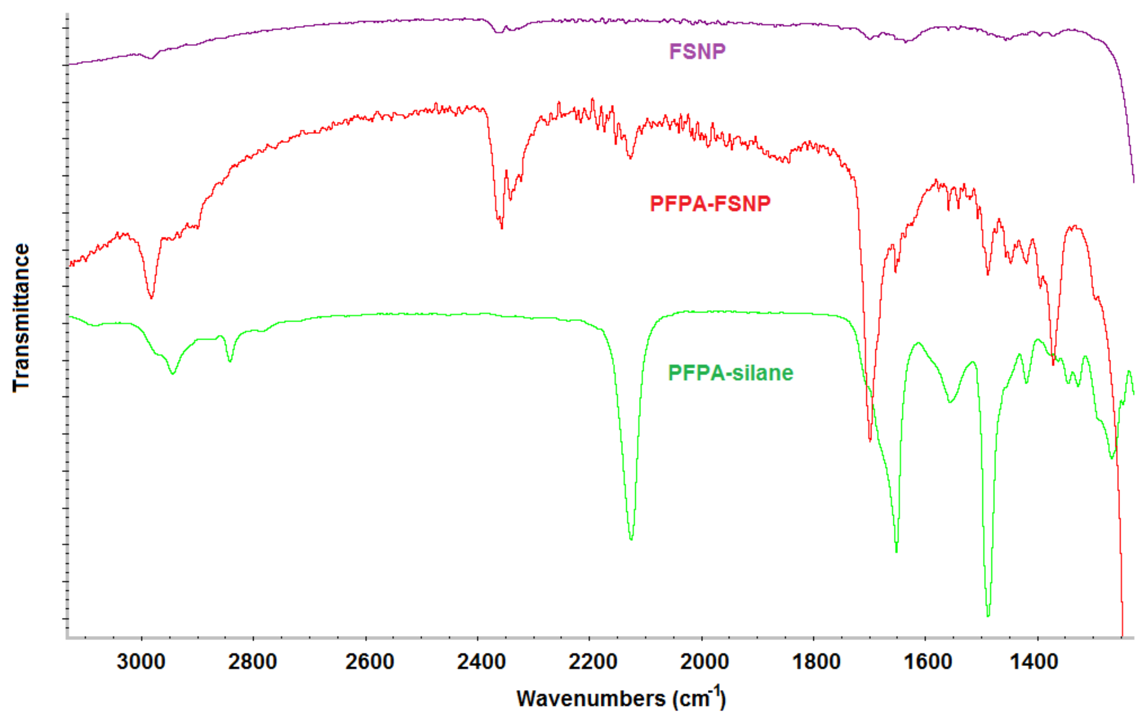


Figure S3. FTIR spectra of FSNPs (purple), PFPA-silane (green) and PFPA-functionalized FSNPs (red).

2. AFM images of lectin microarray spot after incubation with FSNP-Man2

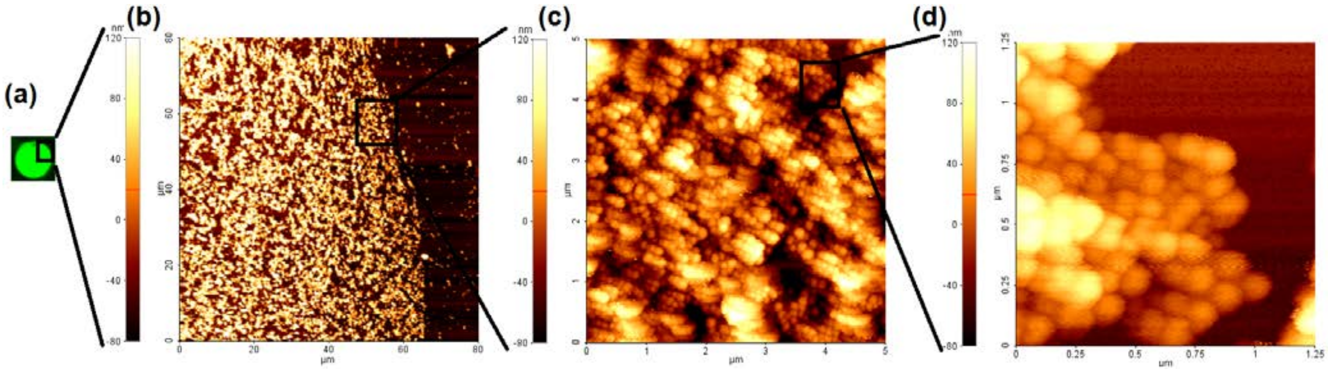


Figure S4. Fluorescence (a) and AFM images (b-d) of a Con A spot after incubating with FSNP-Man2 (1.5 mg/mL) for 2 h. The concentration of the Con A printing solution was 1 mg/mL.

3. Lectins and their corresponding carbohydrate binding ligands

Table S1. Lectins and their corresponding carbohydrate binding ligands.

Lectin		**Origin**	**Glycan specificity**
CVN^{Q50C}	Cyanovirin-N mutant	*Cyanobacteria*	Man(α1,2)Man
CVN^{MutDB}	Cyanovirin-N mutant	*Cyanobacteria*	Man(α1,2)Man
SBA	Soybean Agglutinin	*Glycine max* (soybean)	GalNAc
Con A	Concanavalin A	*Canavaliaensiformis*	Man
PNA	Peanut Agglutinin	*Arachishypogaea* (peanut)	Gal(β1,3)GalNAc /Gal
BS-I	*Bandeiraeasimplicifolia* lectin I	*Griffonia (Bandeiraea) simplicifolia*	GalNAc/Gal
OAA	*Oscillatoriaagardhii* Agglutinin	*Cyanobacteria*	Man(α1,6)Man
PFA	Homolog of OAA	N/A	Man(α1,6)Man
W77	Mutant of OAA	*Cyanobacteria*	Man(α1,6)Man
DBA	*Dolichosbiflorus* Agglutinin	*Dolichosbiflorus* (horse gram)	GalNAc
UEA	*Ulexeuropaeus* Agglutinin I	*Ulexeuropaeus* (Furze gorse)	Fuc
WGA	Wheat Germ Agglutinin	*Triticum vulgaris* (wheat germ)	GlcNAc
RCA-I	*Ricinuscommunis* Agglutinin I	*Ricinuscommunis* (castor bean)	Gal/GalNAc/Lac

4. Glycan densities of FSNP-glycans

Table S2. Glycan densities on FSNP-glycans

Glycan	**Number of glycan per FSNP ($x10^4$)**
Man	5.5
Glc	5.4
Gal	5.4
GlcNAc	5.8
GlcNAc	5.7
Fuc	5.4
2α-Man2	2.9
6α-Man2	3.0
2α,2α-Man3	2.4
3α,6α-Man3	2.3

5. Association constants (K_a) of lectins and glycans

Table S3. Reported association constants (K_a) of lectin-glycan pairs.

Lectin	**Glycan**	**K_a (M^{-1})**
CVN^{Q50C} [3]	2α-Man2	1.43×10^{3} (Domain A) 1.56×10^{4} (Domain B)
	2α,2α-Man3	2.94×10^{5} (Domain A) 2.33×10^{4} (Domain B)
CVN^{MutDB} [4]	2α-Man2	1.32×10^{3}
	2α,2α-Man3	2.94×10^{5}
Con A [5]	Man	8.2×10^{3}
	2α-Man2	4.17×10^{4}
	2α,2α-Man3	3.79×10^{5}
	Glc	1.96×10^{3}
SBA [6]	GalNAc	1.51×10^{6}
PNA [7]	Gal	0.98×10^{3}
	GalNAc	2.43×10^{3}
BS-I [8]	Gal	2.1×10^{4}
	GalNAc	1.87×10^{5}
DBA [9]	GalNAc	4.2×10^{3}
WGA [10]	GlcNAc	0.4×10^{3}
RCA-I [11]	Gal	2.2×10^{3}
	GalNAc	4.84×10^{4}

6. References

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