Online supporting material

Quantitative glycomics from fluidic glycan microarrays

X.-Y. Zhu, Bryan Holtz, Yini Wang, Lai-Xi Wang, Paul E. Orndorff, and Athena Guo

EXPERIMENTAL METHODS

Mannose linked lipid: The α-O-linked mannose-lipid (**3**) was prepared from the conjugation of a functional lipid, 1,2-Dipalmitoyl-*sn*-Glycero-3-Phosphothioethanol (16:0 PTE) (Avanti Polar Lipids, **1**) with a maleimide-activated mannose (**2**), as shown in Fig. S1. The synthesis of (**2**) has been detailed elsewhere.[si] Briefly, we mixed 10mM of (**2**) in d-chloroform with stoichiometric amount of (**1**). The reaction mixture was stirred for 4 days at room temperature. After the completion of the reaction, the solvent was evaporated to give a final product in > 95% yield as monitored by ^{1}H NMR, Fig. S2-4. After the reaction, Fig. S4, we see the disappearance of the ^{1}H peak at 6.73 ppm from sp2 C-H in the maleimide group and appearance of two new ^{1}H peaks at 2.90 & 3.20 ppm, respectively, for the CH2 & CH groups linked to S.[sii]

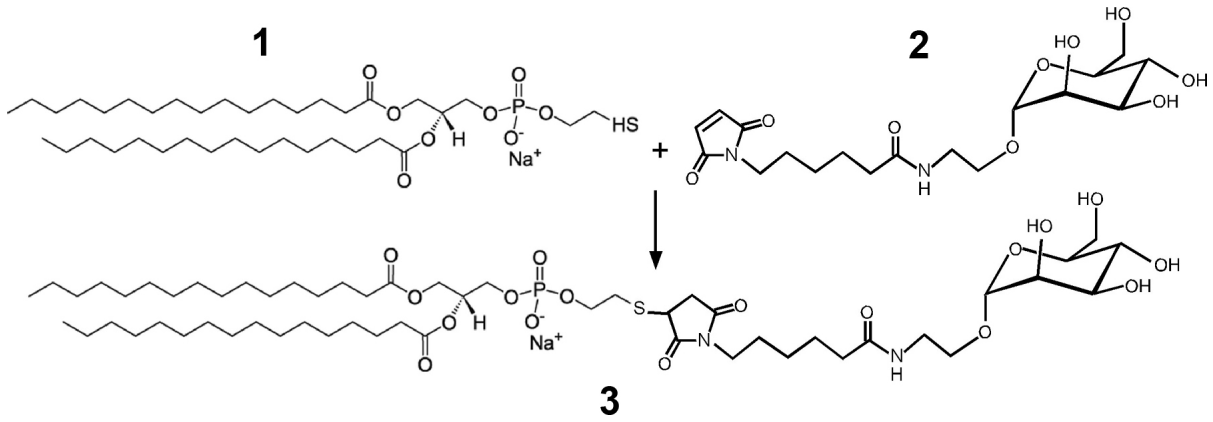

Fig. S1. Schematics for conjugating mannose to the lipid, PTE.

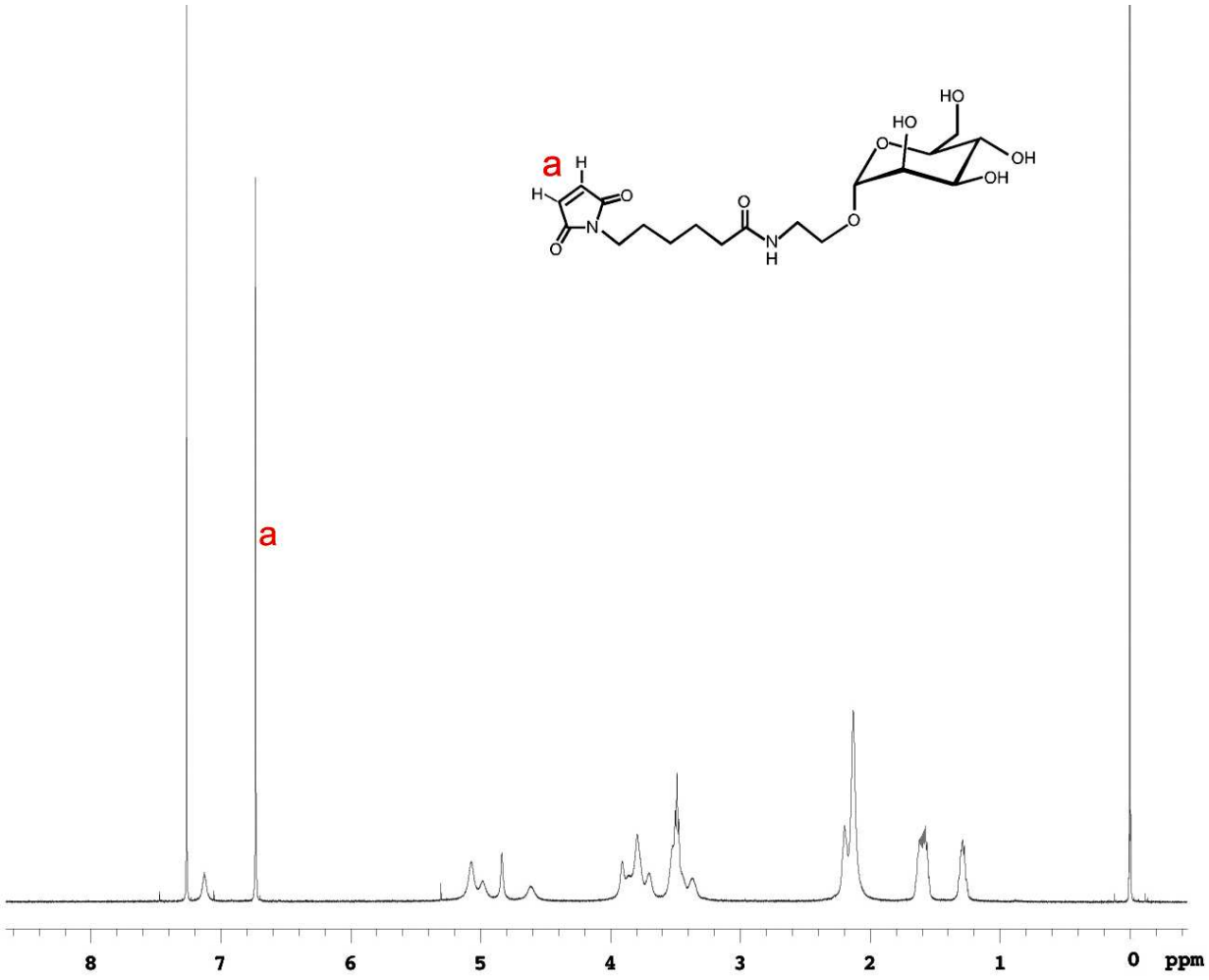

Fig. S2. ^{1}H NMR spectrum of the maleimide-activated mannose. One of the signature peaks on the maleimide group is labeled.

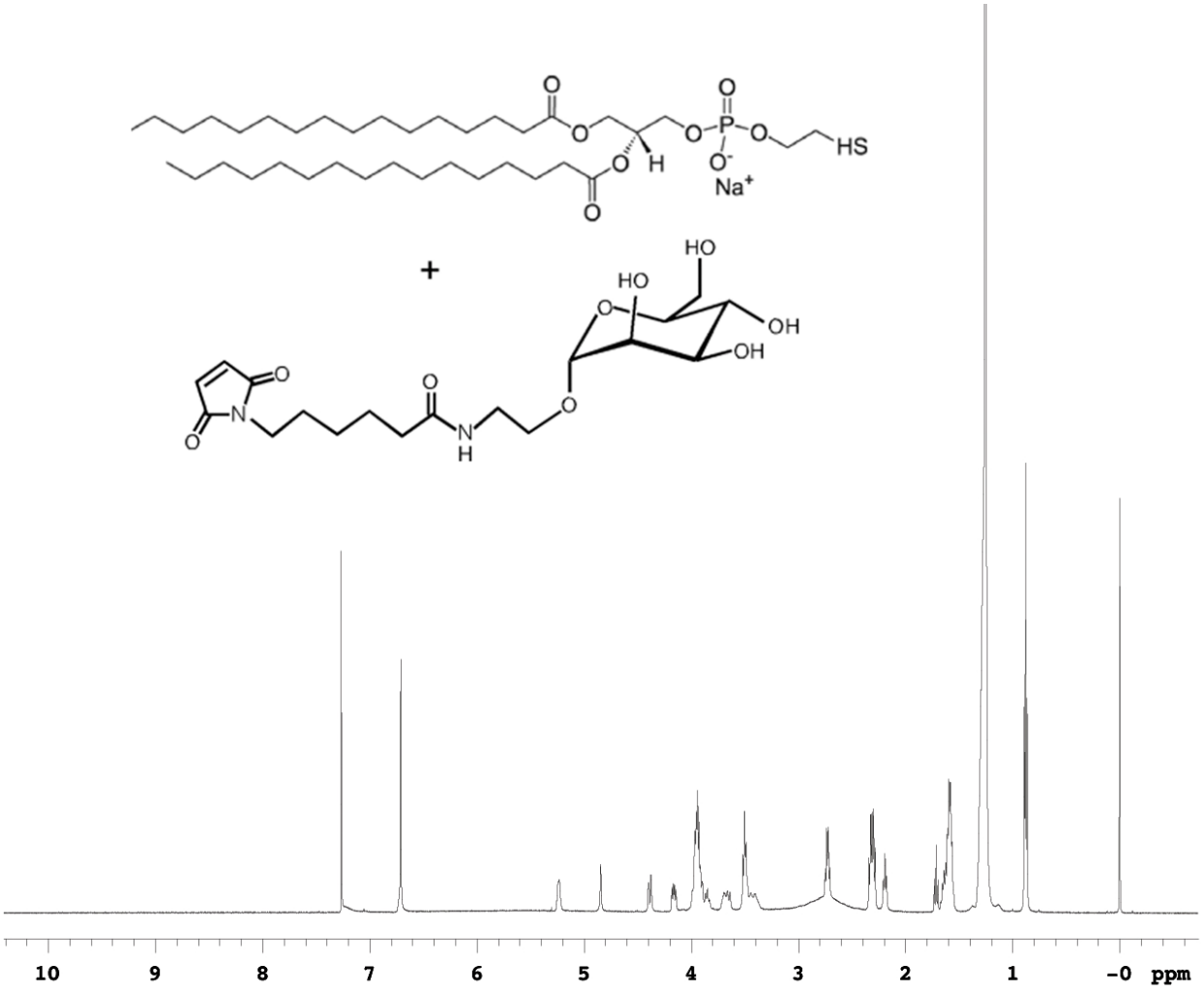

Fig. S3. ^{1}H NMR spectrum of the reaction mixture at time zero of maleimide-activated mannose and the lipid, 1,2-Dipalmitoyl-*sn*-Glycero-3-Phosphothioethanol.

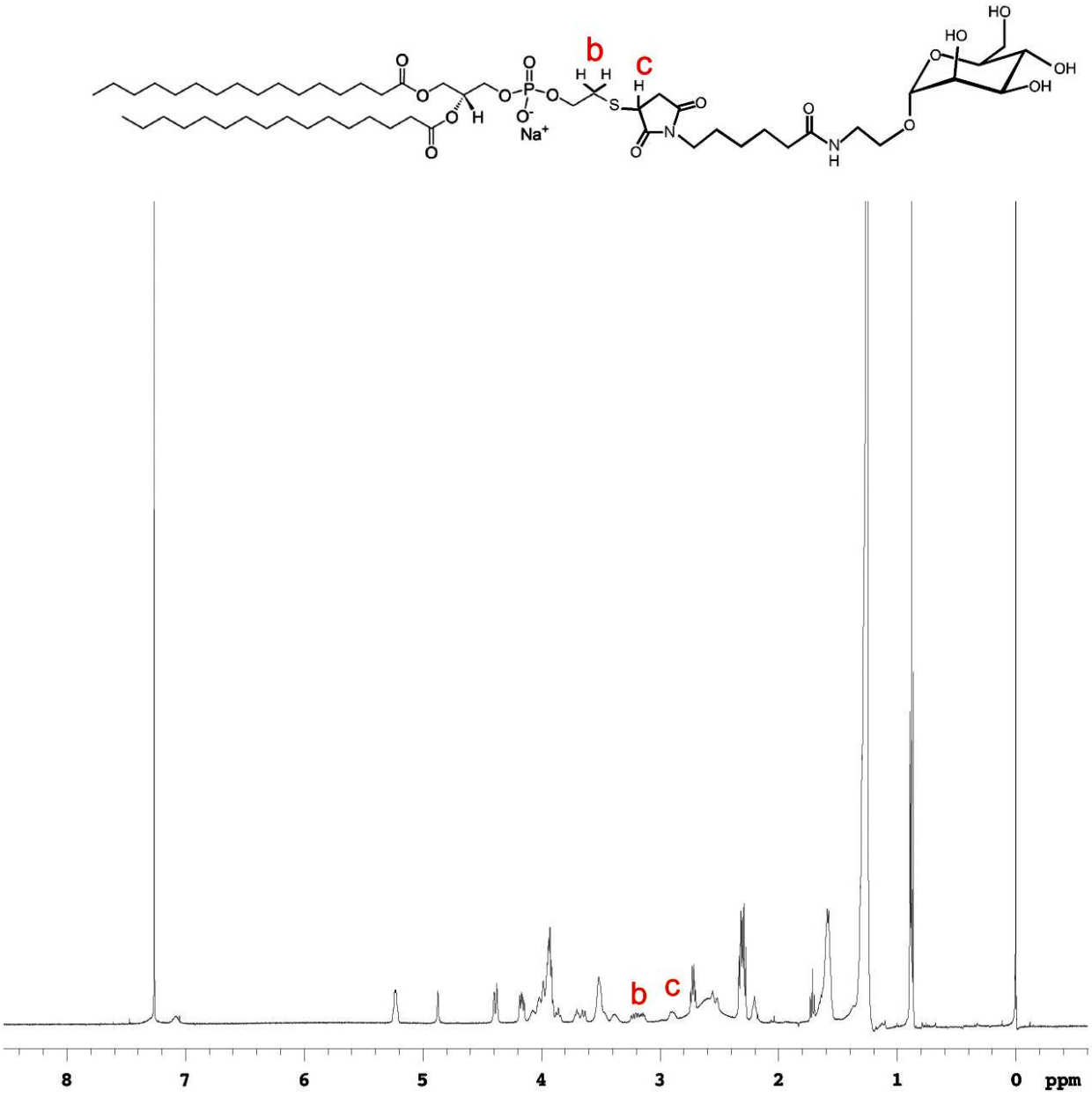

Fig. S4. ^{1}H NMR spectrum of the same reaction mixture as in Fig. 3s, but after 4 days of reaction time. The spectrum is nearly 100% attributed to the α-O-linked mannose-lipid. Two of the signature peaks from the covalent link are labeled.

Mannose presenting nanoparticles. We mixed 85nM of iron oxide nanoparticles (Ocean Nanotech, Springdale, AR, USA) in 10 mM of sodium borate buffer (pH=5.0) with 260 μM of dimethylaminopropyl] carbodiimide hydrochloride (EDC) and 433 μM of N-hydroxysuccinimide (NHS) in a final volume of 0.4 ml. The reaction mixture was gently stirred for 20 min at room temperature and then added 0.4 mL sodium borate buffer (20 mM, pH=8.0). To another vial, 14 μmol of D-Mannosamine hydrochloride (Sigma-Aldrich) was dissolved in 50 μL borate buffer (20 mM, pH=8.0) and the pH was adjusted to 8 with 1M sodium hydroxide. The mannose solution was then added to the nanoparticle reaction mixture to give a final concentration of 17.5 mM. The reaction was carried out for 2 h under stirring and then stopped by the addition of 34 μL Tris buffer (10 mM, pH=7.4) and stirred for an additional 10 min. We decanted the liquid using a magnetic separator. The particles were washed with 0.8 mL Tris buffer twice, re-suspended in 0.2 mL Tris buffer and stored at 4 ^{o}C. We used Fourier transform infrared spectroscopy (FTIR) to estimate the percentage of surface –COOH groups that were conjugated with mannose via the reaction in Fig. S5. Figure S5 shows FTIR spectra before and after the conjugation reaction. Base on changes in the COO spectral region, we estimated that ~30% of surface –COOH groups were conjugated to mannose groups. This estimate was mainly based on the intensity loss of the COOH peak at 1700 cm^{-1} after the conjugation reaction.

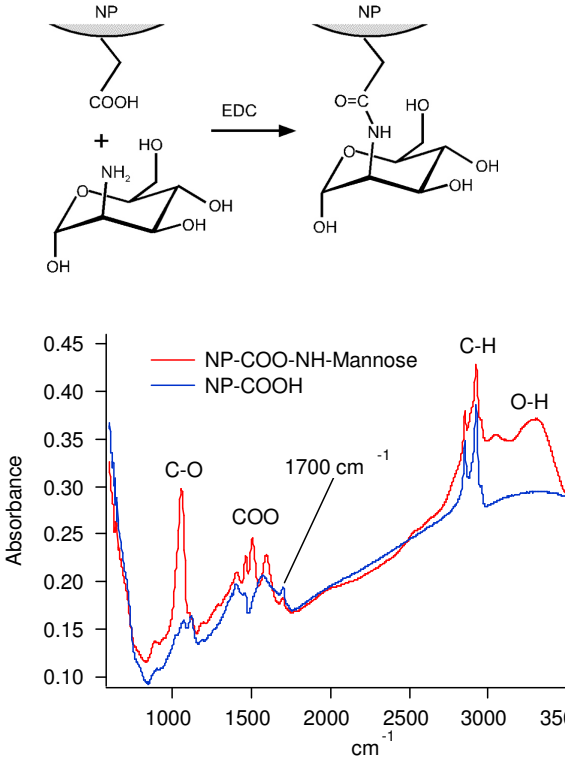

Fig. S5. Upper: Reaction for conjugating mannose to the carboxylic acid groups on the nanoparticle; Lower: FTIR spectra of COOH terminated magnetic nanoparticles before and after conjugation to mannose. The intensity of the COOH peak at 1700 cm-1 decreases by ~30% after the rection. The successful conjugation of mannose to the nanoparticle is also verified by new peaks assigned to mannose (C-O, O-H, & new COO peaks).

Preparation of supported lipid bilayers. Lipids (eggPC, Ma-PTE, and TR-DHPE) were dissolved/mixed in CH_2Cl_2 and, after the evaporation of CH_2Cl_2, re-suspended in 50 mM tris(hydroxymethyl)aminomethane (Tris) buffer (pH=7.4) containing 25 mM NaCl to reach a total lipid concentration of 1mg/ml. Suspension of the lipid mixture after prefiltration through 0.45 μm pores was forced through a polycarbonate filter with 50 nm pores more than 11 times to prepare the SUV solution. $CaCl_2$ (5 mM) and glycerol (10-20%) was added to the SUV solution; the former facilitated SUV rapture and SLB formation while the latter increased viscosity and

decreased drying rate during microarray fabrication. The SUV solution was arrayed onto the cholesteryl-PEG surface (MicroSurfaces, Minneapolis, MN), incubate at R.T. in a humidified chamber (RH ~80%) for 30 minutes, and then transferred to a beaker containing >100 mL of washing buffer (50 mM Tris, 25 mM NaCl, pH = 7.4). We carried out washing by gently moving the sample (with a pair of tweezers) under the washing solution for ~3 min. The mannose microarray was kept hydrated (under a thin film of buffer solution) until use (within ~ 1 hour). As detailed before, lipids in the SLB in each arrayed spot remain fluidic in the hydrated state, as shown by fluorescence recovery after photobleaching (FRAP). Details on FRAP results have been reported in our previous work.[siii] Fig. S6 shows FRAP images taken at 60s and 900s after photobleaching for an SLB containing 0.5% mannose-PTE. Nearly complete recovery of fluorescence intensity is observed, thus establishing the fluidic nature of lipids in the SLB.

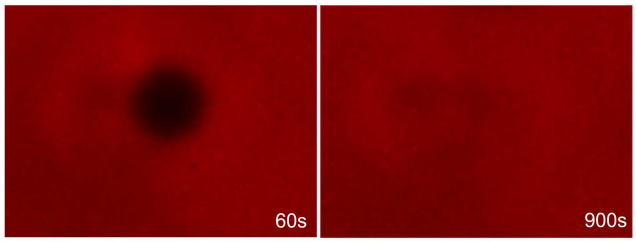

Fig. S6. Fluorescence images (240 μm x 300 μm) taken at 60s and 900s after photobleaching for a hydrated SLB containing 0.5% mannose-PTE, 0.5% TR-DHPE, 99% eggPC.

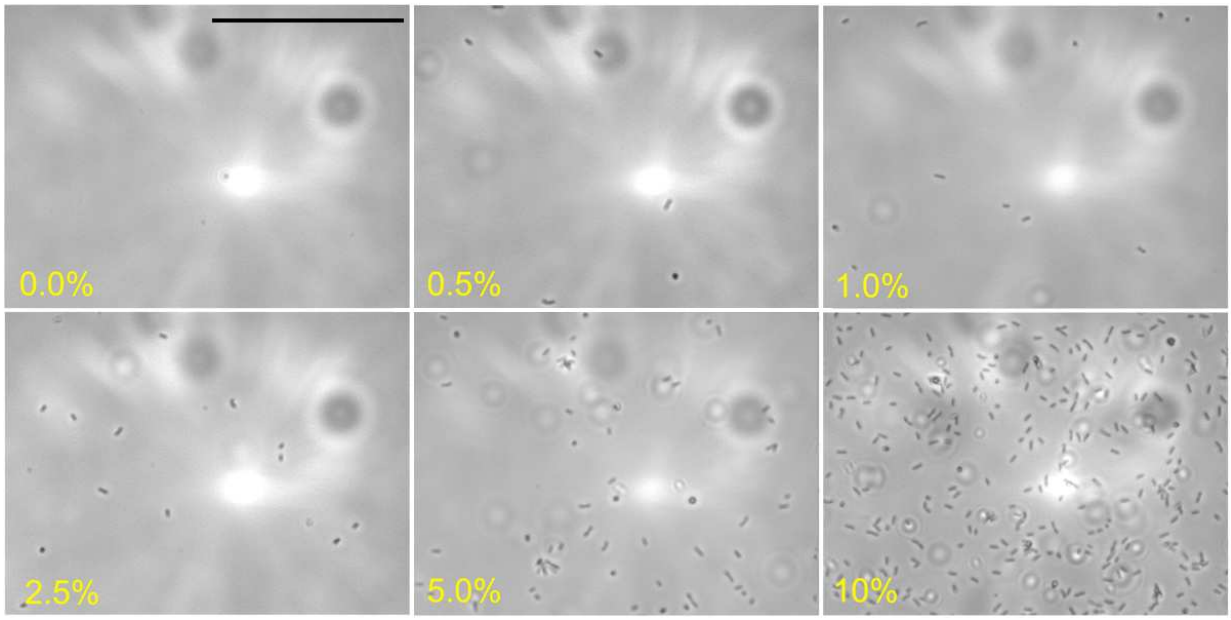

Fig. S7. Optical microscope images of E-coli 178 adsorbed on the supported lipid bilayer containing 0.0-10.0% and 10% mannose. Each image is zoomed inside the boundary of each SLB spot. The adsorbed E-coli appears as elongated spots. Scale bar = 50 μm.

E. coli adsorption. We took 1mL of bacteria cell suspension, span down cells, washed at least three times with 1 mL of 50mM Tris (pH7.4) containing 25mM NaCl, 1mM $MnCl_2$ and 1mM $CaCl_2$, and then resuspended the cells in 1 mL of the same buffer and diluted 12.5 fold (final volume = 1.5 mL) to get a total of $6x10^6$ of bacteria cells for binding experiment. The mannose

microarray presenting surface (coverslip) was incubated with the *E.coli* bacteria solution under static conditions (no shear flow) at room temperature in a humidified chamber for 2.5 hours. The sample was then transferred to a beaker containing >300 mL of washing buffer, gently moved around under the washing solution for ~5 minutes, and then laid-down on a glass side with a well for imaging (60x) under an optical microscope (Nikon 50i). Representative images of E. coli adsorption on the SLBs with different percentages of mannose (0-10%) are illustrated in Fig. S7. We repeated each experiment at least 10 times to perform rigorous statistical analysis. As negative control, we used the ORN208 strain, as shown in Fig. S8. We observe no ORN208 adhesion for mannose percentage $\leq$ 5%. A low density of ORN208 adhesion was observed only for mannose percentage > 5%.

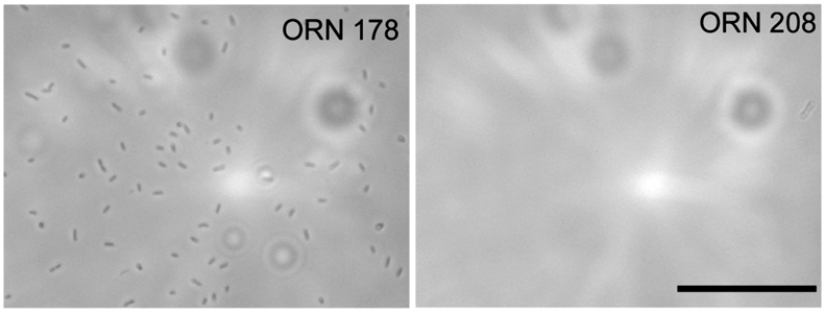

Fig. S8. Specific binding of *E. Coli* ORN 178, but not ORN 208, to a fluidic SLB presenting 5% mannose. Scale bar = 50μm.

Nanoparticle inhibition assay. We prepared SLB containing 7.5% mannose on the cholesteryl-PEG coated coverslip. The coverslip was incubated with 300 μL of *E. coli* bacteria solution mixed containing certain concentration (0-20 pM) of mannose-conjugated nanoparticles. at R.T. for 1 hr and 45' in a humidified chamber. The coverslip was washed with >10 ml of buffer to remove excess bacteria and nanoparticles, laid-down on a glass side with well, and imaged (60x) under an optical microscope. Images such as those in Fig. S7 and Fig. S8 are used to quantify the density of adsorbed E. coli as a function of solution phase nanoparticle concentration.

Complete citation of Ref. 8.

Blixt, A.; Head, S.; Mondala, T.; Scanlan, C.; Huflejt, M. E.; Alvarez, R.; Bryan, M. C.; Fazio, F.; Calarese, D.; Stevens, J.; Razi, N.; Stevens, D. J.; Skehel, J. J.; van Die, I.; Burton, D. R.; Wilson, I. A.; Cummings, R.; Bovin, N.; Wong, C.-W.; Paulson, J. C. *Proc. Natl. Acad. Sci. USA*. **2004**, *101*, 17033-17038.

[si] Ni, J.; Singh, S.; Wang, L. X. *Bioconj. Chem.* **2003**, *14*, 232-238.

[sii] Pretsch, E. ; Buhlmann, P.; Affolter, C. *Structure Determination of Organic Compounds* (Springer, Berlin, **2000**)

[siii] Deng, Y. , Wang, Y., Holtz, B., Li, J.-Y., Traaseth, N., Veglia, G., Stottrup, B., Elde, R., Pei, D., Guo, A.; Zhu, X.-Y. *J. Am. Chem. Soc.* **2008**, *130*, 6267-6271.