Electronic Supplementary Information

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Deposition of nanoparticles onto polysaccharide-coated surfaces: Implications for nanoparticle-biofilm interactions

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Materials and Methods

Materials. Hematite (α-Fe_2O_3) NPs (pseudo-hexagonal platelets) were prepared as described by Schwertmann and Cornell (2000)[1]. Spherical non-functionalized silica (SiO_2) NPs were obtained from nanoComposix, Inc. (San Diego, CA). NPs were characterized for their sizes and zeta potentials as described in the SI. Polysaccharides used in this study were sodium alginate, dextran sulfate, dextran, and chitosan. Their characteristics are provided in Table S1. All chemicals used in this study were obtained from VWR International (Radnor, PA) or Sigma-Aldrich (St. Louis, MO) unless specified otherwise.

NP characterization. The hydrodynamic diameters by dynamic light scattering (DLS) and zeta potentials of hematite and silica NPs were determined using a Malvern Zetasizer NS (Worcestershire, U.K.). All measurements were performed under test conditions used in the NP deposition experiments by QCM as described below. The zeta potentials of NPs were calculated from measured electrophoretic mobility values using the Smoluchowski approximation.

Polysaccharide-coated surface characterization. Silica sensor surfaces coated with polysaccharides were characterized as described below. Detailed information for each procedure is provided below.

Surface zeta potential. The surface zeta potentials of polysaccharide-coated silica sensors in 10 mM NaCl (pH 5.7) were determined using the Surface Zeta Potential Cell (ZEN1020) from Malvern Instruments (Worcestershire, UK) following the protocol as described by Corbett et al. (2012)[2]. In brief, small sections of clean QCM silica sensors were immobilized onto sample holders and

exposed to 10 mM HEPES (pH 7.4) for 10 min. Then the surfaces were treated with poly-L-lysine as necessary and the polysaccharide solution with 10 mM HEPES rinses after each treatment step. All solution conditions were the same as the QCM experiment setup described below. After the final clean buffer rinse, samples were placed in the measurement cell. Mineral oil emulsified in 10 mM NaCl (pH 5.7) through mixing by sonication was used as the tracer particles in each measurement. The ZEN 1020 measurement cell was placed in the Malvern Zetasizer NS and the electrophoretic mobility of the tracer particles were measured at six different displacements from the sample surface. The values were used to calculate the surface zeta potential by the Zetasizer software.

Contact angle measurements. The surface wettability of silica sensors coated with each polysaccharide was determined through contact angle measurements using the KRÜSS EasyDrop (Hamburg, Germany). Silica sensors with adsorbed polysaccharide layers were prepared on a Q-Sense E1 quartz crystal microbalance with dissipation monitoring (QCM-D) as described below in "NP deposition experiments by QCM". Upon washing loosely bound polysaccharides with the background buffer solution, the sensors were removed and dried under a gentle flow of nitrogen gas. Contact angle measurements were performed on the KRÜSS EasyDrop (Hamburg, Germany) by dropping 50 μL of distilled H_2O onto the surface and capturing an image within 10 s. Values reported are averages of at least three measurements.

Atomic force microscopy (AFM) and Kelvin probe force microscopy (KPFM). The surface topography of silica sensors coated with each polysaccharide was evaluated by contact mode AFM in air using a Pacific Nanotechnology Nano-R2 microscope. The surface potential variability of

polysaccharide-coated silica sensors were determined by KPFM using a MFP-3D AFM (Asylum Research, Santa Barbara, CA) with a lift height of 50 nm as described by Reitzel et al. (2001)[3]. Silica sensors with adsorbed polysaccharide layers were prepared by QCM as described below followed by drying under a gentle flow of N_2 gas and used in the microscopy analyses. At least six separate areas were scanned per sample. Surface areas and surface roughness (root mean squares) were determined from AFM images using the surface area calculation in the *Gwyddion* software package (http://gwyddion.net/).

Charge density measurements by QCM. The negative charge densities of silica sensors coated with alginate or dextran sulfate were determined by QCM as described by Perry and Coronell (2013)[4]. In brief, polysaccharide layers were adsorbed onto silica sensors as described below. Following rinsing of the sensors with clean 10 mM HEPES buffer and subsequent rinsing with milliQ H_2O, the polysaccharide-coated sensors were subjected to alternate exposure to 1 mM CsOH solution and milliQ H_2O. Adsorbed masses of Cs from the second exposure were used to calculate the average negative charge density of each surface. Moles of adsorbed CsOH per nm^2 as measured by QCM were converted to sites/nm^2 of negative charge density using the Avogadro's number.

NP deposition experiments by QCM. Silica quartz crystal sensors (14 mm diameter) with a fundamental resonant frequency of 5 MHz (QSX 303, Q-Sense, Gothenburg, Sweden) were used in the QCM experiments. Sensors were cleaned by soaking in 2% sodium dodecyl sulfate solution for at least 30 min followed by UV-ozone treatment for 10 min. Upon mounting a silica sensor into the QCM module, 10 mM sodium 4-(2-hydroxyethyl) piperazine-1-ethanesulfonate (HEPES) (pH 7.4) was flowed through the system for at least 10 min. After obtaining a stable baseline (± 0.05 Hz/s),

the polysaccharide layer was adsorbed with or without precoating with poly-L-lysine (PLL). As silica sensors are negatively-charged, the PLL coating provided a positive charge surface for favorable adsorption of anionic and neutral polysaccharides. For alginate (1 mg/mL), dextran sulfate (0.5 mg/mL), and dextran (5 mg/mL), 100 mg/L PLL solution was flowed through followed by rinsing with the background buffer solution, and then the polysaccharide solution was introduced. For chitosan (2 mg/mL), the polysaccharide solution was flowed through immediately following the clean buffer. All organic solutions were made in 10 mM HEPES buffer (pH 7.4) at concentrations that resulted in an organic layer of similar average thickness on the QCM sensor as determined by the Voigt viscoelasticity model (assuming even coverage). All solutions were flowed through as long as necessary to obtain a stable reading. The system was rinsed with clean buffer for at least 10 min following polysaccharide adsorption. Subsequently, 10 mM NaCl (pH 5.7) was introduced until a stable baseline was obtained. Then a 10 mg/L working suspension of NPs in 10 mM NaCl was pumped through to interact with the substrate surface. Changes in resonance frequency (Δf) and in resonance dissipation (ΔD) were monitored over time as NPs deposited onto the surface, while constant and intermittent alternating voltages were applied to the quartz crystal. The mass deposited on the QCM sensor was calculated using the Sauerbrey equation. With the best signal-to-noise ratio, Δf and ΔD obtained from the third overtone are presented in this study. The extent of deposition was determined after the sensor electrode was washed with background electrolyte solution to remove unbound NPs and a new stable frequency reading was reached. The initial deposition rate was determined by calculating the slope of the change in adsorbed mass over the first ten minutes of deposition. The temperature of the solutions in the flow module was maintained at 25 ± 0.02°C for all experiments. The flow rate was kept constant at 100 µL/min. All test conditions were run at least in triplicate. Real time dynamic light scattering (DLS)

measurements were run simultaneously during QCM adsorption experiments to monitor NP size changes over time in each test solution.

Statistical analysis. A two-tailed Student's *t* test was used to compare sample (i.e. treatment) values to control values. Results were reported as significantly different with a p-value ≤ 0.05 (n=3, unless otherwise noted).

TABLES AND FIGURES

Table S1. Characteristics of polysaccharides used in this study.

Name	Monomer	Size (Da) and other characteristics	pK_a
Alginate	D-mannuronate L-glucuronate	Approx. 70,000 Da	3.3 (-COOH)[5]
Dextran sulfate	Sulfated glucose	9,000-20,000 Da	4.0 ($-SO_3H$)[6]
Dextran	D-glucose	74,000 Da	11.8 (-OH)[7]
Chitosan	*N*-acetyl-glucosamine	Medium range, 75-85% deacetylated	6.63-6.78 ($-NH_2$)[8]

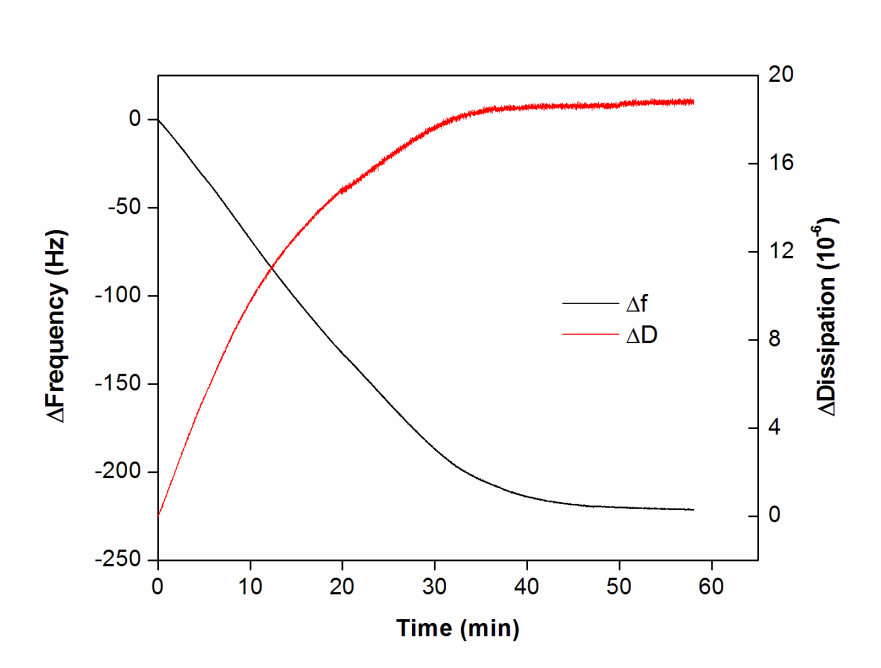

Figure S1. Representative third overtone QCM-D data for hematite NP deposition onto a polysaccharide-coated silica sensor. Flow through of 10 mg/L hematite NP in 10 mM NaCl (pH 5.7) started at t=0 after a stable baseline in 10 mM NaCl was obtained (not shown).

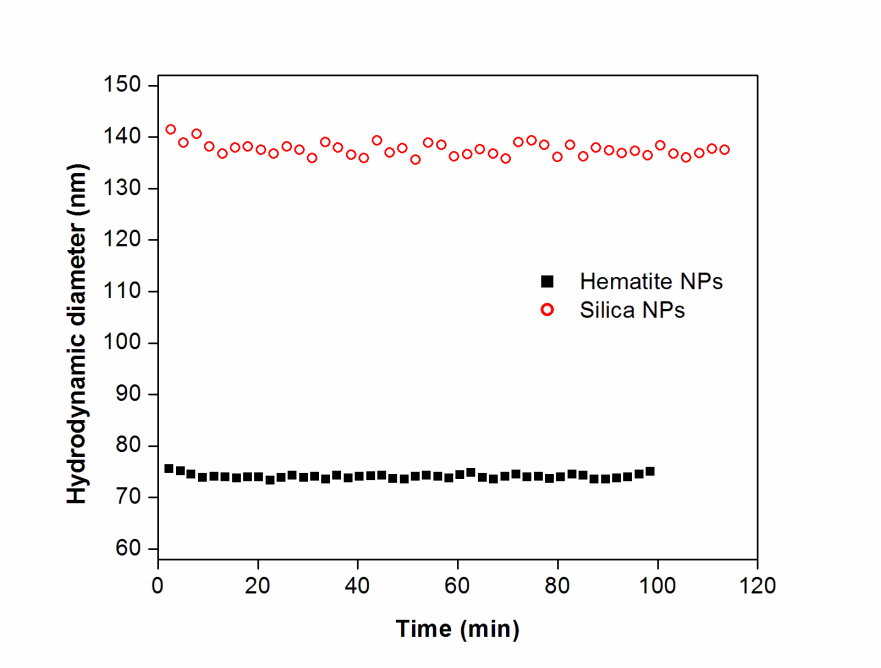

Figure S2. Change in hydrodynamic diameters of hematite and silica NPs in 10 mM NaCl (10 mg/L; pH 5.7) measured by DLS over the duration of NP deposition experiments. Data shown are from representative experiments.

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