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Supporting Information

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Metallic striped Nanowires as Multiplexed Immunoassay Platform for Pathogen Detection**

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Materials and Methods

Materials. Ovalbumin (Ova) and Bovine serum albumin (BSA) proteins were obtained from Sigma (St. Louis, MO), *Bacillus globigii* (Bg) spores were obtained from US Army Dugway Proving grounds (Dugway, UT), and *E-coli* MS2 bacteriophage was purchased from American Type Culture Collection (ATCC; Manassas, VA). Solutions of antigens were freshly prepared in 10 mM PBS buffer (pH 7.4), from obtained stock solution of Bg spores at 10^9 cfu/ml, and stock solution of MS2 bacteriophage at 10^{10} pfu/ml. Both biotin-labeled and unlabeled antibodies (Ab), which include anti-Bg Ab (monoclonal), anti-MS2 Ab (polyclonal) and anti-Ova Ab (polyclonal), were obtained from Tetracore (Gaithersburg, MD). The design and production of the nanowires (nanobarcodes) has previously been described,[7] and they are commercially available from Nanoplex Technologies Inc. (Mountain View, CA). Briefly, alternating layers of gold and silver (and nickel) are electroporated into the pores of an alumina template, resulting in the formation of striped nanowires. Following synthesis of the nanowires, the alumina template is removed using a strong base. The particles are subsequently coated in mercaptoundecanoic acid (MUA), allowing the formation of a carboxyl terminated self-assembled monolayer (SAM) on the particle surface. For this investigation, nanowires containing 6-striped patterns of gold and silver were used for

striping decoding analysis. The nanowires have dimensions of ~6.0 μm length and ~0.25 μm diameter. A 50 nm stripe of Ni is included at both ends of the nanowire to confer magnetic properties. The Ni-tipped nanowires are usually employed in the assay within the next 2-3 weeks and are observed to be stable in 4°C MUA-coated solution within that time-frame.

Magnetophoretic Mobility Analysis. The magnetophoretic mobility describes the ratio of the velocity induced on the particle by the magnetic field, v_{MP}, and magnetic energy density, $S_m = (\mathbf{B}\cdot\nabla)\mathbf{B}/2m_o$, a function of the magnetic field, $\boldsymbol{B}$, and the permeability in a vacuum, m_o.[1] For a cylindrical-shaped particle moving perpendicular to its long axis, the mobility of the nanowire is,

$$m_{MP} = \frac{(c_p - c_m)r^2 L_{Ni}}{4hL_p}\left(\ln(L_p / r) + .5\right)$$

where c_p and c_m are the susceptibilities of the particle and surrounding medium respectively, h is the viscosity of the medium, r is the radius of the nanowire, L_p, is the full length of the nanowire, and L_{Ni}, is the total length of the Ni segments.[2,3] The magnetophoretic mobility of the nanowires was measured for each Ni segment length. The nanowires were imaged in a 200 μm by 2 mm rectangular cross section borosilicate capillary (Vitrocom; Mountain Lakes, NJ). The capillary was mounted vertically in an acrylic fixture to minimize interaction of particles with capillary walls during settling. The capillary was filled with DI water (pH 5.5, 2 μS/cm) and capped at the ends to ensure negligible fluid flow. The magnetic field was applied using NdFeB permanent magnets aligned with the field lines parallel to the capillary and the field gradient in the transverse direction. The nanowires aligned vertically in the capillary and migrated in a horizontal direction toward the magnet. The imaging system consisted of a Nikon inverted epifluorescent microscope with a ninety-degree mirror attachment between the turret and objective to rotate the image plane from horizontal to vertical. Back illumination through the channel was provided by an external halogen light

source with a fiber optic light guide (Carl Zeiss MicroImaging). A Nikon ELWD air immersion objective (M=60, NA 0.7) with a working distance of 2.1 mm was used to view the center plane of the capillary. Images of the particles within the capillary were recorded using a Cooke Pixelfly CCD camera (Cooke Corporation; Romulus, MI) with a 640 by 480 pixel array and 12-bit readout resolution. The camera was externally triggered with a pulse generator (Berkeley Nucleonics Corporation; San Rafael, CA) to obtain images at a fixed frame rate of 2 fps. A 0.6x demagnifying lens was included in the optical path on the camera port to enable the CCD array to capture a larger field of view with a negligible loss in image resolution. A total of 1061 images were taken for each experiment.

Magnetophoretic velocities were determined from the particle images using particle-tracking techniques to locate particles and match them between images. The slope of the velocity versus magnetic energy density curve was calculated from five magnetic energy density values to determine the average mobility. To measure the magnetic energy, magnetic field was applied through the channel using Neodymium-Iron-Boron (NdFeB) permanent magnets. A Ni-alloy cone attached to the magnets focused the field to a point 1 mm from the wall of the channel. This focusing provides high field gradients across the channel while maintaining high field strength. The magnetic field and field gradient were measured using a F.W. Bell 5080 Gauss/Teslameter (F.W. Bell, Orlando, FL) with a flat-end probe. Changing the permanent magnets varied the field strength and gradient and therefore the magnetic energy density.

The residual nanowire clumping effect after exposure to magnets was analyzed by exposing the nanowires in DI water to a 300 mT magnetic field for 30 min and then placing them in a 96 well plate with PBS buffer to simulate the assay conditions. The nanowires were imaged at the bottom surface of each well. Approximately twenty images were taken of each well containing nanowires with different Ni segment lengths. On average, each image

included on the order of 100 nanowires. The segmentation scheme used to analyze the images utilized a Prewitt edge-detection algorithm to detect particles and clumps of particles. The feature outlines were eroded and dilated with a three by three pixel diamond-shaped structuring element (with pixel values at four corners set to zero and other pixel values set to unity) to remove spurious pixels. Unbroken particle or clump outlines were then filled and size and eccentricity thresholds distinguished single particles from those in clumps. The population of clumps was determined by dividing the total clump area by the theoretical area of a single particle. The ratio of single nanowire to those in clumps was then calculated for each Ni segment length.

References:

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