

Selective Noncovalent Adsorption of Protein to Bifunctional Metallic Nanowire Surfaces

Nira S. Birenbaum,[†] Bert T. Lai,[†] Christopher S. Chen,[‡] Daniel H. Reich,[§] and Gerald J. Meyer^{*,†}

Departments of Chemistry, Biomedical Engineering, and Physics and Astronomy, Johns Hopkins University, 3400 North Charles Street, Baltimore, Maryland 21218

Received September 5, 2003

Metallic nanowires composed of nickel and gold as well as bimetallic nickel–gold nanowires were fabricated via templated electrodeposition in nanoporous alumina membranes. Gold surfaces were functionalized with alkanethiols with terminal hexa(ethylene glycol) groups (EG₆), while nickel surfaces were functionalized with palmitic acid, a 16-carbon fatty acid. When exposed to a fluorescently tagged protein, hydrophobic nickel wires exhibited bright fluorescence while EG₆-terminated gold wires did not, indicating that the protein did not adhere to the EG₆-functionalized nanowires. Nickel–gold nanowires presenting distinct segments of alkyl and EG₆ surfaces were also exposed to the fluorescent protein. Intense fluorescence was only observed on the nickel segment of these wires, demonstrating that proteins selectively adsorbed to one portion of these multicomponent nanostructures.

Metallic nanoparticles can be derivatized by binding organic molecules to the surface.¹ This surface functionalization often occurs via the formation of self-assembled monolayers (SAMs) similar to those that have been so well characterized on planar surfaces.² These monolayers can then be further modified either by formation of new covalent bonds³ or through noncovalent interactions.⁴ Noncovalent interactions are of critical importance in many biological systems, including the complex tertiary structure of proteins and the hydrogen bonding network that holds together complementary strands of DNA. Hydrophobic and hydrophilic interactions are involved in passive adsorption of proteins to surfaces. Prime and Whitesides showed that SAMs composed of a mixture of hydrophobic and hydrophilic compounds on planar gold were more resistant to protein adsorption as the fraction of hydrophilic molecules in the monolayer increased.⁵ They also demonstrated that monolayers which terminated in oligo(ethylene glycol) groups were the most effective at preventing the noncovalent attachment of a variety of proteins. We have succeeded in applying these same principles to nanowire surfaces. These high-aspect-ratio nanoparticles can be composed of different metal components in distinct segments, allowing for spatial separation of functional groups.⁶ Two types of functionalized nanowire surfaces were used in this study: alkyl-terminated monolayers on nickel and hexa(ethylene glycol) (EG₆)-terminated monolayers on gold.⁷ We present here

the selective adsorption of protein to one segment of a bifunctional metallic nanowire.

The nanowires were fabricated by templated electrodeposition into nanoporous alumina membranes, as has been previously described.⁸ A layer of copper was sputter-deposited on one side of the membrane to serve as the working electrode, and a small amount of copper was electrodeposited inside the membrane to ensure that the pores were sealed. The metal for the nanowires was then deposited from aqueous solution.⁹ The main advantage of this fabrication method is the ability to create multicomponent nanowires, simply by depositing one metal in the template, replacing the electrodeposition solution, and depositing the second metal on top of the first. Using membranes with a nominal pore diameter of 200 nm, 10–25 μm long gold, nickel, and bimetallic nickel–gold nanowires were isolated.¹⁰

Clean and isolated metallic nanowires can be functionalized by exploiting the unique surface chemistry of the metal. Thus, gold wires are functionalized with thiols,¹¹ while nickel is functionalized with carboxylic acids, which bind to the native oxide layer on the metal.¹² For the hydrophobic, alkyl-terminated surfaces, nickel wires were combined with a 1 mM solution of palmitic acid (Aldrich), a 16-carbon fatty acid, in ethanol overnight. To create the protein-resistant gold surfaces terminated in EG₆, (1-mercaptopundec-11-yl)-hexaethylene glycol (EG₆–SH) was synthesized according to the literature.¹³ An ethanolic solution of this compound was reacted with gold wires in

[†] Department of Chemistry.

[‡] Department of Biomedical Engineering.

[§] Department of Physics and Astronomy.

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(9) Copper was deposited from 1 M CuSO₄. The nickel deposition solution contained 20 g/L NiCl₂·6H₂O, 515 g/L Ni(H₂NSO₃)₂·4H₂O, and 20 g/L H₃BO₄. Gold was deposited from a commercially purchased solution (Technic, Inc.) containing potassium aurocyanate. All electrodeposition was carried out at a constant potential of –1.0 V vs Ag/AgCl.

(10) The nanowires were isolated by removing the copper from the membrane using a 0.05 M solution of CuCl in 25% HCl and dissolving the alumina membrane in warm 0.5 M KOH.

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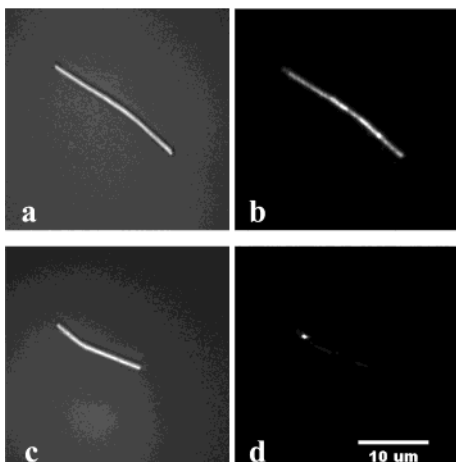


Figure 1. (a) Optical image of a Ni nanowire functionalized with palmitic acid and tagged with fluorescent protein. (b) Fluorescence image of the same nanowire. (c) Optical image of a Au nanowire functionalized with EG₆-SH and tagged with fluorescent protein. (d) Fluorescence image of the same nanowire.

the same manner as described for nickel. Bimetallic nanowires with both a nickel and a gold component were functionalized in one step, by adding the wires to a solution containing both EG₆-SH and palmitic acid.¹⁴

To investigate the interaction of proteins with these functionalized nanowire surfaces, wires were immersed in an aqueous solution of the Alexa Fluor 594 goat anti-mouse IgG protein, an antibody covalently bound to a fluorescent molecule (Molecular Probes, Inc.). The protein was added to a 1 mL suspension of nanowires in water to a final concentration of 20 μg/mL. The nanowires were exposed to the protein solution for time periods ranging from 1 min to 1 h. After the protein was allowed to adhere, the wires were rinsed and observed under a fluorescence microscope. For reaction times greater than 10 min, there did not appear to be a significant difference in the appearance of the nanowires. Therefore, 20 min was chosen as a convenient reaction time for subsequent experiments. In general, Ni|CH₃ nanowires showed bright fluorescence while Au|EG₆ nanowires were very dim or completely nonfluorescent (Figure 1). This finding demonstrates that protein preferentially adsorbs to the methyl-terminated nickel surfaces, but the EG₆-terminated gold wires resist protein adherence. Previous studies of protein adsorption on patterned planar surfaces generally showed no measurable fluorescence from the EG₆-terminated regions.¹⁵ The occasional detection of dim fluorescence, and hence adsorption of protein, on the gold nanowires would suggest that the EG₆ monolayer contains defects which affect its resistance to protein.¹⁶ The source of such defects may be imperfection in the electrodeposited gold lattice or the inherent imperfections that arise from the geometry of the curved nanowire surface.

Since there appeared to be a significant distribution of fluorescence intensities for both the Ni|CH₃ and Au|EG₆ wires, a quantitative analysis was also carried out. Using commercial image analysis software (Meta Imaging), the area of the nanowire was outlined and an average count

Table 1. Fluorescence Intensity of Protein-Modified Nanowires

type of wire	fluorescence intensity (au) ^a
Ni CH ₃	340
Au EG ₆	70
bare Ni	90
bare Au	210

^a Average intensity from fluorescence microscopy calculated for a sample of greater than 25 nanowires.

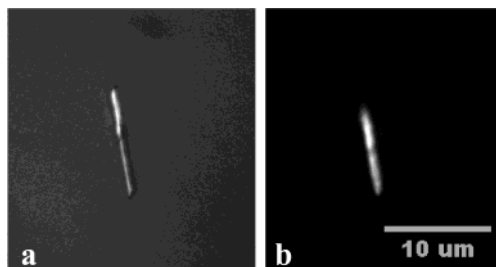


Figure 2. (a) Optical image of a functionalized Ni-Au nanowire showing distinct nickel and gold segments. (b) Fluorescence image of the same nanowire, showing that protein is localized on the nickel segment.

of pixel intensity was calculated. To account for exceptionally bright spots of fluorescence from aggregated or precipitated proteins, 5% of the brightest pixels were eliminated from the average. The average intensity of the background was subtracted from this value to give the average fluorescence intensities. Table 1 shows a comparison of the average intensity values for different populations of nanowires. The analysis showed that methyl-terminated nickel wires tagged with the fluorescent protein were on average approximately 5 times brighter than EG₆-terminated gold wires. This verifies the empirical observation that the protein did not adhere to the EG₆-functionalized nanowires. In a control experiment, unfunctionalized gold wires were also combined with the fluorescent protein. Fluorescence on these wires was 3 times more intense than on EG₆-functionalized wires, indicating that the protein does adhere to bare gold. Interestingly, unfunctionalized nickel wires exhibited very low fluorescence intensities after being exposed to the protein. This probably results from more efficient energy transfer quenching by the proximate Ni surface.¹⁷

Two-part nickel-gold nanowires functionalized with palmitic acid and EG₆-SH were subjected to the same protein adhesion assay as the nickel and gold wires. Because of the different surface chemistries of the two metals, these bimetallic nanowires present two functional groups spatially segregated on different regions of the nanowire.⁶ Figure 2a shows a reflectance image of one of these nanowires, where the shiny upper segment is gold (with an EG₆-terminated monolayer) and the dull lower segment is nickel (with an alkyl-terminated monolayer). Figure 2b shows a fluorescence image of the same nanowire after being exposed to the fluorescent protein. It is clear that only the nickel portion of the nanowire fluoresced, indicating the presence of protein only on the nickel segment. This demonstrates that multicomponent nanostructures can be modified at the molecular level to yield materials to which proteins adsorb selectively in specific regions.

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In this communication, we have established the ability to restrict protein adsorption to a defined section of a nanowire. By analogy with studies of similar SAMs on planar surfaces^{5,18} and because there is a strong precedent for the broad application of poly(ethylene glycol) coatings in protein-resistant materials,¹⁹ we believe that this result is general and can be extended to other proteins and biomolecules, as well as to application with living cells.²⁰

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The selectivity observed here demonstrates that the utility of EG functionalization in preventing protein adsorption applies to both planar and nonplanar surfaces. Although there has been much research involving the use of EG-terminated monolayers to pattern proteins on planar substrates, this characteristic has only recently been applied to spherical nanoparticles.^{19b} The localization of two functionalities on different regions of the nanowire cannot be easily mimicked with spherical nanoparticles and is the key feature that leads to the selective adsorption of protein. The magnetic, optical, and chemical anisotropies of these wires make them useful probes for biological systems.

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