

Multicomponent Magnetic Nanorods for Biomolecular Separations**

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Nanomaterials have been used extensively in the development of high sensitivity and high selectivity biodetection schemes.^[1–4] Designer particles, including noble-metal polyhedral structures, quantum dots, nanopatterns, and nanorods have found application in many forms of biological tagging schemes, including DNA and protein detection, cell sorting, and histochemical staining.^[5–12] Significant advantages over conventional molecule-based fluorophore strategies have been identified for several of these structures.^[13–15] Other applications for nanomaterials in biology, beyond diagnostics, include therapeutics and separations.^[9] A key area for researchers working with proteins involves separation and purification. Traditionally, nickel columns have been used in conjunction with histidine tagged proteins to separate such structures from a matrix of other undesirable biological elements. We and others have been working with nanorod structures prepared by the porous template synthesis approach pioneered by Martin and the group of Moskovits.^[16,17] This synthetic procedure allows one to prepare rods electrochemically with uniform diameters and with predefined block lengths of inorganic and organic materials with excellent control.^[18,19] We hypothesized that if nickel were introduced as one of the blocks in a single or multicomponent nanorod structure, it could be used as a magnetic nanosized affinity template for histidine tagged proteins and, therefore, an appropriately applied magnetic field could be used to effect separation of the protein–rod complex from a multicomponent solution. Herein, we demonstrate how one can use two-component triblock ≈ 330 nm diameter rod structures with gold end blocks and a nickel interior block as materials that can very efficiently separate His-tagged proteins from non-his tagged structures. This work builds on our work with the transport of His-tagged structures by dip-pen nanolithography (DPN) to bulk solid-state nickel oxide substrates and the work of others involving the generation of microscopic arrays of His-tagged proteins on bulk nickel oxide substrates.^[20,21]

Magnetic multisegment nanorods composed of nickel and gold blocks were synthesized by using the method of electro-deposition into a porous alumina membrane (experimental conditions are described in the Supporting Information). The gold portions of the three-component structure were used to prevent nickel domain etching during the silver dissolving procedure. Prior to use, the rods were repeatedly rinsed with distilled water until the pH of the solution was 7.

The multicomponent nanorods were washed with methanol and ethanol to remove contaminants from their surfaces. This was done by using a magnetic field (BioMag, Poly-science, Inc.) to pull the rods to the sidewalls of a plastic vial while rinsing them with the appropriate solvent. The gold portions of the nanorods were passivated with 11-mercaptoundecyl-tri(ethylene glycol)(PEG–SH) by incubating the rod samples in 1 mL, 10 mM ethanolic solution of the surfactant for 2 h followed by copious rinsing with ethanol and then nanopure water (Barnstead International, Dubuque, IA, USA). Others have shown that alkylthiols preferentially modify the gold surface in such two component structures.^[5] The gold surface was modified with PEG–SH for two reasons. First, the PEG–SH minimizes nonspecific binding of proteins to the nanorod structures.^[22,23] Second, it stabilizes the rods by minimizing bare gold surface–surface interactions.


The specific interaction of polyhistidine (His $\times 6$) with bulk oxidized nickel surface is well known.^[20,21] Similarly, fluorescein-tagged poly-His (His $\times 6$) binds specifically to the Ni portions of the substrate as evidenced by confocal fluorescence microscopy. In a typical experiment, Au-Ni-Au nanorods (10^9 – 10^{10}) were incubated in a 63 μM fluorescein labeled poly-His solution (1 mL, 0.1M PBS(phosphate buffered saline), pH 7.4) for 12 h at room temperature (22 °C). Then the nanorods were vigorously rinsed with phosphate buffered saline (PBS) solution followed by nanopure water. During each rinsing step, the rods were separated from the supernatant by using magnetic force. Fluorescence imaging shows that the fluorescein-tagged polyhistidines efficiently bind to the nanorod structures (Figure 1b). This reaction between the poly-His and Au-Ni-Au rods can be monitored with the naked eye simply by watching the color of the solution decrease in intensity as a function of reaction time (Figure 1c). A quantitative analysis of the efficiency of poly-His adsorption was performed by preparing a standard calibration curve from the fluorophore-labeled poly-His over a range of concentrations starting with the experimental poly-His initial concentration of 63 μM and going to 0.16 μM (inset Figure 2). The fluorescence emission intensity of supernatant solution isolated from the reacted nickel nanorods shows that $\approx 90\%$ of the poly-His was captured by the rods from the starting solution (Figure 2). As a control experiment, pure Au nanorods (no Ni), passivated with PEG–SH, were incubated in the poly-His solution (63 μM in 0.1M PBS, pH 7.4) under nearly identical conditions, and little interaction between the poly-His molecules and PEG–SH modified Au particles was observed (i.e., no detectable change in the emission of the fluorescein as measured by fluorescence spectroscopy, data not shown).

The Au-Ni-Au rods can be used in a novel scheme for separating His-tagged proteins from structures without His-

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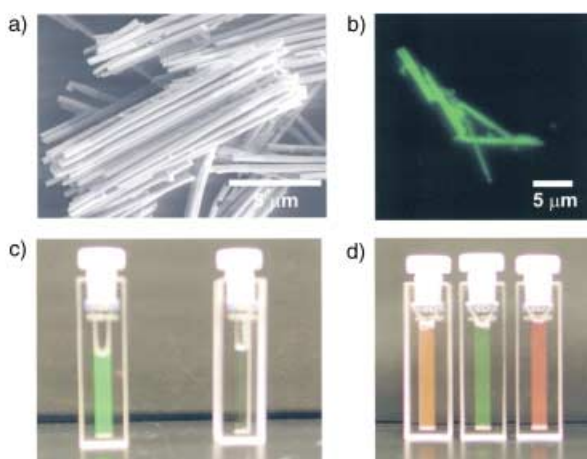


Figure 1. a) A field emission SEM image of Au-Ni-Au rods. b) A confocal fluorescence image of Au-Ni-Au rods after modification of Ni blocks with fluorescein-tagged poly-His. c) A picture of two cuvettes showing solutions of fluorescein-tagged poly-His before (left-hand side) and after (right-hand side) exposure to the Au-Ni-Au rods. d) Left: Orange solution containing dye-labeled His-tagged (Red, Alexa 568-tagged ubiquitin) and untagged (green, Alexa 488-labeled anti-rabbit IgG) proteins before exposure to nanorods. Middle: After exposure to the Au-Ni-Au rods, the solution is green, indicating removal of the red-dye labeled His-tagged ubiquitin. Right: The red solution formed after release of the red-dye labeled His-tagged ubiquitin in the eluent buffer.

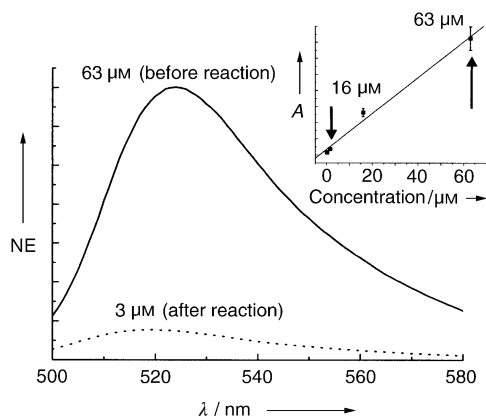
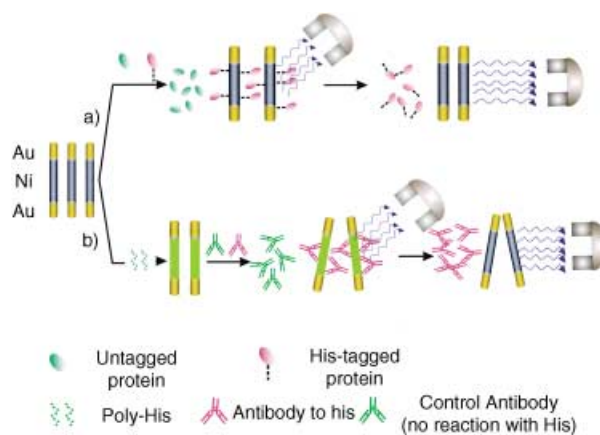


Figure 2. Fluorescence spectra corresponding to Figure 1 c, before and after separation of poly-His with Au-Ni-Au rods: Inset shows the standard curve and the arrows represent each concentration of poly-His before and after separation. NE is normalized emission, arbitrary units; A is the integrated area, arbitrary units.

tags (Scheme 1a). For example, a 1 mL solution of two different proteins with different dye labels (anti-rabbit IgG without a His tag but labeled with Alexa 488 and His-tagged ubiquitin labeled with Alexa 568; concentration of each protein = $100 \mu\text{g mL}^{-1}$, in 0.1M PBS, pH 7.4) were prepared (Figure 1 d, far left, orange solution). The orange solution containing the mixture of proteins was added to a vial containing PEG-passivated nanorods (10^9 – 10^{10}). The solution was mechanically shaken for 24 h. Application of a magnetic field caused the nanorods to move to the side walls and the resulting green supernatant (Figure 1 d, middle cuvette) could be collected and studied by fluorescence spectroscopy. Eluent buffer (pH 2.8, ImmunoPure IgG Elution Buffer, Pierce



Scheme 1. a) Separation of His-tagged proteins from untagged proteins. b) Separation of antibodies to poly-His (anti-poly-His) from other antibodies.

Biotech., Inc.) was added to the vessel containing the nanorods coated with His-tagged, Alexa 568-labeled ubiquitin. This addition results in the release of the His-tagged proteins from the nanorods and the formation of a red supernatant (Figure 1 d, right). Each of the solutions discussed above was studied by UV/Vis and fluorescence spectroscopy and compared with each other. The spectrum of the mixture of proteins before the addition of nanorods shows two bands at $\lambda_{\text{max}} = 516 \text{ nm}$ and 600 nm that correspond to the two dye labels. After addition of the nanorods, there is an 86% decrease in the signal ($\lambda_{\text{max}} = 600 \text{ nm}$) for the dye label associated with the His-tagged protein and only 6% for the protein without the His tag ($\lambda_{\text{max}} = 516 \text{ nm}$). After adding the eluent buffer, which is at a pH 2.8 and results in the release of proteins from the Ni surface, we see a strong signal at $\lambda_{\text{max}} = 600 \text{ nm}$ associated with the His tagged proteins. Fluorescence spectroscopy shows that 56% of the original His-tagged ubiquitin has been effectively separated from the two component mixture in pure form (Figure 3).

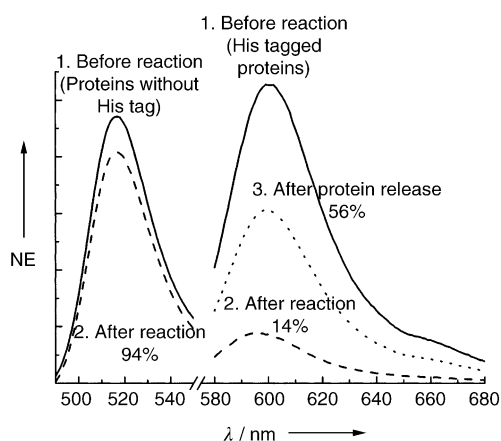


Figure 3. Fluorescence spectra corresponding to Figure 1 d. Solid lines represent a fluorescence spectrum of a mixture of dye labeled His-tagged and untagged proteins. Dashed traces show a spectrum of the supernatant after removal of His-tagged proteins with Au-Ni-Au rods. Dotted traces show a spectrum of the product generated by releasing the His-tagged proteins into eluent buffer.

Finally, Poly-His tagged Au-Ni-Au rods are bioactive and selectively react with antibodies for poly-His, Scheme 1b. Indeed, when a mixture of Alexa 488-labeled anti-human IgG and Alexa 568-labeled anti-poly-His IgG ($100 \mu\text{g mL}^{-1}$ in 0.1M PBS, pH 7.4) was introduced to a PBS solution (pH 7.4) of the Poly-His tagged Au-Ni-Au rods, the dye-labeled anti-poly-His IgG was very efficiently removed from the solution as evidenced by fluorescence spectroscopy (see Supporting Information). After 24 h, 70% of the anti-poly-His ($\lambda_{\text{max}} = 600 \text{ nm}$) was removed from the solution while only 4% of the signal ($\lambda_{\text{max}} = 516 \text{ nm}$) associated with the antihuman IgG is lost. The rods can be attracted to the side of the reaction vessel with a magnetic field allowing one to remove the supernatant. The anti-poly-His can be released from the rods by adding eluent buffer at pH 2.8. Acidic conditions are known to decrease the specific interaction between the antibody and poly-His.^[24]

We have demonstrated that Ni-containing nanorods can be used as novel materials for the efficient separation of mixtures of biomolecules by exploiting the chemical and physical properties of these nanostructures. The Ni portion of the nanostructure provides a docking site for His-tagged proteins and is ferromagnetic, allowing one to selectively and efficiently remove them from solution with an appropriately applied magnetic field. The gold ends are used to chemically protect the nickel interior and provide a second platform for introducing further chemical functionality (PEG, in this case, but in principle, many other thiol-rich molecules and materials). Finally, the nanorods provide increased surface area and are a pseudo-homogeneous system, compared to a bulk structure, thereby increasing the efficiency of the target binding and separation process. Histidine tagging of proteins has been broadly adopted throughout the molecular biology and biochemistry communities, and this novel and straightforward separation scheme provides an alternative to Ni chromatographic columns and potentially has a much greater degree of tailorability through choice of nanorod block compositions and surface coatings.

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