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Fabrication of silica-coated gold nanorods functionalized with DNA for enhanced SPR imaging biosensing applications

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Abstract

A novel method for preparing gold nanorods that are first coated with a thin silica film and then functionalized with single stranded DNA (ssDNA) is presented. Coating the nanorods with 3-5 nm of silica improves their solubility and stability. Amine-modified ssDNA is attached to the silica-coated gold nanorods via a reductive amination reaction with an aldehyde trimethoxysilane monolayer. The nanorods exhibit an intense absorption band at 780 nm, and are used to enhance the sensitivity of surface plasmon resonance imaging (SPRI) measurements on DNA microarrays.

Metallic nanorods are nanoscale materials that possess unique optical and electronic properties which make them extremely useful when incorporated into schemes for the detection of biomolecules such as DNA, RNA and proteins. To successfully integrate these materials into bioaffinity detection assays, the nanoscale surfaces must first be functionalized with biomolecules without altering their stability in solution. For example, thiol-modified single-stranded DNA (ssDNA) can be immobilized onto the surface of gold nanoparticles (NPs) in a single-step displacement reaction of electrostatically absorbed citrate anions. These DNA-modified NPs, first reported by Mirkin *et al.*[1] and Alivisatos *et al.*[2] have been used extensively for the detection and identification of oligonucleotides. The straightforward thiol attachment chemistry is made possible by the anionic character of the nanoparticle surface due to the presence of the citrate. In contrast, gold nanorods produced by the methods developed by either Murphy[3] or El Sayed[4] have a net positive surface charge due to the presence of an adsorbed monolayer of the surfactant, hexadecyltrimethyl-ammonium bromide (CTAB), on the nanorod surface. Thus, the thiol chemistry used to modify gold NPs is very difficult when employed for the attachment of ssDNA to surfactant-coated gold nanorods. The reasons for this are that the high density of the surfactant monolayer decreases the access of the thiol-modified ssDNA to the nanorod surface and the negatively-charged phosphate backbone of the ssDNA interacts with the positively charged CTAB molecules; the net result is typically a rapid aggregation and precipitation of the gold nanorods from solution. This letter describes an alternative strategy for preparing ssDNA-functionalized gold nanorods based on a multi-step process in which the gold nanorods are first modified with a thin silica film and then the ssDNA is attached to the silica shell via an aldehyde coupling reaction. We further demonstrate that these DNA-functionalized silica-coated gold nanorods can be used to greatly enhance the sensitivity of surface plasmon resonance imaging (SPRI) measurements of DNA hybridization adsorption onto DNA microarrays.

The preparation of DNA-functionalized silica-coated gold nanorods requires a sequential surface modification process that is shown schematically in Figure 1. The functionalized gold nanorod synthesis can be divided into three main steps:

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Gold nanorod fabrication. To make surfactant-coated gold nanorods, the well-documented seed mediated procedure developed by El Sayed[4] was employed. This method yields gold nanorods that are stabilized by absorbed cationic CTAB from the reaction mixture, with an aspect ratio of 5:1 (average length of 50 nm and average width of 10 nm) as verified from TEM analysis (Supporting Information, Figure S1).

Silica shell formation. The gold nanorods were coated with a very thin (ca. 3-5 nm) silica film that i) improved the colloidal stability of the nanorods by reducing aggregation, ii) improved the shape stability of the nanorods and iii) allowed for further modification of the nanorod surface. This silication method was first developed for citrate stabilized gold NPs,[5-7] and has been applied successfully to gold nanorods.[8-13] In this procedure, a silane coupling agent, 3-mercaptopropyl trimethoxysilane (MPTMS, Sigma), was used as the metal surface modifier to enhance the affinity of gold for silica that was deposited from a sodium silicate solution (Sigma, 27 wt.%) for a duration of four days. Briefly, this was accomplished by reacting a freshly prepared 10 mL nanorod solution with an ethanolic MPTMS solution (10 mM 100μL) for 45 min. under gentle stirring. Then the aqueous sodium silicate solution (0.54 wt.% 200 μL) at pH 10 was added and left to react with the nanorods for four days. Excess MPTMS, sodium silicate, CTAB and reaction by-products were removed by centrifugation at 7000 rpm for 20 min. The supernatant was then discarded and the rods redispersed in water. Figure 2 shows a representative TEM image of the silica coated nanorods with the inset showing an enlarged image of the rods possessing a thin surface silica shell of approximately 4 nm thickness.

DNA functionalization. A ssDNA monolayer was attached to the silica-coated gold nanorods by first converting the silica shell surface into an amine reactive surface by reaction with a trimethoxysilane aldehyde (TMSA, United Chemical Technologies). A reductive amination reaction was then used to conjugate surface bound aldehyde moieties to amine-modified ssDNA molecules via the formation of an intermediate Schiff base that was converted to a highly stable secondary amine bond in the presence of a cyanoborohydride reducing agent. Specifically, an aqueous 700 μL solution of silica-coated rods at pH 8 were reacted with an ethanolic solution of TMSA (10 mM, 7 μL) at room temperature for two hours to insure the condensation reaction of TMSA with the surface silanol groups. Amine-modified ssDNA, T_{25} (1 mM, 2μL) was then added together with the reducing agent, sodium cyanoborohydride ($NaCNBH_3$, Sigma, 2 μL, 0.2 M) to the aldehyde-silane-modified gold nanorod solution and left to react for 24 hours at room temperature. (Caution: sodium cyanoborohydride is a highly toxic compound). The DNA functionalized gold nanorods were then centrifuged for 20 min at 7000 rpm to eliminate excess TMSA, DNA and any reaction by-products. The precipitate was redispersed in Tris buffer (50 mM Tris-HCl, 2 mM MgCl) of pH 7.6.

When an equimolar mixture of A_{25} and T_{25} DNA-functionalized silica-coated gold nanorods were allowed to react at room temperature, these aggregated within an hour. Both, transversal and longitudinal surface plasmon absorption bands at 517 and 780 nm in the UV-visible absorption spectra (Supporting Information, Figure S2) decreased in intensity due to hybridization of the complementary nanorods resulting in aggregation and eventual loss of the nanorods from solution by precipitation. The nanorods aggregation was also indicated by TEM imaging (Supporting Information, inset in Figure S2).

The application of DNA-functionalized silica-coated gold nanorods to enhance SPRI measurements was demonstrated by the sequence specific adsorption of gold nanorods onto a DNA microarray. Briefly, a two component ssDNA microarray was created on a set of 16 gold thin film spots (1 mm diameter, 45 nm thickness) on an SF10 glass slide. The details of the DNA microarray fabrication process and surface attachment chemistry have been published previously elsewhere.[14-15] Two sequences were used in the DNA microarray: T_{25} and A_{25}.

Exposure of the microarray to a solution of T_{25} DNA-modified silica-coated gold nanorods in an SPRimager instrument and flow cell (GWC Technologies) for approximately 10 min. led to the SPRI differential reflectivity image and line profile shown in Figure 3. A very large differential reflectivity change ($\Delta\%R = 28 \pm 2.6\%$) was observed due to the hybridization adsorption of the T_{25} gold nanorods onto the A_{25} DNA microarray elements. This large increase in the $\Delta\%R$ is due to the strong optical properties of the nanorods, which are governed by the wavelength dependent complex refractive index of the metal. The adsorption of the gold nanorods produces large changes in the local electromagnetic fields in the vicinity of the interface. The reflectivity change observed for the adsorption of a full monolayer of gold nanorods was comparable to the value previously reported for the adsorption of a full monolayer of gold nanoparticles[16] and was approximately 15 times larger than the response observed for the hybridization adsorption of a full monolayer of DNA. The optical response from a dilute monolayer of gold nanorods is expected to differ from the optical response of a dilute monolayer of gold nanoparticles due to the differences in their optical properties; a full study of the wavelength and surface coverage dependence of these optical properties will be detailed in a later paper. Non-specific adsorption of the nanorods onto the remaining T_{25} elements was minimum.

In summary, the experiments reported here show that silica-coated gold nanorods modified with an aldehyde silane monolayer can be successfully reacted with amine-terminated ssDNA via a reductive amination reaction to create stable solutions of DNA-functionalized silica-coated gold nanorods. Additionally, the DNA-functionalized gold nanorods are capable of hybridization with the complementary DNA either immobilized onto a planar gold surface or attached to another nanorod. Future experiments will employ DNA-functionalized gold nanorods in conjunction with enzymatic amplification methods for the ultrasensitive detection of DNA and RNA with nanorod-enhanced SPRI.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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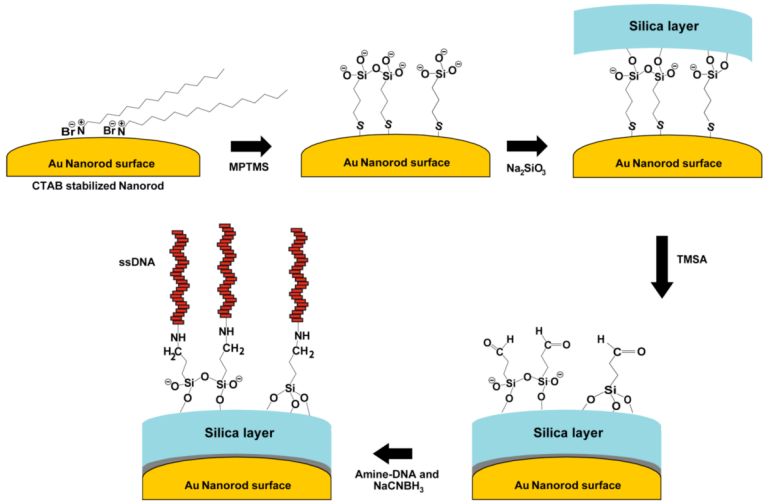

Figure 1.

Schematic diagram of the surface reactions employed to obtain DNA-functionalized gold nanorods. First the nanorods were coated with a thin silica layer using the silane coupling agent, 3-mercaptopropyl trimethoxysilane, followed by reaction with sodium silicate. Surface bound aldehyde functional groups were attached to the silica film by using trimethoxysilane aldehyde and then used to conjugate the amine modified DNA in a reductive amination reaction.

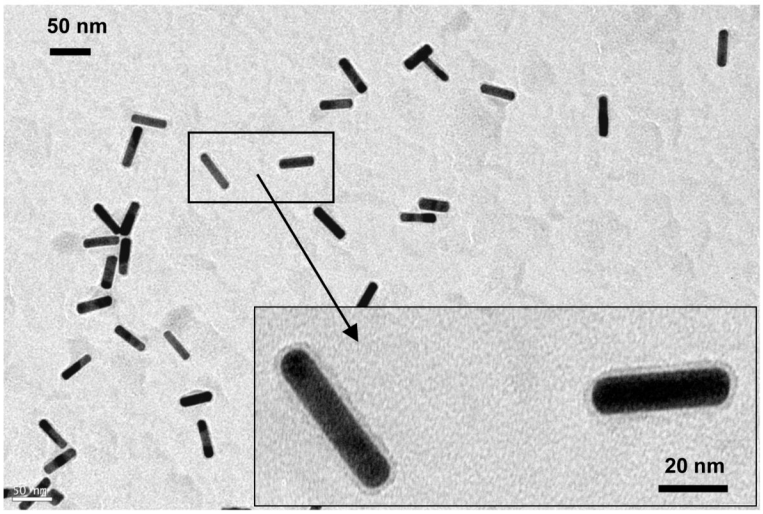

Figure 2.
TEM image of silica stabilized gold nanorods that have an aspect ratio of 1:5 (average width: length of 10 nm : 50 nm). A silica layer of approximately 4 nm, which was deposited onto the rods surface from a sodium silicate solution, can be observed in the magnified image.

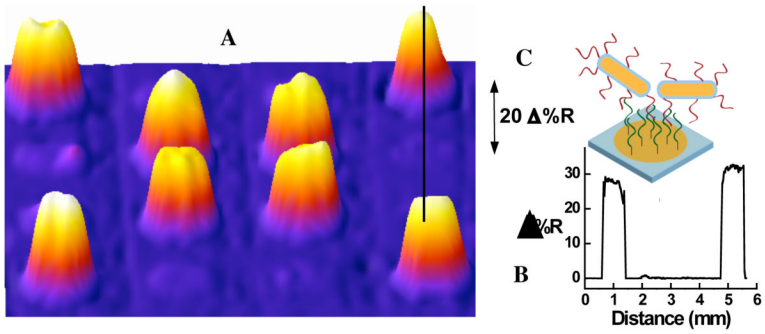

Figure 3.

(A) SPR difference image showing the hybridization adsorption of T_{25} ssDNA-functionalized silica coated gold nanorods onto immobilized ssDNA, A_{25}, on gold spotted glass. This was acquired by subtracting the images taken before and after exposure to the T_{25}-coated Au nanorods. (B) Line profile plotted as the change in percent reflectivity obtained from the area indicated by the black line in the SPR image. (C) Schematic of the surface assembly after the hybridization absorption of the DNA-functionalized nanorods.