ADVANCED FUNCTIONAL MATERIALS

Supporting Information

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SUPPORTING INFORMATION

Synthesis, Functionalization and Bioconjugation of Monodisperse Silica-coated Gold Nanoparticles as Robust Bioprobes

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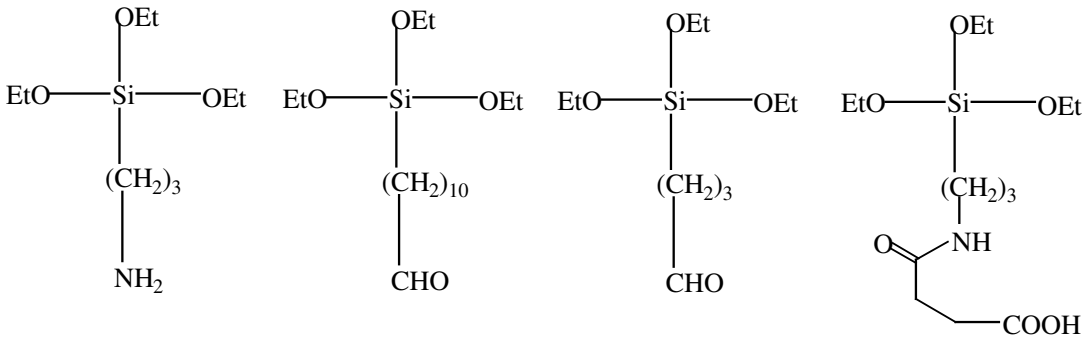
Scheme I. Functional silane coupling reagents for the surface-functionalization of Au@SiO_2 nanoparticles



Materials. 3-aminopropyltriethoxysilane (APTES, >99%, Merck), succinic anhydride (>99 %, Merck), fluorescamine (>97%, Sigma), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC·HCl, 99%, Sigma), and *N*-hydroxysulfosuccinimide sodium salt (sulfo-NHS, Aldrich) were used as received.

NH_2-functionalization of $Au@SiO_2$ Nanoparticles

The procedure for grafting amino silane onto silica nanoparticles was adopted for the surface modification of $Au@SiO_2$ nanoparticles (*T. Pham, J. B. Jackson, N. J. Halas, T. R. Lee, Langmuir 2002, 18, 4915.*). Briefly, 10 μL APTES was added into 2 mL ethanol solution containing ~ 1.3×10^{11} $Au@SiO_2$ particles, and the mixed solution was shaked at room temperature for 2 h followed by heating at 50 °C for 1 h. The resulting amino-modified $Au@SiO_2$ particles were purified by centrifugation and re-dispersion with the use of 10 mL ethanol four times. Eventually the purified $Au@SiO_2$ particles were dispersed into 2 mL deionized water for characterization and bioconjugation.

The density of surface-grafted amino groups was measured by fluorometric method using non-fluorescent fluorescamine reagent for rapid amino assay (*S. Udenfriend, S. Stein, P. Bohlen, W. Dairman, W. Leimgruber, M. Weigele, Science 1972, 178, 871.*). Firstly, 0.1 mL APTES solution (10 μL APTES in 10 mL ethanol) was dissolved into 0.9 mL ethanol and 0.5 mL of 50 mM borate buffer with a pH of 9.2. Secondly, 0.1 mL of the purified amino-modified $Au@SiO_2$ solution was also dissolved into 1 mL ethanol and 0.5 mL of the borate buffer. Then 0.1 mL fluorescamine solutions (5 mg/mL in ethanol) were then quickly mixed with the above two solutions respectively for one minute before fluorescence measurement (λ_{ex} = 390 nm, λ_{em} = 470 nm). The reaction of primary amines with fluorescamine can result in fluorophore products, and the excess fluorescamine can be hydrolyzed into non-fluorescent products very fast. By the comparison of fluorescence intensity of the above two systems, the density of grafted amino groups can be achieved.

COOH-functionalization of $Au@SiO_2$ Nanoparticles

A carboxyl silane agent, i.e. 3-(triethoxylsilylpropylcarbamoyl)butyric acid was prepared according to the reported method (*L. Levy, Y. Sahoo, K. S. Kim, E. J. Bergey, P. N. Prasad, Chem. Mater. 2002, 14, 3715.*). Briefly, 4.5 mmol APTES (1.05 mL) and 4.5 mmol succinic anhydride (0.45 g) were dissolved in a mixed solution of 1 mL ethanol and 0.5 mL dimethylformamide. The reaction mixture was then stirred overnight at room temperature and further used directly in the following grafting process. Furthermore, 1.125 mL of as-prepared COOH-terminated silane (2.2 mmol) was mixed with 2 mL of $Au@SiO_2$ aqueous solution (containing ~ 1.3×10^{11} $Au@SiO_2$ particles) and 8 mL ethanol. After shaked for 2 h, the reaction mixture was heated at 50 °C for 1 h under a nitrogen environment. Subsequently, the mixture was washed thoroughly with 10 mL ethanol four times followed by dispersing it into 2 mL deionized water for characterization and bioconjugation. A carbodiimide coupling reagent (EDC) was used to determine the density of surface-grafted carboxyl groups (*G. T. Hermanson, Bioconjugate Techniques, Academic Press, CA 1996.*). 0.2 mL COOH-grafted $Au@SiO_2$ solution (pH ~ 5, ~ 1.3×10^{10} $Au@SiO_2$ particles in total) was mixed with 0.1 mL A-1318 (0.58 mM in ethanol) followed by adding 10 mg EDC·HCl and 20 μL of 125 mM sulfo-NHS in water. After shaked for 2 h, the sample was washed thoroughly with 10 mL ethanol four times followed by dispersing it into 1 mL ethanol for fluorescence measurement (λ_{ex} = 520 nm, λ_{em} = 570 nm) as compared to the reference sample of A-1318 in ethanol after reacted with 3-(triethoxylsilylpropylcarbamoyl)butyric acid.