SUPPORTING INFORMATION

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

Shuhua Liu and Mingyong Han

Scheme I. Functional silane coupling reagents for the surface-functionalization of Au@SiO₂ 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.
NH₂-functionalization of Au@SiO₂ Nanoparticles

The procedure for grafting amino silane onto silica nanoparticles was adopted for the surface modification of Au@SiO₂ 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 × 10¹¹ Au@SiO₂ 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@SiO₂ particles were purified by centrifugation and re-dispersion with the use of 10 mL ethanol four times. Eventually the purified Au@SiO₂ 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@SiO₂ 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<sub>ex</sub> = 390 nm, λ<sub>em</sub> = 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\textsubscript{2} Nanoparticles

A carboxyl silane agent, i.e. 3-(triethoxysilylpropylcarbamoyl)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\textsubscript{2} aqueous solution (containing ~ 1.3 \times 10^{11} Au@SiO\textsubscript{2} particles) and 8 mL ethanol. After shaken 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\textsubscript{2} solution (pH ~ 5, ~ 1.3 \times 10^{10} Au@SiO\textsubscript{2} particles in total) was mixed with 0.1 mL A-1318 (0.58 mM in ethanol) followed by adding 10 mg EDC\cdot HCl and 20 \mu L of 125 mM sulfo-NHS in water. After shaken 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 ($\lambda_{ex} = 520$ nm, $\lambda_{em} = 570$ nm) as compared to the reference sample of A-1318 in ethanol after reacted with 3-(triethoxysilylpropylcarbamoyl)butyric acid.