Electronic Supplementary Material (ESI) for Analytical Methods.

Supplementary Information

Methods to Functionalize Gold Nanoparticles with Tandem-Phosphorothioate DNA: Role of Physicochemical Properties of Phosphorothioate Backbone in DNA Adsorption to Gold Nanoparticles

Abbas Karami, Masoumeh Hasani*

Faculty of Chemistry, Bu-Ali Sina University, Hamedan 65174, Iran.

**Corresponding author:hasani@basu.ac.ir*

Preparation of the buffers

500 mM HEPES buffer pH = 7.6 was prepared by dissolving 11.915 grams of HEPES powder (Mw = 238.3 g/mol) in 90 mL of ultrapure water, pH adjusted to 7.6 with HCl and NaOH, and the volume was diluted to 100 mL with deionized (DI) water. Buffer solutions were autoclaved at 125 °C for 45 min, filtered through a syringe head filter with a 0.22 µm cellulose acetate membrane, and stored in different volumes at -20 °C or 4 °C. Lower buffer concentrations were obtained by diluting the stock buffer (500 mM) with autoclaved DI water. 500 mM citrate buffer pH 3.1 was prepared by dissolving 14.705 g of trisodium citrate dihydrate salt (Mw = 294.10 g/mol) in 90 mL DI water, and then adjusting the pH to 3 by adding concentrated HCl and diluting the volume to 100 ml with DI water. The buffer solution was then sterilized by filtration and autoclaving.

Characterization of the synthesized AuNPs

Figure S1A shows the UV-vis extinction spectra of the as-prepared AuNPs before and after 3-fold dilution. The concentration of the nanoparticle solution was estimated according to the Beer–Lambert law using UV–Vis absorbance spectra.[1] The average particle sizes of the as-prepared AuNPs were measured using Dynamic Light Scattering (DLS) technique (Figure S1B). The synthesized AuNPs were 13 nm in diameter and had an extension coefficient of 2.7×10^{8} $M^{-1}cm^{-1}$ [2]. The calculated concentration of the AuNP solution was approximately 10 nM. Figure S1C shows a transmission electron microscopy (TEM) image of the as-prepared AuNPs taken on a Philips EM 208S microscope operating at an electron energy of 100 kV.

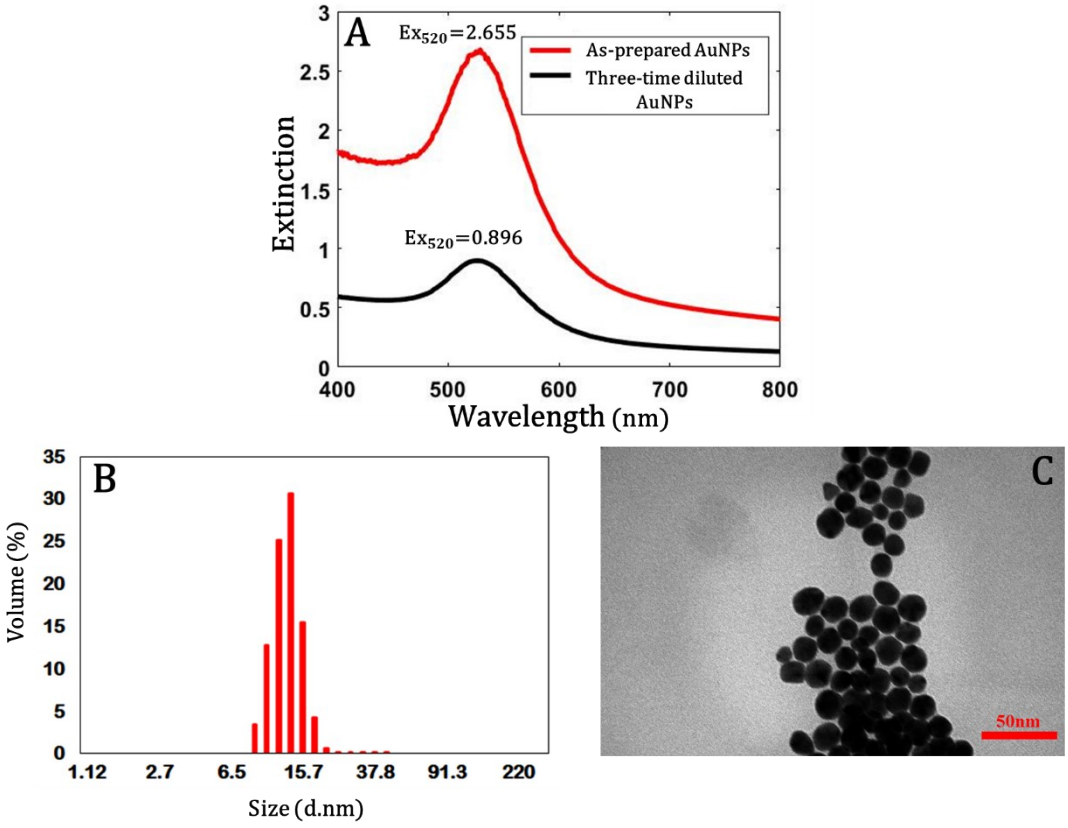

Figure S1. (A) UV-vis extinction spectra of the as-prepared AuNPs before and after 3-fold dilution. (B) DLS spectra of the as-prepared AuNPs at 25°C. (C) A TEM image of the as-prepared AuNPs.

Determination of DNA loading capacity in the synthesized SNAs

In a typical experiment, 50 µL of SNA (16 nM or 0.8 pmol) prepared with different methods (i.e. salt-aging, low pH assisted and freezing methods) was added to 250 µL of 10 mM HEPES buffer containing 24 mM KCN (final concentration of 20 mM KCN in a total volume of 300 µL) and the solution was mixed gently. Five minutes after the solution became colorless, the sample was transferred to a special microcuvette for fluorescence measurement and fluorescence spectra were recorded with a fluorescence spectrophotometer at an excitation wavelength of 493 nm. Fluorescence spectra of KCN-treated SNA samples were collected in a data matrix as an unknown set. In addition, FAM-labeled DNA solutions of known concentrations (20, 40, 60, 80, 100 pmol) were prepared using the same buffer, pH, ionic strength, and KCN concentration as the unknown samples. Fluorescence spectra of these standard FAM-DNA solutions were collected as a calibration set. The fluorescence spectra of the calibration and the unknown sets were then augmented into one column-wise matrix. Multivariate curve isolation method with alternating least squares algorithm (MCR-ALS) was used for calibration modeling and analysis of the augmented data [3]. The known value of FAM-tagged DNA in the calibration set can be applied as a correlation constraint during the implementation of the ALS algorithm. Only one compound (i.e., FAM-tagged DNA) is varied within the system, resulting in one significant eigenvalue determined by singular value decomposition (SVD). One of the pure FAM-tagged DNA spectra was chosen as the initial estimation. In addition, the non-negativity constraint was applied in both column and row spaces during ALS iterations. Finally, the amounts of FAM-labeled DNA in the unknown sets were predicted at the end of the repetitions. The number of DNA attached to each particle was calculated by dividing the total amount of fluorescent oligonucleotides (predicted values) by the total amount of particles in the solution.

Sample preparation for FTIR spectroscopic measurements

FT-IR measurements were performed using the KBr pellet technique. First, the PO-DNA1/AuNP and full-PS-DNA/AuNP conjugates were prepared using the low pH method (at a DNA: AuNP ratio of 300:1). Subsequently, the unbound DNAs were eliminated through the post-binding washing procedure, which was repeated three times. The resulting conjugates were centrifuged to remove the supernatant, and the deposited gelatinous pellets were dried by placing them in a vacuum oven at 60 °C for 45 minutes. In addition, a sample of bare AuNPs was centrifuged

and dried. The dried pellets were mixed and ground with 100 mg of KBr powder to prepare the KBr disks and were then instantly utilized for FTIR measurements.

References

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[2] W. Haiss, N.T. Thanh, J. Aveyard, D.G. Fernig, Determination of size and concentration of gold nanoparticles from UV− Vis spectra, Analytical chemistry 79(11) (2007) 4215-4221.

[3] J. Jaumot, A. de Juan, R. Tauler, MCR-ALS GUI 2.0: New features and applications, Chemometrics and Intelligent Laboratory Systems 140 (2015) 1-12.