

Supplementary Material

Effect of Modification Protocols on the Effectiveness of Gold Nanoparticles as Drug Delivery Vehicles for Killing of Breast Cancer Cells

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FTIR analysis of thiol@AuNPs attached to Taxol conjugate (Method A)

Figure S1 shows the FT-IR spectra of thiol@AuNPs-Taxol conjugate (Method A). Figure 1.a represents the LA capped AuNPs. The main characteristic bands of pure LA were monitored. A characteristic peak at 3021 cm^{-1} due to OH stretching vibration was observed. The splitting bands at 1644 and 1621 cm^{-1} confirm the capped of LA onto the surface of gold nanoparticles. The peak at 1406 cm^{-1} is due to symmetric stretching vibrations of COO^- . A broad band between 3582 - 3108 cm^{-1} is ascribed to O-H stretching vibrations because of the water molecules. Also, asymmetric and symmetric vibrations of methylene group (CH_2) at 2925 and 2860 cm^{-1} are strongly visible in Figure 1.a.

Figure S1 b) shows the bands for LA exchanged by 16-MHDA attached AuNPs (thiol@AuNPs). The characteristic absorption bands between approximately 3536 and 3230 cm^{-1} due to O-H stretching vibrations result from residual water molecules while the peaks at 2918 and 2849 cm^{-1} are attributed to asymmetric and symmetric stretching vibrations of

methylene groups, at 1705 cm^{-1} due to C=O stretching vibrations of the ester, at 1640, 1560 and 1410 cm^{-1} due to asymmetric and symmetric stretching vibrations from O–C–O while the band at 2553 cm^{-1} assigned to S-H vibrations disappeared confirming the successful attachment of 16-MHDA onto the AuNPs surface (Figure S1 b). Several absorbance bands from pure LA and pristine 16-MHDA disappeared after the attachment into the surface of gold nanoparticles. This can be attributed to the forming of new bands between the thiol ligands and the AuNPs molecules.

On the other hand, Figure S1 c) displays NHS attached to thiol@AuNPs. The bands at 2917 cm^{-1} and at 2849 cm^{-1} are due to asymmetric and symmetric stretching vibrations of methylene groups. A weak band at 1782 cm^{-1} is assigned to the vibrational stretch of ester carbonyl C=O group which indicates the presence of ester bond between NHS and carboxylic group of alkanethiol molecules (16-MHDA). The splitting bands at 1637 cm^{-1} and 1613 cm^{-1} confirm the forming of ester group. The peak at 1402 cm^{-1} is due to symmetric stretching vibrations of COO^- . The peak located at 1255 cm^{-1} due to an ester bond C-N stretching vibration while the band at 1096 cm^{-1} is contribution from C-O stretching vibrations.

Furthermore, the main characteristic bands of Taxol conjugated to thiol functionalised AuNPs were observed (Figure S1 d and Table S1). The bands at 2921, 2873, and 2849 cm^{-1} are attributed to asymmetric and symmetric stretching vibrations of methylene groups. The band at 1735 cm^{-1} is due to carbonyl group C=O stretching vibrations from the ester group. Other peaks are detailed in Table S1.

Table S1. FTIR peak positions of Taxol conjugated to functionalised AuNPs (Figure S1 d).

Wavelength (cm^{-1})	Peak position
2921, 2873, 2849	C-H stretching
1735, 1637, 1613, 1402	C=O stretching
1104	C-O
948, 832	C-H in-plane deformation
1465	aromatic groups in Taxol
1250	C-N stretching
3580-3232	N-H /O-H stretching

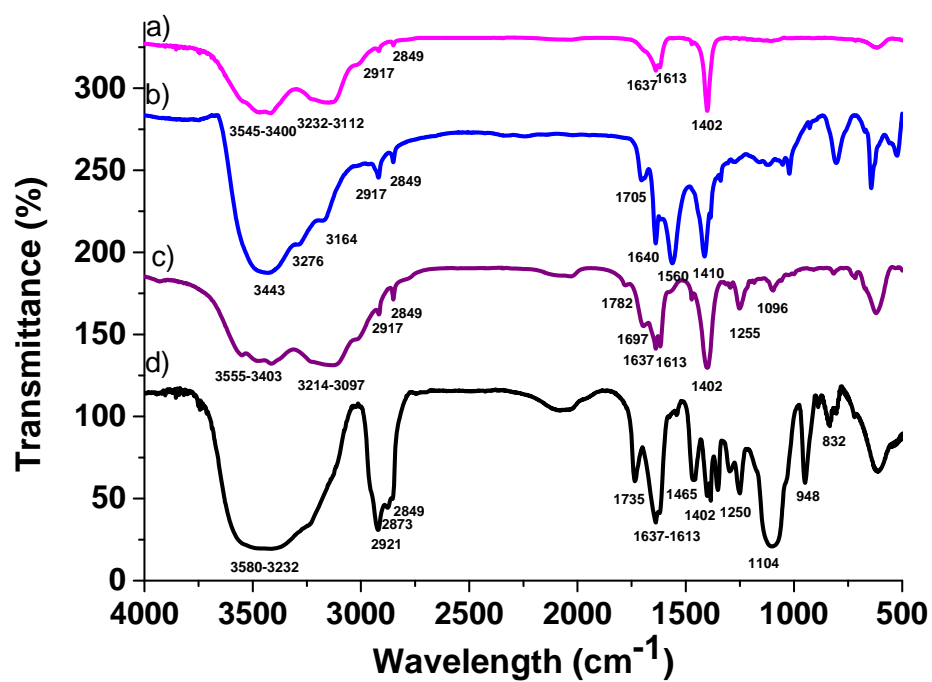


Figure S1. FTIR spectra of LA@AuNPs (curve a), thiol@AuNPs (curve b), NHS-thiol@AuNPs (curve c) and thiol@Au NPs-Taxol (A) (curve d) in KBr.

TEM analysis of various AuNPs

TEM images of the AuNP at each stage of reproduction are provided in Figure S2. The TEM was used to confirm the integrity of monodispersed gold nanoparticles. The particle size stays quite constant through the different steps of the production. The size distribution histogram shows a narrow size distribution for each type of AuNP. Dark spots of gold particles are observed in each sample with sizes of approximately 17 nm. The TEM images indicate the predominant formation of spherically shaped gold nanoparticles with some irregular particles while the light shells were attributed to the molecules functionalised the gold surface.

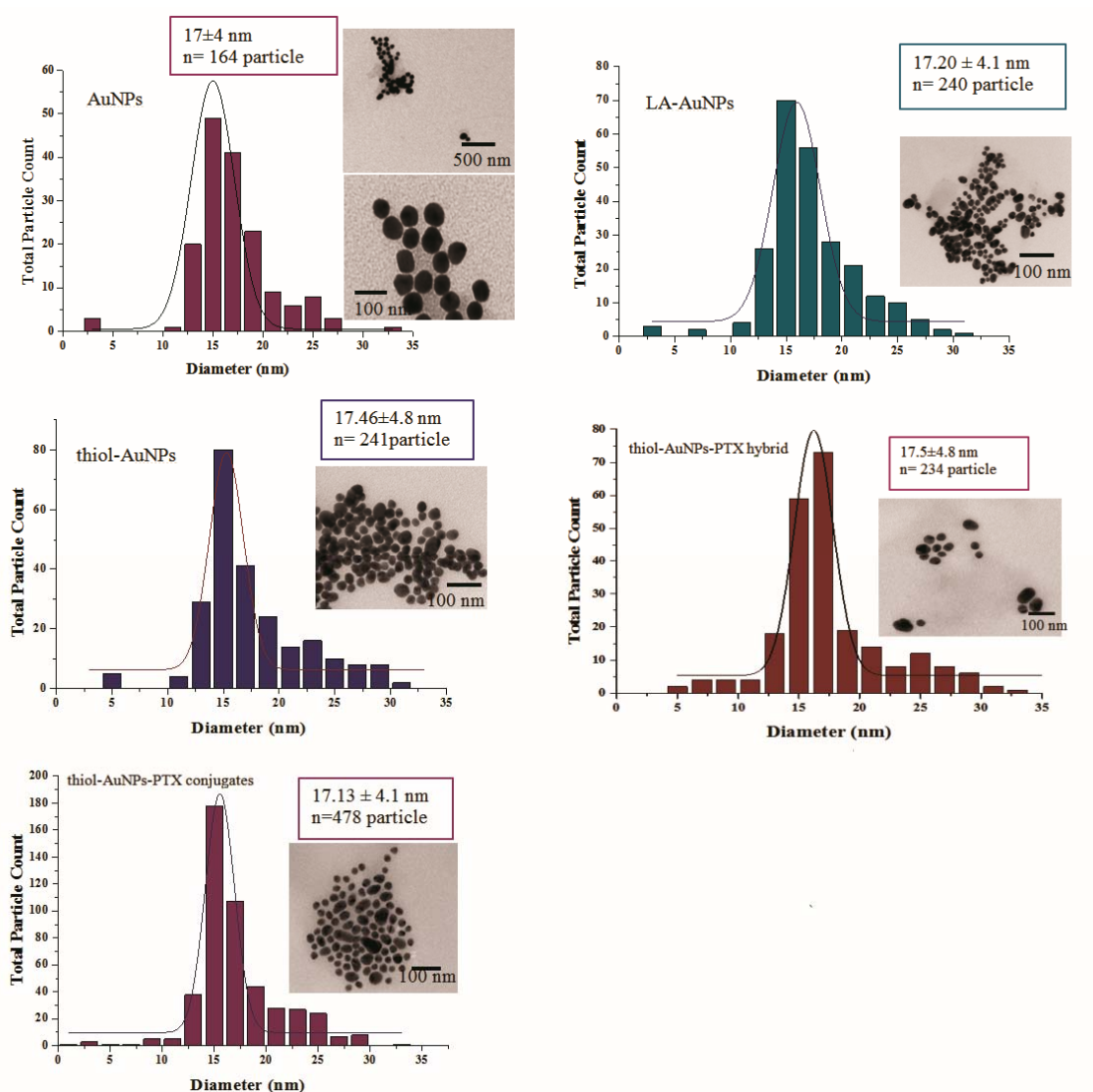


Figure S2. TEM images of the AuNPs.

DLS analysis of various AuNPs

DLS analysis of the various AuNPs was also performed. As with the TEM the particles show a consistent size through each production step.

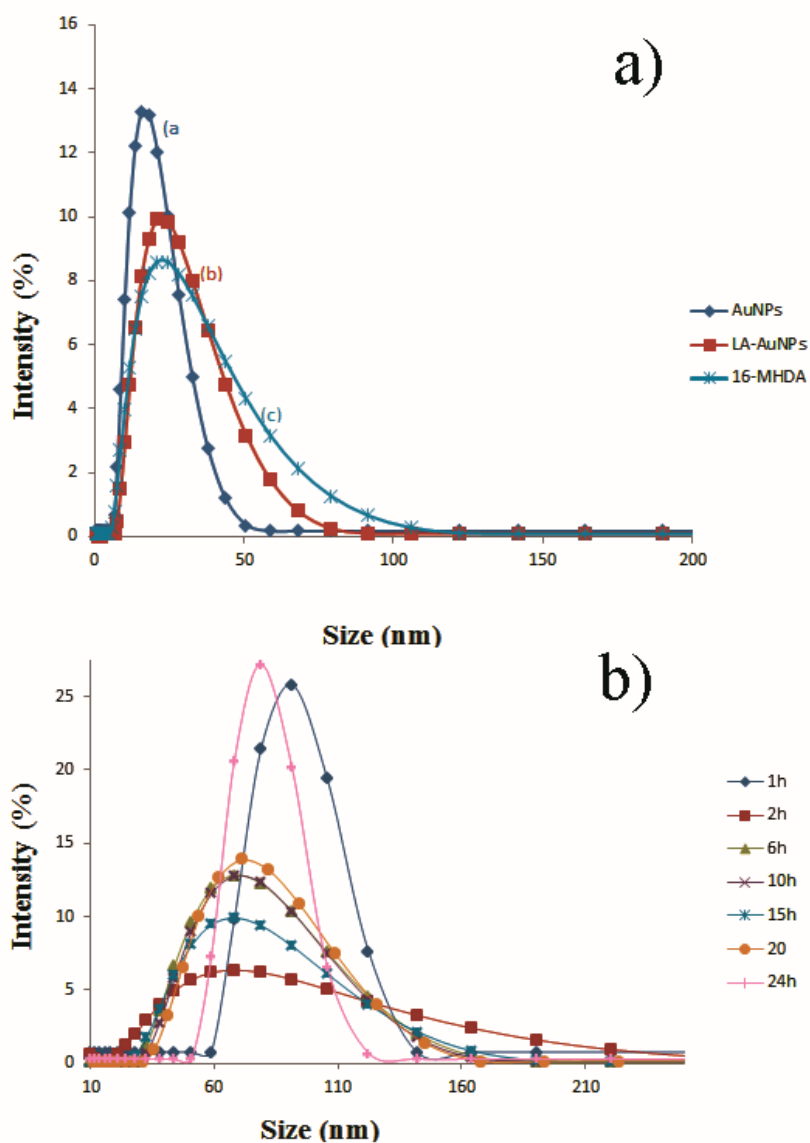


Figure S3. DLS analysis of the AuNPs. a) DLS curves at different preparation stages, b) DLS curves for the hybrid after various exposure times to Taxol.

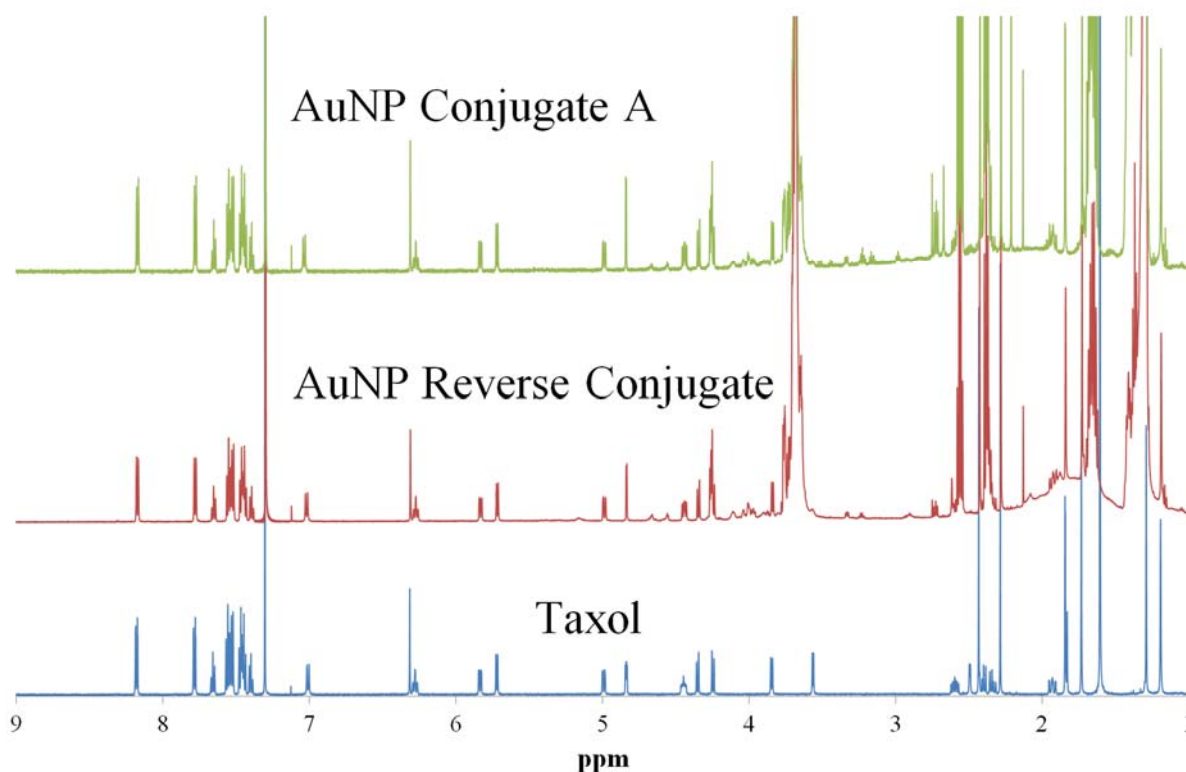


Figure S4: ¹H NMR of Taxol and the AuNP formed through the EDC/NHS coupling reaction for thiol@AuNPs-Taxol (Rev) or thiol@AuNPs-Taxol (Conjugate A) showing that the coupling occurs at C2' - OH and C7-OH groups.

Further, a selective 1D NMR Correlation Spectroscopy (COSY) experiment was used to confirm the locations of ester coupling. As shown in Figure S5a, selective excitation (using a shaped pulse) of the C2' proton resonance at 4.83 ppm in the AuNP reverse conjugate reveals only coupling to the proton C3' signal at 5.83 ppm with no signal at 3.57 ppm demonstrating that the C2'-OH proton is no longer present. In Figure S5b, selective excitation (using a shaped pulse) of the C7 proton resonance at 4.38 ppm in the AuNP conjugate A reveals only coupling to the proton C6 signal at 2.60 and 1.90 ppm demonstrating that the C7-OH proton is no longer present given the absence of any signal at 2.46 ppm.

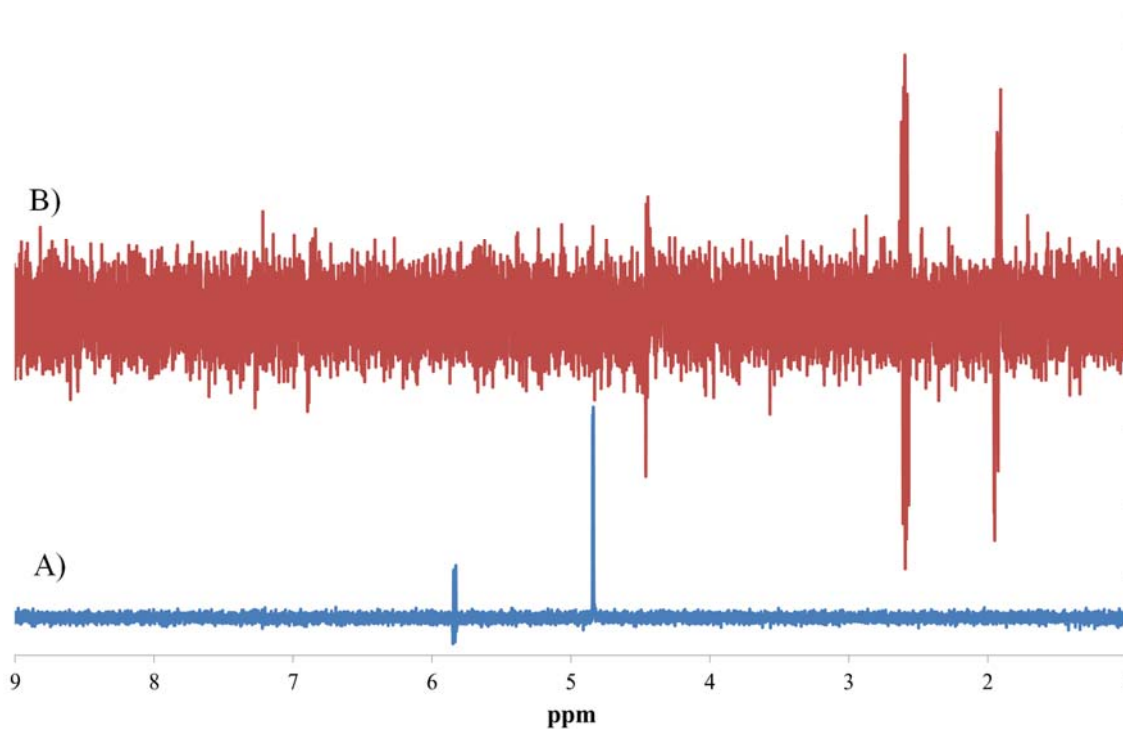


Figure S5: Selective 1D NMR COSY spectra of a) the AuNP reverse conjugate tuning the excitation to the C2' proton resonance at 4.83 ppm and b) AuNP conjugate A tuning the excitation to the C7 proton resonance at 4.45 ppm.

We have examined conjugate A after storage in the fridge in PBS buffer for two months and find no Taxol in the solution after centrifuging to remove the particles. The uv-vis data is shown in Figure S6.

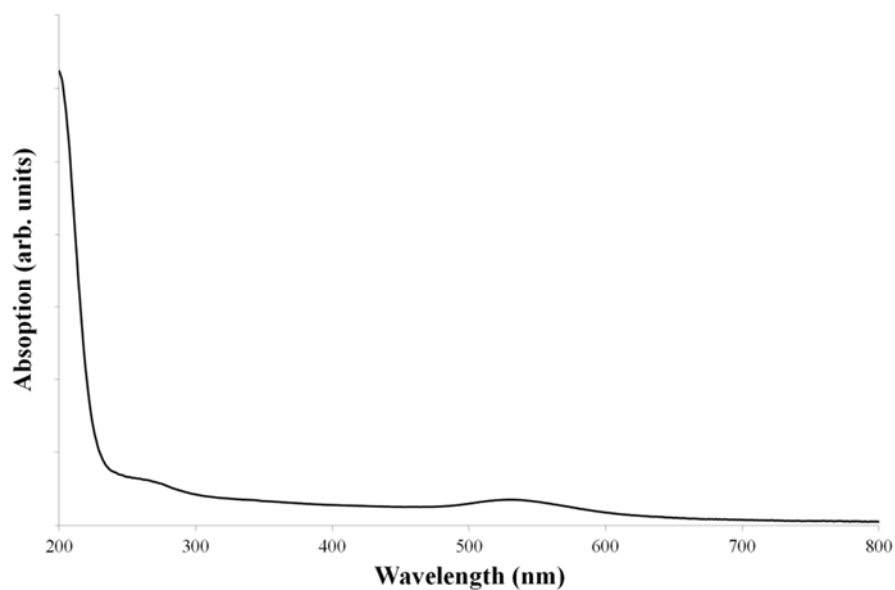


Figure S6: UV-vis spectrum of supernatant after removing conjugate A nanoparticles following two months of storage in the fridge.