Fabrication of Gold Nanoparticles on Biotin -di-Tryptophan Scaffold for Plausible Biomedical Applications †

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SUPPORTING INFORMATION

MATERIAL AND METHODS

General- Methanol, Water, $HAuCl_4$, $NaBH_4$ were purchased from Spectrochem, Mumbai, India, and used without further purification.

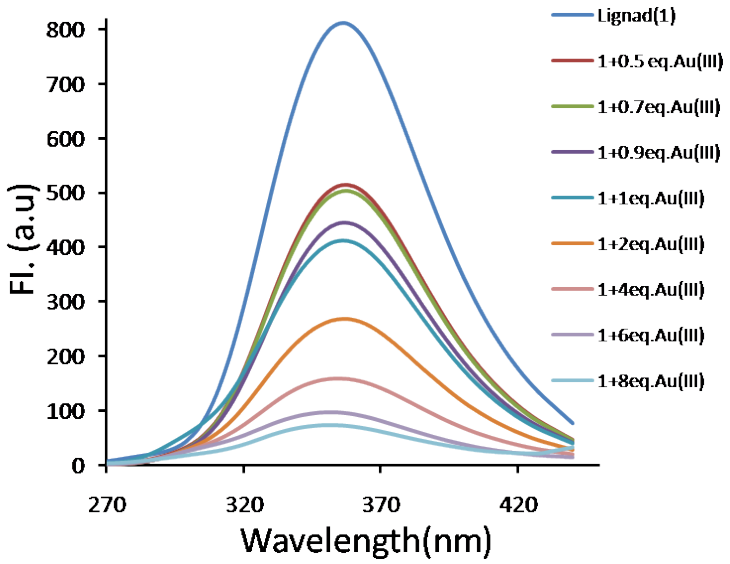

Figure S1: Fluorescence quenching titration of **1** (10^{-5} M) by increasing concentration of gold solution. Solvent methanol water (1:1). E_{em} = 358 nm, E_{ex} = 280 nm.

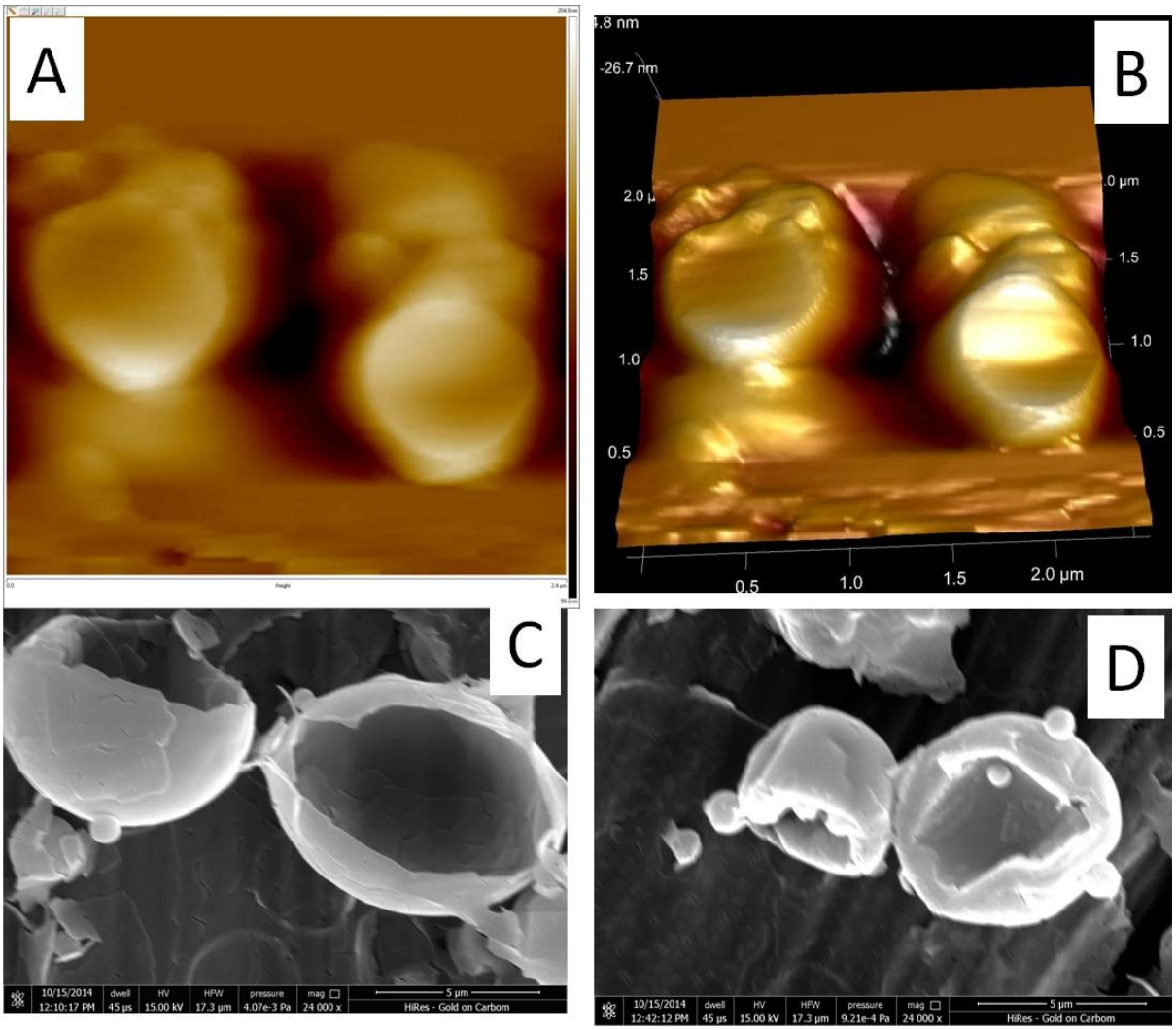

Figure S2: Few more views of ***Top***: AFM and ***Bottom***: SEM images showing effect of plasmonic heating on the AuNPs encapsulated vesicles.

Uv/Vis experiments: -UV-Vis absorption spectra were recorded on VARIAN CARY 100 Bio UV-Vis spectrophotometer with 10 mm quartz cell at 25±0.1 °C.

Atomic Force Microscopy (AFM) – Neat and co-incubated solution of Biotin–Trp-Trp peptide sample (**1**) with gold was imaged with an atomic force microscope. The Samples were placed on

freshly cleaved HOPG and muscovite mica surfaces followed by imaging with an atomic force microscope (INNOVA, ICON Analytical Equipment, Bruker, Sophisticated Instrument Center-Dr. Hari Singh Gour Central University, Sagar-M.P.) operating under the Acoustic AC mode (AAC or Tapping mode), with the aid of a cantilever (NSC 12(c) from MikroMasch, Silicon Nitride Tip) by NanoDrive™ version 8 software. The force constant was 2.0 N/m, while the resonant frequency was 284.60 kHz. The images were taken in air at room temperature, with the scan speed of 1.5-2.0 lines/sec. The data analysis was done using of Nanoscope Analysis Software. The sample-coated substrates were dried at dust free space under 60W lamp for 6h followed by high vacuum drying and subsequently examined under AFM.

Transmission Electron Microscopy (TEM) – The samples were placed on a 400 mesh carbon coated copper grid. After 1 minute, excess fluid was removed and the grid was/wasn't negatively stained with 2% uranyl acetate solution. Excess stain was removed from the grid and the samples were viewed using a FEI Technai 20 U Twin Transmission Electron Microscope operating at 80 kV. The microscope is a STEM and is also equipped with a EDS detector, HAADF detector and Gatan digital imaging system.

Energy dispersive spectroscopy (EDAX) analysis by TEM embedded analyzer- The TEM has an EDAX facility which contains the EDAX unit with a detector Super Ultra Thin Window (Super UTW) used for capturing of x-rays, and the spectroscopic analysis was done by the EDAX GenesisVersion 3.60 software system.

X-Ray diffraction measurements- X-Ray diffraction (XRD) analysis of drop-coated films on glass substrates of the Au(I)NPs capped vesicles of sample **1** was carried out on a ARLX'TRA, X-ray Diffractometer (Thermo electron corporation) instrument operating at 40 kV and a current of 30 mA with Cu-K_α(λ =1.451841Å) radiation.

Fluorescence studies- Fluorescence spectra were recorded on Varian Luminescence Cary eclipsed and CARY win 100 Bio UV-Vis spectrophotometer with a 10 mm quartz cell at 25 ± 0.1 ºC. The solutions of **1** and metal salts were prepared in CH_3OH/H_2O (50:50). Deionized water and methanol (HPLC grade) were used in these studies. The solutions containing **1** (10^{-5} M) and different concentrations of metal salt were prepared in CH_3OH/H_2O (50:50) and were kept at 25±1 ºC for 0-24 h and recorded their fluorescence spectra at fresh as well as aged conditions. All fluorescence scans were saved as ACSII files and further processed in Excel™ to produce all graphs shown.

Fluorescence microscopy – Dye stained structures were examined under a fluorescent microscope (Leica DM2500M), provisioned with a fluorescence illuminator and a fluorescein filter (502/526 nm). This filter optimized visualization of rhodamine B -treated (positive resolution) compared with untreated (negative resolution) spherical structures. 10 μM rhodamine B dye solution was added directly to AuNPs-1 hybrid solution (1 mM) in 50% methanol/water. 20 μL of this solution was spread on a glass slide, dried at room temperature, and imaged under a fluorescence microscope.

Preparation of AuNPs: The $HAuCl_4$-**1** hybrid colloid samples were prepared according to the following procedure: First, 1 mL of 50% aqueous methanol solution of **1** (1mM) was added into 1 mL of water in a 10 mL round bottom flask. Aqueous solution of $HAuCl_4$ (1 mL, 4 mM) was then introduced into the resulting solution under rigorous stirring at room temperature. A gradual color change from colorless to redish orrange was observed and a large amount of precipitate occurred after 1hr. When the stirring was stoped. The solution was further stirred for another 2 h. The colloid samples thus prepared were used directly for characterization without further treatment. The as-prepared colloidal particles can be easily decomposed through redox reaction of $HAuCl_4$ contained therein with $NaBH_4$. The reduction of the colloidal particles gave products mainly containing a large quantity of fused gold nanoparticles with mean diameter 6-7 nm, indicating that the larger networking present at the complete prereduction stage was decomposed after reduction with $NaBH_4$. The XRD and UV-Vis studies further confirm the formation of nanoparticles.

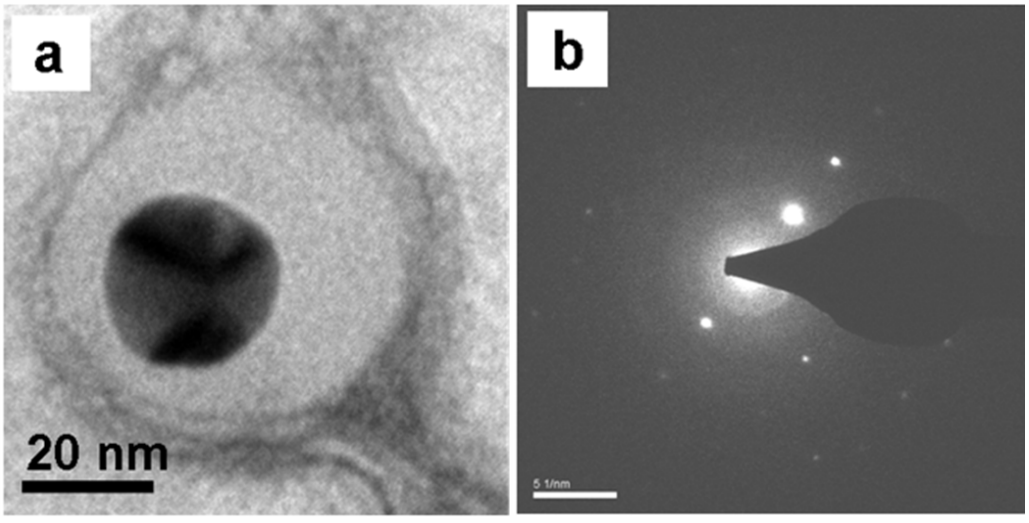

Figure S3: AuNPs coated vesicle and corresponding SAED pattern.

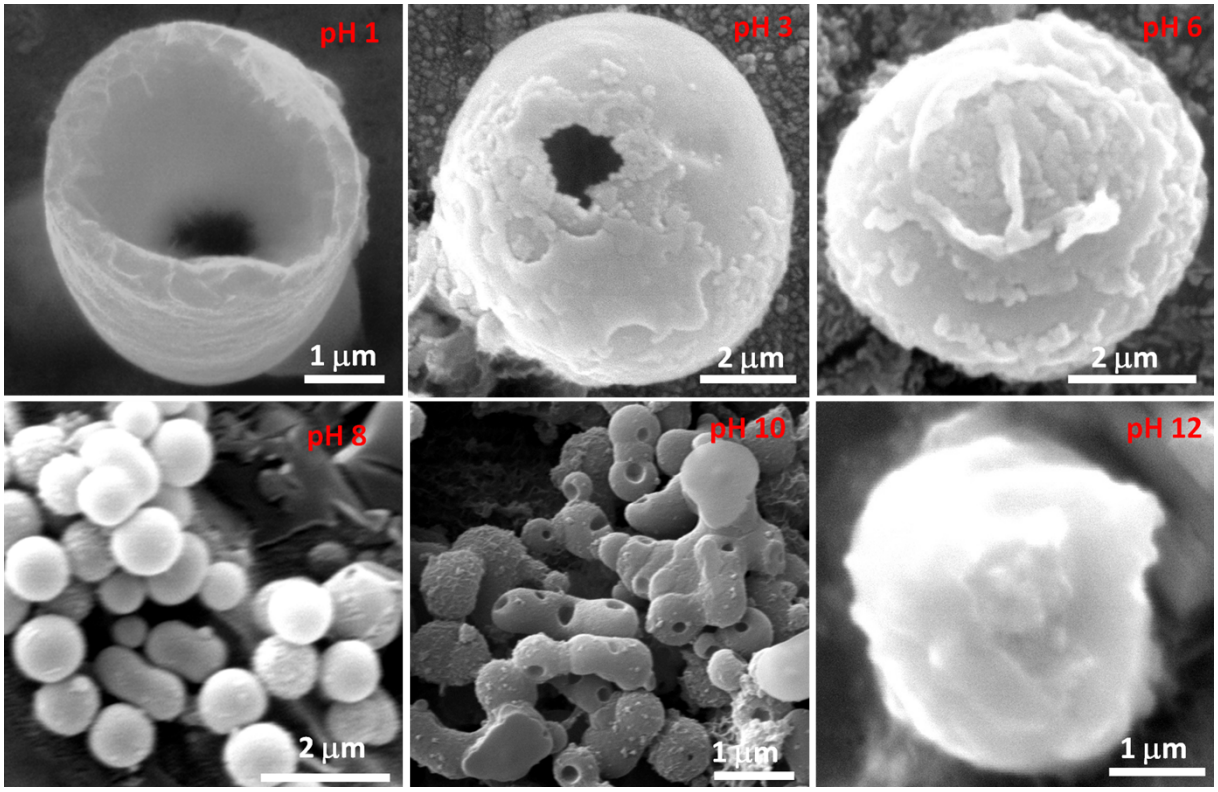

Figure S4: pH dependent study of gold nanoparticle-biotin-Trp-Trp biomaterials. The figure clearly depicts that at highly acidic and basic conditions the vesicular structure of **1** is not stable. The optimum pH at which these biomaterials were stable is in the range of pH 7-9.

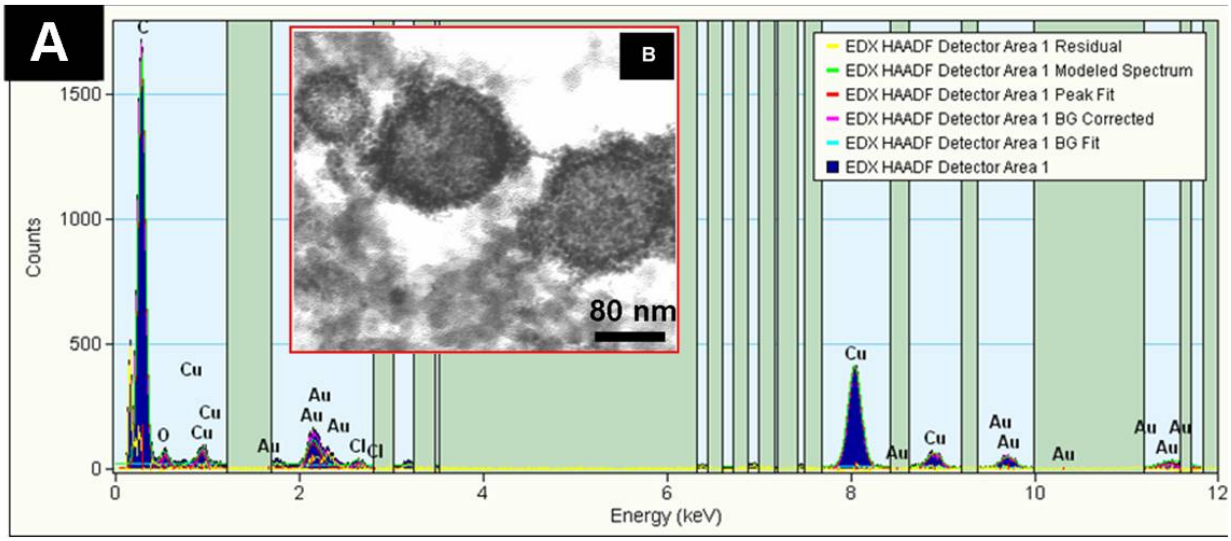

Figure S5: EDAX spectra of AuNPs loaded single vesicle taken from inset B.

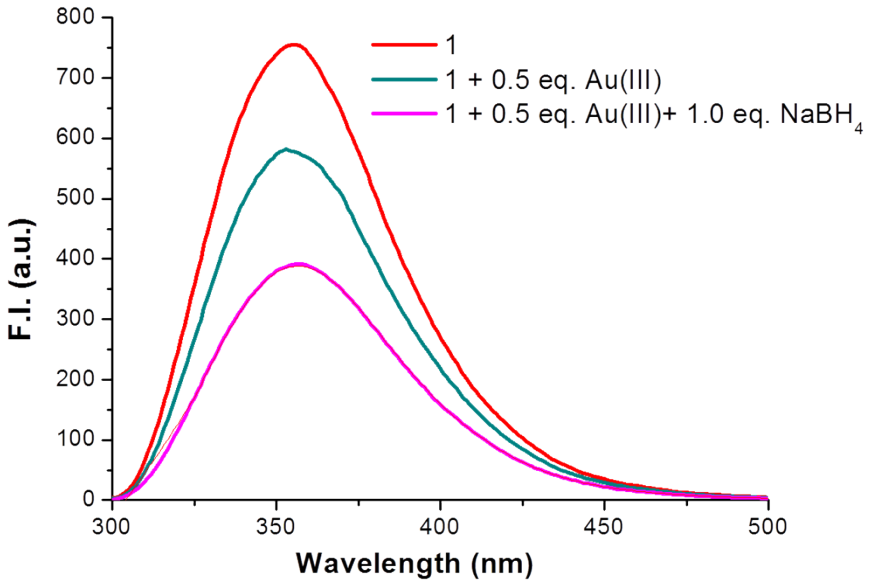

Figure S6: figure depicts that the quenching of Trp Fluorescence is possible after the addition of $NaBH_4$ which lead to the formation of the AuNPs.

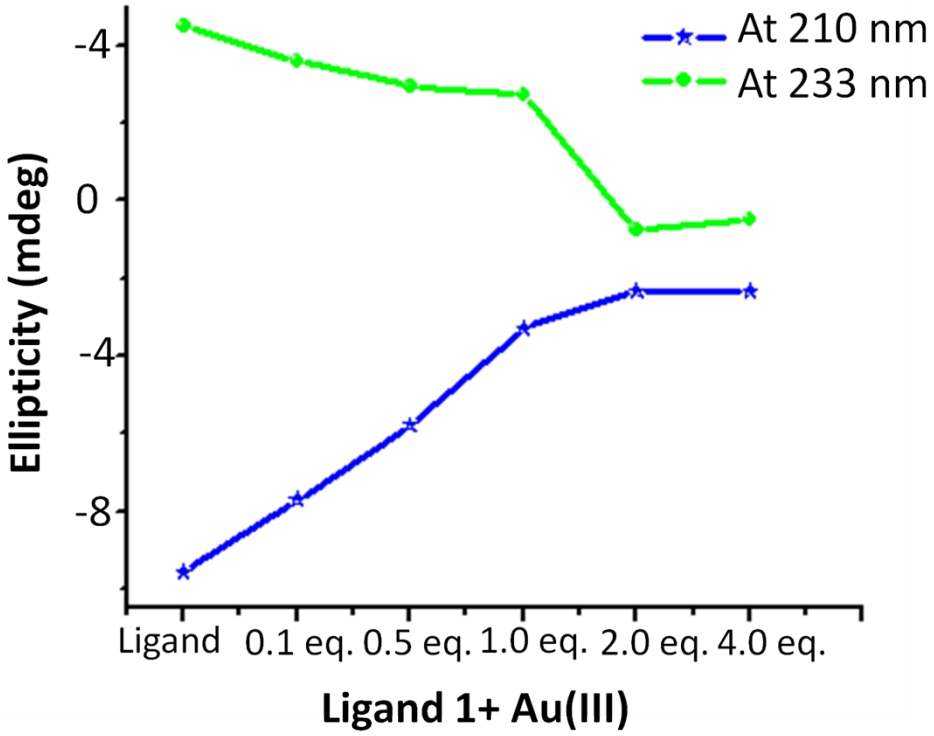

Figure S7: Au(III) ion dependent CD spectrum of 1 (0.5 mmol) at 210 and 233.

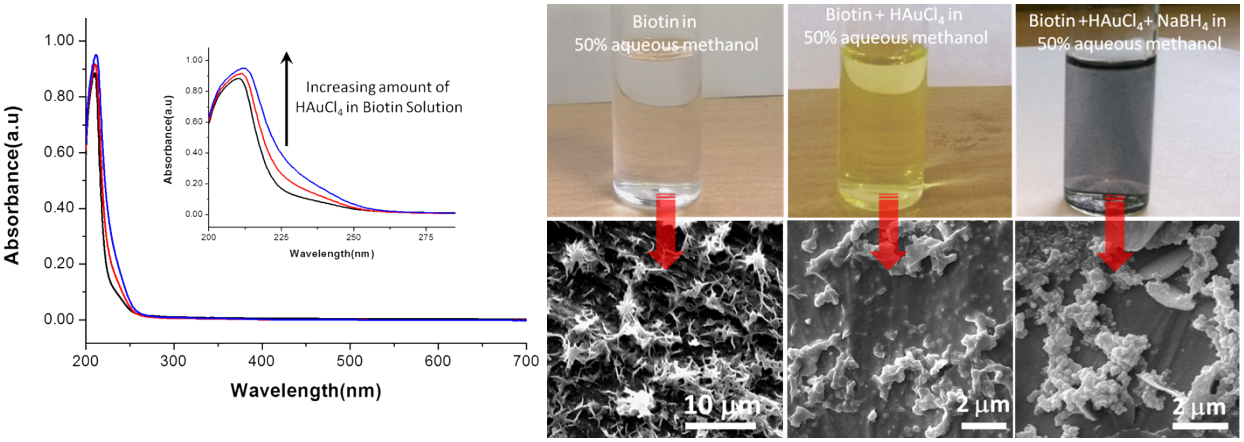

Figure S8: *Left:* UV spectra of Biotin (black trace), with $HAuCl_4$ (red trace) and after the addition of $NaBH_4$ solution (blue trace) depicts that the complex formation with biotin however SPR band was not observed hence unable to synthesized AuNPs. *Right:* photographs and corresponding SEM micrographs show that the reduction of $HAuCl_4$ is not possible in the presences of biotin alone and also the formation of AuNPs.

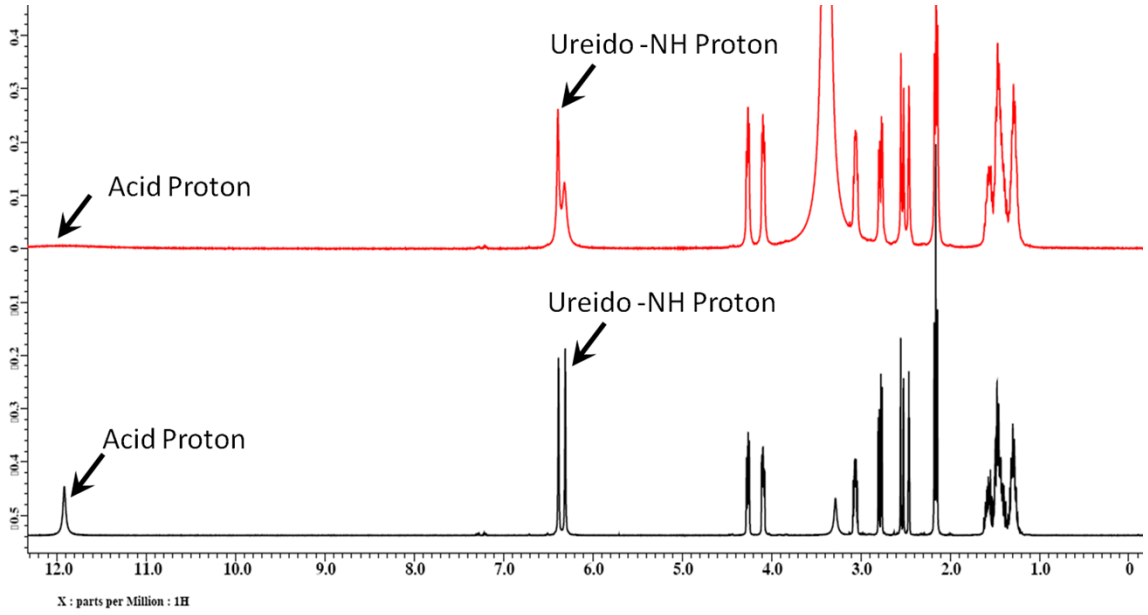

Figure S9: ***Bottom:*** ^{1}H NMR titration experiment spectra of Biotin (black trace) in DMSO-d_6 and ***Top:*** with the addition of $HAuCl_4$ (red trace) depicts that the complex formation between biotin and Au(III) ions is possible and possible sites of these interactions are Ureido NH and carboxyl acids protons.

Additional References:

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