Electronic Supporting Information:

Enzyme-free colorimetric assay of serum uric acid

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Experimental Section

Materials. $HAuCl_4$, $NaBH_4$, 2-TU, and UA were purchased from Sigma-Aldrich. All other chemicals used in this investigation were of analytical grade and used without further purification.

Instrumentation. Electronic absorption spectra were recorded with CARY 5000 UV-visible-NIR spectrophotometer. Fourier transform infrared spectroscopic (FTIR) measurements were performed with Perkin Elmer FTIR spectrophotometer RX1. TEM measurements were performed using JEOL Model JEM-2010 microscope with an operating voltage of 200 kV.

Synthesis of 2-TU functionalized Au nanoparticles. In a typical synthesis, 40 µL of $HAuCl_4$ (30 mM) was mixed with 90 µL of 2-TU (1 mM) and 2.8 mL of water. The reducing agent $NaBH_4$ (70 µL of 22 mM) was added to the mixture under vigorous stirring. The total volume of the sample was 3 mL and the final concentration of $HAuCl_4$, 2-TU and $NaBH_4$ was 0.4 mM, 30 µM and 0.51 mM, respectively. The stirring was continued for 40 min. and the resulting wine red colored 2-TU functionalized Au nanoparticle solution was stored at room temperature. The final concentration of $HAuCl_4$, 2-TU and $NaBH_4$ are 0.4, 0.03, and 0.51 mM, respectively. The molar ratio of 2-TU to $HAuCl_4$ is 0.075:1. The pH of the colloidal nanoparticles was measured to be 5.6.

It should be mentioned here that the nanoparticles synthesized at higher concentration (>30 μM) were not stable, presumably due to the self-aggregation (please refer the spectra given in ESI15 on page no 18) and they cannot be effectively used for the sensing of UA.

Synthesis of unfunctionalized Au nanoparticles: In a typical synthesis, 70 μL of $NaBH_4$ (22 mM) was added to an aqueous solution containing 0.4 mM of $HAuCl_4$ under vigorous stirring and the stirring was continued for 40 min.

Synthesis of citrate-stabilized Au nanoparticles: Citrate-stabilized Au nanoparticles were prepared according to the literature procedure with slight modification.[1] Briefly, 0.9 mL of 39 mM of sodium citrate dihydrate was added to the boiling solution of $HAuCl_4$ (9.1 mL, 0.9 mM) and stir for 30 min and the resulting wine red colored citrate-stabilized Au nanoparticle stored at room temperature.

Sensing of UA. Typically, an aqueous solution of UA (5 μL of 150 μM) was gradually added to the colloidal 2-TU functionalized Au nanoparticle (3 mL) and the spectrum was recorded after 1 min.

Real sample analysis. In order to minimize the matrix effect due to high molecular weight proteins, the serum samples were diluted by 10 times and filtered using 3 kDa molecular weight cut off (MWCO) micro-centrifuge filter. The filtered serum samples (25 μL) were injected into 2-TU functionalized nanoparticles and the optical spectrum was recorded after 1 min. After registering the spectrum, the sample was spiked with known concentration of UA and recovery was calculated from the spectral response after the spike.

1. G. Frens, *Nature Phys. Sci.*, 1973, **241**, 20.

DFT calculations : The density functional calculation in gas phase were performed using model system; 2-(methylthio)pyrimidine-4(3*H*)-one (MeTU) was chosen to represent 2-TU functionalized nanoparticles. Because AA exist as a monoionic species at pH>4.2 (first pK_a = 4.17, J. Am. Chem. Soc. 1935, 57, 1929), the ascorbate anion was considered in the calculation. All geometris were fully optimized using TURBOMOLE program package (TURBOMOLE V6.2 2010, a development of University of Karlsruhe and Forschungszentrum Karlsruhe GmbH, 1989-2007, TURBOMOLE GmbH, 1989-2007, TURBOMOLE GmbH, since 2007 available from http://www.turbomole.com). B97-D functional in conjunction with def2-SVP bais set was used.

Figure ESI1

(a) UV-vis spectrum, (b) TEM image and (c) particle size distribution plot of 2-TU functionalized Au nanoparticles

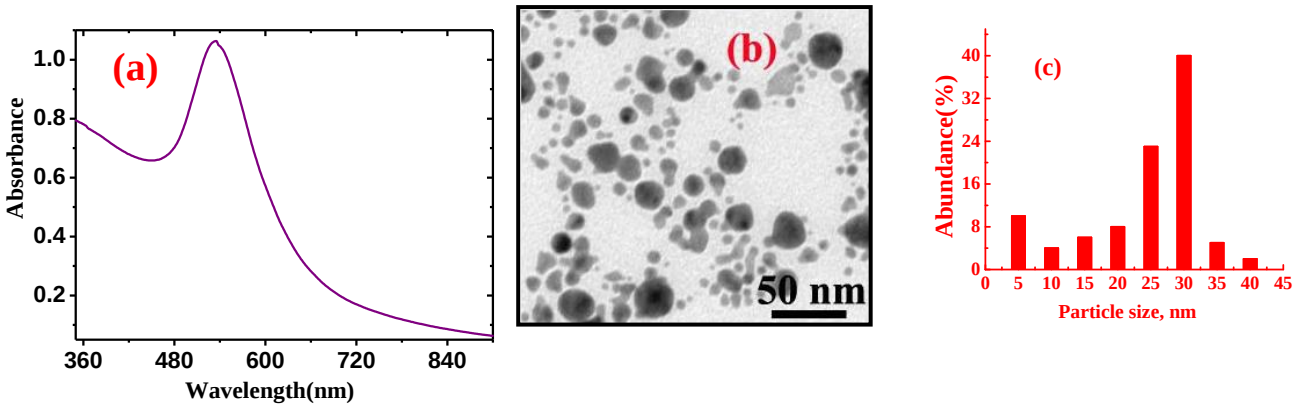
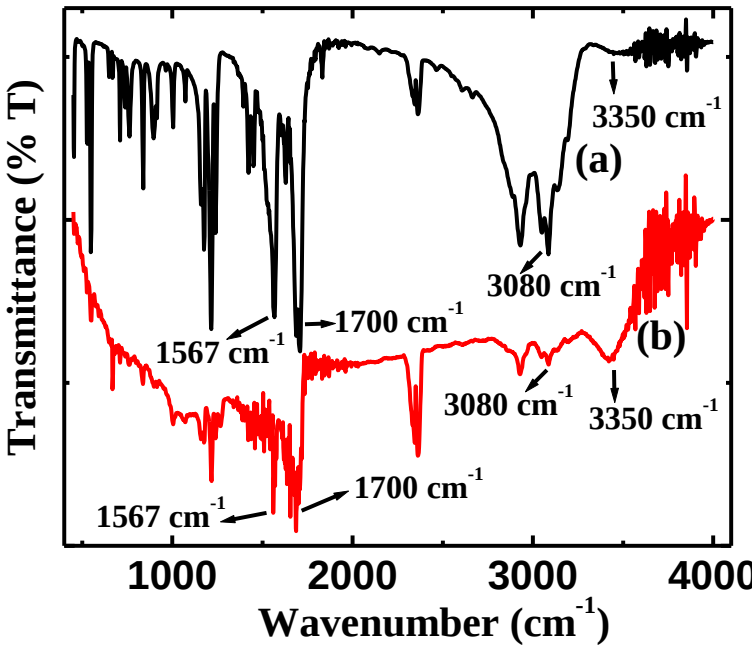


Figure ESI2

FTIR spectra of (a) 2-TU and (b) 2-TU functionalized Au nanoparticles.



Frequency	Assignment
3350 cm^{-1}	N–H stretching
3080 cm^{-1}	C–H stretching
1700 cm^{-1}	C=O stretching
1567 cm^{-1}	N–H in plane bending

Figure ESI3

TEM image of 2-TU functionalized Au nanoparticles in presence of UA (4 µM).

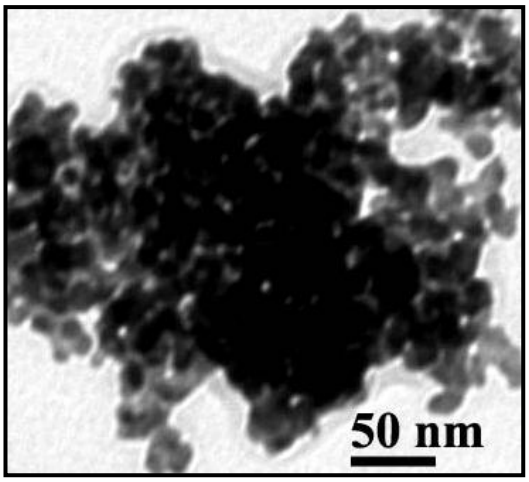


Figure ESI4

UV-vis spectra of (a) citrate-stabilized and (b) unfunctionalized Au nanoparticles in presence and absence of UA (10 µM). Inset shows TEM image Au nanoparticles. The procedure for the synthesis of citrate-stabilized and unfunctionalized nanoparticles are given on page 2 of ESI.

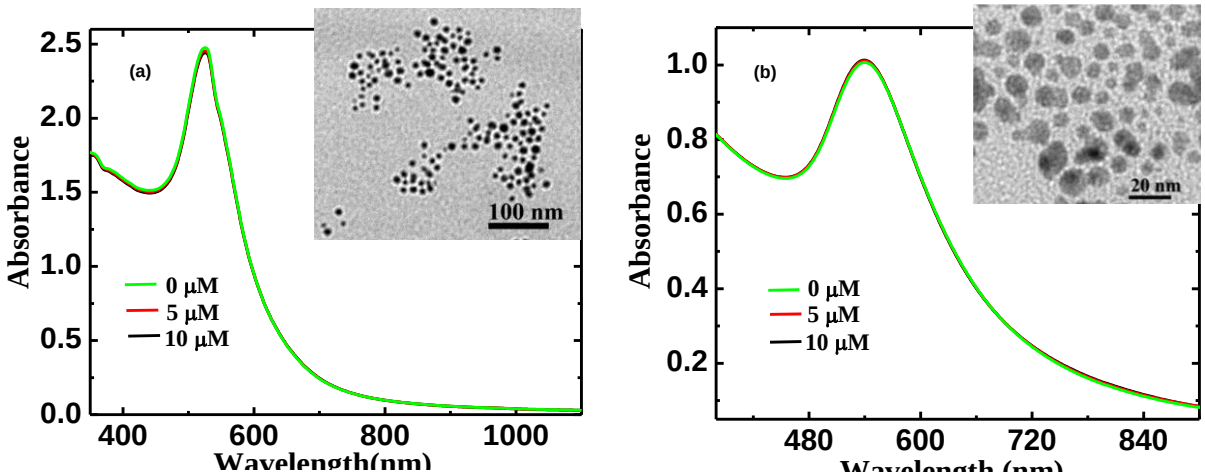
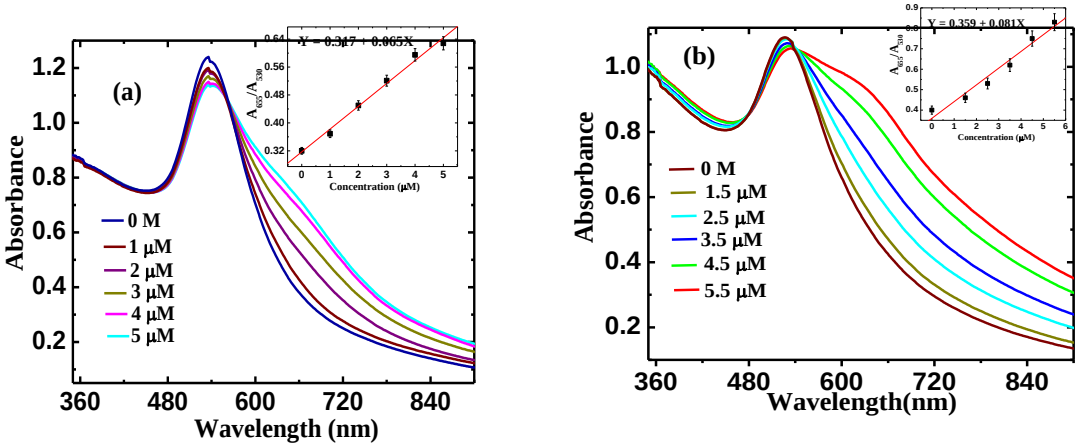


Figure ESI5

UV-vis spectral response of 2-TU functionalized Au nanoparticles towards UA, illustrating the concentration-dependent sensitivity and limit of detection of UA. Concetration of nanoparticles: (a) 0.25 nM and (b) 0.06 nM. Concentration of 2-TU: (a) 30 μM and (b) 10 μM. The cocnetration of the nanoparticles was calculated according to the standard literature procedure (Anal. Chem. **2001,** 73, 5220-5227). The nanoparticles were synthesized according to the following procedures.

(a) Mixing an aqueous solution of 0.5 mM of $HAuCl_4$, 30 μM of 2-TU, and 0.51 mM of $NaBH_4$ and stirring in a magnetic stirrer for 40 min. The total volume of the reaction mixture was 3 mL.

(b) Mixing an aqueous solution of 0.4 mM of $HAuCl_4$, 10 μM of 2-TU, and 0.51 mM of $NaBH_4$ and stirring in a magnetic stirrer for 40 min. The total volume of the reaction mixture was 3 mL.



Analytical data obtained from the measurements:

(a) Concentration of nanoparticle: 0.25 nM

Concentration of 2-TU: 30 μM

LOD (3σ): 1 μM

Slope of the calibration plot (sensitivity): 0.06 μM^{-1} (Y=0.317+0.065 X)

(b) Concentration of nanoparticles: 0.06 nM

Concentration of 2-TU: 10 μM

LOD (3σ): 1.5 μM

Slope of the calibration plot (sensitivity): 0.08 μM^{-1} (Y= 0.359+0.081 X)

Figure ESI6

UV-vis spectra of 2-TU functionalized Au nanoparticle in presence of different concentrations of UA. Inst shows the calibration plot. Different concentrations of UA were individually added to the sample vials of freshly prepared functionalized nanoparticles.

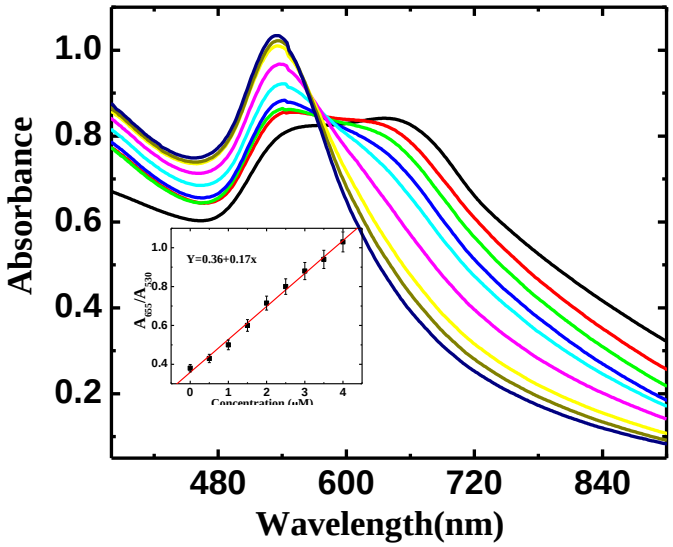


Figure ESI7

UV-vis spectra of 2-TU functionalized nanoparticles in the presence of UA (2.5 μM) at different time intervals.

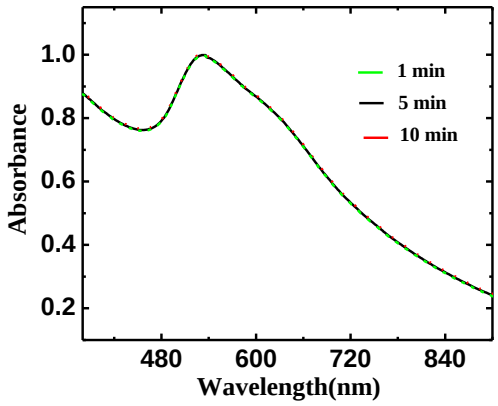


Figure ESI8

(a) Absorption spectra illustrating the selectivity of the optical sensing method towards UA using 2-TU functionalized Au nanoparticles and (b) Plot illustrating the selectivity of UA assay. The ratio of A_{655}/A_{530} is plotted against analyets. Concentration of the analytes: 0.1 mM each.

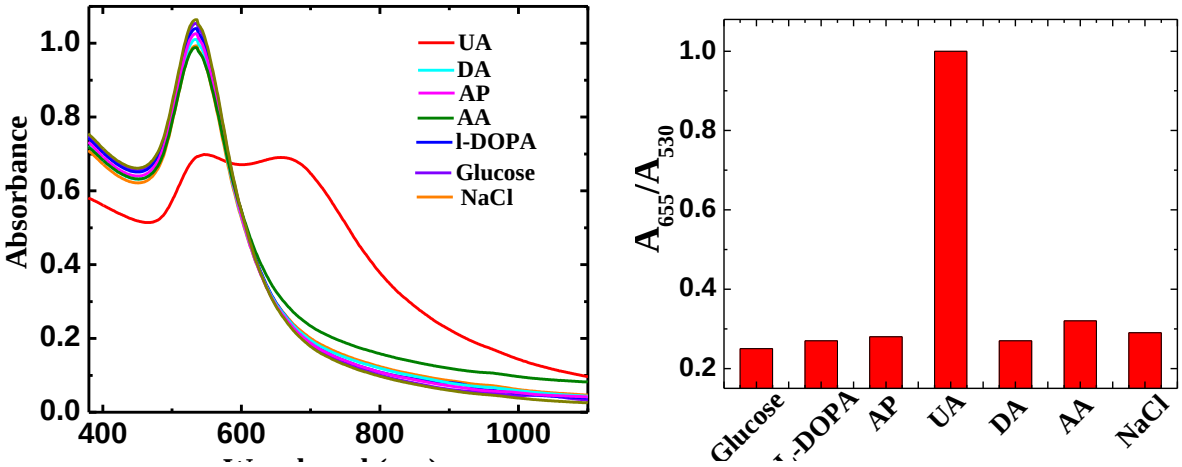
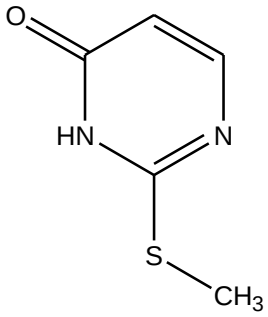
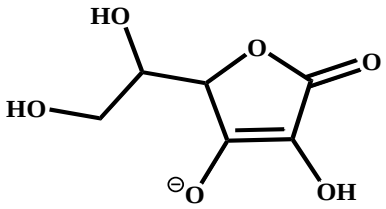


Figure ESI9

Structure of 2-(methylthio)pyrimidin-4(3*H*)-one and ascorbate anion.



2-(methylthio)pyrimidin-4(3*H*)-one



Ascorbate

Figure ESI10

The hydrogen bonding interaction UA with the MeTU based on the DFT calculation. The theoretical calculation indicates that up to 6 units of MeTU can have favorable hydrogen bonding interaction with one molecule of UA.

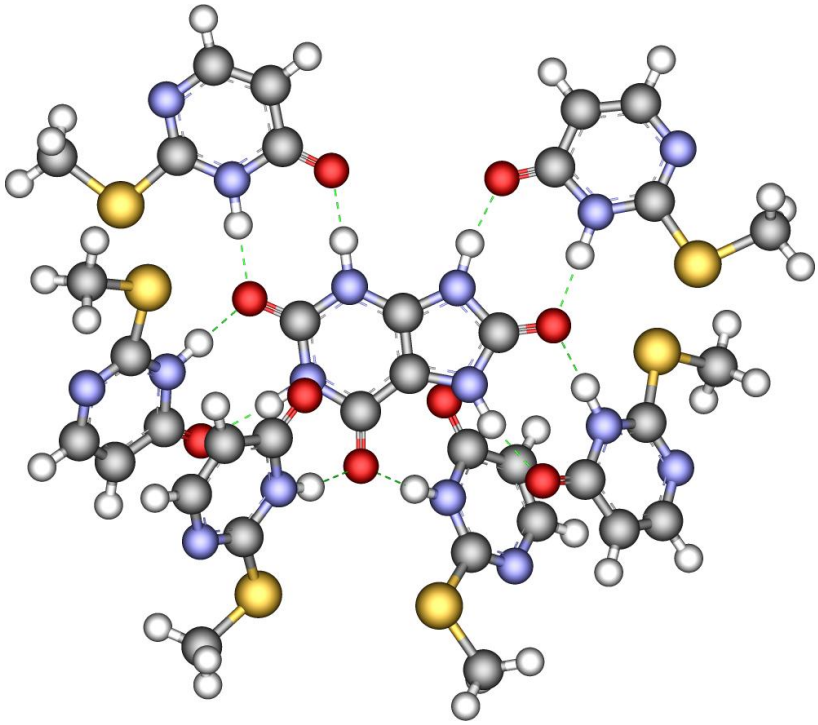


Figure ESI11

The geometry based on the preliminary DFT calculation showing possible π-π interaction of two hydrogen bonded UA units. (Four MeTU molecules per plane are considered). Top-view (left) and side-view (right).

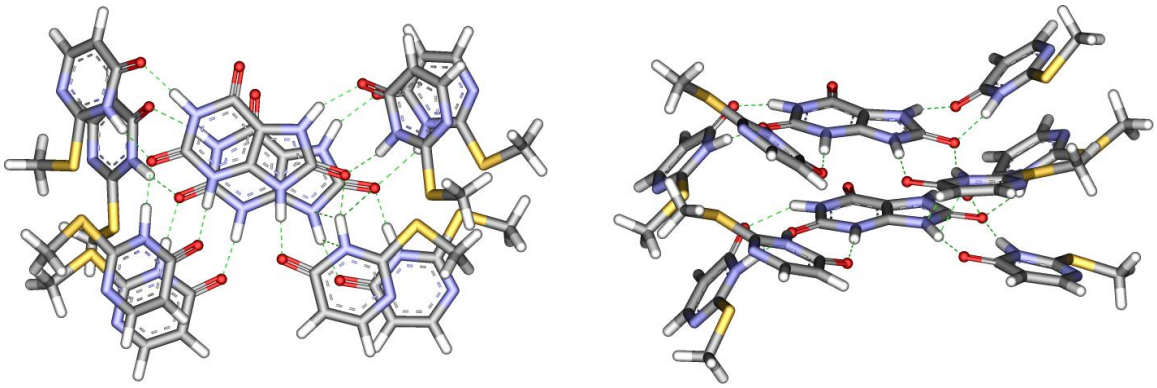


Figure ESI12

Optimized geometry and possible hydrogen bonding of AA with MeTU.

Unlike UA, AA is highly flexible and is not a planar molecule. Although AA can have hydrogen bonding interaction, it could not effectively aggregate the nanoparticles due to the fact that TU units are randomly oriented around AA anion.

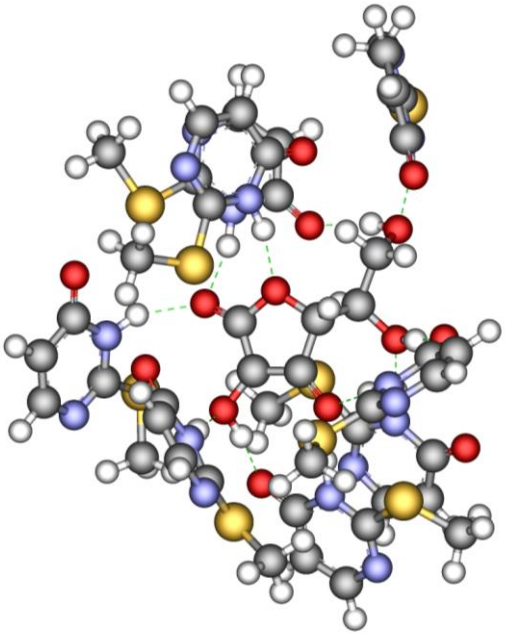


Figure ESI13

UV-vis spectral profile illustrating the selectivity of the colorimetric method. Different concentrations of interfering analyte (a) glucose and (b) 4-acetamidophenol were progressively added to the functionalized nanoparticles.

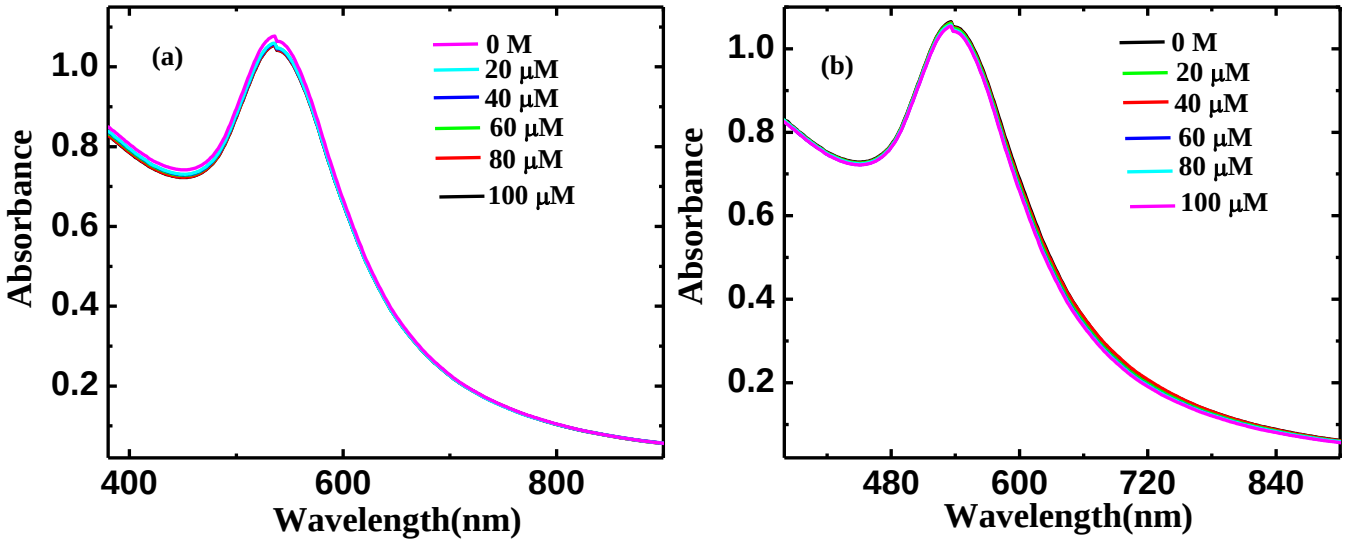


Figure ESI14

Absorbance vs time plot at 655 nm in presence of real sample analysis.

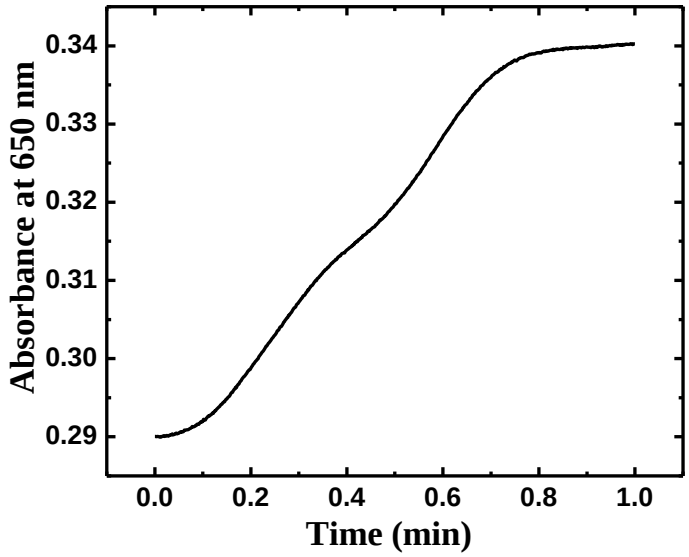
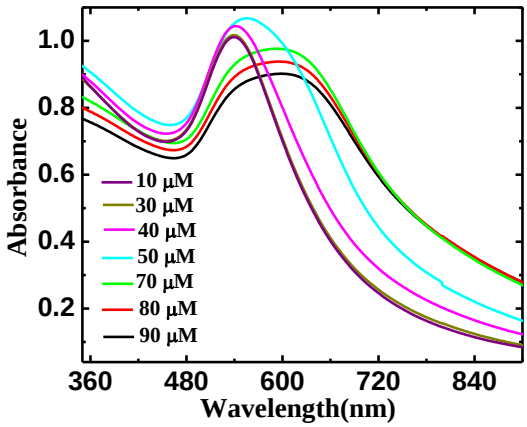


Figure ESI15

UV-vis spectra of Au nanoparticles synthesized at different concentration of 2-TU.



The above spectra illustrate the self-aggregation of nanoparticles while increasing the concentration of 2-TU. The surface plasmon band gradually shifts to higher wavelength side and the spectral response is very broad, possibly due to the self-aggregation of the nanoparticles. The colloidal nanoparticle is not stable for long time; the particles gradually settle down in the sample vial.