Supporting Information

Ultra Small Gold Nanoparticles Synthesis in Aqueous Solution and their application in Fluorometric Collagen Estimation using Bi-ligand Functionalisation

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SI:1 Calculation of the concentration of optimised LBH-AuNPs.

SI:2 Fluorescence standard graphs of Lysine and FITC

SI:3(a) Fluorescence spectra of recovered fluorescence of FITC and Lysine from AFL NPs using different concentration of collagen (2μg/ml to 10μg/ml)

SI:3(b) TEM and optical image of collagen-AFL nanoparticles

SI:3(c) Standard graph of extracted rat tail collagen by Sirius red test.

SI:3(d) Rat tail collagen estimation tables.

SI:3(e) Fluorescence spectra of recovered fluorescence of FITC and Lysine from mono-functionalised AuNP-Lysine (AL) and AuNP-FITC (AF) particles using different concentration of collagen (2 μg/ml to 10μg/ml)

SI:1 Calculation of concentration of LBH-AuNPs

The concentration of the nanoparticle was calculated by an experimental model [1] involves multistep evaluation. First, average diameter of the AuNP was estimated from TEM micrograph of LBH-AuNPs. According to this value, molar extinction coefficient (ε), was calculated from this standard experimental model. Next, AuNP concentration was determined using extinction intensity *vs* extinction coefficient at 450 nm. The concentration of optimum LBH-AuNP synthesized at 2.64mM of $LiBH_4$ was evaluated as 1.3 μM. The calculations are given below.

Average size of LBH-AuNP = 2.25±0.30nm

$C=A_{450}/\varepsilon_{450}$ = 0.5528/4.25Ã—10^5 = 1.3μM

SI: 2 Fluorescence standard graphs of FITC and Lysine

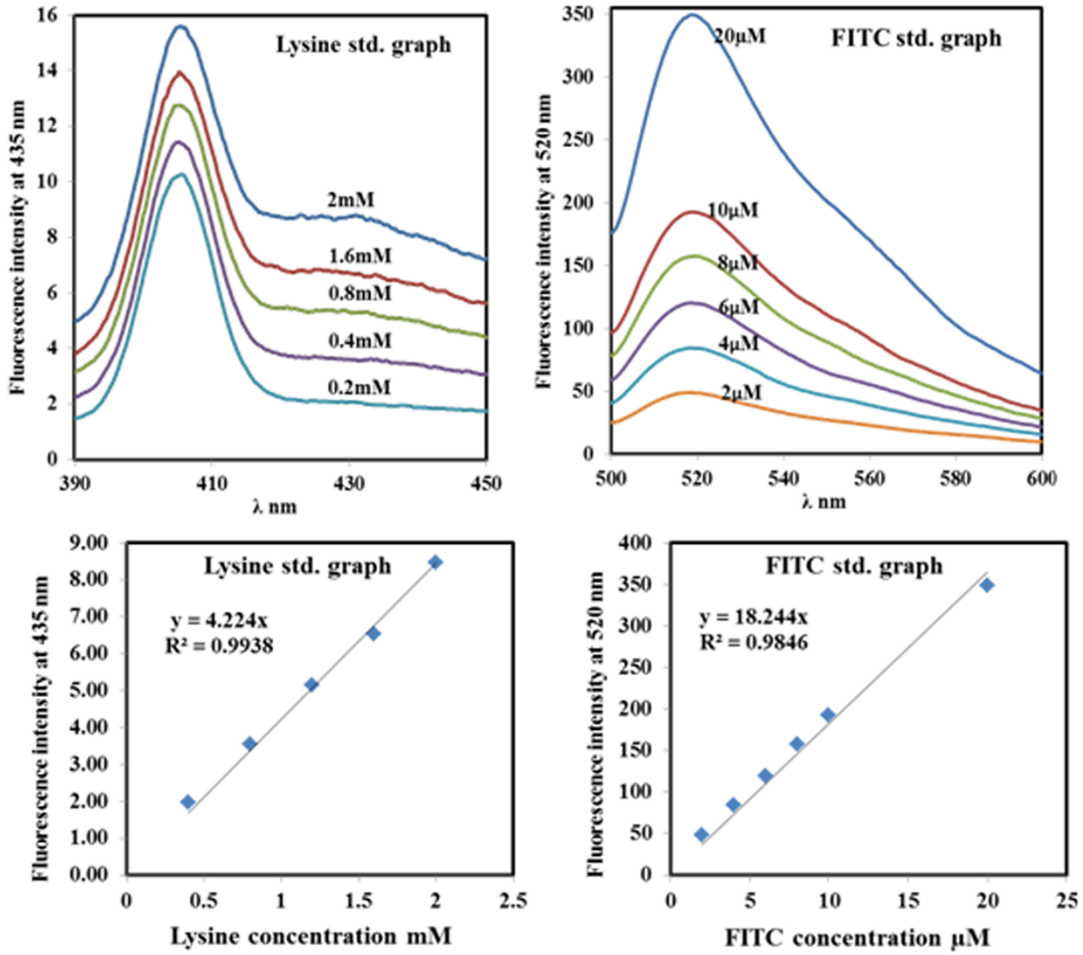

Fig SI:2 Standard graphs of fluorescence of FITC and lysine

SI: 3 (a) Fluorescence spectra of recovered fluorescence of FITC and Lysine from AFL NPs using different concentration of collagen (2 µg/ml to 10µg/ml)

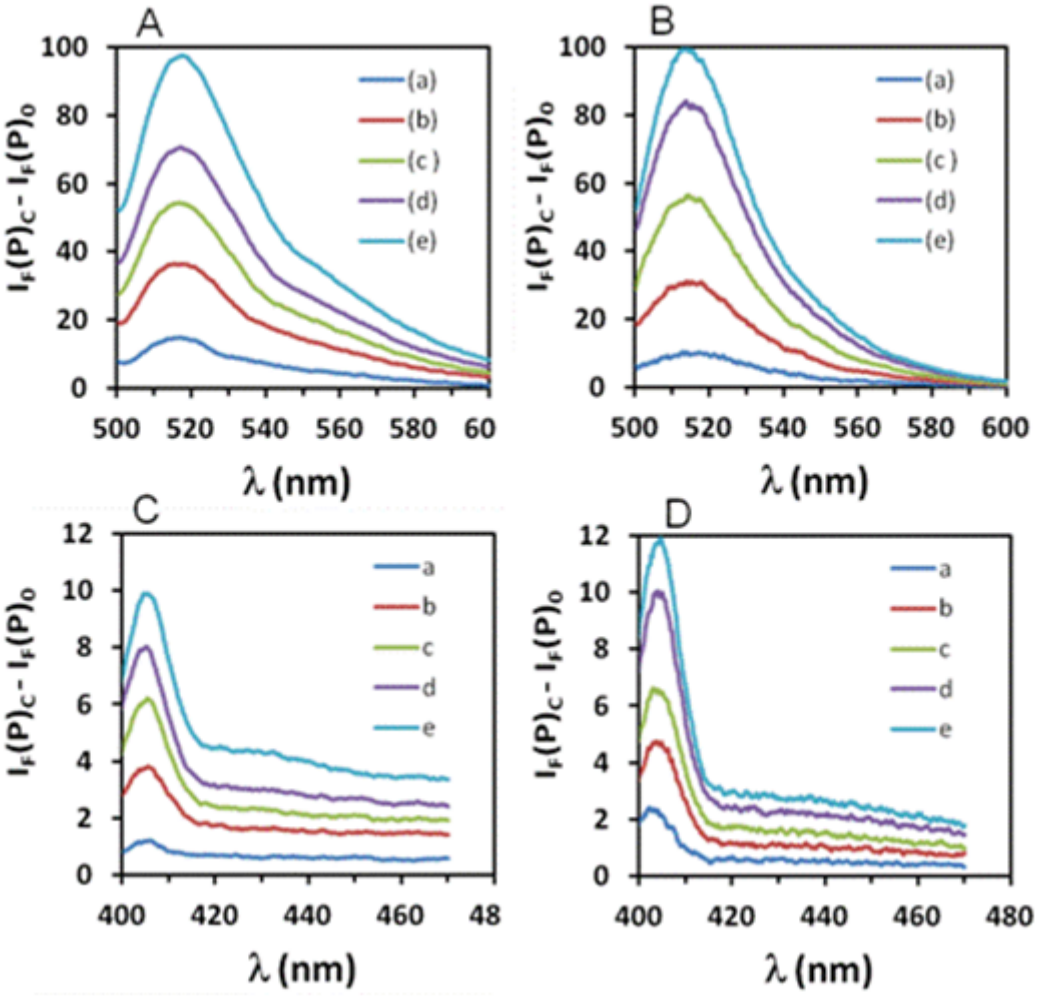

Fig SI: 3(a) Reappearance of fluorescence of FITC and Lysine from AuNP-FITC- Lysine (AFL) nanoaprticles with different concentration of collagen (a) 2µg/ml, (b) 4 µg/ml , (c) 6µg/ml, (d) 8µg/ml, (e) 10µg/ml. A, B, C and D are Fluorescence of FITC (standard collagen), FITC (rat tail collagen), lysine (standard collagen) and lysine (rat tail collagen) respectively.

SI: 3(b) TEM and optical image of collagen-AFL nanoparticles

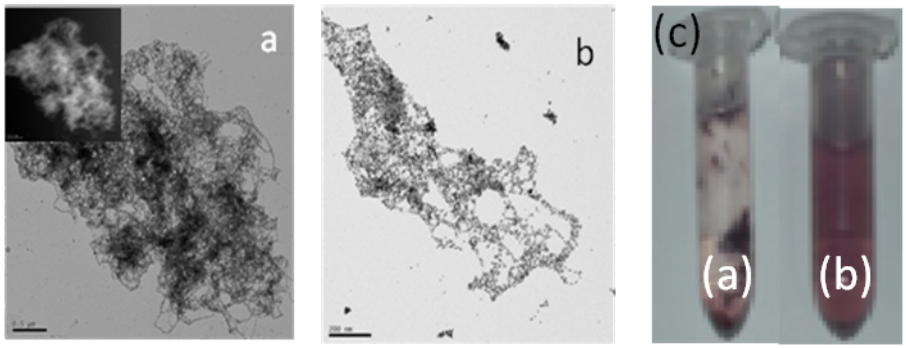

Fig SI: 3(b) TEM and optical image of Collagen-AFL nanoparticles : (a) 20µg/ml, (b) 2µg/ml and (c) corresponding COL-AFL images .

SI: 3(c) Standard graph of extracted rat tail collagen adjusted by Sirius red test

Collagen was extracted from procedure followed by Navneeta Rajan et. al [2]and estimated by Sirius red test[3].

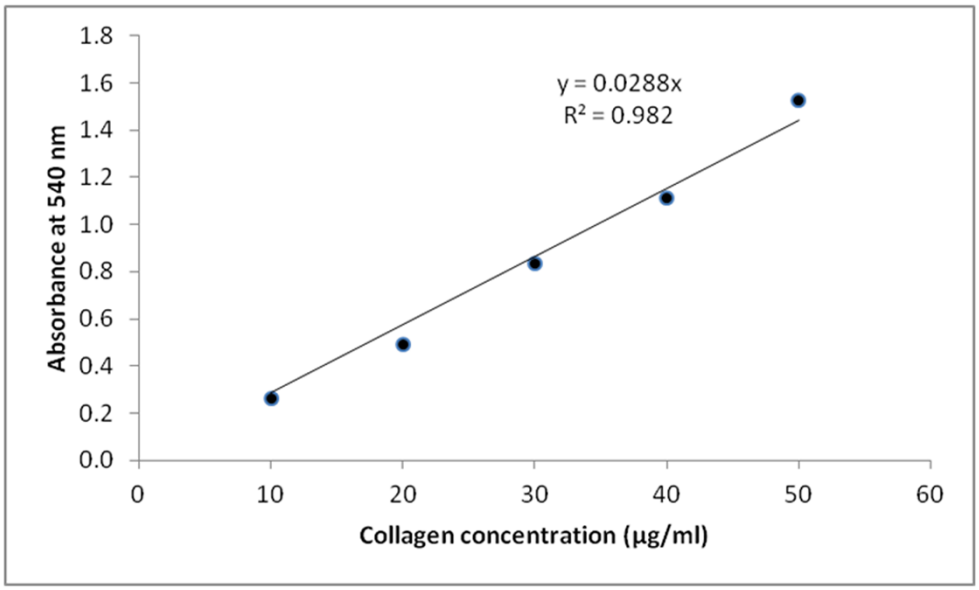

Fig SI: 3(c) Sirius res test standard graph of Rat tail extracted collagen.

SI: 3(d) Rat tail collagen estimation tables

(I)

Collagen Conc. (µg/ml)	Collagen found (µg/ml, n=3)	Recovery(%, n=3)	RSD(%, n=3)
2	1.63	81.59	2.34
4	2.81	70.34	1.36
6	4.09	68.17	0.93
8	5.92	73.97	0.65
10	7.07	70.66	0.54

(II)

Collagen Conc. (µg/ml)	Collagen found (µg/ml, n=3)	Recovery(%,n=3)	RSD(%,n=3)
2	1.15	57.47	2.34
4	3.31	82.83	1.69
6	5.94	99.00	2.82
8	8.91	111.34	4.67
10	10.56	105.61	4.70

Fig SI: 3(d) Table (I) and (II)showed the estimation of rat tail collagen by restored lysine and FITC fluorescence respectively.

SI: 3(e) Fluorescence spectra of recovered fluorescence of FITC and Lysine from mono-functionalised AuNP-Lysine (AL) and AuNP-FITC (AF) particles using different concentration of collagen (2 µg/ml to 10µg/ml)

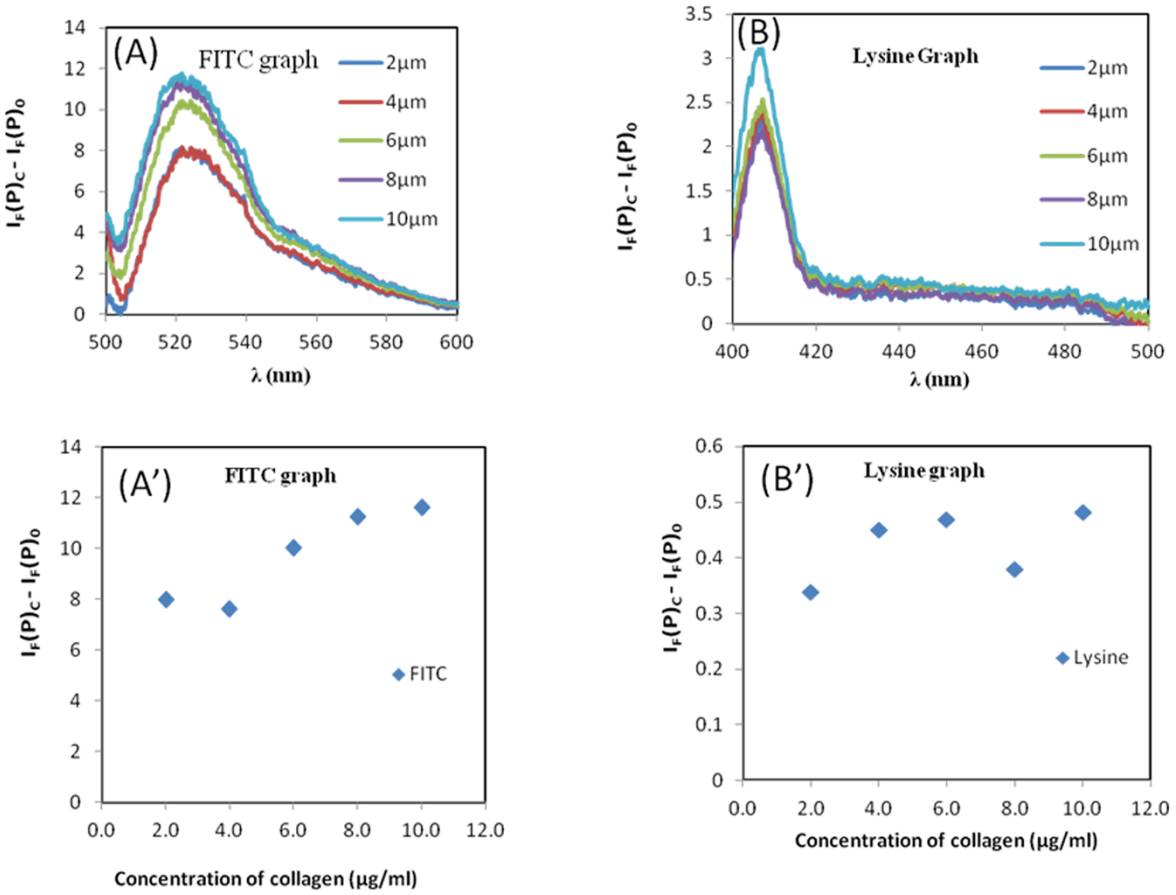

Fig SI: 3(e) Reappearance of fluorescence of FITC and Lysine from AuNP-FITC (AF) and AuNP-Lysine (AL) nanoparticles with different concentration of pure standard collagen from 2µg/ml to 10ug/ml. (A) and (B) represent the fluorescence of FITC and lysine respectively, and (A') and (B') represent the corresponding fluorescence of FITC and Lysine at 520nm and ~435nm respectively.

References:

1. Wolfgang Haiss, Nguyen T. K. Thanh, Jenny Aveyard, and David G. Fernig, Anal. Chem. 2007, 79, 4215-4221.
2. Navneeta Rajan, Jason Habermehl , Marie-France Coté , Charles J Doillon & Diego Mantovani, *Nature Protocols,* 2007, **1**, 2753 – 2758.
3. Marcello marota,Guglielmo martino, *Analytical biochemistry,* 1985,**150,** 86-90.