The biocompatible validity of amino acid ionic liquid mediated gold nanoparticles for enhanced activity and structural stability of papain

Sumit Kumar and Pannuru Venkatesu*
Department of Chemistry, University of Delhi, Delhi - 110007, India

Instrumentations

Differential scanning calorimetry (DSC) NANO DSC instrument (TA Instruments, USA) has been used to monitor temperature effect on the thermal stability of papain in the presence of different concentration of [CHO][Trp] and [CHO][Trp]IL mediated AuNPs. The 1 mg/mL of papain has been loaded for measuring all the measurements. For every measurement, approximately 0.650 μL of sample was loaded into the sample capillary provided in the instrument. All samples were degassed for nearly 10 min at degassing station attached with the instrument. The PBS buffer of pH 7.02 has been used for preparing and maintaining the pH of all the samples. The heat flow change with temperature was recorded in the range from 30 to 90 °C at fixed pressure of 3 atm and equilibration time of 300 s. The papain protein shows no change below 50 °C, therefore the finalized data have been recorded for the range of 50 to 90 °C. Finally, all the data have been analyzed with NANO analyzer software using sigmoidal curve fitting and two state model. The NANO analyzer software in the instrument was efficinet to analyse, retrieve and store the thermal denaturation data. The denaturing temperature (T_m) values for all the measurements were calculated from plot of heat capacity (C_P) v/s temperature.

Concentration of [CHO][Trp] and [CHO][Trp]IL mediated AuNPs

The concentration of bulk [CHO][Trp] and [TEA][Trp]IL mediated AuNPs has been calculated with the help of TEM and UV-visible spectroscopy using a protocol provided by Liu et al[1]. The extinction coefficient of [CHO][Trp] and [TEA][Trp]IL mediated AuNPs was calculated to be $3.52 * 10^7$ and $2.80 * 10^7$ $M^{-1}cm^{-1}$, respectively. Therefore, the concentration of prepared solution of [CHO][Trp] and [TEA][Trp]IL mediated AuNPs has been calculated to be 24.75 and 23 nM, respectively. A standard bulks of 10 nM concentration has been prepared for both [CHO][Trp] and [TEA][Trp]IL mediated AuNPs by diluting the above solutions. Moreover, for the thermal and various studies of papain with AuNPs, the amount of 100, 200, 300, 400 and 500 μL/mL was taken from these standard bulks of [CHO][Trp] and [TEA][Trp]IL mediated AuNPs to make the final AuNP concentration of 1, 2, 3, 4 and 5 nM, respectively.

Transmission electron microscopy (TEM)

Morphological characterization of papain in the presence of different concentration of [CHO][Trp] and [TEA][Trp]IL mediated AuNPs was recorded on cryo TEM 200 kV (Fei, electron optics). The instrument is equipped with digital imaging and 35 mm photography system. For TEM measurements Carbon-coated 200 square mesh copper grids were used. The 20 µL sample of $6.30*10^{-6}$ M papain solution was loaded to carbon side of TEM grid followed by negative staining with phosphotungstic acid (PTA). It was left undisturbed for 1 hr to make grid moisture free in a silica desiccator. For the better understanding of size and interactions taking place between papain and [CHO][Trp] and [CHO][Trp]IL mediated AuNPs, images were taken at different magnifications with varying the scale.

UV-visible spectroscopy measurements

Absorption spectra of Papain were recorded on Shimadzu UV-1800 (Japan) spectrophotometer. The instrument has the highest resolution of 1 nm and the spectra were recorded using 1 cm path length quartz cuvette. The spectra were recorded at room temperature in the wavelength range of 250–650 nm. The blank solutions (sample without papain) subtraction was done for each sample and each spectrum was obtained after averaging the values from three scans.

Steady state fluorescence measurements

Steady state fluorescence emission spectra measurements were carried out at 25 °C on Cary Eclipse fluorescence spectrofluorimeter (Varian instruments, Mulgrave, Australia). It is equipped with Peltier-type temperature controller with a precision of ±0.05 °C and an intense xenon flash lamp. The emission spectra were recorded using the excitation wavelength at 295 nm and the concentration of papain was fixed at 1 mg/mL. Both the excitation and emission slit widths were set at 5 nm respectively.

Fourier transform infrared spectroscopy (FTIR)

FTIR spectra of papain in the absence and presence of [CHO][Trp] and [CHO][Trp]IL mediated AuNPs were recorded in the range of 4000–400 cm^{-1} with the help of Thermo- Fisher Scientific FTIR spectrometer. For the preparation of samples, buffer was prepared in D_2O and maintained

at pD ~7.0. The concentration of papain was fixed at 2.5 mg/mL and was pre-equilibrated for 1-2 hour at room temperature. Before taking the spectra for samples, spectra for background and buffer were collected under the same condition. About 40 μL was placed between ZnSe windows by using a spacer of 50 μm path length. Total of 256 interferometer scans were performed with 2 cm^{-1} resolution. For analyzing the data Omnic software was used.

Circular dichroism (CD) measurements

All the circular dichroism (CD) spectral studies were performed on Jasco-185 spectrophotometer (USA) equipped with a Peltier system for controlling the temperature having an accuracy of ±0.1 °C. The system was calibrated with (1S)-(+)-10-camphorsulfonic acid (Aldrich, Milwaukee, WI) possessing molar ellipticity (Θ) of 2.36 M cm−1 at 295 nm and molar extinction coefficient of 34.5 M cm^{-1} at 285 nm. The far UV (240− 190 nm) and near UV (250-350) spectra of protein in different concentrations of [CHO][Trp] and [CHO][Trp]IL mediated AuNPs was obtained using 0.1 and 1 cm path length quartz cells respectively. Through Dichroweb software the structural parameters: α-helix, β-sheet, β-turns and unordered were obtained. All measurements were repeated thrice to ensure reproducibility.

Dynamic light scattering and Zeta potential calculations of papain

For determining the hydrodynamic diameter (d_H) and Zeta-potential, the Zetasizer Nano ZS90 (Malvern Instruments Ltd., UK) was employed. The Zetasizer instrument is equipped with a 4 mW He−Ne laser with a laser attenuator (automatic) having the transmission of 100−0.0003%. For Zeta potential measurement, DTS1070 disposable cuvettes were used. For all the measurements, detection rate was set from 0.1 nm to 10 μm and to obtain thermal equilibrium, the temperature was set to 25.0 °C. Each measurement was recorded thrice, to improve the signal-to-noise ratio. Ultimately, Stokes−Einstein equation was employed for determining the diffusion coefficient and thereby calculating d_H. The data was analyzed using the Malvern Zetasizer Software version 7.01.

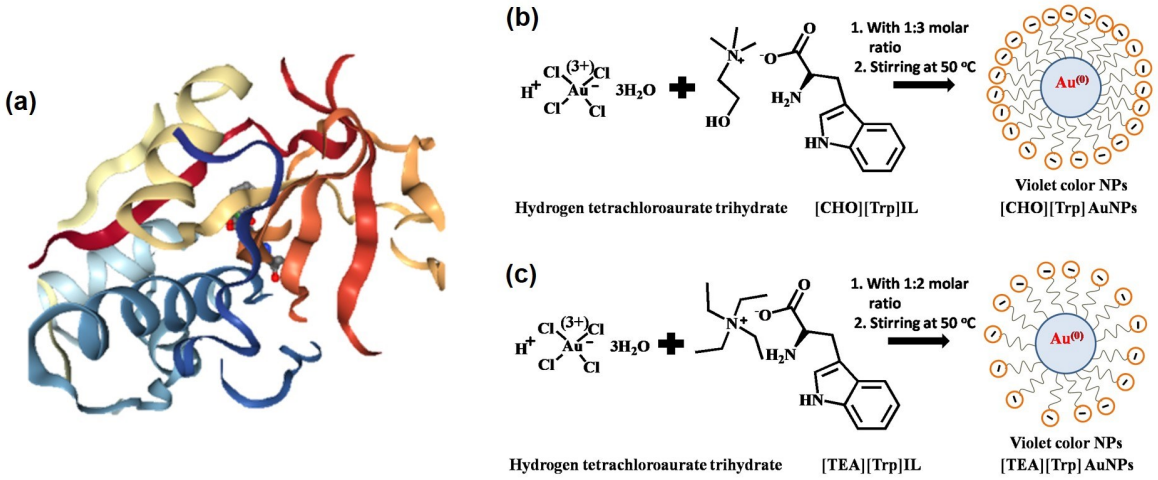

Figure 1S (a) The structure of papain has been downloaded from protein data bank and been finalized using PyMOL software. The brief information about the synthesis and structure of [CHO][Trp] and [TEA][Trp]IL-mediated AuNPs has been given in Figs. 1S b and c, respectively.

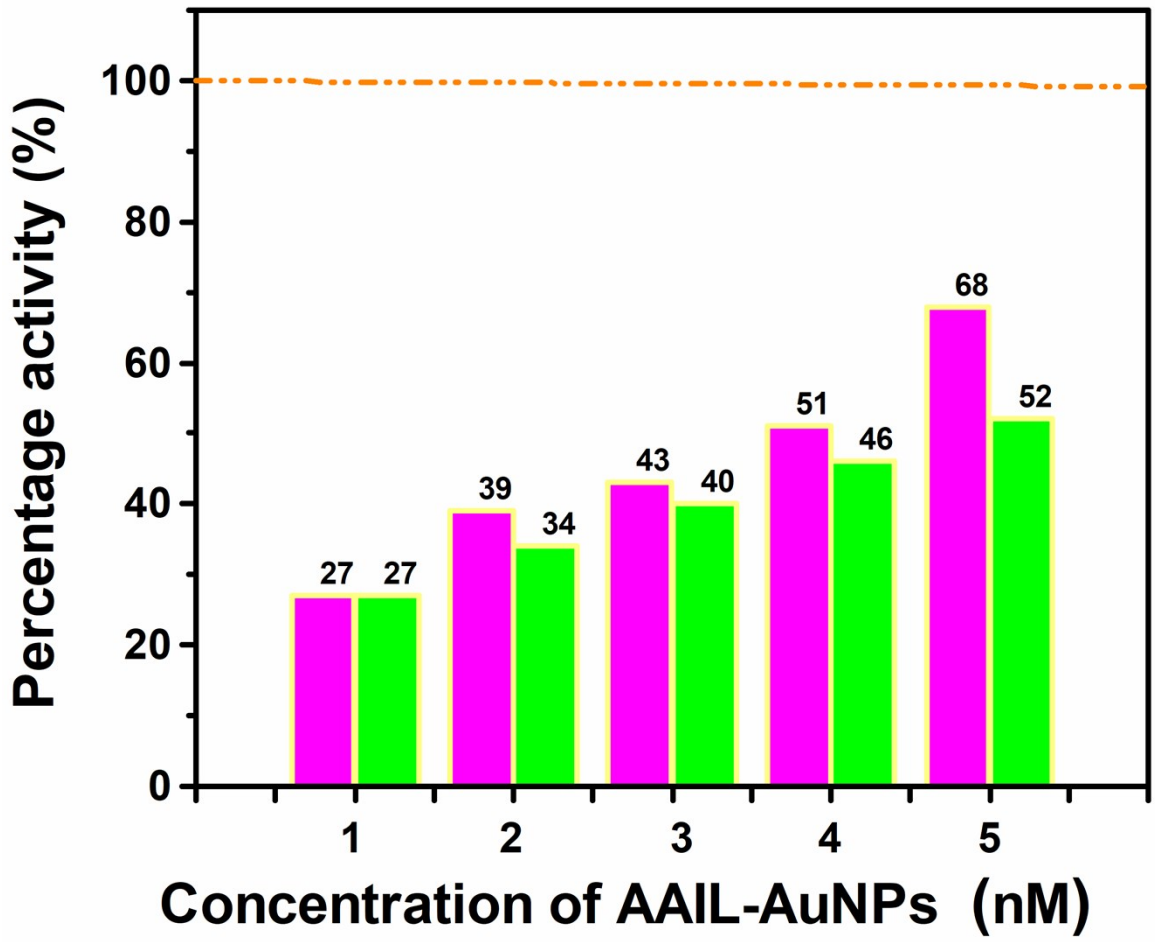

Fig. 2S The Proteolytic activity measurements of [CHO][Trp] (magenta color) and [TEA][Trp] (green color) modified AuNPs in the absence of papain. The orange dashed and dotted line is representing the activity of pure papain. The decomposition of AAIL-AuNPs in the presence of TCA has been observed in the proteolytic activity in the absence of papain.

Table 1S The denaturing temperature (T_m) of papain in the absence and presence of various concentration of [CHO][Trp] and [TEA][Trp]IL mediated AuNPs.

S. No.	Concentration of AAIL-mediated AuNPs (nM)	T_m values (^oC) of papain (1 mg/mL) in various concentration of AAIL-mediated AuNPs

		T_m in [CHO][Trp]IL mediated AuNPs	T_m in [TEA][Trp]IL mediated AuNPs
1.	0	76.90 ± 1.83	
2.	1	76.91 ± 1.26	76.50 ± 1.38
3.	2	74.20 ± 1.52	75.11 ± 1.71
4.	3	74.67 ± 0.85	75.53 ± 0.62
5.	4	73.92 ± 1.06	75.30 ± 0.96
6.	5	73.48 ± 0.64	75.20 ± 1.14

Table 2S The change in heat capacity, $\Delta_u C_p$ ($kJmol^{-1}K^{-1}$) values of papain in the absence and presence of various concentration of [CHO][Trp] and [TEA][Trp]IL mediated AuNPs.

S. No.	Concentration of AAIL mediated AuNPs (nM)	Heat capacity, $\Delta_u C_p$ ($kJmol^{-1}K^{-1}$) values of papain (1 mg/mL) in various concentration of AAIL mediated AuNPs	
		Papain in [CHO][Trp]IL mediated AuNPs	Papain in [TEA][Trp]IL mediated AuNPs
1.	0	13 ± 0.01	13 ± 0.01
2.	1	2 ± 0.03	60 ± 0.02
3.	2	60 ± 0.02	100 ± 0.03
4.	3	20 ± 0.01	20 ± 0.05
5.	4	20 ± 0.04	12 ± 0.03
6.	5	24 ± 0.02	13 ± 0.01

Table 3S The change in enthalpy, $\Delta_u H$ ($kJmol^{-1}$) values of papain in the absence and presence of various concentration of [CHO][Trp] and [TEA][Trp]IL mediated AuNPs.

S. No.	Concentration of AAIL mediated AuNPs (nM)	Enthapy, $\Delta_u H$ (kJmol^{-1}) values of papain (1 mg/mL) in various concentration of AAIL mediated AuNPs	
		Papain in [CHO][Trp]IL mediated AuNPs	Papain in [TEA][Trp]IL mediated AuNPs
1.	0	533.3 ± 0.04	533.3 ± 0.04
2.	1	541.9 ± 0.01	618.7 ± 0.02
3.	2	655.8 ± 0.02	438 ± 0.03
4.	3	438.6 ± 0.04	429.1 ± 0.02
5.	4	402.8 ± 0.05	393.8 ± 0.03
6.	5	385.5 ± 0.01	435.41 ± 0.05

Table 4S The change in entropy, $\Delta_u S_p$ (kJmol^{-1}K^{-1}) values of papain in the absence and presence of various concentration of [CHO][Trp] and [TEA][Trp]IL mediated AuNPs.

S. No.	Concentration of AAIL mediated AuNPs (nM)	Entropy, $\Delta_u S_p$ (kJmol^{-1}K^{-1}) values of papain (1 mg/mL) in various concentration of AAIL mediated AuNPs	
		Papain in [CHO][Trp]IL mediated AuNPs	Papain in [TEA][Trp]IL mediated AuNPs
1.	0	1.52 ± 0.04	1.52 ± 0.04
2.	1	1.55 ± 0.01	1.77 ± 0.02
3.	2	1.89 ± 0.02	1.26 ± 0.03
4.	3	1.26 ± 0.04	1.23 ± 0.02
5.	4	1.16 ± 0.05	1.13 ± 0.03
6.	5	1.11 ± 0.01	1.25 ± 0.05

Table 5S The change in hydrodynamic diameter (d_H, nm) of papain in the presence of various concentration of [CHO][Trp] and [TEA][Trp]IL mediated AuNPs has been measured using DLS.

S. No.	Concentration of AAIL mediated AuNPs (nM)	Hydrodynamic diameter (d_H, nm), values of papain (1 mg/mL) in various concentration of AAIL mediated AuNPs	
		Papain in [CHO][Trp]IL mediated AuNPs	Papain in [TEA][Trp]IL mediated AuNPs
1.	0	100.36 ± 0.89	100.36 ± 0.89
2.	1	142.19 ± 0.97	232.88 ± 0.93
3.	2	182.04 ± 0.81	163.53 ± 0.74
4.	3	189.00 ± 0.82	210.78 ± 0.78
5.	4	147.50 ± 0.94	222.59 ± 0.83
6.	5	162.13 ± 0.95	164.51 ± 0.53

Table 6S The change in zeta potential (mV) values of papain in the presence of various concentration of [CHO][Trp] and [TEA][Trp]IL mediated AuNPs has been measured using DLS zetasizer.

S. No.	Concentration of AAIL mediated AuNPs (nM)	Zeta potential (mV), values of papain (1 mg/mL) in various concentration of AAIL mediated AuNPs	
		Papain in [CHO][Trp]IL mediated AuNPs	Papain in [TEA][Trp]IL mediated AuNPs
1.	0	1.89 ± 0.51	1.89 ± 0.51
2.	1	- 0.23 ± 0.20	1.46 ± 0.44
3.	2	- 2.38 ± 0.74	2.11 ± 0.03
4.	3	- 3.74 ± 0.04	2.41 ± 0.94
5.	4	- 3.83 ± 0.05	3.34 ± 0.10
6.	5	- 4.57 ± 0.50	4.38 ± 0.53

Table 7S The length of different dimensions of complex C-1 (papain – [CHO][Trp]AuNPs) and C-4 (papain – [TEA][Trp]AuNPs) has been shown using imageJ.

The dimensions (nm) for the complex C-1 and C-4	
C-1 (papain – [CHO][Trp]AuNPs)	C-4 (papain – [TEA][Trp]AuNPs)
I , 58.20	I , 104.32
II, 64.14	II, 130.67
III, 21.69	III, 129.65
IV, 20.21	IV, 63.41
V, 23.89	V, 61.69
VI, 22.52	VI, 63.15
VII,43.54	

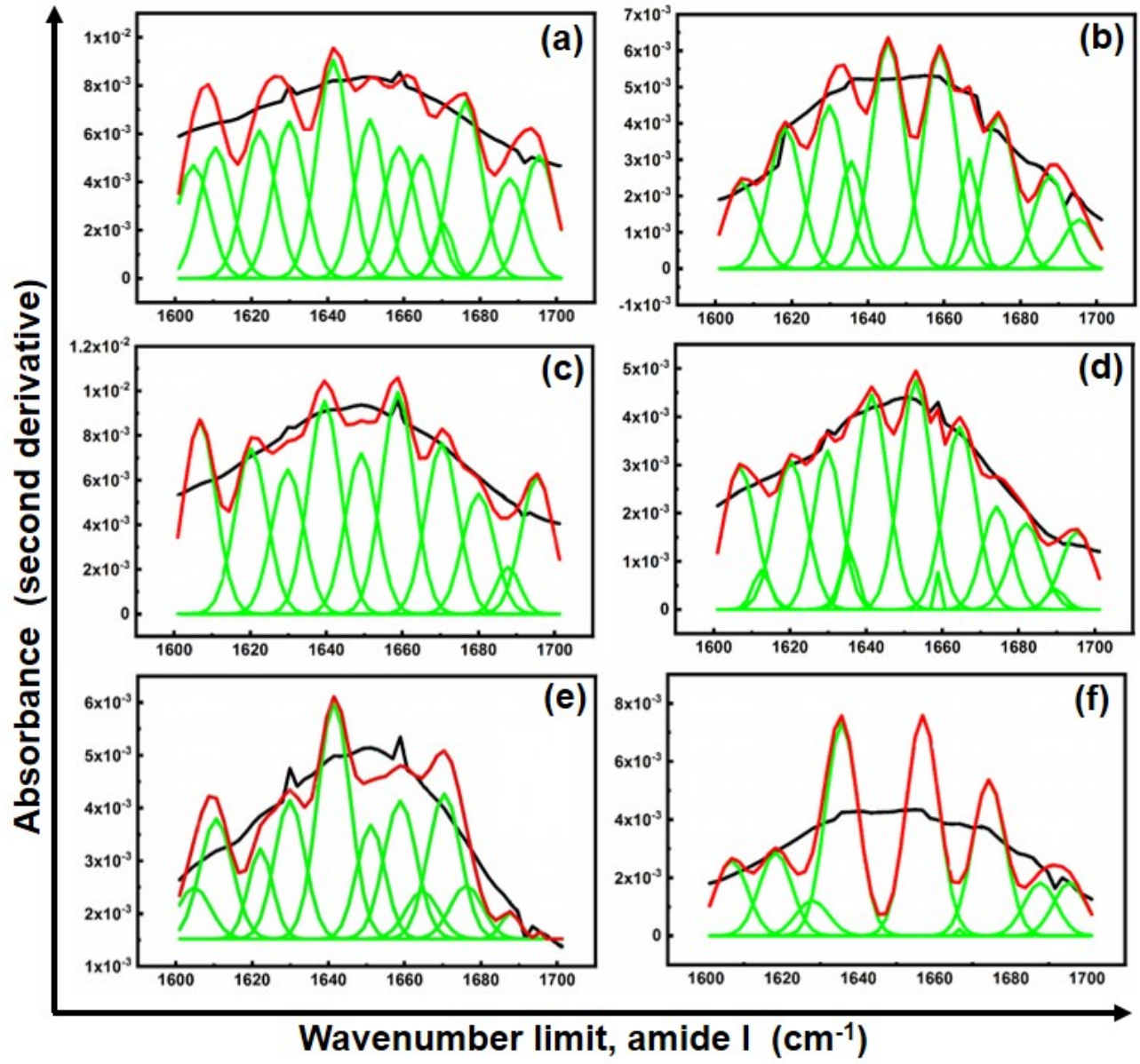

Figure 3S The deconvolution of amide I peak of pure papain in medium concentration of AAIL-mediated AuNPs. a, c and e are showing the deconvolution amide I peaks for papain in the 2, 3 and 4 nM concentration of [CHO][Trp]-mediated AuNPs, respectively. Whereas, b, d and f are showing the deconvolution amide I peaks for papain in the 2, 3 and 4 nM concentration of [TEA][Trp]-mediated AuNPs, respectively.

Table 8S Deconvoluted second derivative amide I peaks frequencies and secondary structure assignments papain-AAIL mediated AuNPs.

Mean frequency (cm^{-1})						Secondary structure assignment
Papain in [CHO][Trp]IL- AuNPs (nM), Amide I peak			Papain in [TEA][Trp]IL- AuNPs (nM), Amide I peak			
2	3	4	2	3	4	
1604,1610	1606	1604,1610	1606	1606, 1612	1606	β – sheets
1622	1620	1622	1618	1620	1618	β – sheets
1629	1629	1629	1629	1629	1627	β – sheets
-	1639	-	1635	1635	1635	β – sheets and extended chain
1641	-	1641	1645	1641	-	Un-ordered
1651	1649	1651	-	1652	1656	α-helics
1658,1664	1658	1668, 1664	1658	1658, 1664	1666	β – turn
1670, 1676	1670, 1679	1670, 1676	1666, 1674	1674	1674	β – turn
1687, 1695	1687, 1695	1687, 1695	1687, 1695	1681, 1689, 1695	1687, 1695	β – sheets

Table 9S The evaluation of secondary structure change of papain protein in the presence of various concentration of [CHO][Trp] and [TEA][Trp]IL mediated AuNPs using dichroweb software.

Sample name	Secondary structure composition (%)				Total	NRMSD (normalised residual mean standard deviation)
	Total Helical structure	β- Sheet	Turn	Unordered		
Pure Papain	34.9	11.1	22.11	31.3	100.1	2.32
[CHO][Trp]AuNP - 1 nM	38.5	5	25.2	32.3	100	1.25
[CHO][Trp]AuNP - 2 nM	34.38	11.18	23.18	31.28	100.1	2.58
[CHO][Trp]AuNP - 3 nM	34.4	10.4	22.4	31	100	2.28
[CHO][Trp]AuNP - 4 nM	34.7	10.8	23	31.5	100	2.51
[CHO][Trp]AuNP - 5 nM	34.78	10.38	23.18	31.68	100.2	2.54
[TEA][Trp]AuNP - 1 nM	34.78	10.78	22.98	31.48	100.2	2.55

[TEA][Trp] AuNP - 2 nM	34.6	10.6	23.4	31.4	100.0	2.25
[TEA][Trp] AuNP - 3 nM	36.12	5.63	25.52	32.72	99.99	1.24
[TEA][Trp] AuNP - 4 nM	38.8	4.1	25.1	32.1	100.1	1.87
[TEA][Trp] AuNP - 5 nM	34.42	10.92	23.23	31.42	99.99	1.55

References :-

1. X. Liu, M. Atwater, J. Wang, Q. Huo, *Colloids Surf. B,* 2007, **58**, 3-7.