Strategically designed antifibrotic gold nanoparticles to prevent collagen fibril formation

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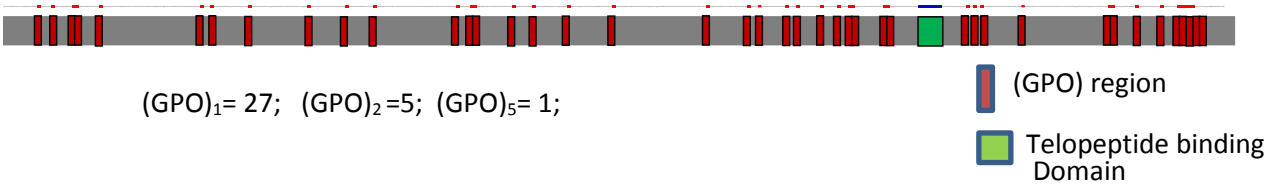
SUPPLEMENTARY MATERIAL

a



b

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RGEPGPPGPAGFAGPPGADGQPGAKGEPGDAGAKGDAGPPGPAGPAGPPGPIGNVGAPGAKGAR
GSAGPPGATGFPGAAGRVGPPGPSGNAGPPGPPGPAGKEGGKGPRGETGPAGRPGEVGPPGPPG
PAGEKGSPGADGPAGAPGTPGPQGIAGQRGVVGLPGQRGERGFPGLPGPSGEPGKQGPSGASGE
RGPPGPMGPPGLAGPPGESGREGAPGAEGSPGRDGSPGAKGDRGETGPAGPPGAPGAPGAPGPV
GPAGKSGDRGETGPAGPAGPVGPVGARGPAGPQGPRGDKGETGEQGDRGIKGHRGFSGLQGPPG
PPGSPGEQGPSGASGPAGPRGPPGSAGAPGKDGLNGLPGPIGPPGPRGRTGDAGPVGPPGPPGP
PGPPGPPSAGFDFSFLPQPPQEKAHDGGRYYRA

FIGURE S1. Analysis of collagen sequence for GPO stretches. (a)A proportionate graphical representation of α1 collagen (type 1) sequence displaying the frequent occurrence of GPO sequences (red) throughout the triple helical region of the peptide. The green area shows the telopeptide binding region within the collagen sequence. **(b)**. Sequence of α1 chain of type 1 collagen.

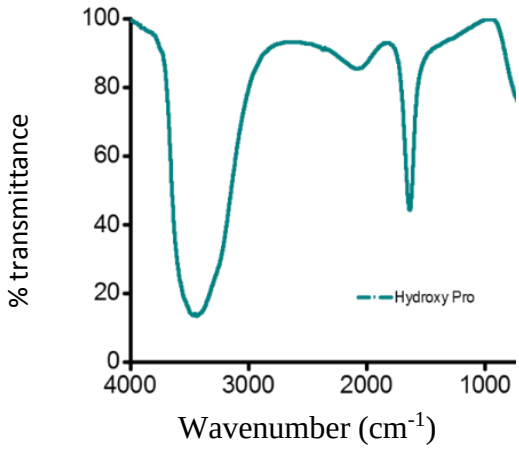


FIGURE S2. FTIR Spectrum of AuNPs^{HYP} sample.

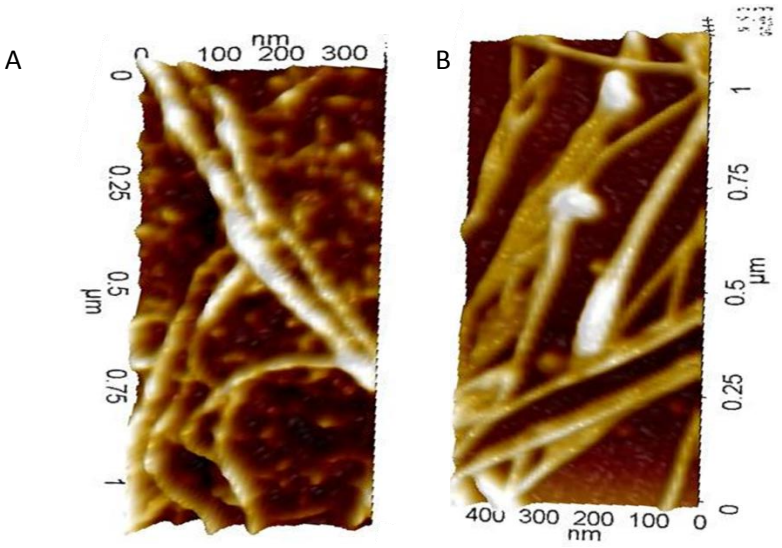


Figure S3. AFM imgages of the collagen fibrils obtained from both inhibited (A)and control (B)reactions.

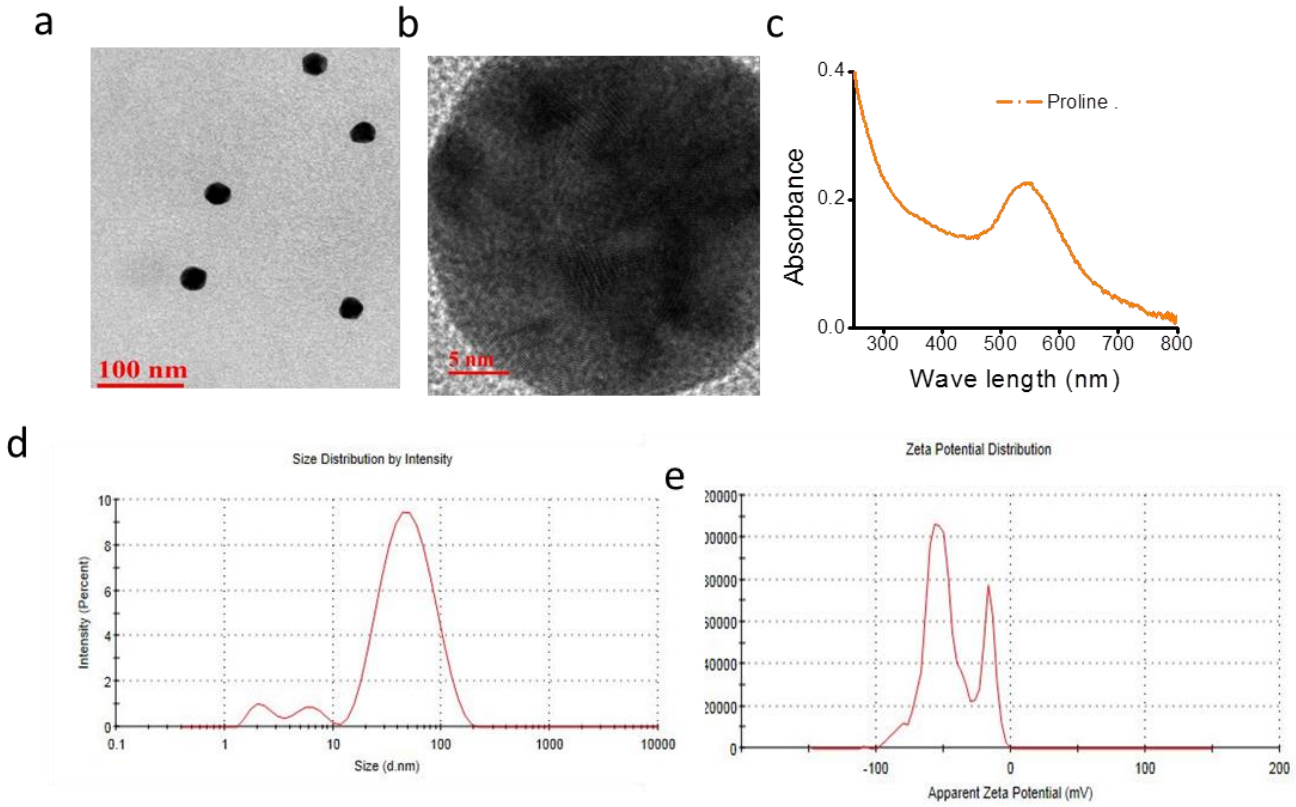


FIGURE S4.Characterization Proline coated gold nanoparticles (AuNPs^{PRO}) (a)TEM displaying the evenly sized (~20nm) spherical AuNPs^{PRO}**(b)**HRTEM images of the AuNPs^{PRO}**(c)**UV-Vis spectrum of AuNPs^{PRO}**(d)** Dynamic Light Scattering (DLS) peak of AuNPs^{PRO}**(e)**Zeta potential peak of AuNPs^{PRO}.

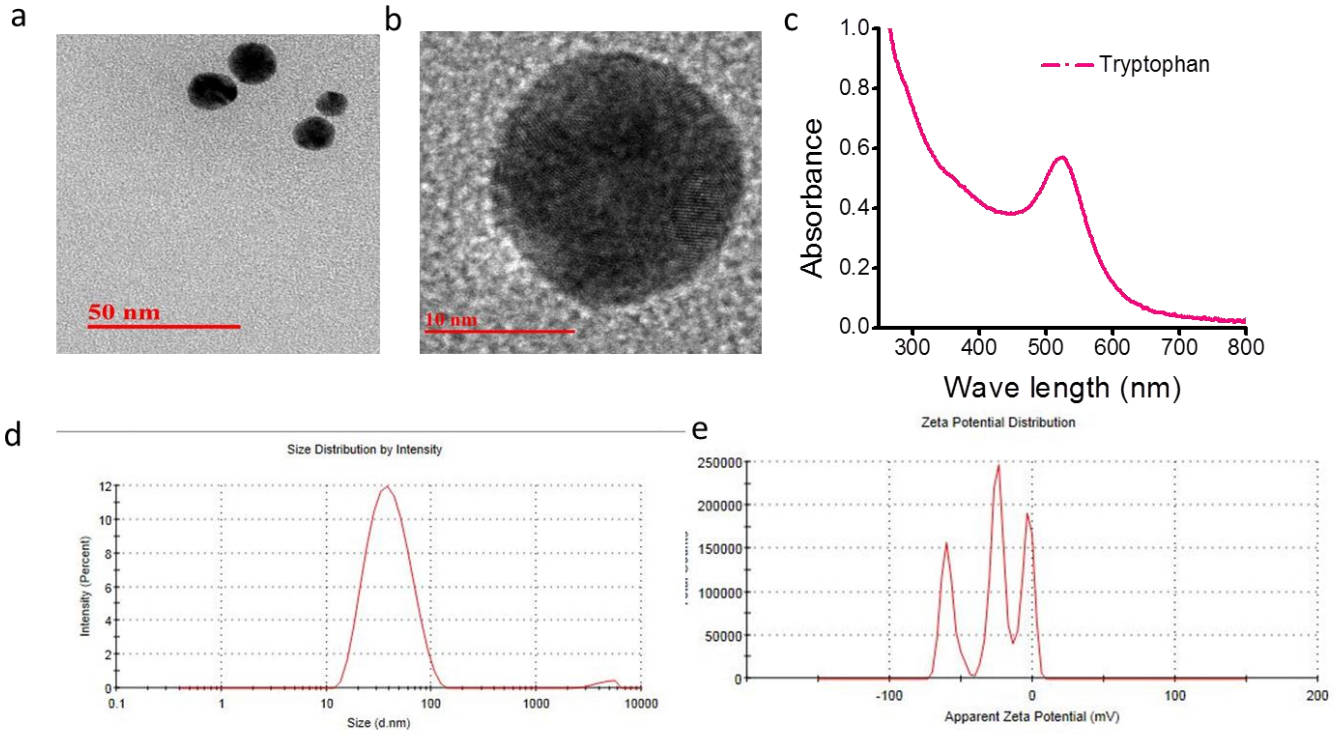


FIGURE S5. Characterization tryptophan coated gold nanoparticles (AuNPs^{TRP}) (a)TEM displaying the evenly sized (~20nm) spherical AuNPs^{TRP}**(b)**HRTEM images of the AuNPs^{TRP}**(c)**UV-Vis spectrum of AuNPs^{TRP}**(d)** Dynamic Light Scattering (DLS) peak of AuNPs^{TRP}**(e)**Zeta potential peak of AuNPs^{TRP}.

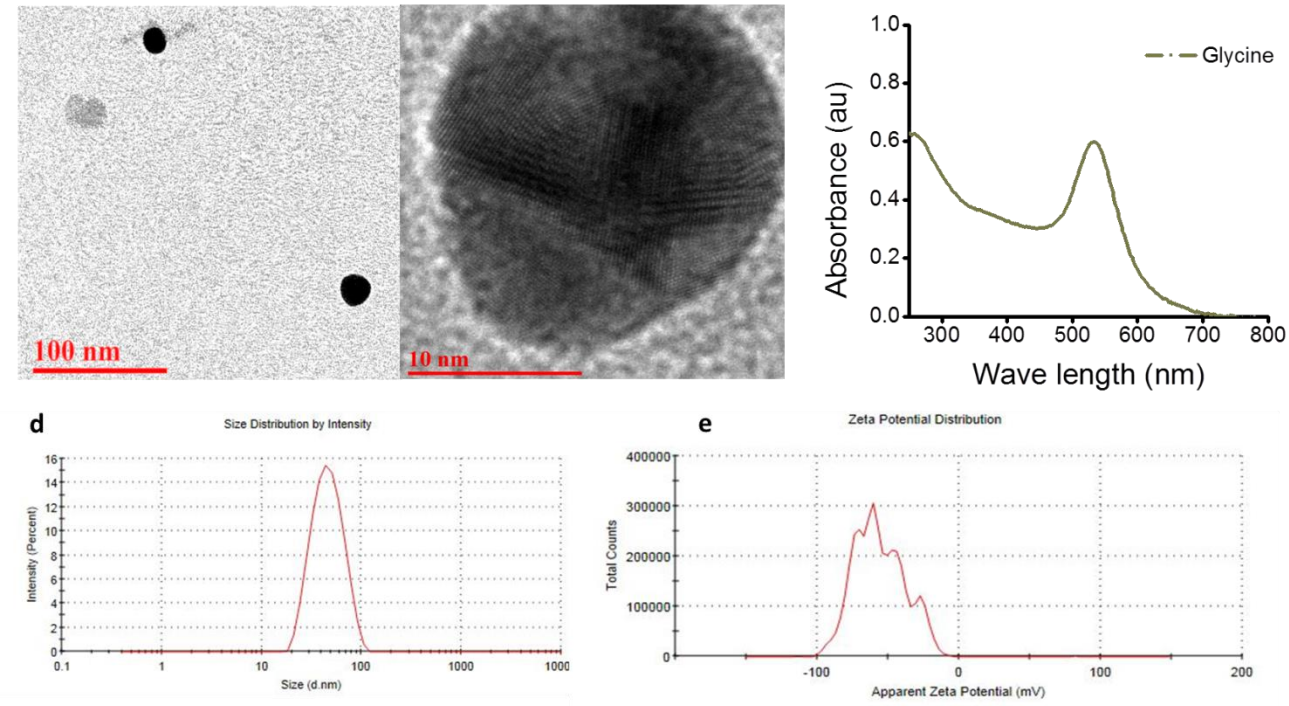


FIGURE S6.Characterization glycine coated gold nanoparticles (AuNPs^{GLY}) (a)TEM displaying the evenly sized (~20nm) spherical **AuNPs^{GLY}(b)**HRTEM images of the **AuNPs^{GLY}(c)**UV-Vis spectrum of **AuNPs^{GLY}(d)** Dynamic Light Scattering (DLS) peak of **AuNPs^{GLY}(e)**Zeta potential peak of **AuNPs^{GLY}**.

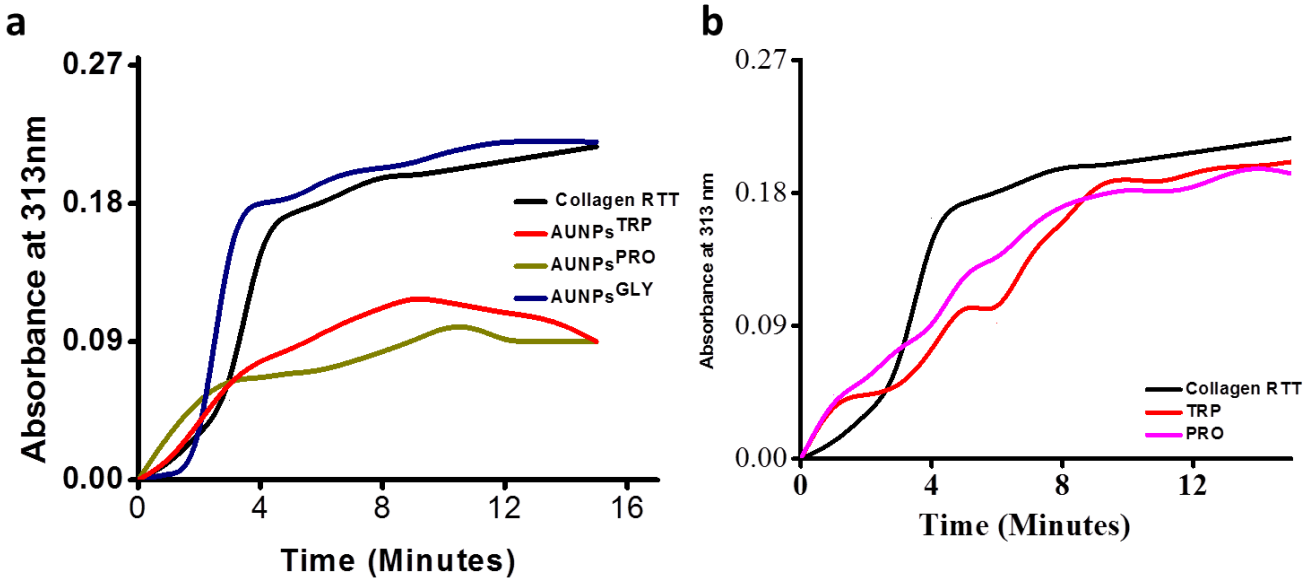


FIGURE S7.*Effect of proline, tryptophan and glycine coated nanoparti5cles on fibril formation process of type 1 collagen*. **(a)** Turbidity data showing fibril formation of calf skin collagen in the presence and in the absence of inhibitors under physiological temperature 37°C and in PBS at pH 7: (——) control collagen; (——) collagen + AuNPs^{TRP} at 1:100 molar ratio; (——) collagen + AuNPs^{PRO} at 1:100 molar ratio; (——) collagen + AuNPs^{GLY} at 1:100 molar ratio. **(b)**Effect of free uncoated amino acids on collagen fibril formation; (——) control collagen; (——) collagen+ free Trp at 1:100 molar ratio; (——) collagen+ free Pro 1:100 molar ratio.

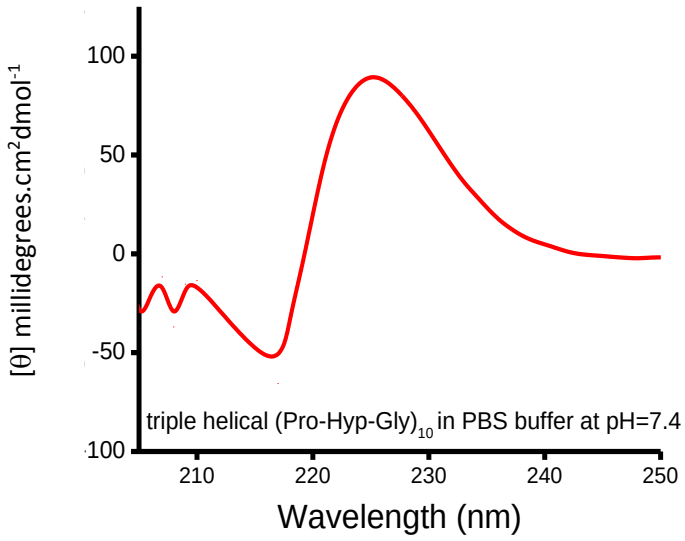
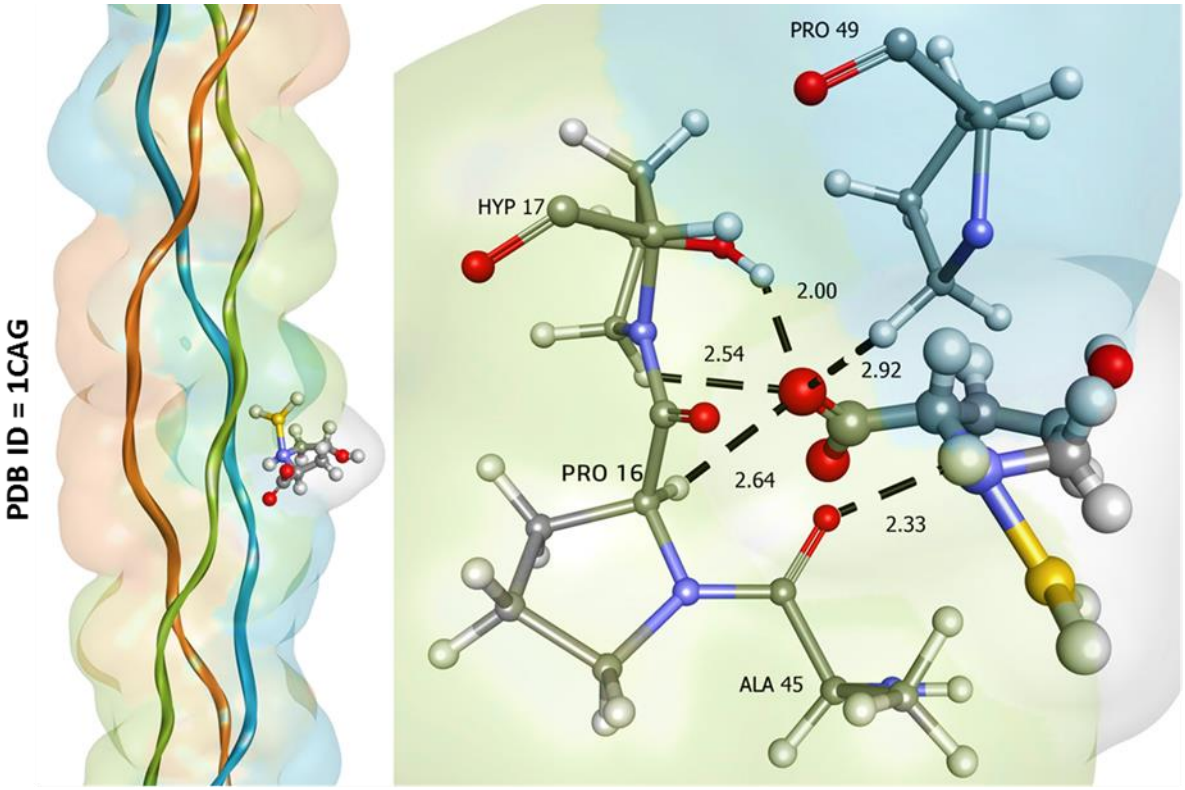


FIGURE S8. Circular Dichroism signal of the collagen model peptide $(Pro\text{-}Hyp\text{-}Gly)_{10}$ in PBS buffer at pH 7 (concentration = 1 $mg.ml^{-1}$). The data confirmed the formation of triple-helical molecules of the peptide POG10. This sample was used for binding studies with $AuNPs^{HYP}$ nanoparticles.

1CAG	>1CAG:A|PDBID|CHAIN|SEQUENCE PPGPPGPPGPPGPPAPPGPPGPPGPPGPPGPPG >1CAG:B|PDBID|CHAIN|SEQUENCE PPGPPGPPGPPGPPAPPGPPGPPGPPGPPGPPG >1CAG:C|PDBID|CHAIN|SEQUENCE PPGPPGPPGPPGPPAPPGPPGPPGPPGPPGPPG
1Q7D	>1Q7D:A|PDBID|CHAIN|SEQUENCE XGPPGPPGFPGERGPPGPPGPPX >1Q7D:B|PDBID|CHAIN|SEQUENCE XGPPGPPGFPGERGPPGPPGPPX >1Q7D:C|PDBID|CHAIN|SEQUENCE XGPPGPPGFPGERGPPGPPGPPX

FIGURE S10. Sequence details of different collagen peptides used in our study



Binding Affinity = -

-16.44 kcalmol^{-1}

Interacting Residues	Bond Length(Å)	Interaction
A: ALA15:O - HYP:H17	2.32	Hydrogen Bond
A:PRO16:HA - HYP:O4	2.63	Hydrogen Bond
B:PRO49:HD1 - HYP:O4	2.91	Hydrogen Bond
A:HYP17:H1 - HYP:O4	2.0	Hydrogen Bond
A:HYP17:HD2 - HYP:O4	2.5	Hydrogen Bond

Figure S11. Interaction of the modified Hyp (in which a gold atom is attached to the –NH moiety) with collagen model peptide 1CAG. See supplementary methods below .

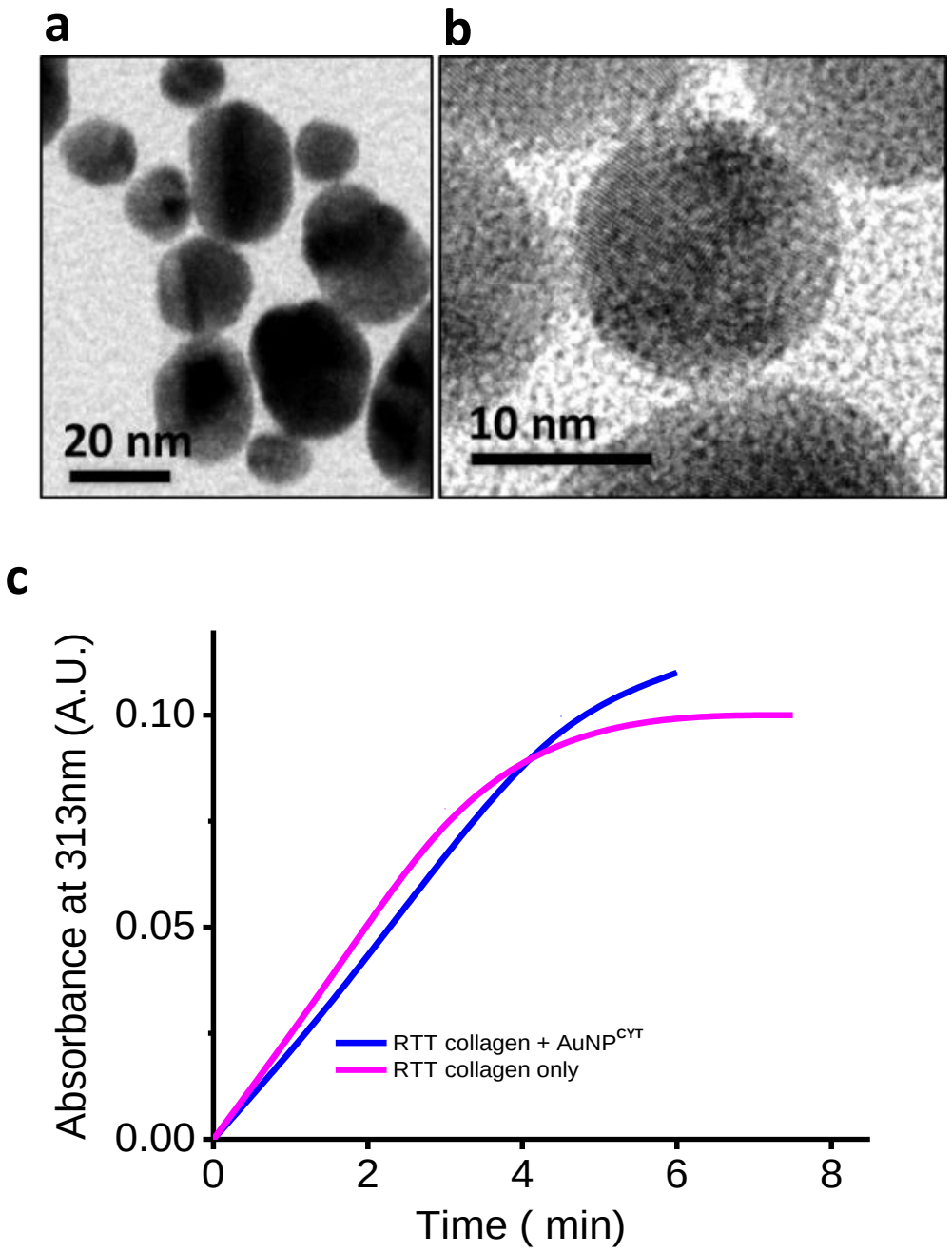


FIGURE S12. **a)** TEM images displaying formation of (10~30nm) spherical $AuNPs^{CYT}$ **b)** HR TEM data $AuNPs^{CYT}$. **c**) Turbidity data showing fibril formation of RTT collagen (0.32 $mg.ml^{-1}$) in the presence and in the absence of inhibitors under physiological temperature 37°C and in PBS buffer. Molar ratio of collagen:inhibitor is given in the parentheses: (——) control collagen; (——) collagen + $AuNPs^{CYT}$ (at 1:100).

SUPPLEMENTARY INFORMATION ON METHODS:

Methods for Hemolysis.

The percentage lysis was calculated by the given formula as shown below.

$$\%\ \boldsymbol{Lysis} = \frac{(\boldsymbol{A\ sample - A\ Negative\ control})}{(\boldsymbol{A\ positive\ control - Anegative\ \ control})} \times \mathbf{100}$$

The cell pellet that has the RBC cells were examined initially by Leishman staining procedure where the RBC sample was placed over the glass slide and smeared. The smear was then washed with 100% methanol for 30 seconds after which the Leishman stain was added to the fixed smear region and the slide was left undisturbed for 15 minutes. Then the slides were washed immediately by water and viewed under Leica microscope.The exact morphology of RBCs was confirmed by Scanning Electron Microscopy. The cell pellets washed in PBS and was fixed by incubating with 1% of glutaraldehyde for 2 hours after that the samples are treated with 2% osmium tetrachloride for an hour. The cell was then washed thrice with 10% ethanol and dehydrated by incubating with 10%, 20%, 30%, 50%, 70% and 80% ethanol for an hour each and then desiccated. Thus, obtained RBC pellet was sputtered with gold/palladium and viewed and then in SEM.

All hemolysis experiments were carried out in accordance with relevant guidelines and regulations of Ethics Committee of Indian Institute of Technology Jodhpur, India. All the experimental protocols were approved by Ethics Committee of Indian Institute of technology Jodhpur (Approval letter no IITJ/EC/2016/02-D).

Extraction of collagen from rat tail tendon.
Tendons were carefully teased from the tails of ~6-month-old male albino Wistar rats. The isolated tendons were washed thoroughly with saline at least three times and the then again repeatedly washed with distilled water. The temperature of both saline and distilled water was maintained at 4 °C during washing. Acid-soluble type-I collagen was isolated from the tendons by use of the method of Chandrakasan et al. (1976)The purified type-I collagen was then dialyzed extensively against 50 mM acetic acid, to remove all traces of salts, then the pure collagen was lyophilized and stored at -20°C

References:
Chandrakasan G, Torchia DA, Piez KA (1976) Preparation of intact monomeric collagen from rat tail tendon and skin and the structure of the non-helical ends in solution. J BiolChem 251(19):6062–6067.

SUPPLEMENTARY METHODS

Protocol for Docking Studies using Discovery Studio.

Molecular docking studies of gold atom conjugated hydroxyproline molecule was done using Discovery studio 4.0 (DS4). Initially the ligand was incorporated with CHARMm force field and then prepared using 'prepare ligand wizard' of DS4. The collagen peptide (PDB ID: 1CAG) was obtained from Protein Data Bank (PDB). The peptide molecule was then prepared by 'protein preparation wizard' as follows.

- The protein molecule was cleaned by removing water and heteroatoms leaving behind the nascent molecule
- The DS4 with an inbuilt algorithm protonates and preprocesses the protein molecules by its predefined parameters at an ionic strength of 0.145 and at pH 7.4.
- Finally, these protein molecules were typed with CHARMm force field

Then the docking of was performed for the input ligand molecule with the processed stable static protein using CDocker docking tool. CDocker is a powerful CHARMm based docking method that has been shown to generate highly accurate docked poses.

During the process of docking the ligand molecule will be in a dynamic state with different orientation (10 ligand poses with a pose cluster of 0.1Å – predefined in CDocker protocol with 10 dynamic steps, 10 ligand orientation having 2000 heating and 5000 cooling steps while docking with the nascent protein structure).

Each orientation will be subjected to simulated annealing by molecular dynamics protocol. After successful docking, the CDocker ranks the poses based on CHARMm energy and highest interaction energy scores (considering more negative as more favorable binding).