Electronic supplementary information (ESI) for Nanoscale
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Unprecedented inhibition of tubulin polymerization directed by gold nanoparticles inducing cell cycle arrest and apoptosis†

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Electronic Supplementary Information 1

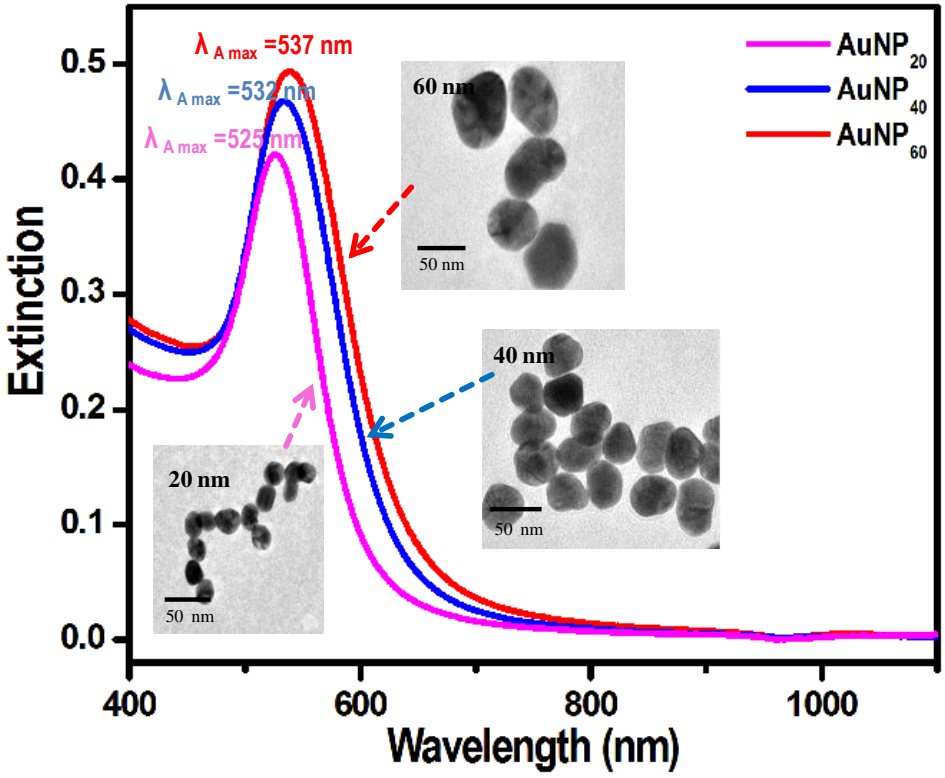

Fig. S1 Extinction spectra of three different sized (20 nm, 40 nm and 60 nm) citrate capped AuNPs with corresponding TEM images, namely $AuNP_{20}$, $AuNP_{40}$ and $AuNP_{60}$. Scale bar for all TEM images is 50 nm.

Electronic Supplementary Information 1A: Calculation of molarity of AuNP

Molarity of AuNPs in solution was calculated using the following formula,

$$M_{NP} = \frac{(Molarity\ of\ Au^{3+}\ in\ the\ solution) \times (Volume\ of\ one\ gold\ atom)}{(Volume\ of\ one\ nanoparticle)}$$

$$M_{NP} = \frac{6MA_r}{\pi D^3 \rho N_A}$$

$= (6 * 250 * 197 * 7) / (22 * (40 * 10^{-7})^3 * 19.3 * 6.023 * 10^{23})$

$= 2068500 / (22 * 64000 * 10^{-21} * 19.3 * 6.023 * 10^{23})$

$= 2068500 / (22 * 64 * 19.3 * 6.023 * 10^{5})$

$= 2068500 / (163671.4112 * 10^{5})$

$= 12.64 * 10^{-5}$ µM = 126.4 pM which gives molarity of $AuNP_{40}$ in the stock solution as 126.4 pM.

Where,

M = Molarity of Au^{3+} stock in µM

A_r = Atomic weight of Au in g

D = Diameter of nanoparticle in cm

ρ = Density of gold in g/cm^3

N_A = Avogadro number

For $AuNP_{40}$ molarity of stock solution was found to be, 126.4 pM.

Electronic Supplementary Information 2

The extents of polymerization inhibition were around 28.6 ± 2.7%, 40.32 ± 1.7% and 60.47 ± 3.5% in presence of $AuNP_{40}$ at 5, 12.5 and 25 pM, respectively (for 30 minutes). Hence the calculated IC_{50} value (i.e. 50% inhibitory concentration) for $AuNP_{40}$ was 18.6 ± 0.9 pM.

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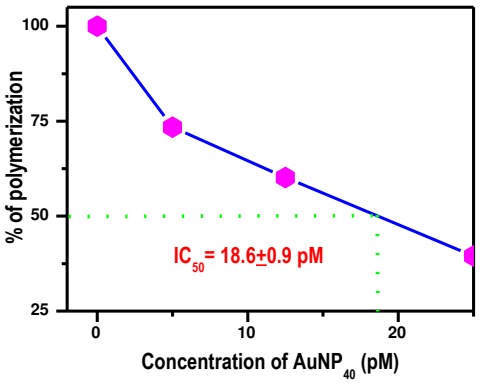

Fig. S2 Plot showing the calculation of IC_{50} concentration of $AuNP_{40}$ for purified mammalian tubulin polymerization. Error in the determination of % polymerization is given in supporting information 1. From the above plot (**Fig. S2**) we can infer that at the IC_{50} dose of $AuNP_{40}$ for polymerization inhibition of tubulin (12 µM) is ~18.6 pM and the molar ratio of $AuNP_{40}$: tubulin is around 1 : 3.16 X 10^5.

Electronic Supplementary Information 3

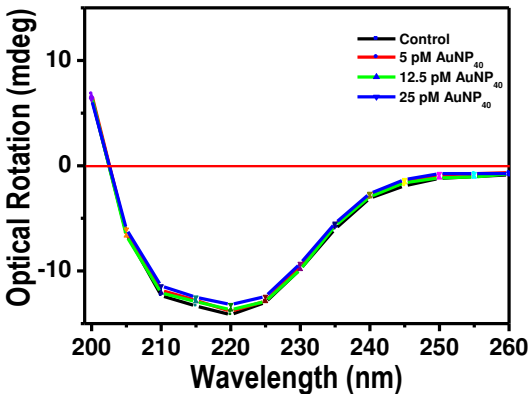

Fig. S3 Circular dichroism spectroscopic measurements revealing no significant change in the secondary structure of tubulin upon interacting with $AuNP_{40}$.

Electronic Supplementary Information 4

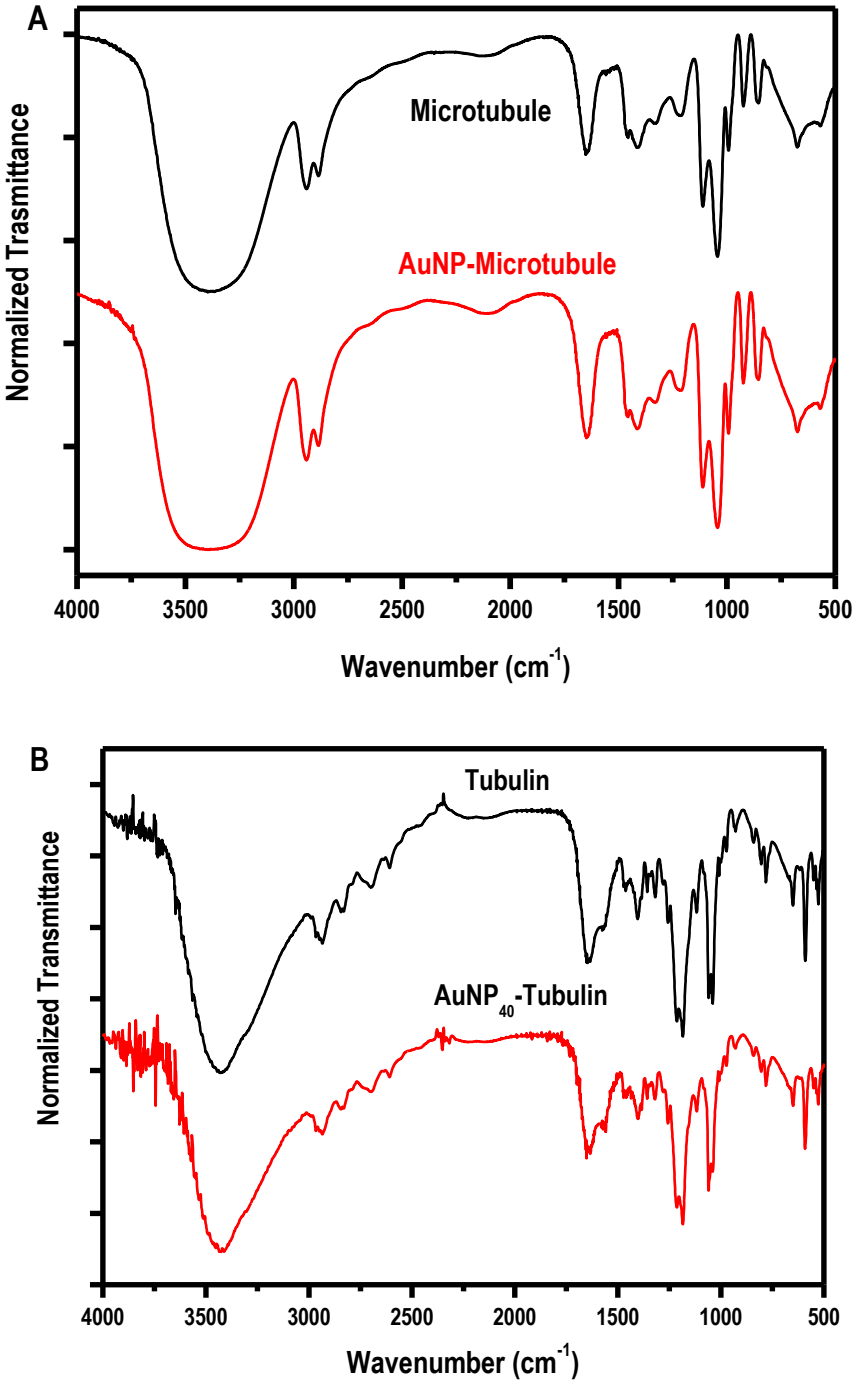

Fig. S4 FTIR spectra of microtubule and tubulin (solid black line in A and B) and $AuNP_{40}$ treated tubulin and microtubule (red solid line in A and B).

Electronic Supplementary Information 5

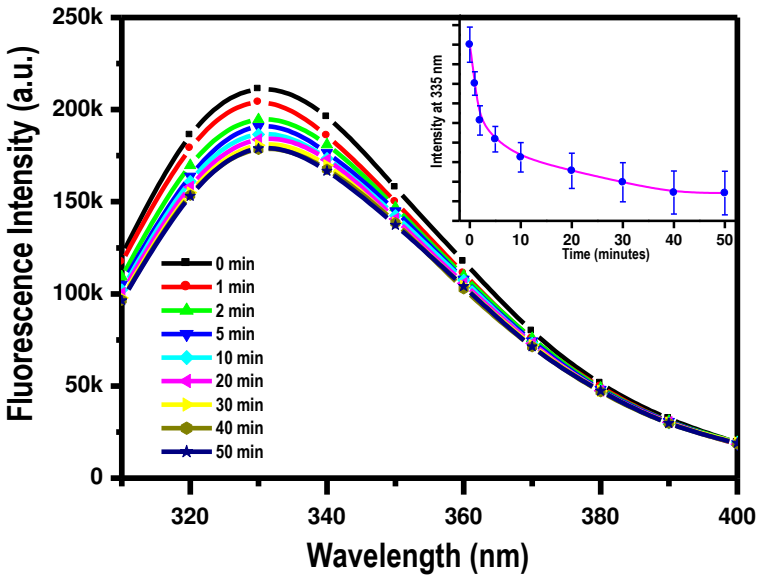

Fig. S5 PL spectra showing the quenching of intrinsic fluorescence of tryptophan upon interaction with $AuNP_{40.}$

Electronic Supplementary Information 6

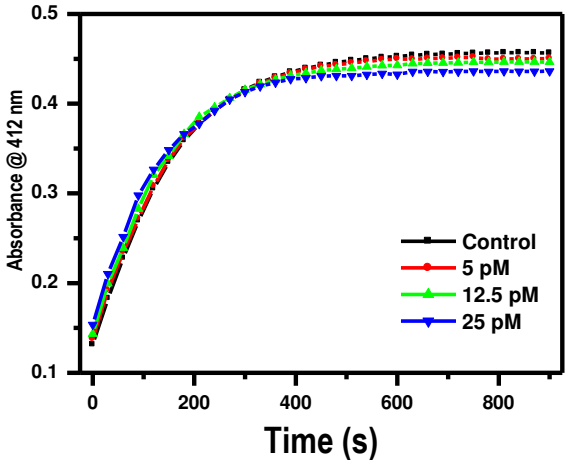

Fig. S7 Thiol estimation with control and tubulin incubated with $AuNP_{40}$ at different concentrations. Results indicated 3-5% loss of cysteine content per heterodimer.

Electronic Supplementary Information 7

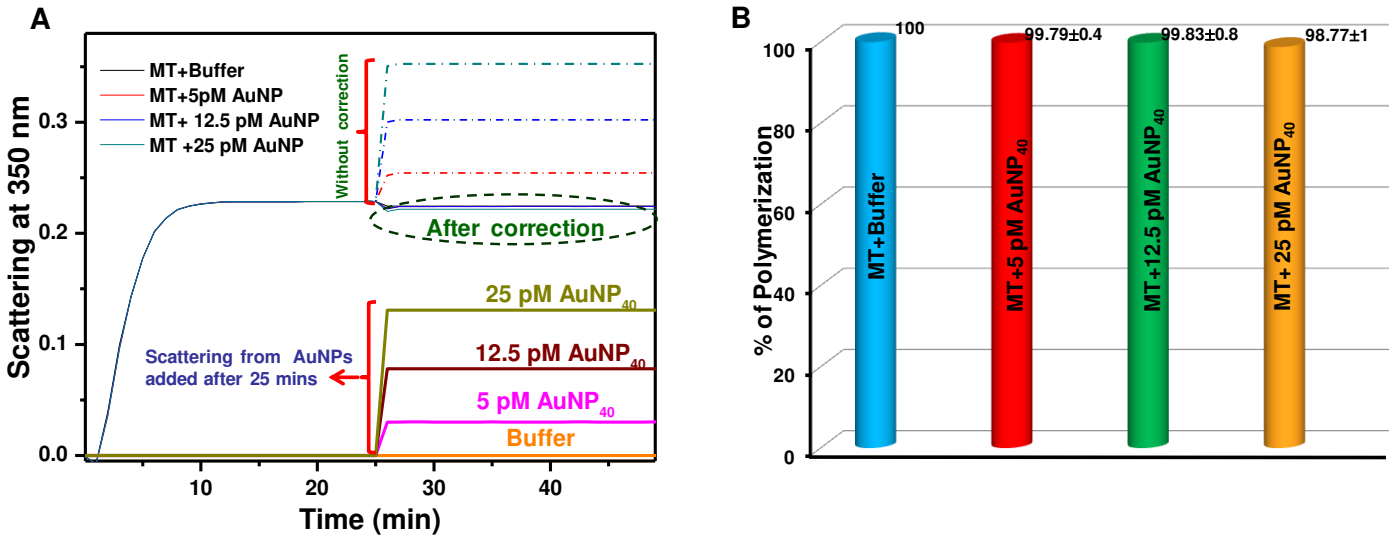

Fig. S7 A) UV-vis spectroscopic study of effect of Au_{NPs} on the polymerized tubulin. After 25 minutes of polymerization, upon addition Au_{NPs} did not depolymerise the polymerized microtubules *in vitro*, as observed from the spectra (highlighted spectral region with dotted ellipse; the original spectra were subtracted with the corresponding scattering spectra of Au_{NPs} for clarity). B) Bar-diagram showing the percentage of retention of polymerized tubulins with various concentrations of Au_{NP}.

Electronic Supplementary Information 8

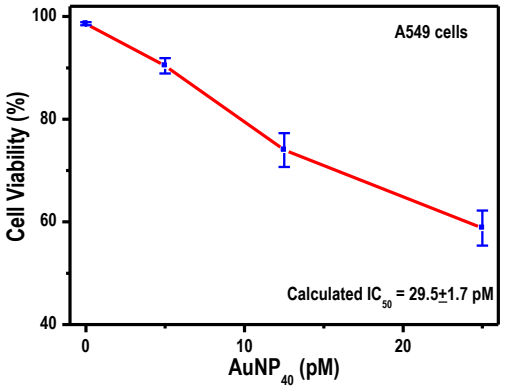

Fig. S8. Cell viability assay results of A549 cells upon 72 h $AuNP_{40}$ treatment. The calculated IC_{50} value was 29.5±1.7 pM.

Electronic Supplementary Information 9A

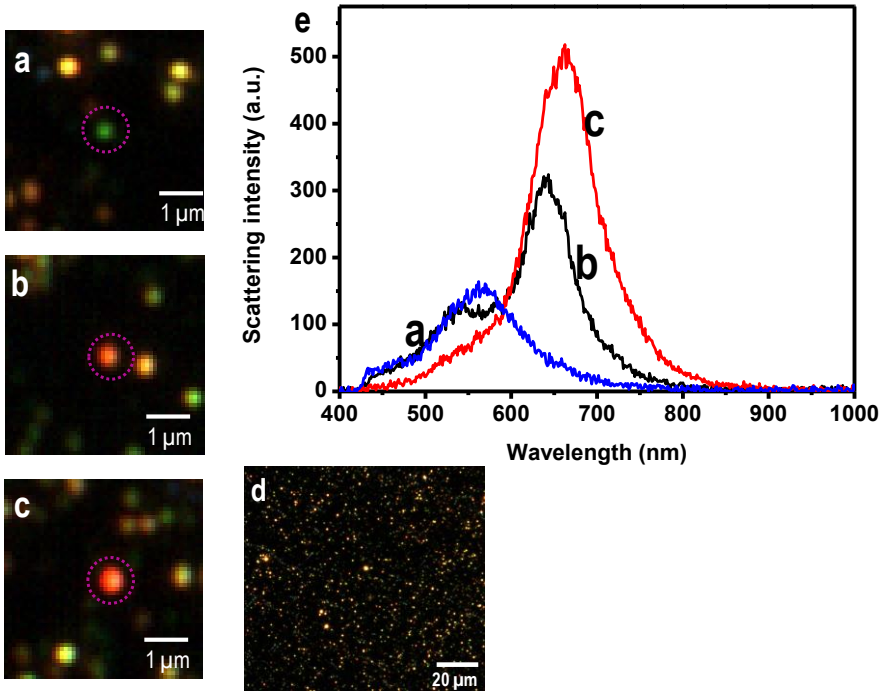

Fig. S9A. Dark field microscopic (DFM) images of $AuNP_{40}$. Left side: images a, b and c are DFM images of $AuNP_{40}$ and d is the large area image from which a, b and c were selected. Right side (e): the corresponding Plasmon Resonance Raleigh Scattering (PRRS) spectra of nanoparticles: a (blue solid line representing AuNP in the image a), b (black solid line representing AuNP in the image b), and c (red solid line representing AuNP in the image c). These particles are labelled in images a, b and c.

Electronic Supplementary Information 9B

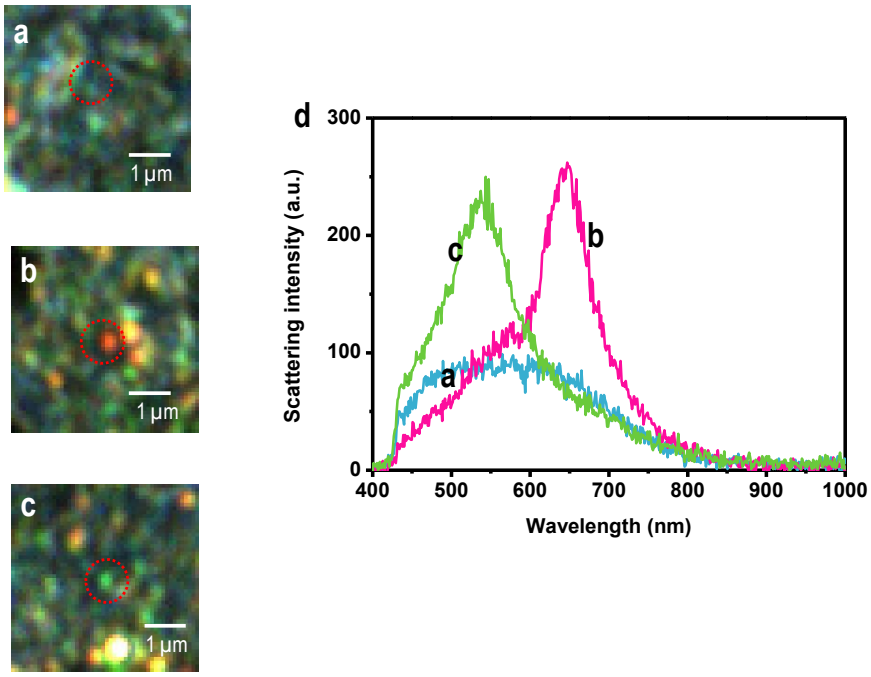

Fig. S9B. AuNPs in the aggregated tubulin matrix. Left: images a, b and c are selected area from Figure 2D. a is part of tubulin aggregate without nanoparticle, b and c are nanoparticles in the aggregated protein matrix. Right (d): the corresponding scattering spectra of a, b and c. a (solid cyan line) is the scattering spectra of protein aggregate in the image a and is broad and low in intensity. b (solid magenta line) and c (green solid line) are scattering spectra (sharp and high in intensity) of nanoparticles in the aggregated protein matrix in the image b and c, respectively. The difference between b and c in the scattering peak position may be due to the surrounding environment. The particles from which spectra are collected are labelled.

Electronic Supplementary Information 9C

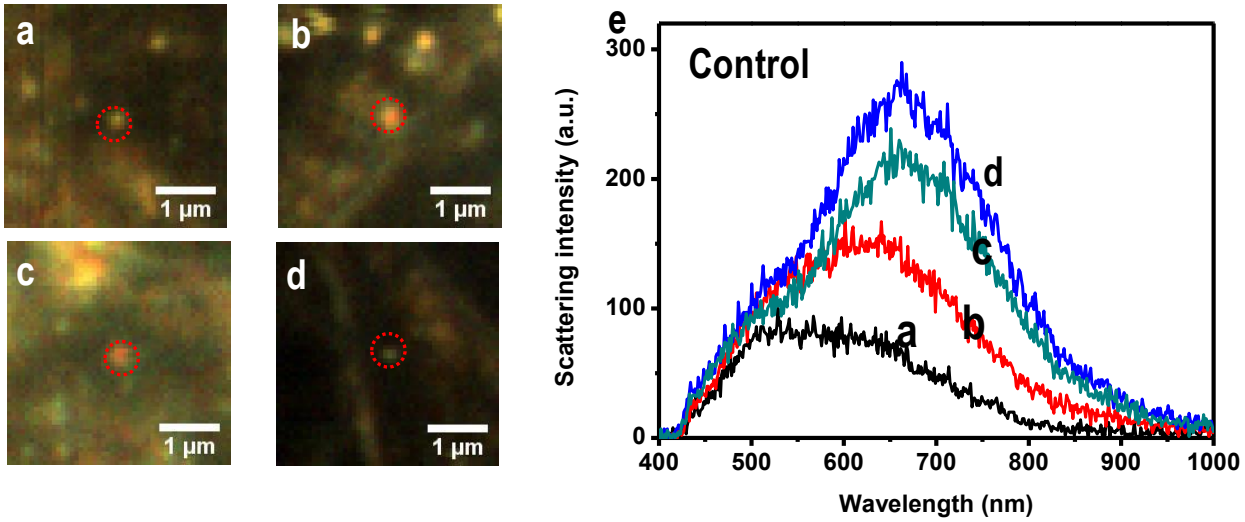

Fig. S8C Scattering from vesicles were observed in the control untreated cells. Left: Images a, b, c and d are four selected area images from the topmost image of Figure 6C. Right (e): Corresponding scattering spectra of vesicles in a, b, c and d. Here the spectra are broad and lesser in intensity unlike those of plasmonic nanoparticles which is a key factor to distinguish nanoparticles from vesicles. The vesicles from which spectra are collected are marked.

Electronic Supplementary Information 9D

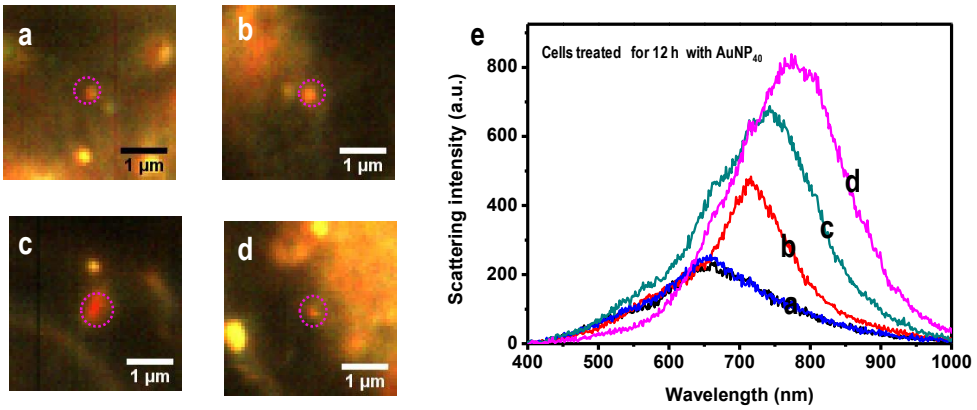

Fig. S9D. Scattering images and spectra of particles uptaken by $AuNP_{40}$ treated cells. Left Side: Images a, b, c and d are selected area images from the middle image of Figure 6C, showing the presence of scattering from AuNPs. Right (e): Corresponding scattering spectra of AuNPs in the right side images a (solid blue line representing AuNPs in image a), b (solid red line representing AuNPs in image b), c (solid green line representing AuNPs in image c) and d (solid magenta line representing AuNPs in image d), respectively. The particles from which spectra are collected are marked.

Electronic Supplementary Information 10

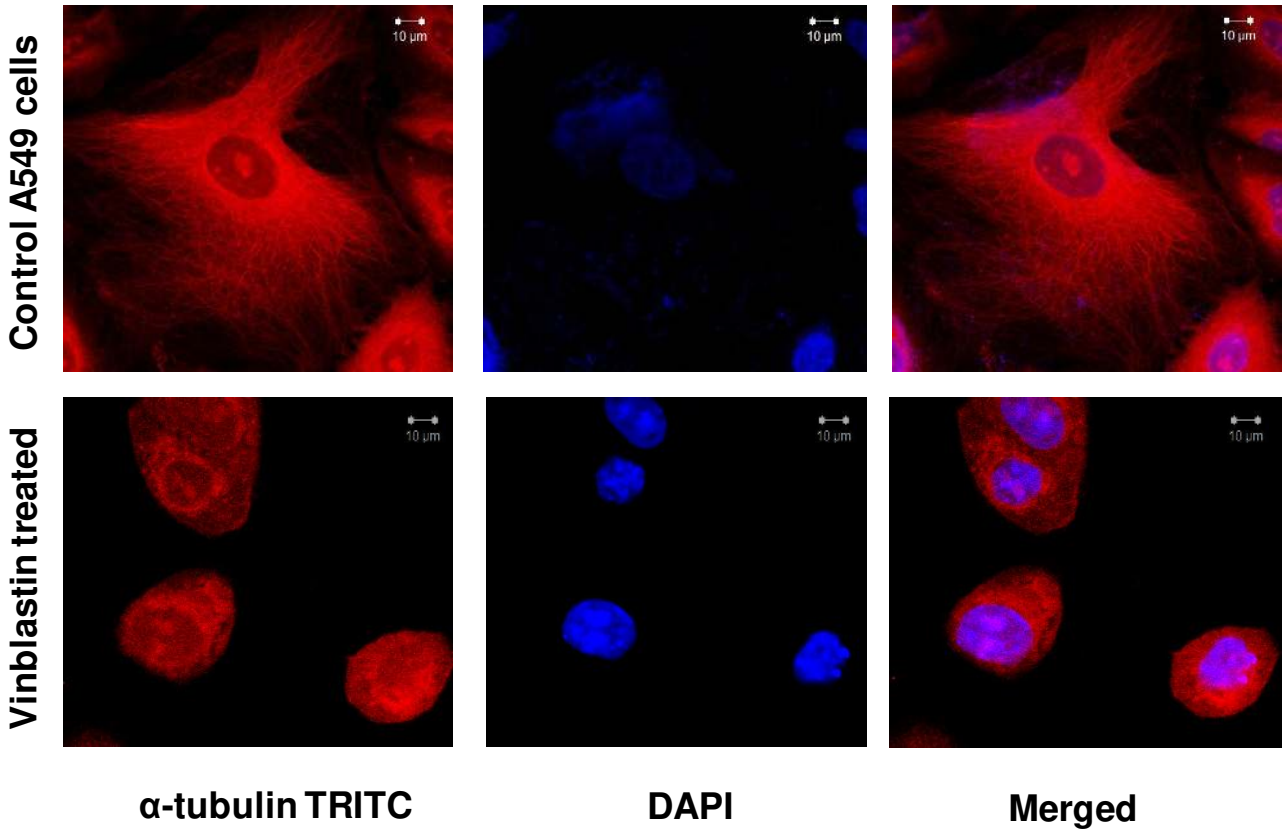

Fig. S10 Positive control: Vinblastin (500 nM) treated A549 cells for 24 hours showing microtubule damage. Upper panel images show control cells (non-treated). Lower panel images show vinblastin treated cells. Nucleus is stained with DAPI (blue) and microtubule is stained with anti-tubulin antibody conjugated with TRITC (red). Scale bar is 10 µm.

Electronic Supplementary Information 11

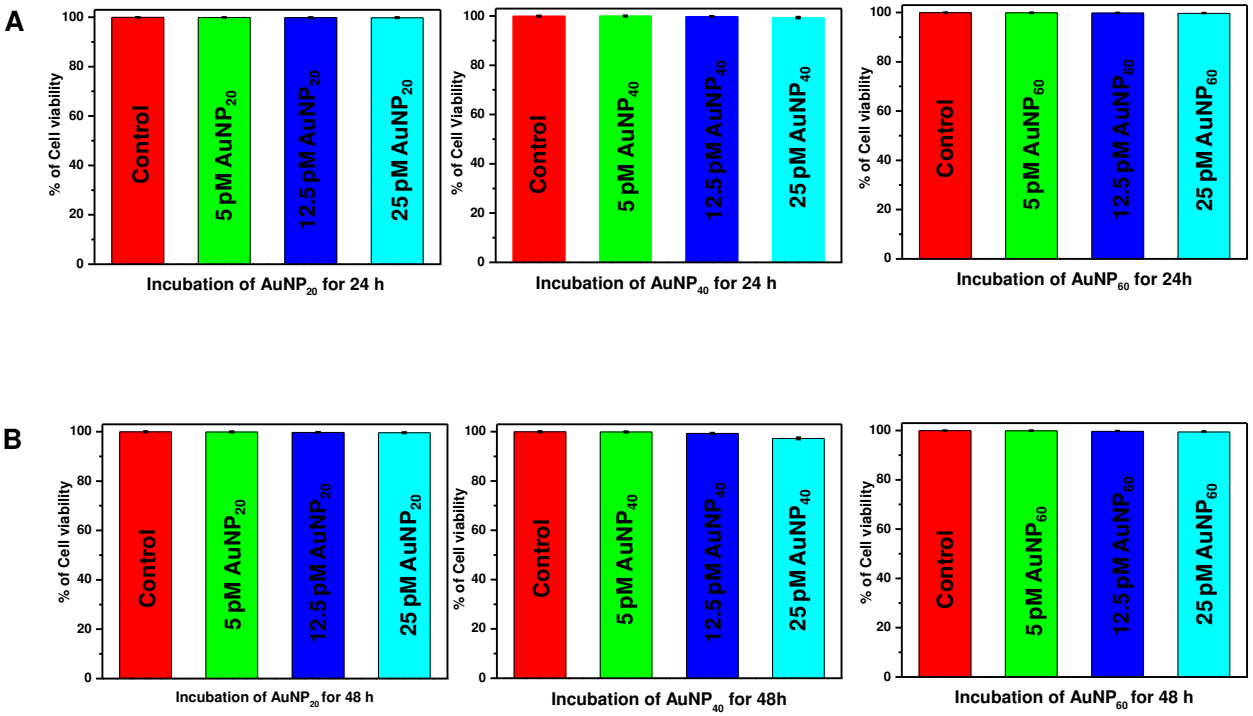

Fig. S11 A) Bar diagram showing % of cell viability for the three different sized AuNPs at different concentrations at 24 h. B) Bar diagram showing % of cell viability for the three different sized AuNPs at different concentrations at 48 h

Electronic Supplementary Information 12

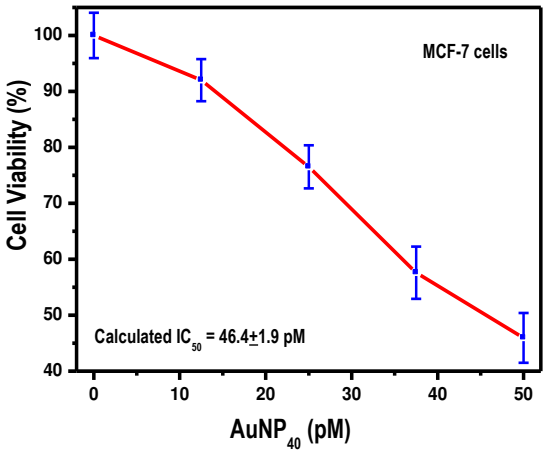

Fig. S12 Cell viability assay of MCF-7 after 72 h treatment with $AuNP_{40}$. The calculated IC_{50} value was 46.4±1.9 pM.

Electronic Supplementary Information 13

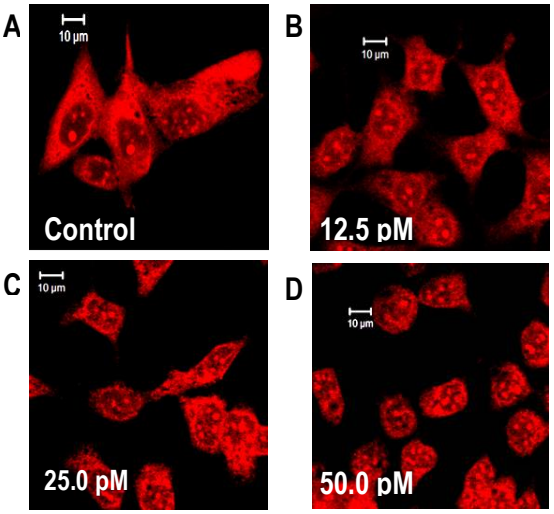

Fig. S13 Observation of a similar effect of AuNPs interacting with MCF-7 cells. The cells were stained with TRITC against anti-α-tubulin antibody. (A) Control MF7 cells, not treated with AuNPs. (B) Treated with 12.5 pM $AuNP_{40}$, (C) treated with 25.0 pM $AuNP_{40}$ and (D) treated with 50.0 pM $AuNP_{40}$. Cellular microtubule structure was monitored after 72 h of incubation.