

Supplementary Information

Laser irradiation affects biological identity and cellular uptake of plasmonic nanoparticles

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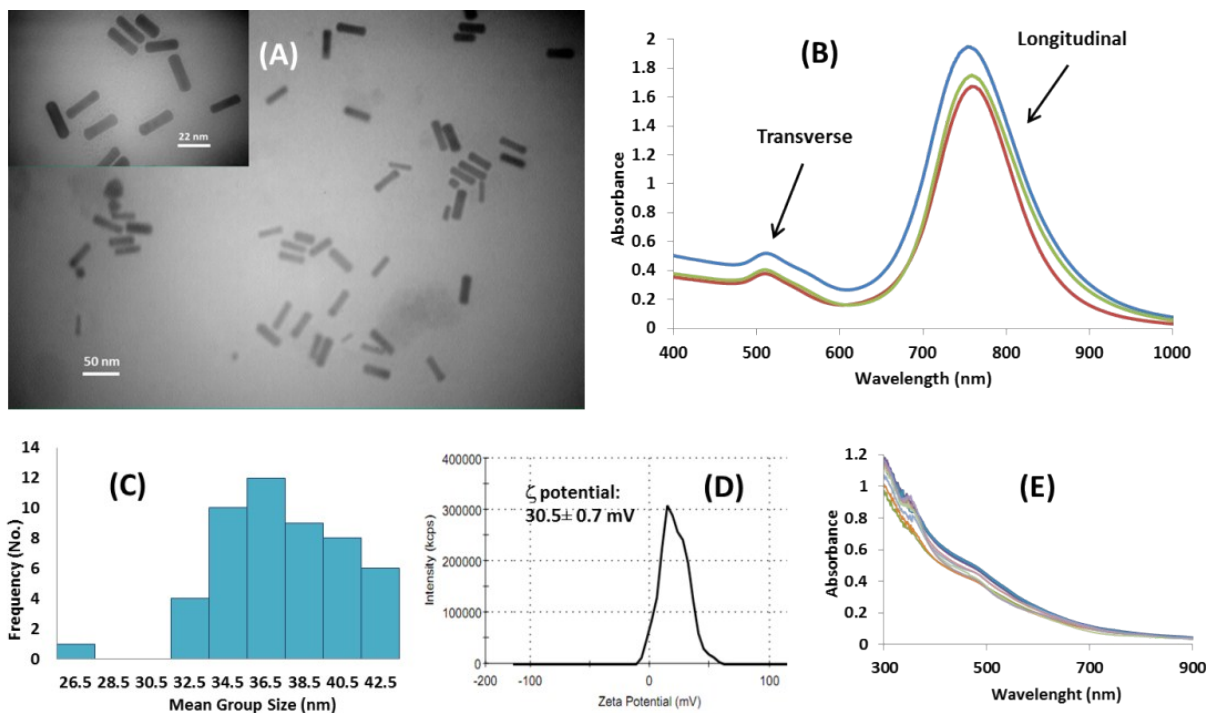
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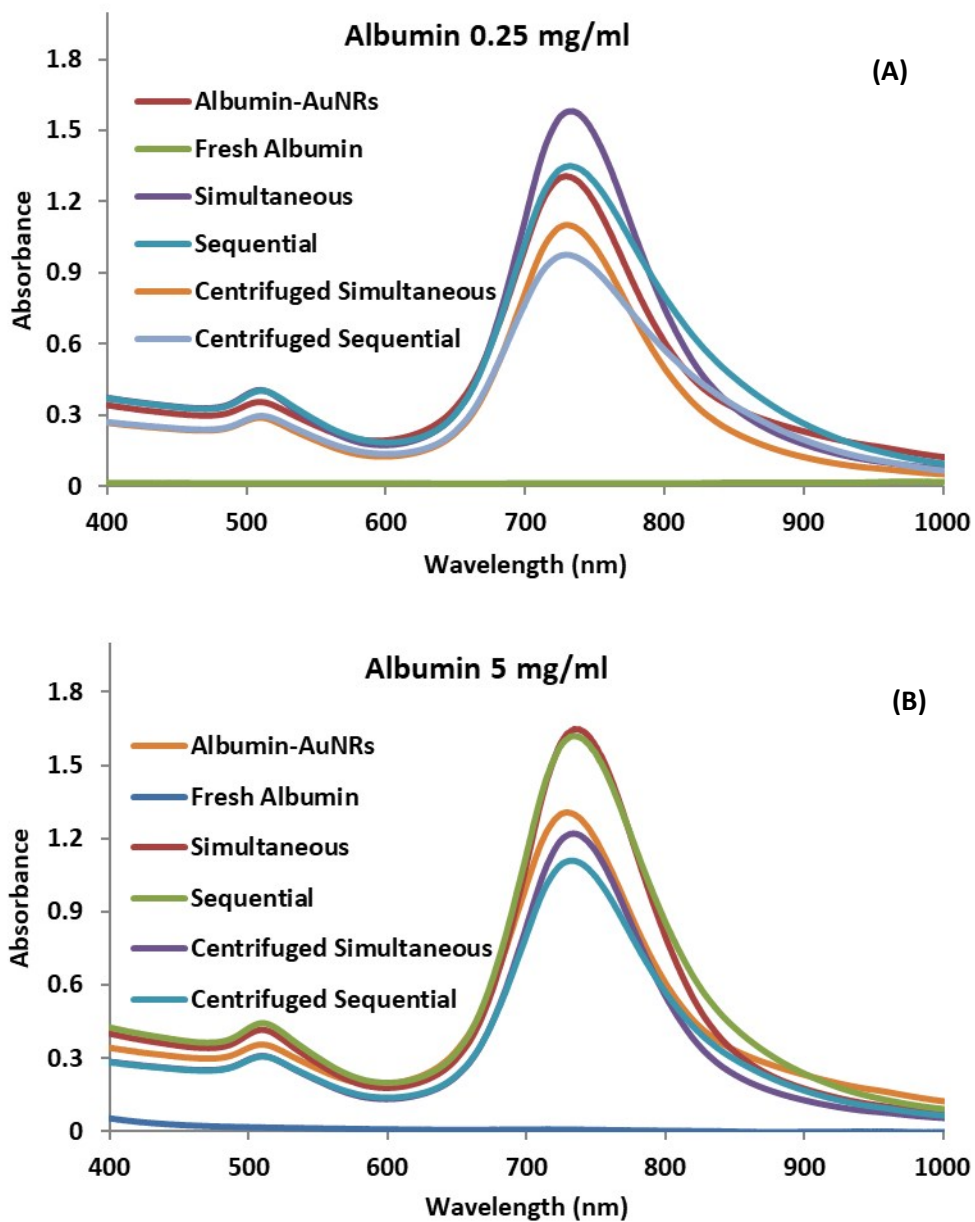
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Physicochemical characterization of synthesized bare AuNRs is shown in SI-Figure 1A-D including TEM images (A), absorbance spectra (B), histogram of longitudinal size distribution (C) and zeta potential (D). It can be seen that synthesized AuNRs are uniform enough in shape and size. The zeta potential is 30.5 ± 0.7 mV which is expected due to CTAB groups (quaternary ammonium) on the surface of AuNRs. No aggregation is seen in TEM images and absorbance spectrum. Reproducibility and acceptability of the prepared seed solutions were evaluated and are shown in Figure 1E. No identifiable peak approves the desired size of seeds.



SI-Figure 1. Physicochemical characteristics of synthesized bare AuNRs. (A) TEM images (B) absorbance spectra of repeated synthesized batches, (C) histogram of size distribution (longitudinal) (D) zeta-potential of bare AuNRs, and (E) absorbance spectra of various batches of prepared seed solutions

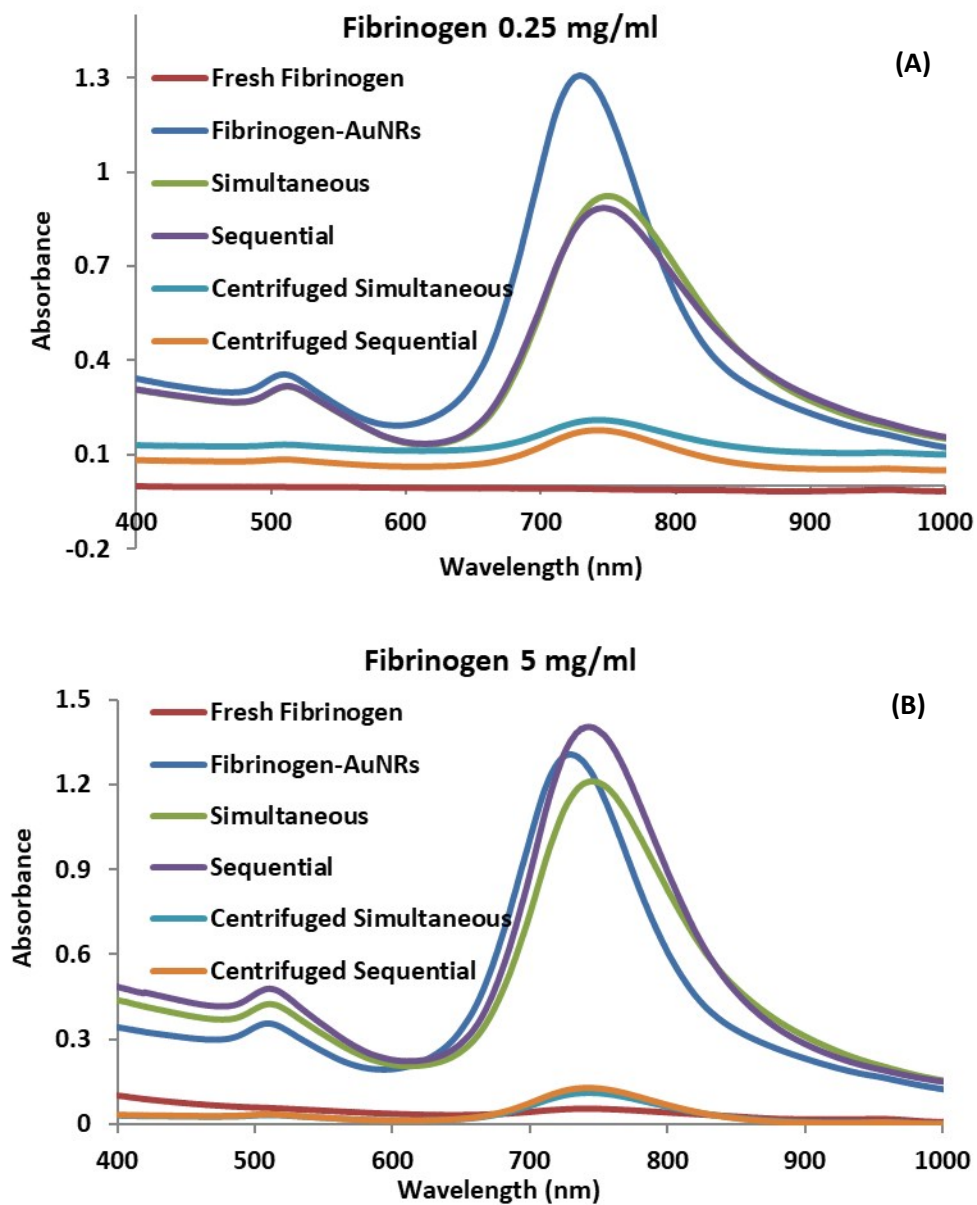


SI-Figure 2. (A) UV-Vis-NIR absorbance spectra of Albumin-coated AuNRs for 0.25 mg/ml of Albumin concentration subsequent to laser induction (simultaneous mode and sequential mode); (B) UV-Vis-NIR absorbance spectra of Albumin-coated AuNRs for 5 mg/ml of Albumin concentration subsequent to laser induction (simultaneous mode and sequential mode).

“Simultaneous”: laser induction concomitant with protein addition to AuNRs; “Sequential”: laser was induced to protein layer already formed on the surface of AuNRs. “Centrifuged” samples are those after centrifugation.



SI-Figure 3. Aggregation seen in some fibrinogen samples which resulted in adsorption of AuNRs onto microtube wall (observable in the inset in bottom right corner).

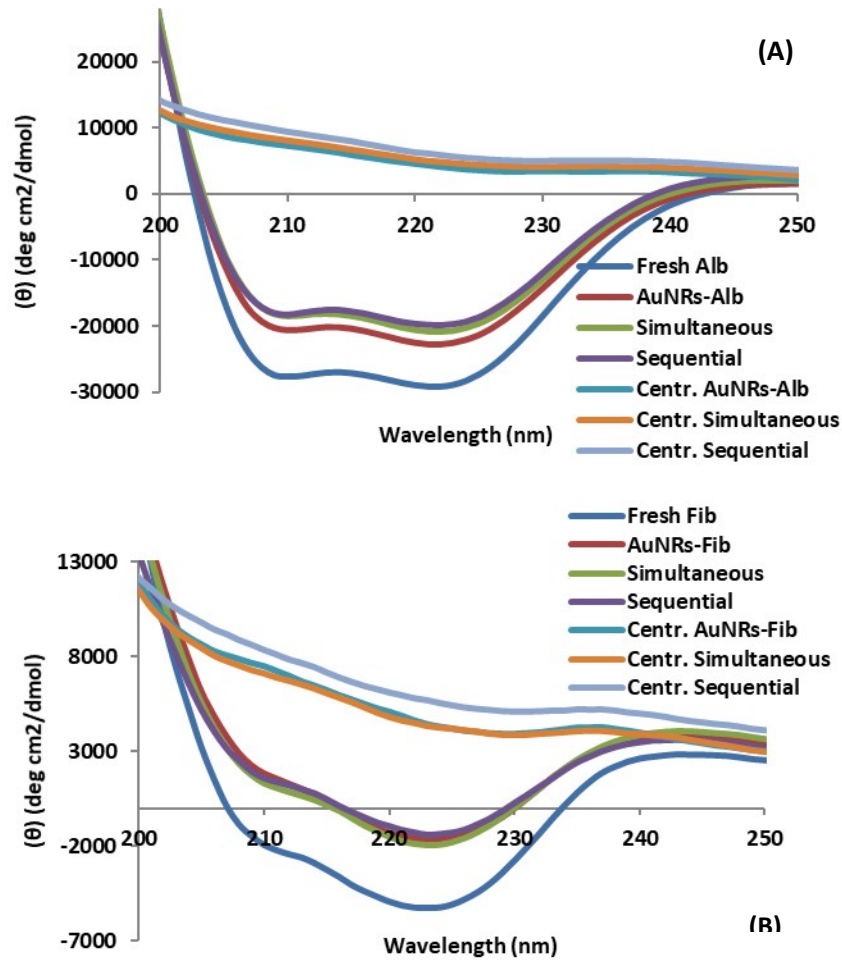


SI-Figure 4. (A) UV-Vis-NIR absorbance spectra of Fibrinogen-coated AuNRs for 0.25 mg/ml of fibrinogen concentration subsequent to laser induction (simultaneous mode and sequential mode); (B) UV-Vis-NIR absorbance spectra of Fibrinogen-coated AuNRs for 5 mg/ml of fibrinogen concentration subsequent to laser induction (simultaneous mode and sequential mode).

“Simultaneous”: laser induction concomitant with protein addition to AuNRs; “Sequential”: laser was induced to protein layer already formed on the surface of AuNRs. “Centrifuged” samples are those after centrifugation.

SI-Table1 . The wavelength of longitudinal surface plasmon resonance (SPR) peak in UV-Vis-NIR spectra of Alb-coated and Fib-coated AuNRs with simultaneous and sequential laser irradiation

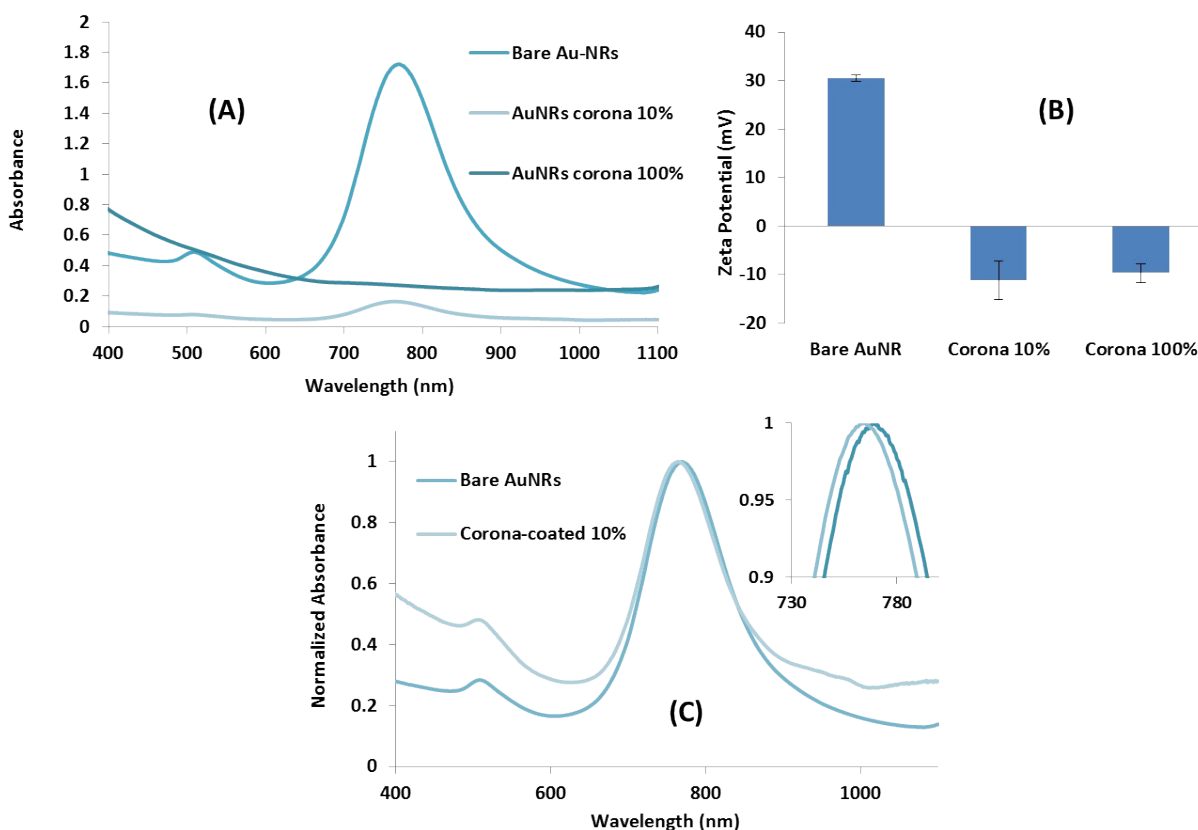
	Laser mode	Wavelength (nm)
Bare AuNR	-	730
Alb-coated AuNRs 0.25 mg/ml	Simultaneous	730.5
	Sequential	730.2
Alb-coated AuNRs 5 mg/ml	Simultaneous	732.5
	Sequential	732.5
Fib-coated AuNRs 0.25 mg/ml	Simultaneous	750.5
	Sequential	746.5
Fib-coated AuNRs 5 mg/ml	Simultaneous	745.5
	Sequential	742.5



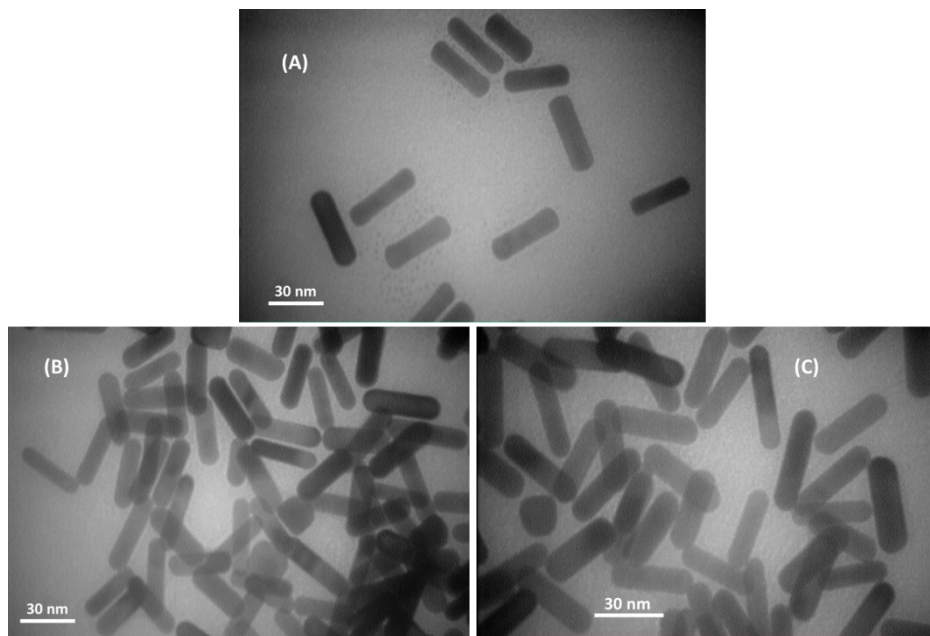
SI-Figure 5. Far-UV CD Spectrographs of Alb-coated AuNRs (A) and Fib-coated AuNRs (B) following simultaneous and sequential plasmonic heating, before and after centrifugation

“Simultaneous”: laser induction concomitant with protein addition to AuNRs; “Sequential”: laser was induced to protein layer already formed on the surface of AuNRs; “Centr.” shows centrifuged samples.

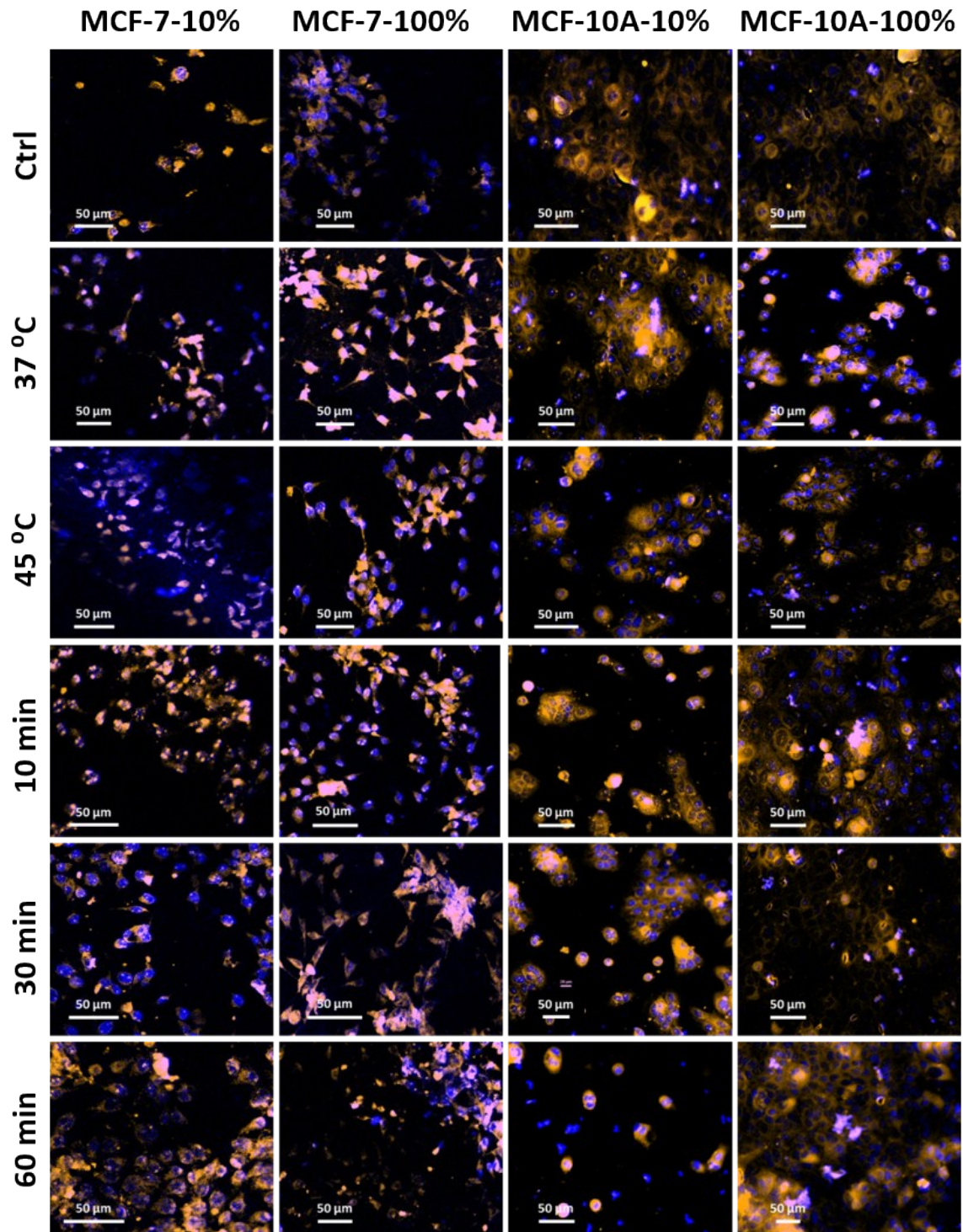
To evaluate the formation of protein layer on the surface of AuNRs and seeking any aggregation, absorption spectra, zeta potential and TEM images of the samples were taken. As it is shown in SI-Figure 2A, the intensity of the SPR peaks are diminished in corona 10%-coated nanorods and suppressed in corona 100%. This phenomenon could be due to the change of dielectric constant of the medium which is more remarkable in 100% corona forming a more dense protein layer on the NPs. Besides, a blue shift (from 769.5 up to 764.5 nm) is seen in SPR peak consistent with the coverage of AuNRs with a dense layer of proteins (SI-Figure 2C).(1, 2) There is no considerable aggregation based on the shape of spectra as no shoulder, asymmetry or extra peak is seen. Another proof of uniform corona layer formed on the surface of nanoparticles can be their zeta potential. The positive zeta potential (30.5 mV) of bare AuNRs is reduced to -12.3 ± 1.7 and -9.3 ± 2.4 mV for corona 10% and 100%, respectively (SI-Figure 2B) which is consistent with previous reports.(1, 2) No aggregation in corona-coated AuNRs samples was further confirmed via TEM (SI-Figure 3).



SI-Figure 6. (A) Absorbance spectra of bare and corona-coated (10% & 100%)-Au-NRs, (B) their zeta potentials and (C) plasmonic red shift of longitudinal SPR peak for corona 10%



SI-Figure 7. TEM images of (A) bare AuNRs, (B) HP-corona-coated (100%) AuNRs, and (C) 1 hour irradiated corona-coated (100%) AuNRs. As it is seen there is not any aggregation after PC formation nor any deformation of the AuNRs subsequent to laser irradiation.



SI-Figure 8. Confocal microscopic images of two immortalized and normal cell lines (MCF-7 and MCF-10A, respectively) for corona 10% and 100% in samples exposed to either thermal incubation (37 and 45 °C) or plasmonic heating (10, 30 and 60 min laser irradiation)

References:

1. Mirsadeghi S, Dinarvand R, Ghahremani MH, Hormozi-Nezhad MR, Mahmoudi Z, Hajipour MJ, et al. Protein corona composition of gold nanoparticles/nanorods affects amyloid beta fibrillation process. *Nanoscale*. 2015;7(11):5004-13.
2. Casals E, Pfaller T, Duschl A, Oostingh GJ, Puntès V. Time evolution of the nanoparticle protein corona. *ACS nano*. 2010;4(7):3623-32.