Supplementary Information

Chitosan-coated triangular silver nanoparticles as a novel class of biocompatible, highly sensitive plasmonic platforms for intracellular SERS sensing and imaging

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The successful use of *p*-ATP labeled Chit-AgNTs for imaging living cells would require proves for their robustness in simulate biological conditions. We have already demonstrated the very good stability of colloidal Chit-AgNTs during storage, under conditions of different ionic strengths and after transfer to various aqueous media.[26] Here we investigated the colloidal stability around physiological temperature of 37 ^{o}C. Fig. S1 A shows the LSPR extinction spectra of colloidal p-ATP labeled Chit-AgNTs recorded at temperatures between 19 °C and 58 °C with 3 °C increments. A decrease in the intensity of the LSPR bands with a concomitant 2 nm blue shift of the in-plane dipolar plasmonic band is observed. The original spectrum is fully recovered after cooling down back to 19 °C (Fig. S1 B). The reversible changes observed in the spectra can not be related to any intrinsic modification of nanoparticle properties but could be explained by the variation of refractive index of water as a function of temperature.

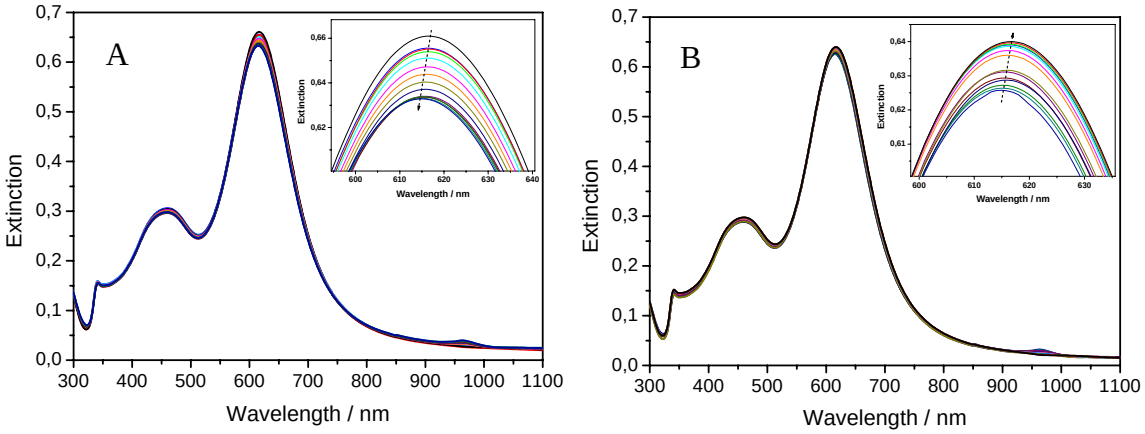

Fig.S1 UV-Vis-NIR extinction spectra of p-ATP labeled Chit-AgNTs recorded at different temperatures: (A) from 19 °C to 58 °C with 3°C increments. (B) from 58 °C to 19 °C with 3°C decrements. The insets show a closer view of the in-plane dipolar plasmonic band.

Comparative experiments were performed to test the internalization of Chit-AgNTs and of *p*-ATP labeled Chit-AgNTs by preparing identically 6 different samples (3 samples incubated with Chit-AgNTs and 3 with *p*-ATP – labeled Chit-AgNTs). More than 90 individual cells (~ 15 cells in each sample) were analyzed by bright field microscopy. Fig. S2 shows six bright field optical images (one image from each sample) representing the cells incubated for 2 hours with Chit-AgNTs (top) and *p*-ATP labeled Chit-AgNTs (bottom), respectively. The nanoparticles internalization and localization inside cell is visible as bright spots generated from the strong light scattering of Chit-AgNTs when the samples are illuminated with a white LED lamp. A careful analysis of the microscopic images in Fig.S2 clearly highlights a difference between cellular uptake of two types of nanoparticles. Specifically, in Figure S2 top we can observe that a proportion of Chit-AgNTs doesn't interact with cells, being present in the cellular environment. In contrast, Fig. S2 bottom shows that almost all p-ATP labeled Chit-AgNTs nanoparticles interact with A549 cells. As result, the cancer cells incubated with *p*-ATP labeled Chit-AgNTs present a higher density of internalized nanoparticles than the cells incubated with unlabeled nanoparticles.

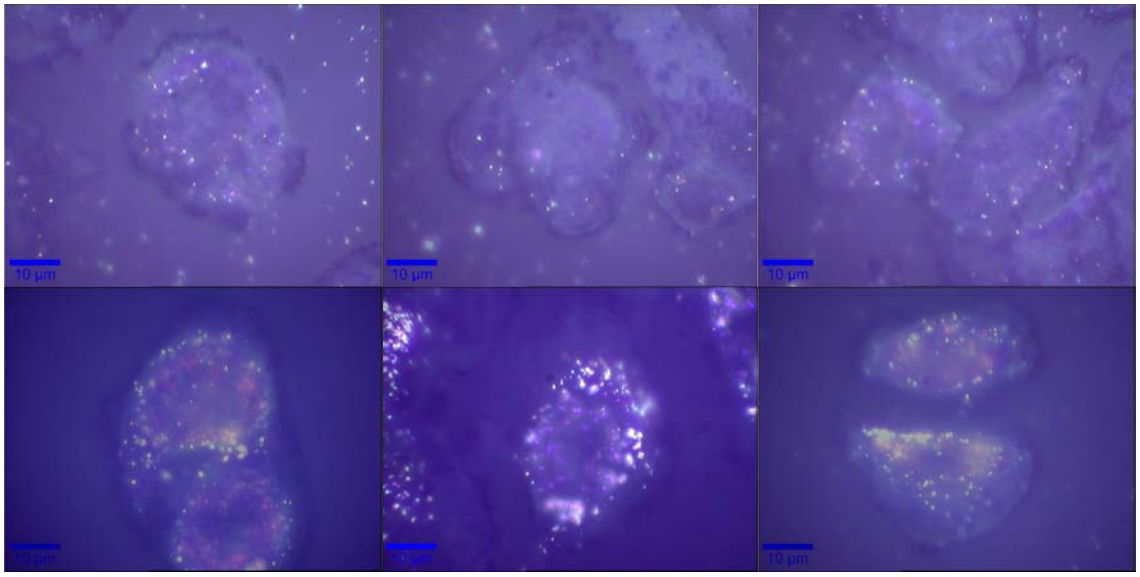

Fig.S2 Selected bright field images of A549 cells incubated for 2 hours in the presence of *p*-ATP labeled chitosan-coated spherical nanoparticles (top) and of *p*-ATP labeled Chit-AgNTs (bottom).

To further demonstrate the advantages of triangular nanoparticles as multiwavelength responsive SERS nano-tags an additional experiment was performed. Spherical silver nanoparticles with a mean diameter of 18-20 nm were prepared using the same synthesis protocol [1]. Briefly, in the first step, a stock aqueous solution of silver particles called 'seeds' was prepared by the reduction reaction of silver nitrate with sodium borohydride. In a typical reaction, one volume of aqueous solution of silver nitrate (2.9×10^{-4} M) was mixed with an aqueous solution of sodium citrate (2.5×10^{-4} M) and cooled in an ice-bath under vigorous stirring. To this mixture, an aqueous solution of sodium borohydride (0.6 ml, 0.1 M) was added dropwise, which resulted in the formation of a bright yellow solution. The resulting seed solution was stored in the dark for 2–3 h before use in order for any excess borohydride to react with water. In the second step, aqueous solutions of seeds (200 μl), trisodium citrate (TSC, 16.5 mM, 200 μl), ascorbic acid (0.1 M, 50 μl) and chitosan (2 mg ml^{-1}, 10 ml) were pre-combined. To this mixture, $AgNO_3$ (0.01 M, 300 μl) was added dropwise under continuous magnetic stirring. The growth of silver nanoplates was carried out at 0 °C. The optical spectrum of the final product exhibits one dominant absorption band around 408 nm (Fig.S3, black curve) which represents the typical signature of the dipolar plasmon resonance of spherical nanoparticles with an average diameter of 18-20 nm [1]. For SERS nanotag preparation, 990 µl colloidal solution of

chitosan-coated silver nanoparticles were incubated at room temperature with 10 µl aqueous solution of *p*-ATP (10^{-3} M). The mixture was kept for 24 h until the system reached equilibrium, after which the nanoparticles were centrifuged and re-suspended in ultrapure water to remove any unused reactants. Compared with the original spectrum of chitosan-coated spherical nanoparticles, a decrease in the intensity of the plasmonic band with a concomitant red shift of 6 nm was observed in the presence of *p*-ATP molecules Fig.S3, pink curve).

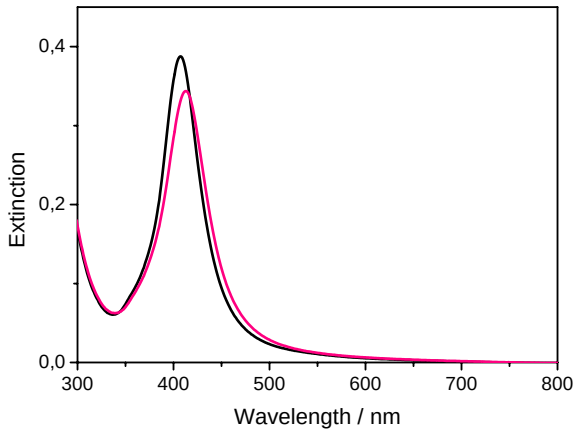

Fig.S3 UV-Vis-NIR extinction spectra of colloidal chitosan-coated spherical nanoparticles: (black) as prepared, (pink) *p*-ATP encoded nanoparticles.

Two samples were identically prepared by incubating A549 cells with the same concentration of *p*-ATP labeled spherical and triangular nanoparticles, respectively. As the NIR availability of any SERS contrast agent is imperative for *in vivo* applications, we focused our experiment on 785 nm laser excitation. Multiple SERS spectra were collected from randomly sized bright spots localized inside cells (see Fig. S4). We found that for the sample incubated with triangular nanoparticles we succeeded measuring SERS spectra from any bright spot visible in the microscopic image (see Fig. S4 B, D). On the contrary, the limited SERS sensibility of spherical nanoparticles under NIR excitation made impossible the collection of spectral signal from smaller bright spots of small dimers or individual nanoparticles (see Fig.S4 A, C). We believe that the study presented here is consistent with demonstration of practical superiority of anisotropic nanoparticles as SERS contrast agents. However a decisive comparison should take in

consideration several unknown parameters hardly to be investigated separately, as for instance number of reporter molecules per nanoparticle, fraction of nanoparticles internalized by the cells (which is a shape dependent property), nanoparticle size and degree of their aggregation.

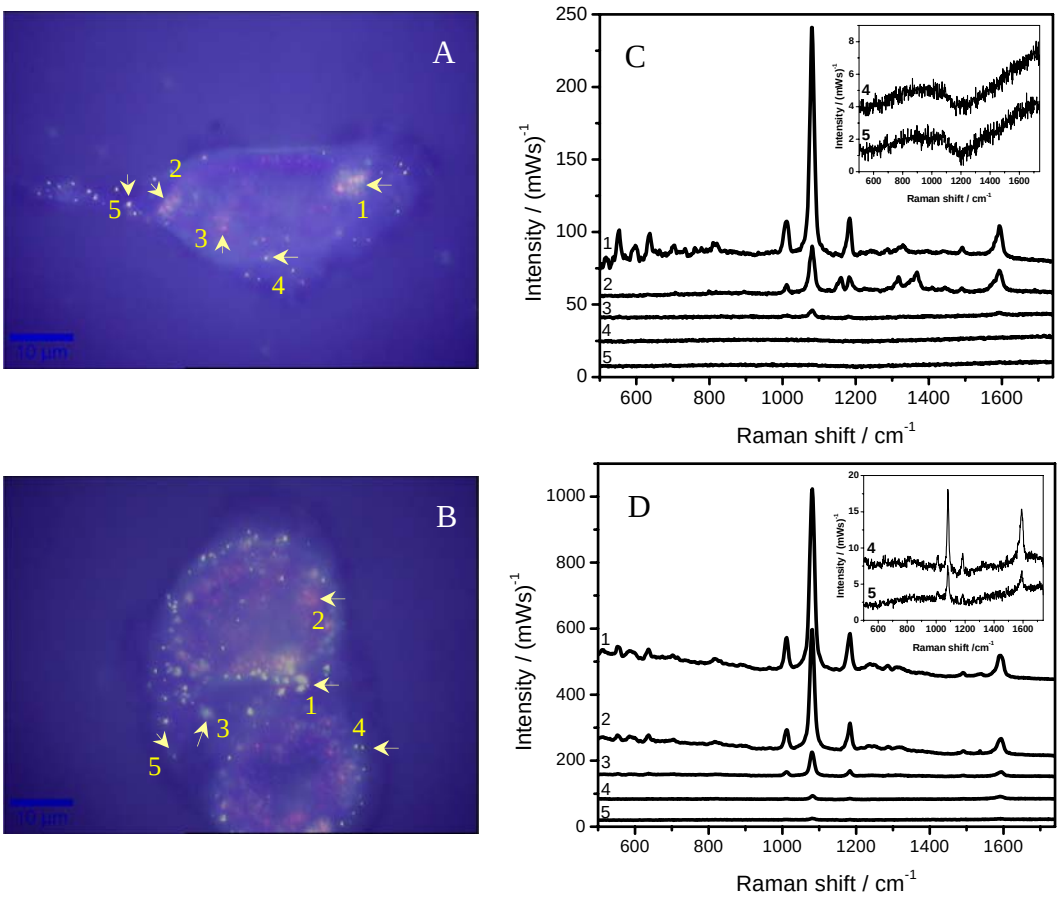

Fig.S4 Left: Bright field images of A549 cells incubated for 2 h in the presence of *p*-ATP labeled chitosan-coated spherical nanoparticles (A) and of *p*-ATP labeled Chit-AgNTs (B). Right: (C) SERS spectra collected from a single A549 cell incubated with *p*-ATP labeled chitosan-coated spherical nanoparticles, recorded at the positions marked in image A. The inset shows a closer view of spectra 4 and 5. (D) SERS spectra collected from a single A549 cell incubated with *p*-ATP labeled Chit-AgNTs, recorded at the positions marked in image B. The inset shows a closer view of spectra 4 and 5. The excitation line was 785 nm.

References:

[1] M. Potara, E. Jakab, A. Damert, O. Popescu, V. Canpean and S. Astilean, *Nanotechnology*, 2011, **22**, 135101.