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Supplementary Information

Peptidic coating for gold nanospheres multifunctionalizable with photostable and photolabile moieties

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Explanation on the motility of the bands in the electrophoresis gels:

The encapsulated and dye-modified nanoparticles show different band motilities due to the interplay between two major effects, which are linked to the hydrodynamic radius (final volume inclusive of the solvation sphere) and to the total charge of the nanostructure. Briefly, at a given total charge, the hydrodynamic radius of a nanostructure is subjected to an increase due to the coating or to the conjugation of the nanoparticle to (bio)molecules (dyes or proteins): the larger the molecule, the larger and slower the nanosystem. Concerning charge variation, the total charge of the functionalized nanostructures at a given pH depends on the protonation state of the superficial groups and of the conjugates. The yields of reaction changes the number of dyes bonded to AuNsKG, which could change significantly, depending on the dye and on the exploited reaction. Despite the choice of similar dyes (fluorescein basic structures), the sum of these effects causes the observed different retention properties in gels.

In order to understand why nanoparticles coated with a mix of G and K peptides could have higher motility and more negative ζ-potential than nanoparticles coated with single peptides (see Fig. S2), we must consider the possibly different density of peptides on the surface of the nanoparticles, and the different charges and possibly dipoles of G and K peptides. We believe that K-peptides can form a denser SAM than G-peptides since the oppositely charged groups on the lysine (both partially cationic amine and anionic carboxylic acid) can facilitate the insertion of more chains. The K peptide can also help to stabilize the final system on its external face (the hydrophilic side) through electrostatic (dipolar) interactions. On the other hand, the more negative G peptides can promote a repulsive effect during the ligand exchange, thus obtaining a less dense (but more negative) SAM with respect to K. It is possible that these two effects interact during the surface replacement with a mix of peptides, and that the presence of K peptides (with their partially positive ammines and their negative carboxylics) may have a screening effect on the repulsive interactions amongst other peptides. The final result is a SAM denser than in the case of pure G-peptides and closer to the density observed with pure K-peptide. Accordingly, a final net charge would be more negative than for the case of AuNs coated with pure peptides. Different organizations in the hydration layers of the nanosystems may also be involved in explaining the reported experimental results. Theoretical simulations about the arrangement of the peptides on the metallic surface are in progress.

Fig. S1

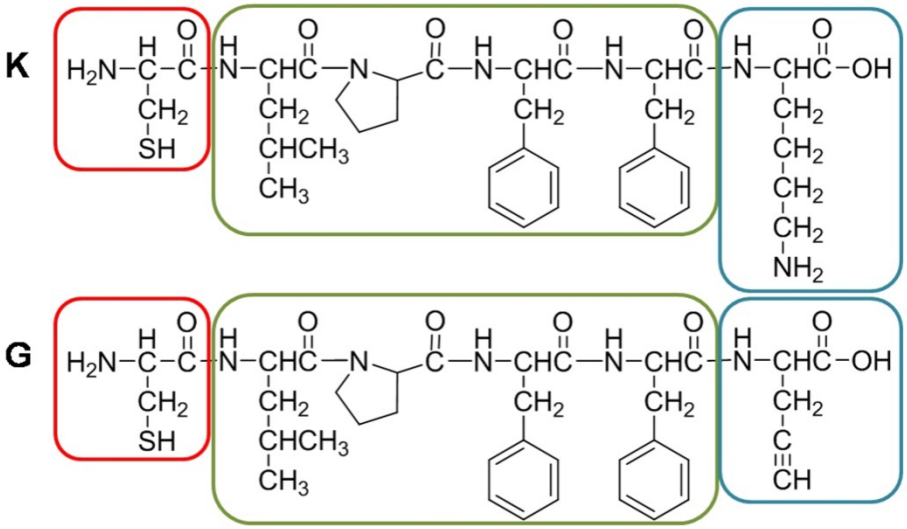


Figure S1. Scheme of the engineered hexa-aminoacids peptides. K-peptide is CLPFFK, and G-peptide is CLPFF-Pra. The only difference between the two peptides is the last aminoacid that is responsible of the final functionalization of the coated-AuNs (blue boxes). The red boxes highlight the cysteine that brings the thiol group, through which the peptides link to AuNs. The green boxes enclose the four amino acids that stabilize the coating thank to the ordering of the peptides in a hydrophobic SAM around the structure.

Fig. S2

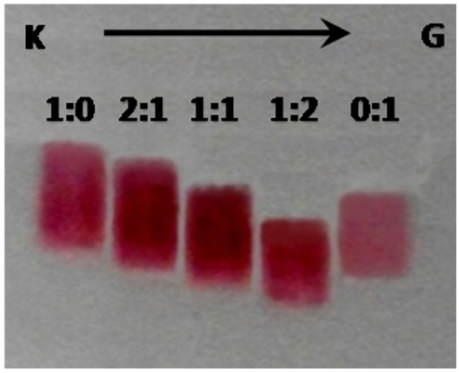


Figure S2. 0.8% agarose gels electrophoresis performed for 30 minutes at 90 V. The samples are encapsulated AuNs with different molar-ratio of peptides K and G (on the left). It's noticeable that 1:2 K to G encapsulated AuNs show a higher motility than AuNs coated with the pure peptides.

Fig. S3

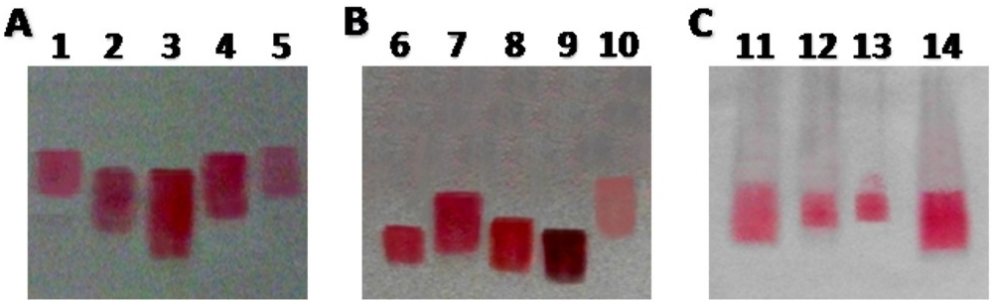


Figure S3. 0.8% agarose gel electrophoresis results of variously encapsulated and functionalized AuNss. A) Effect of click reactions (one by one) on all the three reactive sites on the AuNss encapsulated with a 1:1 (molar ratio in solution) mix of peptides G and K (AuNsKG): 1, AuNsKG; 2, AuNsKGF (amine of F linked to the carboxy of G and K); 3, AuNsKGFn (azide of Fn linked to alkyne of G); 4, AuNsKGA (carboxy of A linked to amine of K); 5, AuNsKG. Lanes 1 and 5 contains the same sample, in order to test the repeatability of the experiment and the homogeneity of the electrical field in the gel. B) Samples functionalized with one, two or three different dyes, in order to test the ability to link by covalent bond three different molecules on the three different active site of AuNsKG system: 6, AuNsGFn; 7, AuNsKG; 8, AuNsKAGFn; 9, AuNsKAGFn-F; 10, AuNsKA. Lanes 6 and 10 are actually the control samples for AuNsKAGFn (lane 8), *i.e.* they are respectively AuNsG and AuNsK who underwent the same processes used to bifunctionalize AuNsKG with both A and Fn. C) In lanes 11-14 are shown the mono-, bi-, and tri-functionalized AuNs encapsulated systems obtained by the process B: 11. AuNsGFn; 12, AuNsGFnKAt; 13, AuNsGFnKAt-Bn; 14, AuNsKAt). The acronyms are: Fn, fluorescein azide; F, fluorescein cadaverine; A, AlexaFluor-488, At, AlexaFluor-647; Bn, norbiotin. See the text for a complete discussion.

Fig. S4

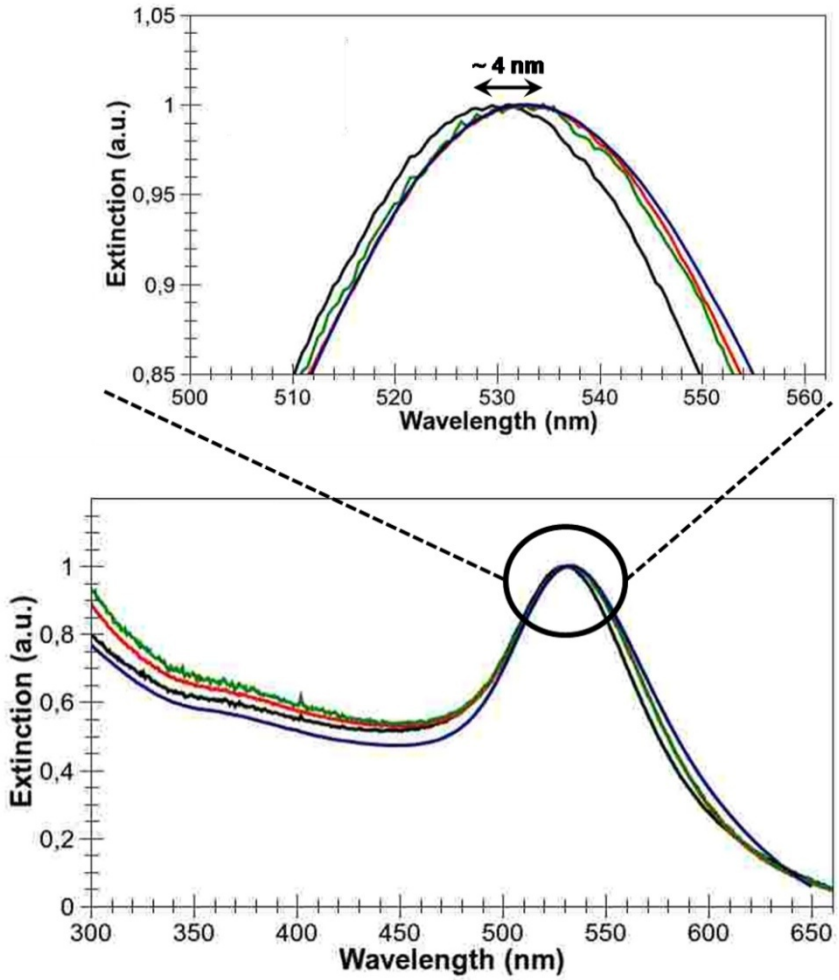


Figure S4. UV-Vis spectra of 30 nm gold nanospheres: AuNs (black line), and the same particles after encapsulation with peptides (AuNsK: green line; AuNsG: blue line; AuNsKG: red line). Note in the zoom in the top panel the 4-nm red shift in the plasmon peak, caused by the change of local refractive index around the metallic surface after the peptide substitution. All spectra are normalized on their plasmon resonance band maximum.

Fig. S5

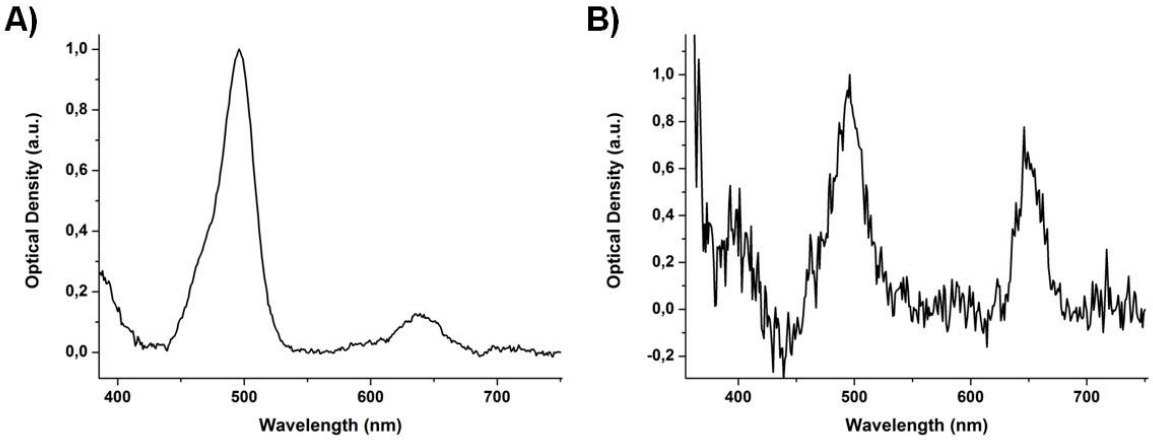
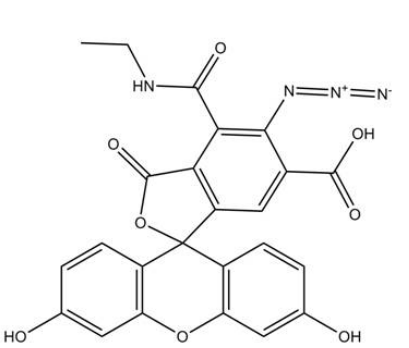
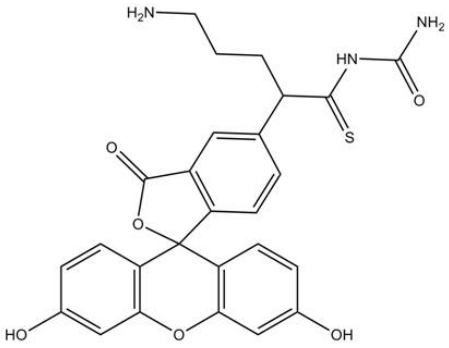


Figure S5. UV-Vis absorption spectra of the stripped coating from systems synthesized by A) process A (AuNsKGFnAtt), and B) process B (AUNsGFnKAt). Note that here Att is the abbreviation for the dye Atto-633. The concentration of the nanoparticles (Nps) was estimated through UV-Vis spectra of the colloids and by the reported extinction coefficient (Liu et al., *Coll Surf B*, 2007, **58**, 3). For systems produced by process A we found ~3500 Fn/Np and ~300 Att/Np (see experimental section). These results are in agreement with the theoretical maximum number of peptides per particle proposed by Puntes et al. (*ACS Nano*, 2009, **3**, 1335; 1 peptide every 0.2 nm^2); the lower experimental numbers could derive by a yield lower than 100% for the final functionalization reaction (Kolb et al., *Angew Chem Int Ed Engl* 2001, **40**, 2004) or by a non-optimum packing of the peptides in the SAM. In particular, even if the molar ratio between peptides K and G in the solution used to substitute the coating of the Nps was 1:1, a lower number of the more negatively charged peptide K (bonded to Att) with respect to peptide G is expected on the surface of the Np. For the systems produced by process B we estimated ~600 Fn/Nps and ~150 At/Nps. These data seems to suggest that a cargo attached on the peptides before the encapsulation of nanoparticles could influence the composition and the density of the coating layer. In particular, a less dense SAM can be explained by the higher negative charge (and maybe the higher steric hindrance) on the fluorophore-substituted peptides; this would explain also the higher concentration of the less charged GFn with respect to KAt.

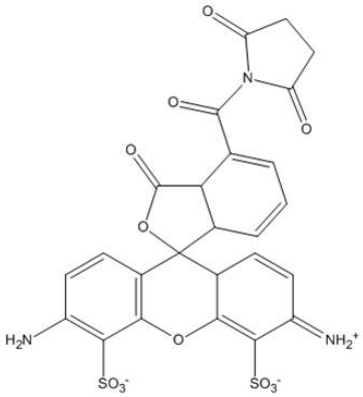
Scheme S6 - Structure of the dyes



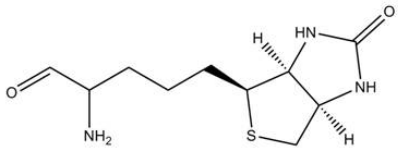
Fluoresceinazide (Fn)



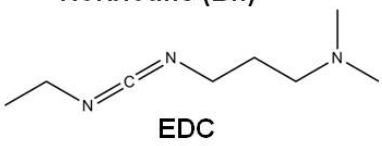
Fluoresceincadaverine (F)



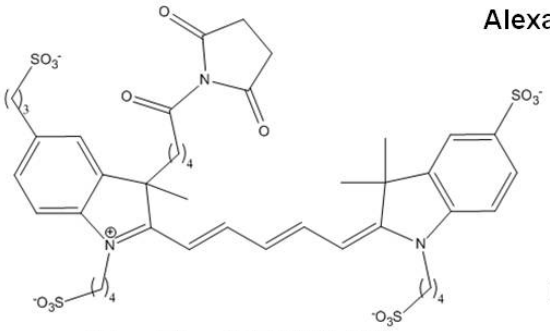
AlexaFluor488-NHS (A)



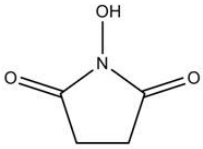
Norbiotine (Bn)



EDC



AlexaFluor647-NHS (At)



NHS
N-hydroxysuccinimide