Force spectroscopy as a tool to determine charge exposition of peptide-functionalized gold nanoparticles

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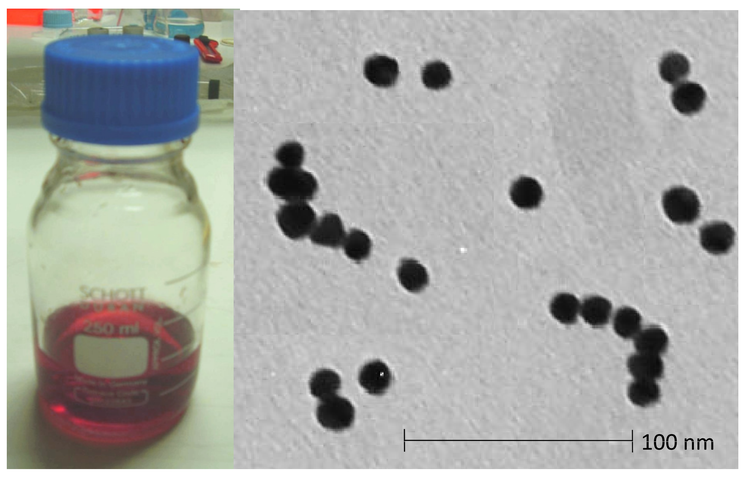
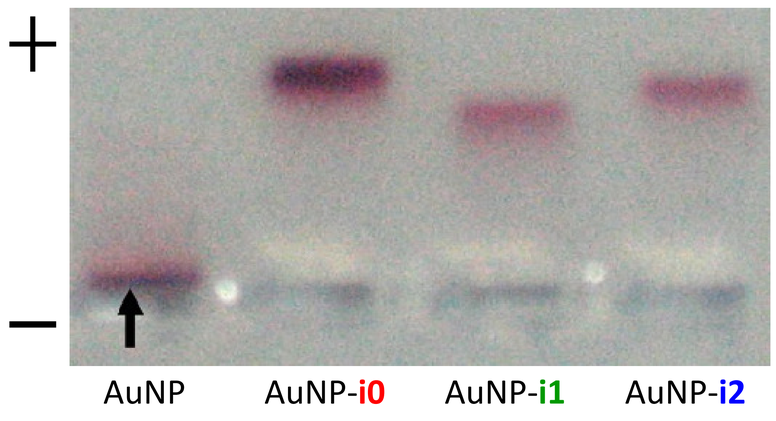
In recent investigations of our group we proposed the use of peptide-functionalized gold nanoparticles (AuNP) showing selectivity for toxic aggregates of amyloid beta protein involved in Alzheimer's disease, and microwaves, to produce the disaggregation of the toxic aggregates, as a tool for treatment of the disease[1]. From the point of view of biological activity, one of the main concerns is how peptides interact and pack on the AuNP surface.

In this work, we have prepared conjugates of AuNP and three isomer peptides capable of molecular recognition for toxic aggregates, namely CLPFFD-NH_2 (**i0**), CDLPFF-NH_2 (**i1**) and CLPDFF-NH_2 (**i2**) and studied the effect of the sequence on the peptide charge exposition through force spectroscopy as well as by electrophoresis, zeta potential and Raman spectroscopy.

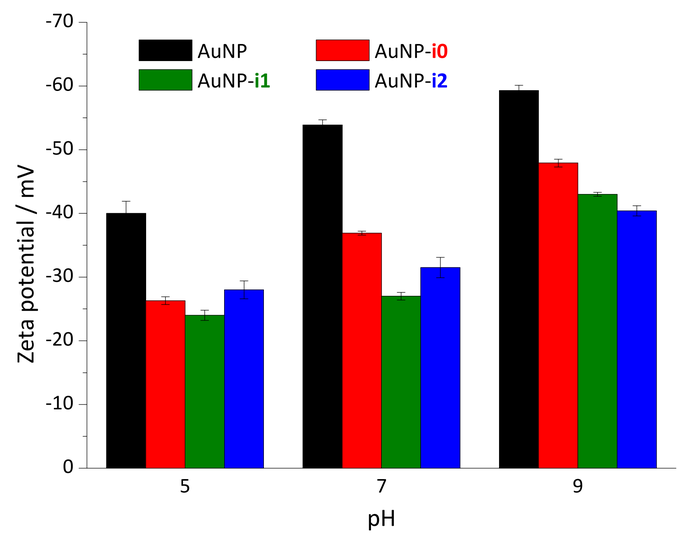
Synthesis and characterization



AuNP are synthesized in colloidal state following Lévy *et al.*[2] and conjugated by adding a 1 mg/mL solution of the peptides under vigorous stirring. **Left:** a gold sol. **Right:** TEM micrograph of AuNP

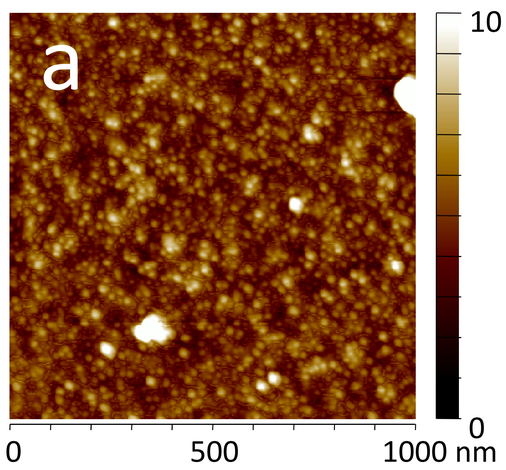


Agarose gel electrophoresis of AuNP and their conjugates[3]. The **i0** conjugates show more mobility, while **i2** and **i1** conjugates show less mobility. Naked AuNP aggregated and showed no mobility.

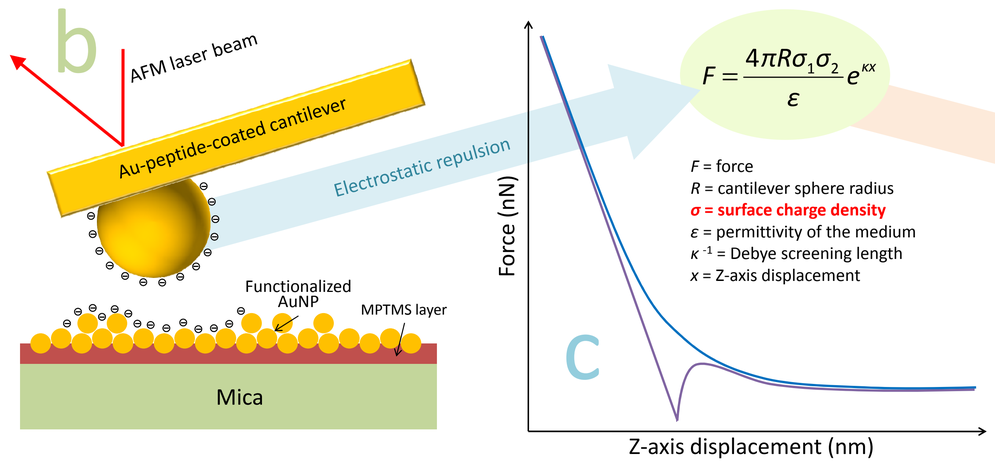


Zeta potential measurements[3]. Conjugates of **i0** show, as a trend, greater zeta values than those of **i1** and **i2**. The functionalization reduces slightly the zeta potential of the colloids.

AFM imaging and force spectroscopy



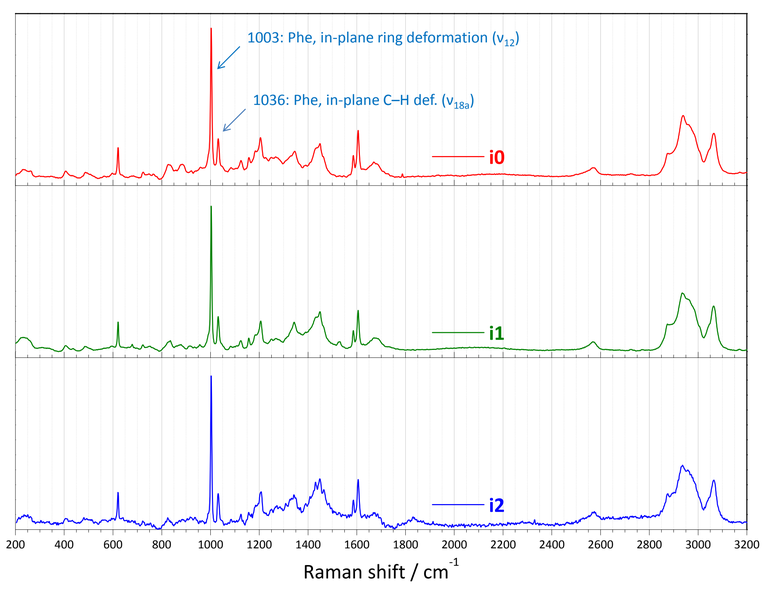
Picture a: AFM image (tapping mode, height) of AuNP-peptide conjugates fixed over mica.

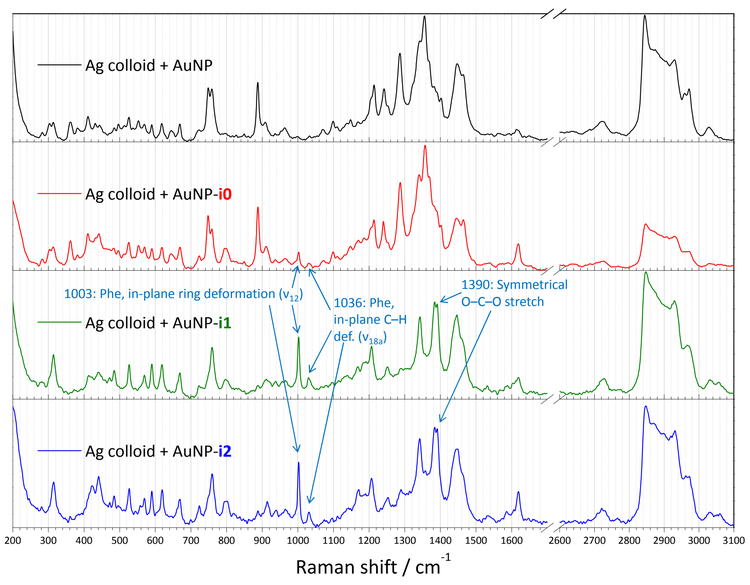


Force spectroscopy AFM experiment. As shown in picture **b**, a ball-tipped gold-coated cantilever is used, and it is functionalized with the peptide in a similar procedure to the AuNP. The AuNP are fixed to a mica surface using 3-amino-propylmethoxysilane (MPTMS). The electrostatic repulsion interaction is measured by putting the AFM in force mode and fitting the resulting curve to the formula[4] described in figure **c**; from this equation we calculated the number of charges dividing by the elemental charge value. Results are shown in figure **d**, indicating similar charge values for **i0** and **i2** conjugates, and less charge in **i1** conjugates.

Raman spectroscopy

Raman spectra of the peptides in solid state and in the colloids, with the aid of a silver colloid to achieve enhancement by SERS, since gold colloids by themselves showed little enhancement (data not shown). The **i0** conjugate has a spectra very similar to that of the naked AuNP, but still shows the typical monosubstituted benzene bands at 1003 and 1036 cm^{-1}, although with very low intensity, unlike the spectra of the solid peptides and the **i1** and **i2** conjugates; the last two only show bands which are characteristic of the peptides, such as the aspartic acid COO^- symmetrical stretching scattering at 1390 cm^{-1} and the many benzene vibrations of the Phe residue. This indicates greater enhancement by SERS in those conjugates, thus confirming the proximity of the carboxyl group and the phenyl rings to the AuNP surface for **i1** and **i2**.

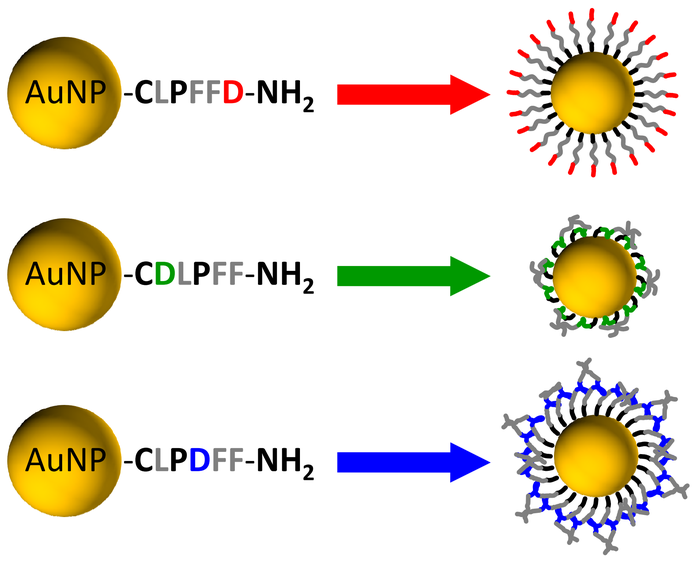




Conclusions

This investigation has provided us with another way of characterizing AuNP not previously reported, which allows to measure the charge exposition of the aspartic acid residue in the peptide.

Furthermore, the results provide further evidence for the effects of a sequence change in the structure of the conjugated peptide, as proposed in our recent investigation[3]: the carboxyl group is closer to the surface of the nanoparticle in **i1**, and to a lesser extent, in **i2**; in **i0** the charged residue is assumed to be farther from the surface, by the greater exposition of the charge in force spectroscopy/zeta potential measurements and less enhancement by SERS, which is greater when closer to the AuNP surface. So **i0**'s peptidic backbone must be near-vertical to the AuNP surface, and **i2** and **i1** are probably more inclined, as shown in the figure at the right.



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References. 1. Kogan M. J.; Turiel A.; Bastus, N. G.; Amigo, R.; Grillo-Bosch, D.; Araya, E.; Turiel, A.; Labarta, A.; Giralt, E.; Puntes, V. F. *Nano Lett.* **2006**, *6*, 110-115. 2. Lévy, R., Thánh, N. T. K., Doty, R. C., Nichols, R. J., Schiffrin, D. J., Brust, M., and Fernig, D. G. *J. Am. Chem. Soc.*, **2004**, *126, 10076-10084.* 3. Kogan M. J.; Olmedo I.; Araya, E.; Sanz, F.; Toledo, P.; Arbiol, J.; Giralt, E.; Álvarez, A.; Medina, E. *Bioconjugate Chem*. [In press]. 4. Heinz W. F.; Hoh J. H. *Trends Biotechnol*. **1999**, 17, 143-150