

# Increased Stability of Oligopeptidases Immobilized on Gold Nanoparticles

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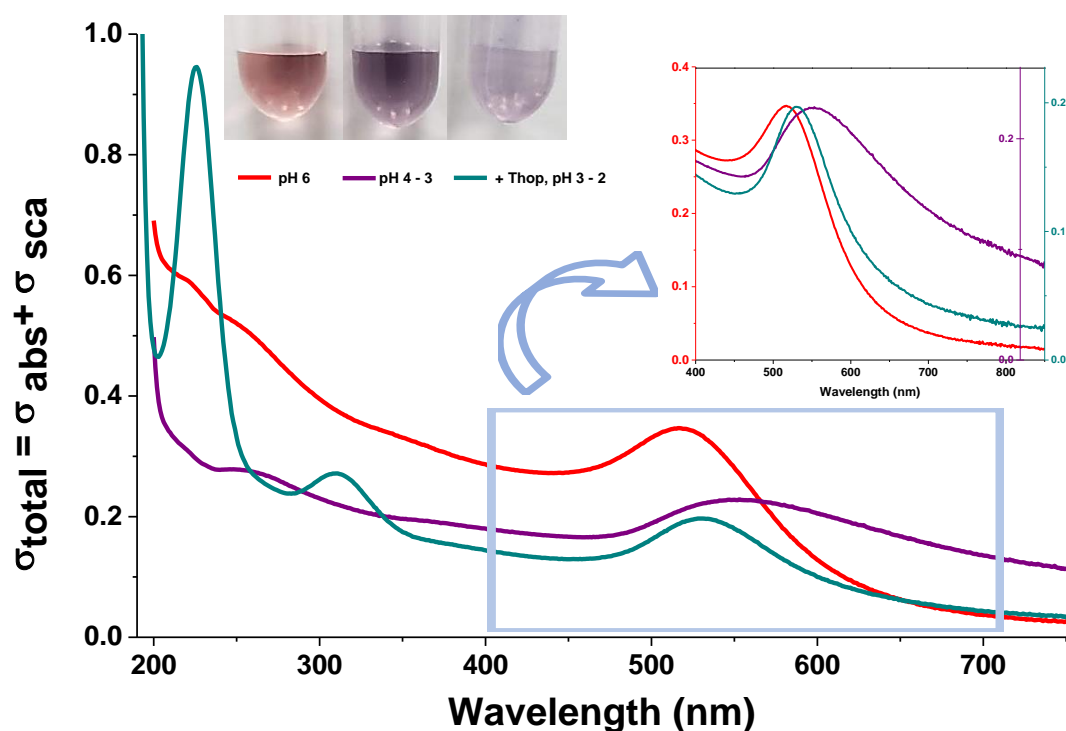
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## 1. Supplemental Material

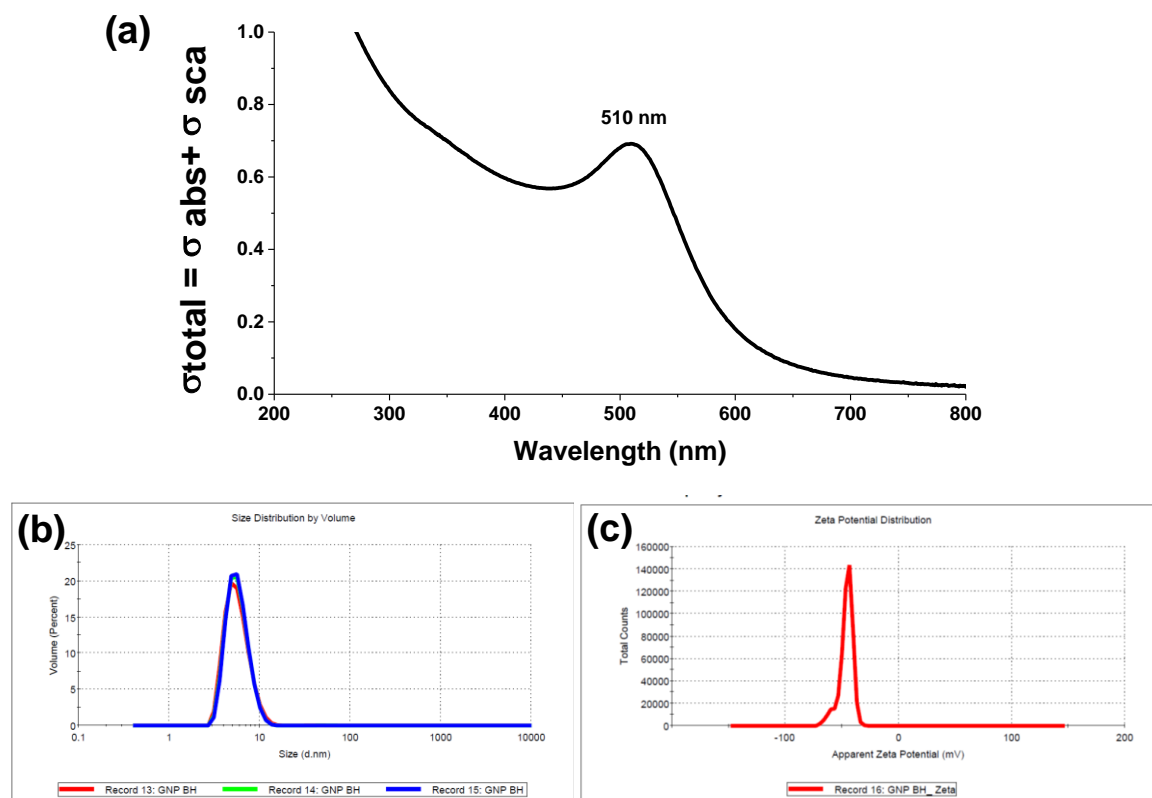
### 1.1. In-situ synthesis of *rTHOP*-GNPs and *rNEL*-GNPs



**Figure S1.** UV-vis spectra of bare GNPs synthesized using sodium borohydride (red line) and the changes in the spectrum, SPR bands (insert) and the visual aspects of the colloidal solution after the addition of 2 nmol.L<sup>-1</sup> of THOP at pH 3.5, which is below their pI values. Bare gold nanoparticles presented spectral changes consistent with nanoparticle aggregation by changing the pH from 6.0 to 3.5 (purple line). The addition of THOP for a final concentration of 2 nmol.L<sup>-1</sup> promoted significant spectral changes that are compatible with nanoparticle disaggregation (dark cyan line). The samples were prepared, and the spectra obtained as described in Materials and Methods.

Table S1. Zeta potential and pH values.

GNP	pH	Zeta Potential (mV)
Hepes	4–5	$-25.4 \pm 5.48$
Hepes – THOP 2nM	4	$-25.9 \pm 4.97$
Hepes – NEL 2nM	4	$-22.2 \pm 5.18$
BH	6	$-46.9 \pm 2.45$
BH	3–4	$-23.5 \pm 0.72$
BH + THOP 2nM	2–3	$-34.2 \pm 1.85$



**Figure S2.** Characterization of GNPs synthesized using  $\text{NaBH}_4$ . (a) SPR band of GNPs peaking at 510 nm (b) The size distribution by volume from DLS (Dynamic light scattering) of the GNP colloidal suspension that determined a predominant population with a mean diameter of  $5.7 \pm 1.7$  nm and with a PdI 0.171 that is consistent with a monodisperse nanoparticles in size. (c) The Zeta Potential distribution indicating a value of  $-46 \pm 6.06$  mV. Both size and zeta potential are in good agreement with literature data about GNP synthesized using  $\text{NaBH}_4$ .

Figure S2 shows the SPR band of GNPs synthesized using  $\text{NaBH}_4$  that were characterized by absorbance spectroscopy, DLS and zeta potential measurements. The SPR peak at 510 nm is consistent with very small GNPs with a diameter around 6 nm as determined by DLS. Zeta potential of  $-46 \pm 6.06$  mV is also in good agreement with previous literature data [1,2].

## 2. References

1. Wang, W.; Ding, X.; Xu, Q.; Wang, J.; Wang, L.; Lou, X. Zeta-potential data reliability of gold nanoparticle biomolecular conjugates and its application in sensitive quantification of surface absorbed protein. *Colloids Surfaces B Biointerfaces* **2016**.
2. Bhattacharjee, S. DLS and zeta potential - What they are and what they are not? *J. Control. Release* **2016**.