



# DISCRIMINATION IN THE AGGREGATION KINETICS OF POLYELECTROLYTE STABILIZED GOLD NANOPARTICLES INDUCED BY CYSTEINE AND HOMOCYSTEINE

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## INTRODUCTION

Cysteine and homocysteine are naturally occurring amino acids structurally similar (only one methylene difference) and metabolically linked. The development of simple and specific methods for their determination is attractive.

Gold nanoparticles (AuNP) have emerged as important colorimetric materials for their strongly distance-dependent optical properties. The analyte-induced aggregation of AuNP shifts the surface plasmon resonance absorption peak towards longer wavelengths. Based on this feature, methods have been investigated for the detection of thiol-containing amino acids which always lack in specificity [1].

The present study shows that the aggregation kinetics of poly(styrenesulfonate) stabilized AuNP can be differentiated significantly by increasing particle size from 10 to 20 nm, allowing the recognition of one methylene difference between Cys and Hcys. Such a differentiation has been optimized by tuning the chain length of the polyelectrolyte which affects its flexibility and therefore its attitude to wrapping around AuNP [2]. The aim was to create a crowded layer able to maximize colloid stability and to discriminate the permeation of species with minimal difference in steric hindrance.

## Experimental conditions

### Materials:

Standard solutions of AuNP having mean diameters of  $4.3 \pm 0.8$  nm,  $9.9 \pm 0.9$  nm and  $20 \pm 1.8$  nm have been employed. The AuNP supplied were produced by a modified tannic acid/citrate method [3].

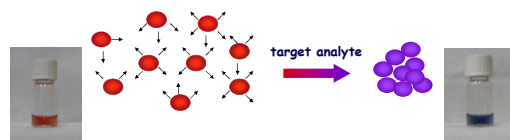
### AuNP aggregation induced by Cys and Hcys:

AuNP aggregation was studied by diluting the standard (1 : 1) with 100 mM phosphate buffer pH 8 and then by acquiring the relevant extinction spectra at different times from the addition of Cys and Hcys at a concentration of  $12.5 \mu\text{M}$ .

### Instrumentation:

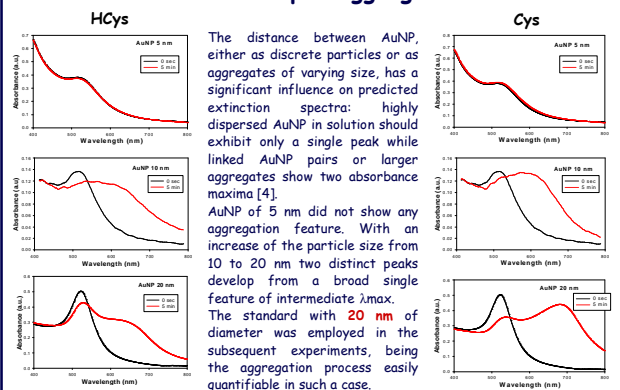
The AuNP extinction spectra were acquired by a CARY 50 UV-Vis spectrophotometer equipped with Cary Win UV software. Conventional quartz cuvette (Optech) with a path length of 1 cm were employed.

## Absorption-based colorimetric assays by using AuNP aggregation



The key to the AuNP-based colorimetric sensing is the control of the colloidal AuNP aggregation stage with the analyte of interest: electrostatic stabilization provided by surface-tethered charged polymers is probably the most effective strategy to stabilize colloidal particles and then to prevent non specific aggregation at high salt concentrations

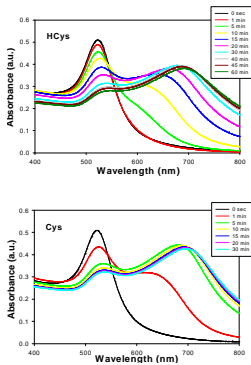
## Influence of AuNP size on spectral features upon aggregation



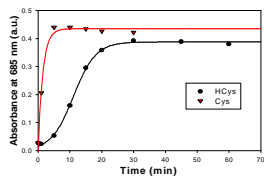
All the AuNP samples were modified by adding PSS (M.W. ~ 70,000) at a concentration of 1% both in the standard and in the buffer before mixing them.

## Optimization of the AuNP modification procedure

The modification procedure, developed upon a careful optimization study, is based on the addition of PSS (M.W. ~ 70,000) at a concentration of 1% to the AuNP standard. The mixture is sonicated for 15 minutes and left to stand another 30 minutes to allow for maximal polyelectrolyte adsorption. PSS is added only to the standard and not to the buffer in order to avoid any polyelectrolyte excess in the reaction solution. The optimization of the modification step allowed to enhance AuNP stability and then to differentiate the aggregation kinetics induced by Cys and Hcys



The kinetics of AuNP aggregation is evidently lowered when induced by Hcys, as it is possible to see by comparing the different evolution of the aggregation peak at 685 nm in the time: after 5 minutes of reaction the absorbance in the case of Hcys is only 12% of the value in the presence of Cys.



Time courses of absorbance at 685 nm of PSS modified AuNP upon the addition of Hcys and Cys

## Influence of PSS molecular weight on the aggregation kinetics

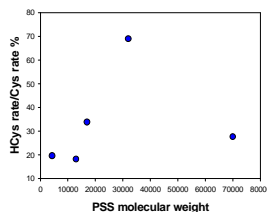
The high surface curvature of NP may restrict polymer adsorption due to limited chain flexibility:

- polymer chain can fully spread on particle's surface with dimensions close to its dimension in a free solution;
- when chain length is increased polyelectrolyte wrap around the particle to optimize the number of contacts;
- by increasing further chain length, excluded electrostatic volume prevents any additional monomer adsorption on the surface via the formation of an extended tail in solution; at least for very high chain lengths flocculation via chain bridging between particles may occur.

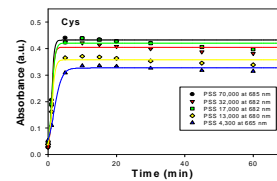
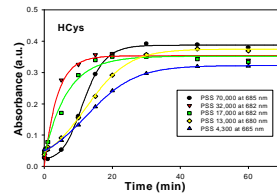
Generally a more efficient wrapping is predicted when the particle size is of the order or greater than the radius of gyration of the polyelectrolyte [5,6].

### Experimental features evidenced by decreasing PSS molecular weight:

- lowering in the kinetics of AuNP aggregation induced by Hcys probably due to a more efficient particles coverage by polymers with shorter chain length; the formation of a compact encapsulation layer favours the permeation of the smallest Cys towards the AuNP surface;
- shift to shorter wavelengths of the aggregation peak induced by both Cys and Hcys imputable to the formation of smallest aggregates for polymers of lower molecular weights;
- PSS with molecular weight of 70,000 showed an anomalous behaviour in the observed trend: the kinetic of aggregation induced by Hcys is lowered with respect to PSS with MW of 32,000 and 17,000.



The ratio between the rate of aggregation induced by Hcys and Cys (derived from the slope of the linear portion of the absorbance vs time plot) outlines the selectivity of the colorimetric evolution at low MW or at very high ones.



Time courses of absorbance at the selected wavelengths of the PSS modified AuNP upon the addition of Hcys and Cys at different PSS molecular weights

## CONCLUSIONS

The colorimetric evolution of AuNP upon aggregation induced by Hcys and Cys is strongly dependent on particle's size: the employment of AuNP with core diameter of 20 nm is associated to the appearance of a well defined aggregation peak with respect to the first peak located near the resonance peak for single particles.

The optimization of the modification procedure, based on the sonication of colloid suspension added with PSS (MW 70,000) at a concentration of 1% and subsequent resting for a certain time, allowed to maximize polymer adsorption and therefore AuNP stability. Due to the enhanced stability of PSS modified AuNP, a differentiation in the kinetics of aggregation induced by Cys and Hcys was observed while the relevant oxidized forms (cystine and homocystine) gave no response (data not shown).

The discrimination between Hcys and Cys in the kinetic of AuNP aggregation can be modulated by varying the molecular weight of PSS: lowering such experimental variable an evident decrease in the kinetic of aggregation induced by Hcys was observed. A similar slowdown was observed at extremely high molecular weights: the notably high dimension of the polymer allows, probably, the formation of a so crowded layer to assure the selective permeation of analytes with minimal difference in steric hindrance.

## References

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