Bovine serum albumin gold nanoclusters as a potential therapeutic platform against Alzheimer disease

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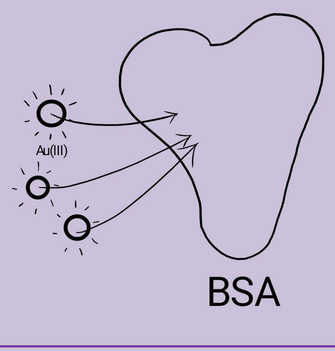
Abstract: Neurodegenerative diseases are the seventh death cause worldwide, being Alzheimer's disease the most common. Serum albumin is the main multifunction protein in the blood stream, taking care of the clearance of waste products from cell metabolism or abnormal machinery. One of such products is A beta peptide, involved in the formation of amyloid fibrils and one of the main hallmarks of Alzheimer's disease. Hence, serum-albumin based therapies may offer an important potential to fight against this disease. Also, small metal nanoparticles with negative surface charge showed an efficient dissembling of preformed amyloid fibrils, so the combination of both components shows a great potential for a possible therapeutic nanoplatform. In the present work we synthesized and characterized small gold nanoclusters embedded in bovine serum albumin by an in-situ synthesis process. Gold metal nanoclusters have unique optical properties due to their small size. They have molecular-like electronic states that produce a large Stokes shift fluorescence phenomenon. Fluorescence Spectroscopy and HRTEM measurements assessed the correct formation of the metal nanoclusters inside the protein. In addition, the colloidal stability of the metal cluster-protein complexes was evaluated under different solution conditions (ionic strength and pH for 7 days). The biocompatibility of the nanoclusters-protein complexes was assessed in vitro in different cell lines by means of the CCK-8 viability assay. Finally, the effect of the nanoclusters in the fibrillation process of serum albumin taken as a model fibrillating protein was evaluated to decipher the therapeutical potential of such complexes for intended Alzheimer's treatment in the future.

Synthesis and storage protocol

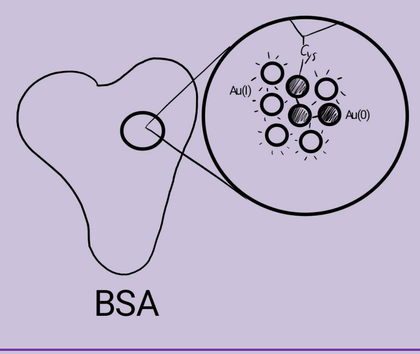
Phase I: Internalization of gold atoms

$5\ ml\ BSA\ 50\ mg/ml + 5\ ml\ HAuCl_4\ 10\ mM$
$37ºC + magnetic\ stirring\ 750\ rpm$
$30\ min$

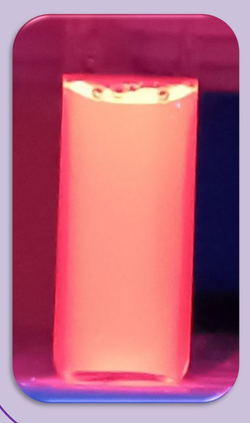


Phase II: Gold atoms reduction

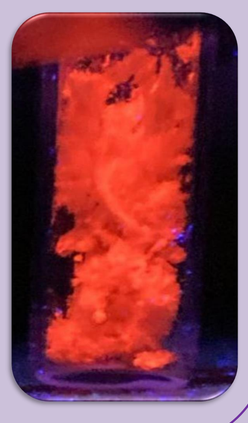
$Phase\ I + 500\ \mu l\ NaOH\ 1M$
$50ºC + magnetic\ stirring\ 1000\ rpm$
$3\ hours$



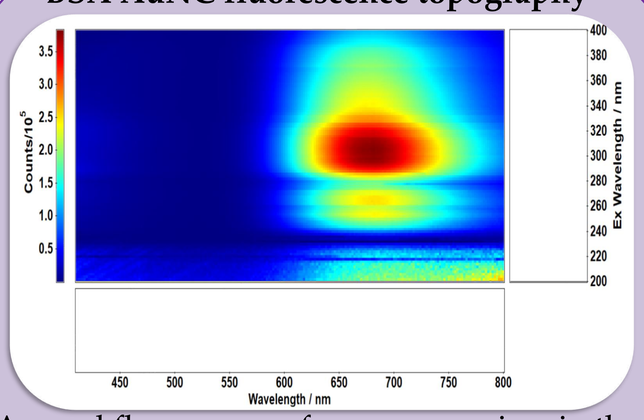
Desalting columns were used to remove the ion excess. Then BSA AuNC were freeze dryed without lossing their optical properties.



Liquid and lyophilized samples show a strong red fluorescence under UV light (λ = 365 nm).

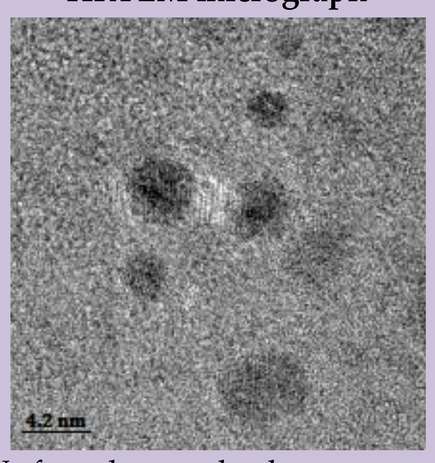


BSA AuNC fluorescence topography



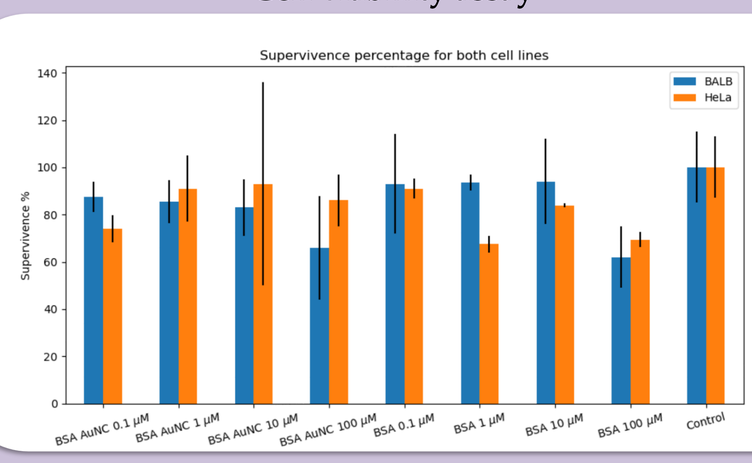
A novel fluorescence fenemenon arises in the protein-metal complex, whit a maximum emission at 670 nm when excited at 310 nm. Red fluorescence is esential in biolabeling to avoid blue/green fluorescence from tissues.

HRTEM micrograph



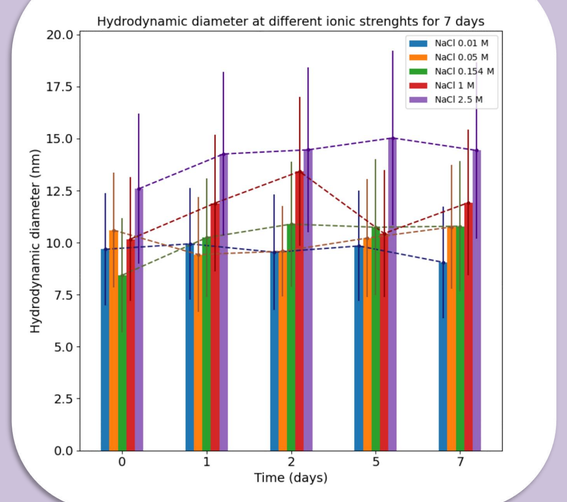
We found several sub-nanometric AuNC (black points) inside the BSA (dark shadows), that ensures the system's proper formation.

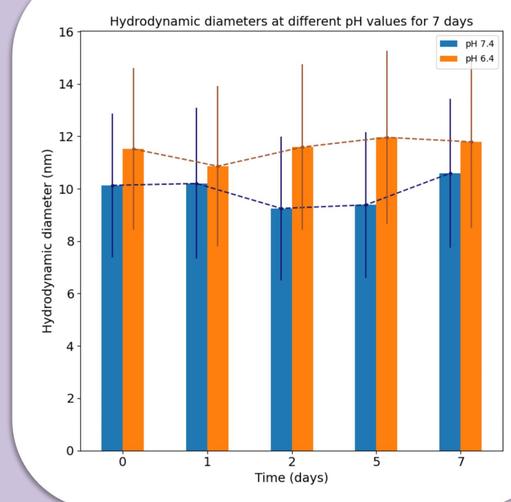
Cell viability assay



CCK-8 Cell Viability Assay of BSA AuNC shows us that AuNC are non toxic at concentrations up to 100 μM in HeLa cell line, and more than 10 μM in Balb cell line, just the same as the BSA on its own.

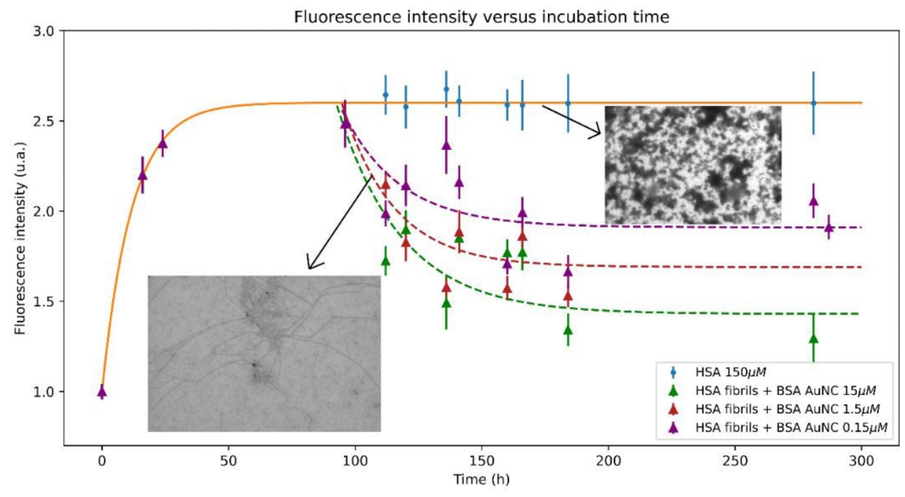
Colloidal stability





Colloidal stability have been analyzed at blood pH and AD patient's brain pH. Also different ionic strenghts were studied. For a week, no changes in the hydrodynamic diameter were found in neither conditions.

HSA amyloid fibrils dissociation



Once BSA AuNC are added to mature amyloid fibrils, ThT fluorescence intensity starts to decay. TEM micrographs shows that this decay is a sympthom from BSA AuNC dissociating amyloid fibrils deposits.