

Interaction between gold nanoparticles and blood proteins

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Materials and methods: *Hanseniaspora guilliermondii* (*H.g.*) were grown from 8 to 48 h in YEPD, centrifuged and washed (ethanol 3% or 6%) prior to incubation with natural the *S.c.* AMPs, at 25 °C for 1 or 2 h. Surface of *H.g.* cells were observed by Atomic Force Microscopy (AFM).

Results: AFM images of *H.g.* cells before and after (Figure 1) exposure to the AMPs show a significant changes on their surface.

Conclusions and discussion: Analysis of the cell's roughness, reveals that untreated cells are smooth, unlike the treated with AMPs. Cells surface roughness increased upon AMPs contact by about 50% from 40.31(±12.87) nm to 58.01(±13.97) nm. Surface morphological details also indicates a destructive effect of saccharomycin on the *H.g.* cell wall.

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Interaction between gold nanoparticles and blood proteins

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ABSTRACT

Introduction: Metallic nanoparticles constitute promising biosensing systems, due to their high affinity to biomolecules such as proteins, which form protein coronas of distinct compositions on their surface [1]. Gold nanoparticles (AuNP) are particularly interesting given their relatively easy, quick and inexpensive synthesis, low toxicity and ease of functionalization with bifunctional molecules. Usually, these molecules have thiol groups bound to the AuNP surface and bio-friendly chemical groups at the opposite end, allowing for controlled protein adsorption. Such functionalised AuNPs may be used as probing agents for a patient's droplet of blood and the health state can be based on the composition of AuNP-adsorbed protein corona. It is thus important to understand the behaviour of each plasma protein in the corona, divided into a tightly-bound inmost monolayer (*hard corona*) and looser outer layers (*soft corona*), removable through centrifugation [2].

Materials and methods: AuNP synthesis was performed according to a modified Turkevich method and AuNP diameter and concentration was determined by UV-Vis spectroscopy. AuNPs were functionalised with 11-mercaptoundecanoic acid and further conjugated with bovine or human serum albumin or fibrinogen. These single protein conjugates were evaluated for hydrodynamic diameter changes after centrifugation by dynamic light scattering (DLS). Agarose gel electrophoresis (AGE) allowed to determine electrophoretic mobility and concentration-dependent conjugation efficiency. Analysis of AGE profiles was by the open source electrophoresis gel image processing software eReus.

Results: DLS showed a decrease in hydrodynamic diameter for centrifuged conjugates of 40 nm gold nanoparticles, with too high polydispersity indexes for the 13 nm ones, suggesting aggregation. AGE revealed a decrease in electrophoretic

mobility as the protein-to-AuNP ratio increases, data fitted to a Langmuir adsorption model. Protein and AuNP concentrations during incubation affect the electrophoretic mobility profile. In fact, depending on the protein-to-AuNP ratio, migration is proportional to the colloidal suspension volume in which conjugation occurred.

Discussion and conclusions: Although centrifugation can induce AuNP aggregation, it appears to affect the protein corona. A decrease in hydrodynamic diameter as determined by DLS appears in centrifuged samples comparatively to its uncentrifuged counterparts, which corroborates the *soft corona* being more loosely bound. Moreover, AGE suggests that for equal protein:AuNP ratios, the volume of sample is determinant for the conjugation process.

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Survey of biogenic amines (histamine and spermidine) in commercial seafood by enzyme linked immunosorbent assay (ELISA)

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ABSTRACT

Introduction: Worldwide there is serious concern about food and consumer safety [1], namely with seafood products. Consequently, there is a major concern regarding food spoilage which make them unsuitable for human consumption. When deteriorating, seafood products suffers a complex series of events that begins when the organism die [1,2]. Therefore, there is a strong need for developing reliable seafood quality analysis. In the present study we surveyed histamine and spermidine in several seafood products (fresh fish and clams), purchased in a Portuguese traditional market.

Materials and methods: Fresh seafood (*Sardina pilchardus*, *Trachurus trachurus*, *Sparus aurata* and *Ruditapes decussata*) were purchased in a market and taken to the laboratory in refrigerated containers. A total of 10 specimens were sampled from each species. Then samples were processed for analysis by homogenising in a phosphate buffer saline solution, centrifuged (10,000×g at 4 °C) for 15 min) and then stored at –80 °C until analysis. Seafood samples were assessed for the presence and content of histamine and spermidine using an indirect Enzyme Linked Immunosorbent assay (ELISA) [3]. The statistical analysis was performed using the Mann–Whitney *U*-test to determine differences between biogenic amine levels in seafood samples. Statistics was performed with a significance level of 5%, using the software Statistica 8.0 (Statsoft, Tulsa, OK, USA).

Results: The results show variable results between species (from < LD to 184104 mg histamine/Kg wet weight). The highest levels were detected in *T. trachurus* samples and the lowest in clams. However, it was possible to detect the presence of the selected biogenic amines (histamine and spermidine) in most samples analysed. The lowest levels of spermidine were determined in *R. decussata* (1504.43 mg/kg w.w.), while the highest levels were determined in *T. trachurus* (184104 mg/Kg w.w.). Regarding histamine, the lowest levels were determined in *R. decussata* (20.23 mg/Kg w.w.) and the highest levels were measured in *T. trachurus* (460.25 mg/kg w.w.).

Discussion and conclusions: Although we are capable to detect the presence of the selected biogenic amines, in most of the samples the levels were below the limits established by Food and Drug Administration [4] and the European Union Commission Regulation (EC) No 1441/2007 [5].

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