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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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Interleukin gene cloning and expression in *E. coli*

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ABSTRACT

Introduction: Cytokines are a large category of protins; they play various functions from inflammation response to cancer development and sepsis. Over-expression or problems in production control of this small peptides can lead to the development of various diseases, including autoimmune diseases, such as rheumatoid arthritis. Inhibition of some of these proteins can result in a therapy that can ease certain symptoms of cases of immunologic diseases.
Material and Methods: This work consists in cloning and expressing six truncated proteins of interleukins: IL-1α, IL-1β, IL-17A, MIF, TNFSF11 and CD20. These synthetic genes were synthesised with the *E. coli* codon usage and inserted in pNZY29 cloning vector (Nzytech). These genes were isolated by amplification by PCR method with specific primers, gel purified and cloned in the pLATE31 (Thermo) expression vector. The purified expression vectors were used to transform the following *E. coli* expression strains: BL21 (DE3), BL21-Gold (DE3), BL21-CondonPlus RIPL (DE3), BL21- Gold (DE3) pLysS, BL21 Star (DE3), BL21 SHuffle, BL21 SHuffle LysY and BL21 XJB (DE3) and expressed and detected according to a previous work [1].
Results: The cloning and expression system used was a directional cloning of PCR-generated fragments and a DNA ligase free method, with this system was achieved a high cloning efficiency (80–90%). The polyacrylamide gel allowed the detection of the strains with higher levels of expression of the recombinant proteins. In this study the level of protein expression was different in each *E. coli* strain tested and was dependent on the protein expressed.
Discussion and Conclusions: We succeeded to produce IL1-α IL1-β IL17A, MIF, TNFSF-11 and CD20 recombinant proteins in different *E. coli* strains but the expression is strain dependent according to the protein. These recombinant proteins are capable of functioning as antigens to produce monoclonal and recombinant antibodies and recombinant peptide ligands. Next steps in the research include culture conditions optimisation to increase recombinant protein yield and the selection and production of recombinant peptide ligands from phage libraries and it is used in an *in vitro* inhibitory and ELISA assay.

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