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**PROTEIN CO-ASSEMBLY FOR THE DELIVERY OF BIOACTIVES**

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**Introduction**

Protection and target delivery of food bioactives are main concerns when the manufacture of food products with health-benefit assets is involved. Natural delivery systems for bioactives for instance casein micelle for calcium phosphate are only encountered in some food products and allow the protection and control release of a limited number of bioactives. For this reason, the design of new devices that allow the encapsulation of any types of bioactives is required. Since a large majority of food products contains proteins in consistent amount, proteins constitute privileged biopolymers for the design of delivery systems for improving food nutritional properties without changing its composition and organoleptic properties (Tavares et al., 2014). However, the way the encapsulation devices is generated and structured is essential in order to optimize the delivery system properties. This constitutes our research concern on the reversible assembly of oppositely charged globular proteins in well-defined microspheres which are putative new delivery systems.

**Materials & methods**

A positively charged protein (Lysozyme, Lactoferrin or avidin) and a negatively charged protein (ovalbumin, bovine serum albumin,  $\alpha$ -lactalbumin or  $\beta$ -lactoglobulin) were mixed together in appropriate concentration, pH and ionic strength conditions. The mechanism of protein co-assembly was investigated from molecular to microscopic scale by a combination of biophysical methods including isothermal titration calorimetry, fluorescence anisotropy, NMR, static and dynamic light scattering, electronic and confocal microscopies, and molecular simulation.

**Results & discussion**

Globular proteins of opposite charge co-assembled when the physicochemical conditions (pH and ionic strength) of the medium were defined adequately. By changing protein concentration it was possible to study the mechanism of co-assembly at various scales. The building block for microsphere formation was an oligomer constituted by a definite number of proteins as shown by isothermal titration calorimetry, fluorescence anisotropy and  $\tau_2$  relaxation time measurements. NMR chemical shift perturbation measurement and protein-protein docking simulation indicated that the oligomer was stabilized by electrostatic interactions involving specific amino acids in the binding sites. At larger scale, the building blocks associated into aggregates following a diffusion limited aggregation mechanism and the aggregates further reorganized into microspheres. For lysozyme- $\alpha$ -lactalbumin suprastructures, the reorganization step was quenched by decreasing temperature suggesting the involvement of hydrophobic contribution. The building blocks moved freely inside the microspheres, where the molecular structure of the proteins was not significantly different from the native one. The microspheres were reversible into protein monomers by increasing ionic strength or adjusting the pH away from the pI of one of the involved proteins.

**Literature cited**

Tavares GM, Croguennec T, Carvalho AF, Bouhallab S (2014) Milk proteins as encapsulation devices and delivery vehicles: applications and trends. Trends in food science and technology, 37: 5-20.