Molecular characterization of tunable microscale protein-based biomaterials

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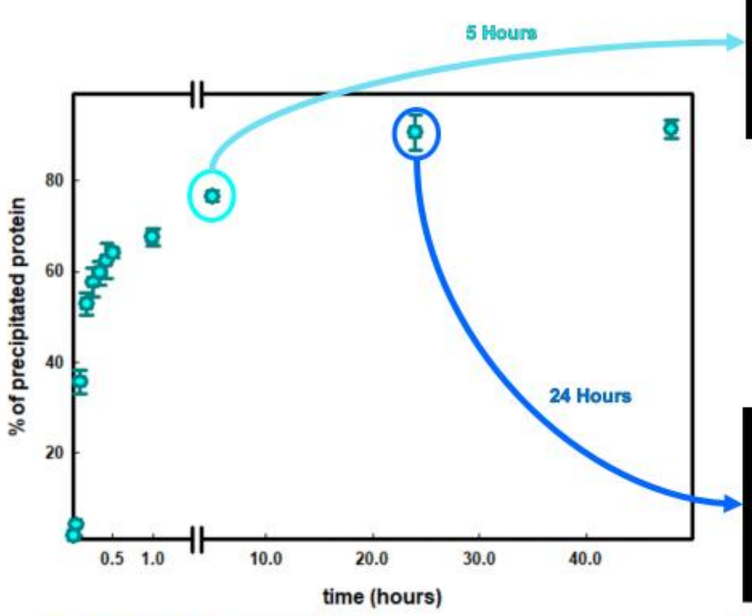
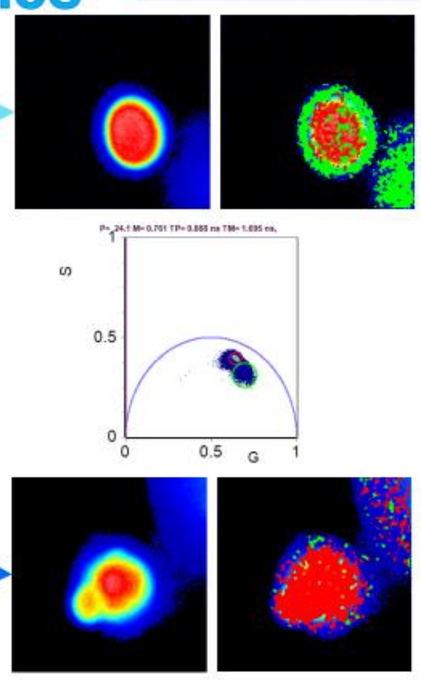
Protein aggregates have been related to many diseases, but recently, there has been a grown interest on using them as biomaterials due to their organic composition and their stability. Specific attention was focused from literature to different types of amyloid aggregates: ordered structures stabilised by a regular pattern of H-bonds. Among all, protein particulates are amyloid superstructures that are formed at a pH near the isoelectric point of the protein they are made of. They have a perfect spherical shape and a size ranging between hundreds of nanometres to a few micrometres, and up to now they weren't related to any disease.

We present an experimental study showing that maturation controls the molecular properties of alphalactalbumin (ALA) particulates. The kinetics of ALA particulates formation is studied at high temperature; at different time points, after massive aggregation is occurred, we select different key points representative of different maturation stages that were characterised with a combination of spectroscopy and microscopy methods. A global view of experimental data clearly proves the possibility of growing biocompatible spherical micro-sized aggregates with different surface properties, stability and size, capable of uptaking from small molecules to proteins, using the same material without any chemical modification but simply by varying the incubation time and the concentration of protein prior incubation.

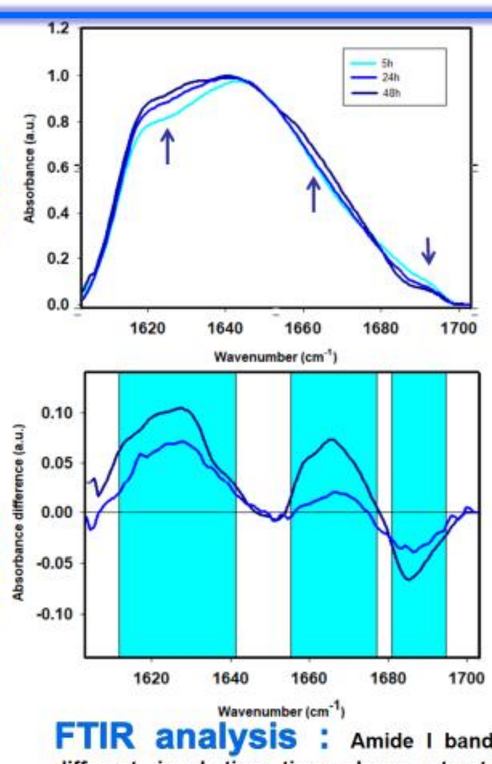
Maturation of microparticles



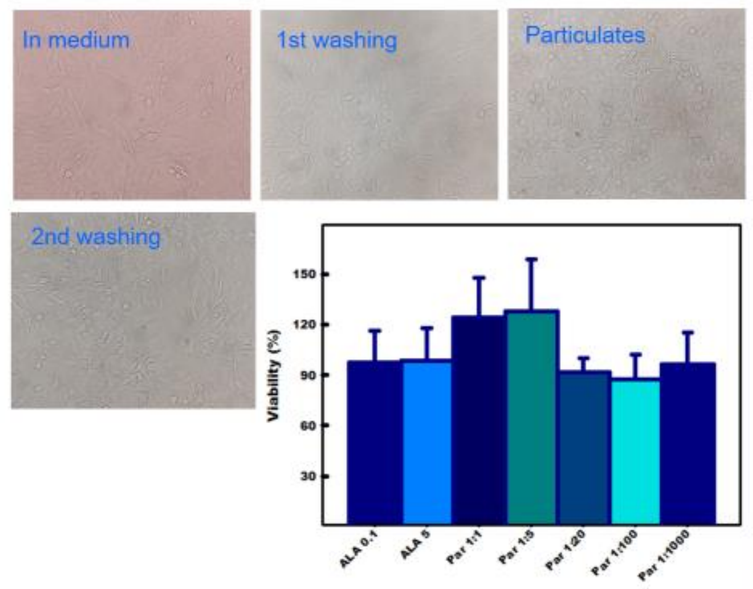
Particulate kinetics: Time evolution of ALA (5mg/ml, pH 5) aggregation at 60° by measuring the concentration of the supernatant after centrifugation.



ThT lifetime: Phasor plot along with fluorescence intensity images and localization images showing lifetimes in different regions of particulates.

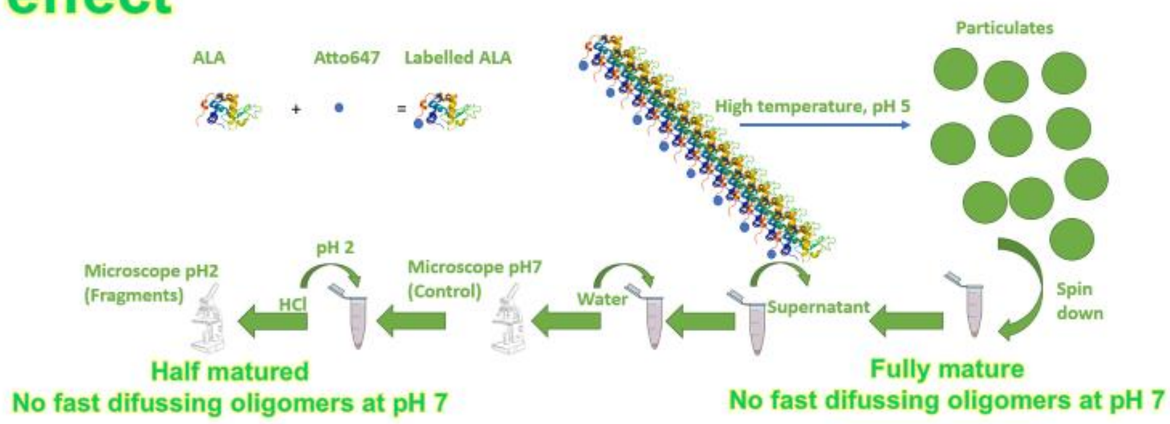


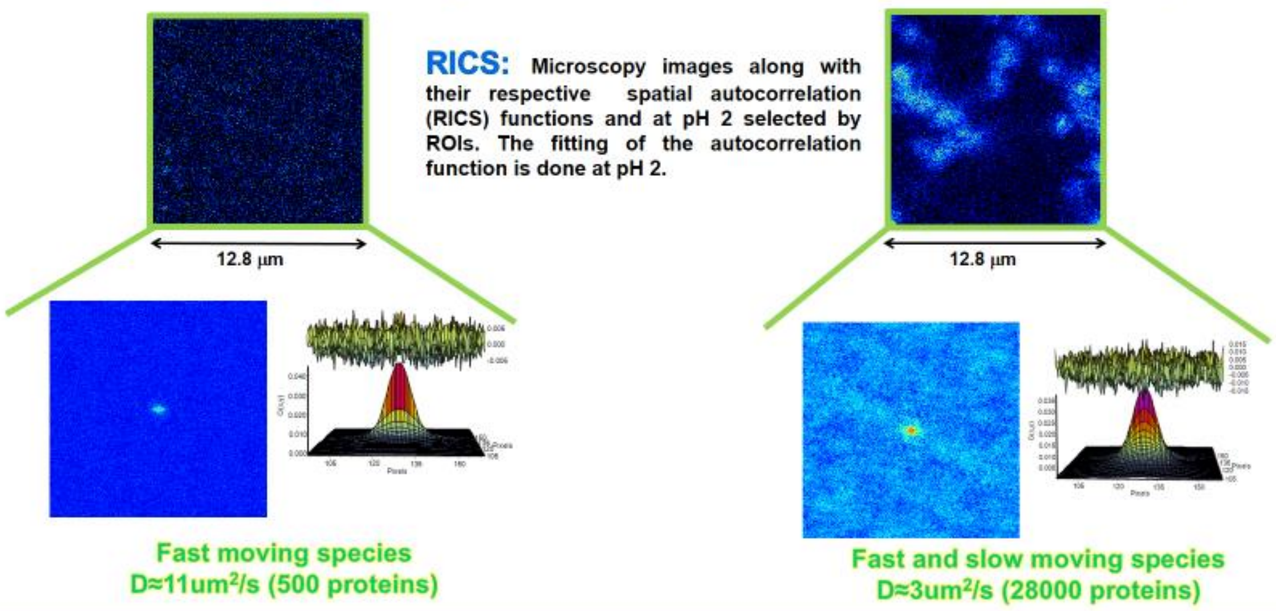
FTIR analysis : Amide I band at different incubation time shows structural differences. Parallel β-sheet content and turns increase with incubation time while antiparallel β-sheet content decreases.



Cell viability: Cell viability in presence of different concentrations of fully matured particulates. Decrease on cell viability or a difference in shape is not appreciated after the addition of particulates.

pH effect





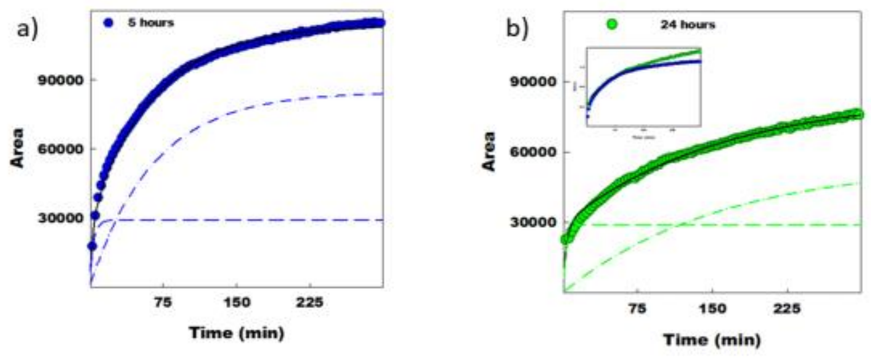
RICS: Microscopy images along with their respective spatial autocorrelation (RICS) functions and at pH 2 selected by ROIs. The fitting of the autocorrelation function is done at pH 2.

Fast moving species
D≈11um²/s (500 proteins)

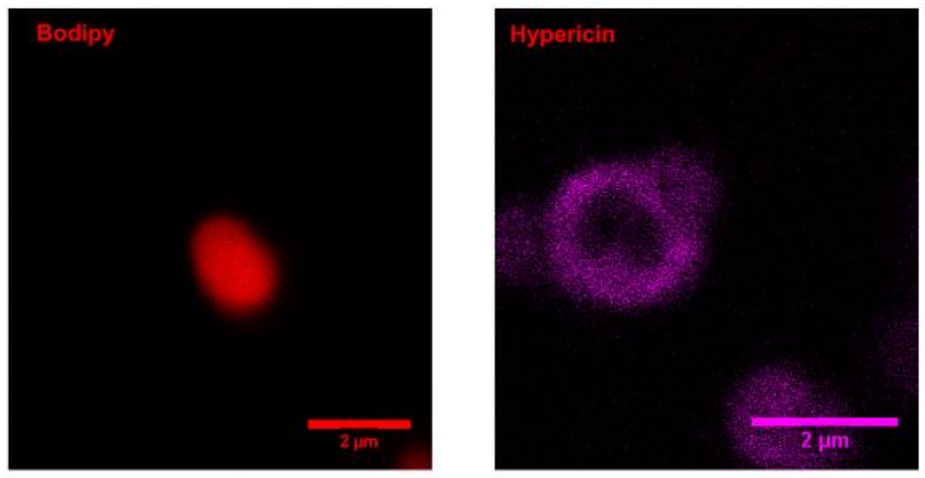
Fast and slow moving species
D≈3um²/s (28000 proteins)

Uptake of molecules

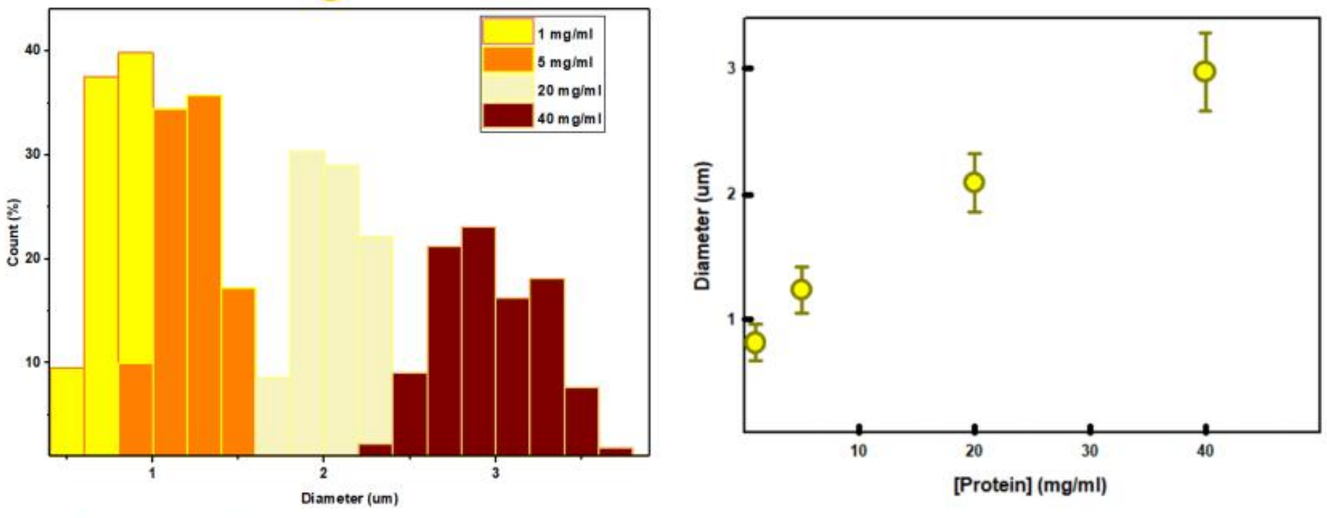
$$y = A\left(1 - e^{(-BX)}\right) + C\left(1 - e^{(-DX)}\right)$$



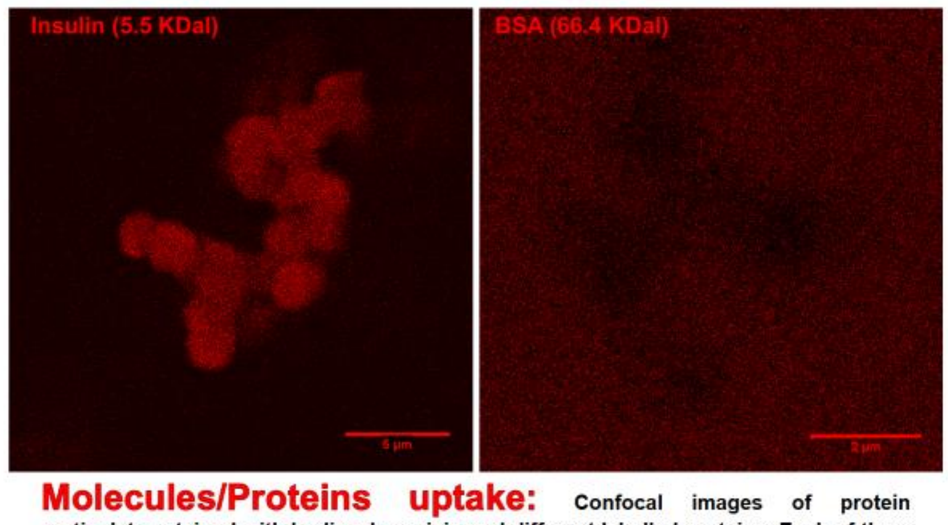
Bodipy uptake: Kinetics of Bodipy upload into protein microparticles. The model shows two components, a fast component identical for both samples and a slow component which is dependent on the maturation.



Controlling the size



Control of the size: Protein particulates were incubated for 5 hours at different concentrations of ALA (1, 5, 20 and 40 mg/ml, pH 5) in acetic acid buffer 20mM. The diameter distributions were extrapolated by analysis of the microscopy images.



Molecules/Proteins uptake: Confocal images of protein particulates stained with bodipy, hypericin and different labelled proteins. Each of them shows a different behaviour.

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