Direct formation of highly tunable and biocompatible protein microparticles.

Dirk Fennema Galparsoro[1,2], Mai Bay Stie[2], Hanne Mørck Nielsen[2], Valeria Vetri[1], Vito Foderà[2]

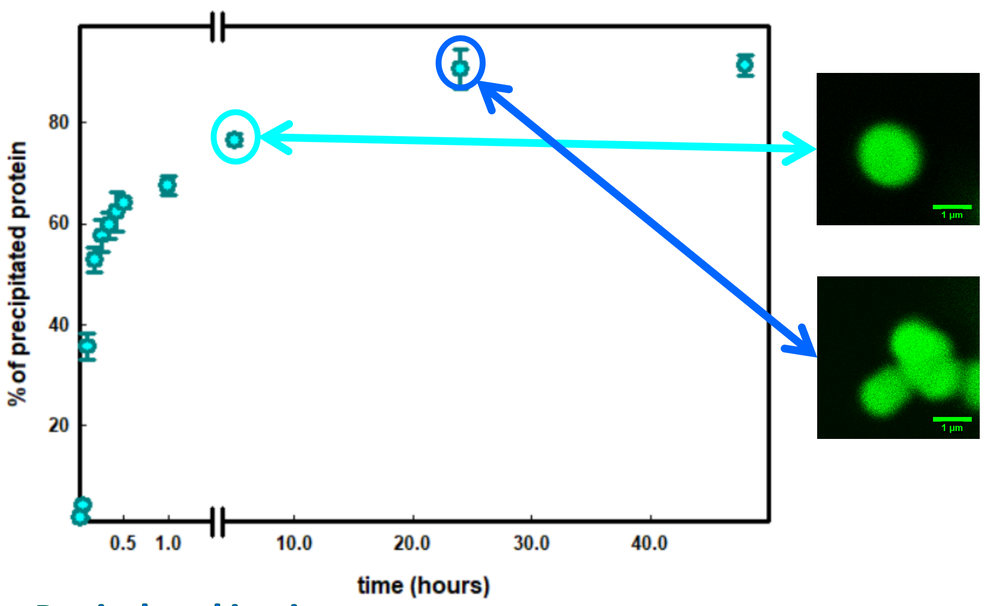
[1]Dipartimento di Fisica e Chimica, Università degli Studi di Palermo, Palermo, Italy.

[2]Department of Pharmacy, University of Copenhagen, Copenhagen, Denmark

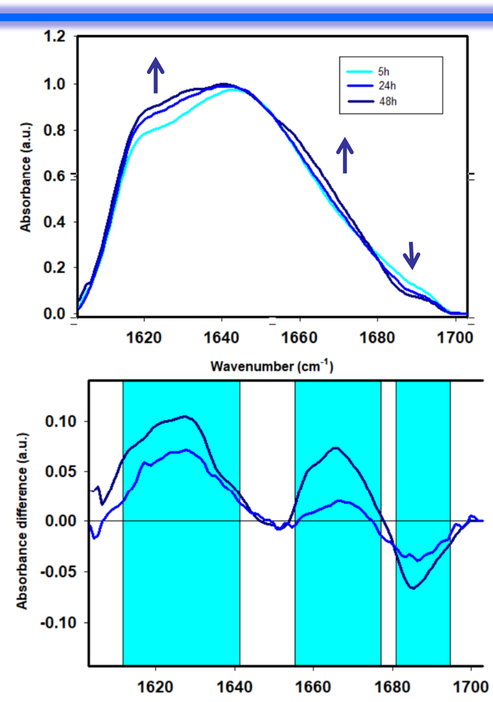
Protein aggregates have been related to many diseases, but recently, there has been a grown interest on using them as biomaterials due to their organic composition and their stability. Specific attention was focused from literature to different types of amyloid aggregates. Among all, protein particulates 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, once formed, alpha-lactalbumin (ALA) microparticles, named particulates, may undergo a maturation process. Fully mature and half-mature particles show differences in terms of the secondary structure composition, capability to incorporate hydrophobic molecules and stability against different stress conditions, all this being related to fundamental forces (intra and inter-protein interactions e.g. electrostatic, hydrophobic, H-bonds). Fully mature particles are not toxic to cells. Finally, the size of the microparticles can be controlled by changing the protein concentration in the initial formulation. The above facts promote ALA particulates as potential biocompatible and sustainable biomaterials for delivery of small molecules.

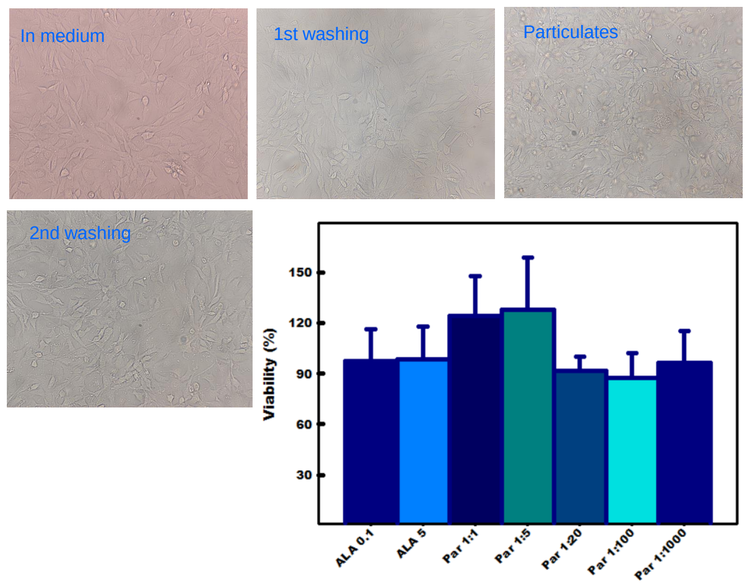
Maturation of microparticles



Particulate kinetics: Time evolution of ALA (5mg/ml, pH 5) formation in acetate buffer coupled with confocal images of different stages of protein particulate formation stained with Thioflavin T (ThT).

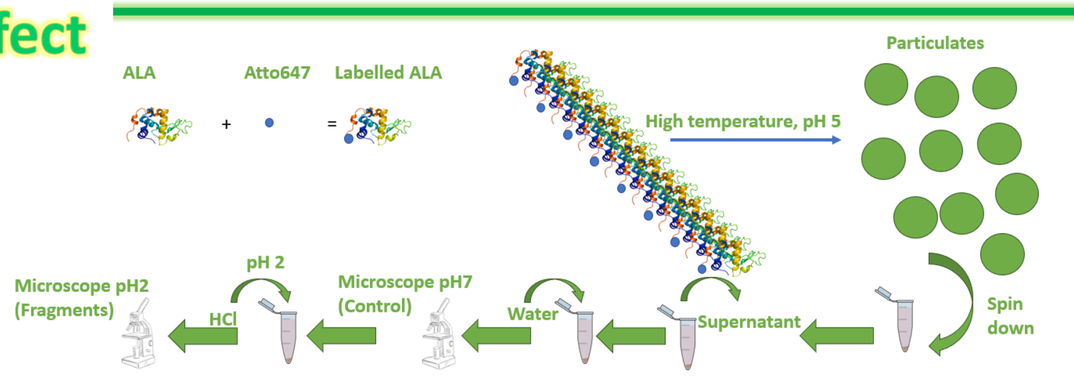


FTIR analysis: Amide I band at different incubation time shows structural differences. Parallel β-sheet content and turns increase with incubation time while antiparallel β-sheet content decreases.



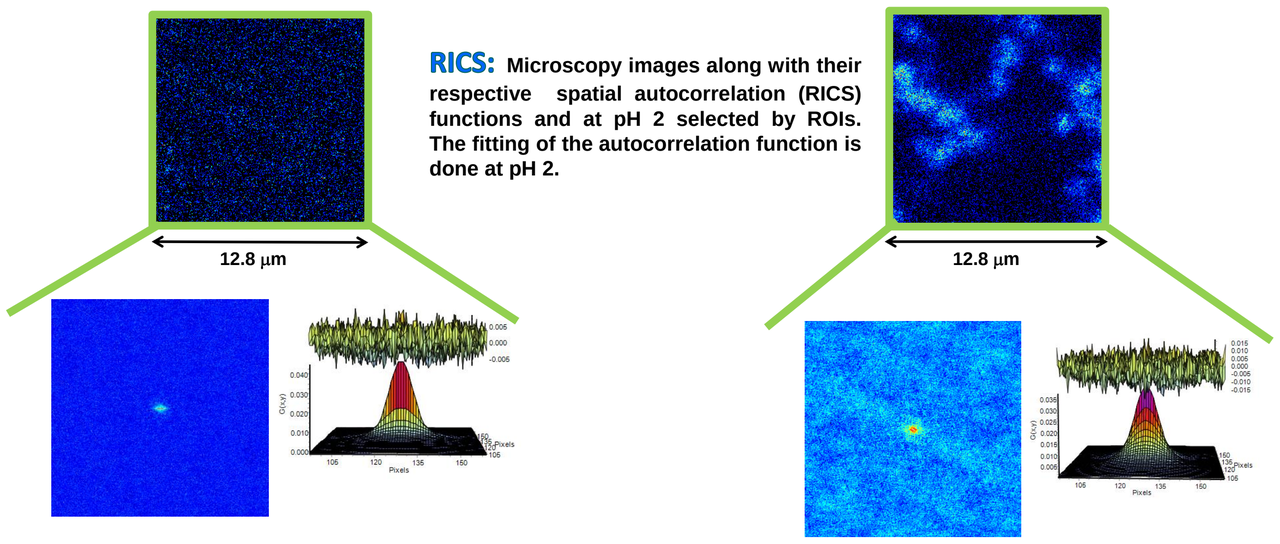
Cell viability: Cell viability in presence of different concentrations of fully matured particulates. Decrease on cell viability or a difference in shape is not appreciated after the addition of particulates.

pH effect



Half matured
No fast difussing oligomers at pH 7

Fully mature
No fast difussing oligomers at pH 7



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.

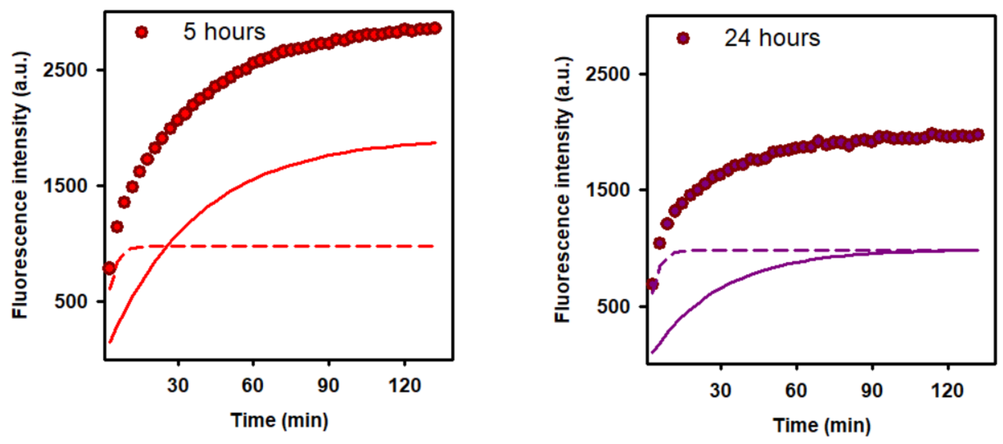
Fast moving species
D≈11um2/s (500 proteins)

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

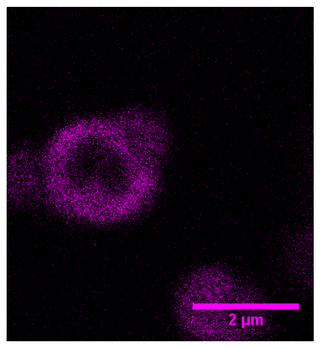
Uptake of hydrophobic fluorophores

Slow component: 36 minutes
Fast component: 10 minutes

Slow component : 28 minutes
Fast component: 10 minutes

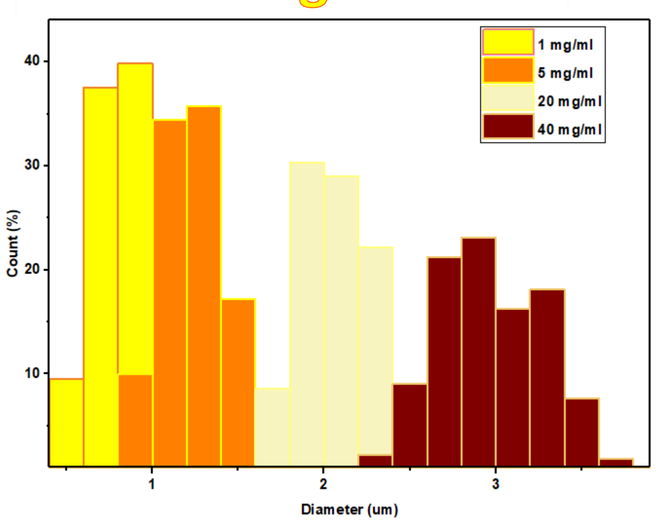


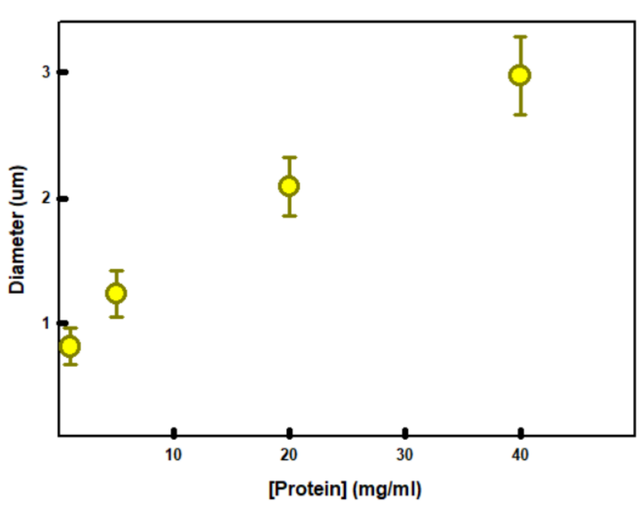
Bodipy uptake: Kinetics of Bodipy upload into protein microparticles.The model shows two components, a fast component identical for both and a slow component different for both samples.

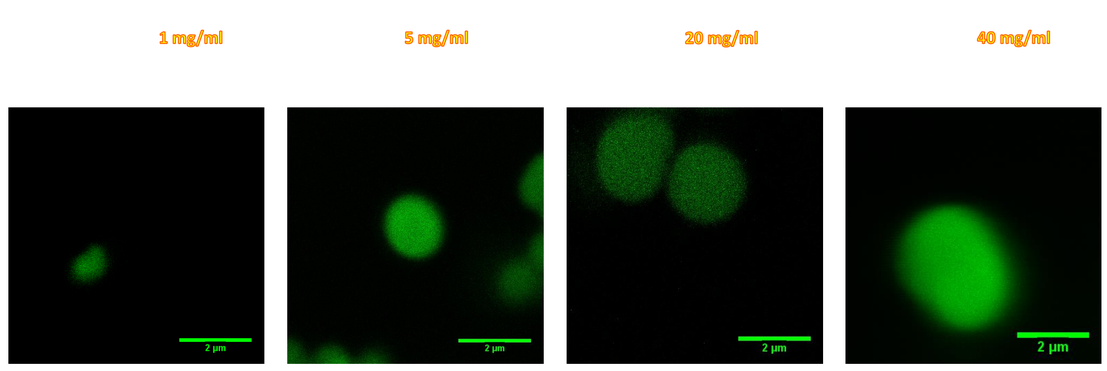


Hypericin uptake: Image of Hypericin stained protein particulates incubated for 24 hours. The staining is slow forming rings in the Surface.

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 and from microscopy measurements staining them with ThT their diameter distribution was calculated and its statistical analysis is developed.

UNIVERSITY OF COPENHAGEN

VILLUM FONDEN

UNIVERSITÀ DEGLI STUDI DI PALERMO