UNIVERSITY OF COPENHAGEN

Highly tunable protein microspheres for drug delivery

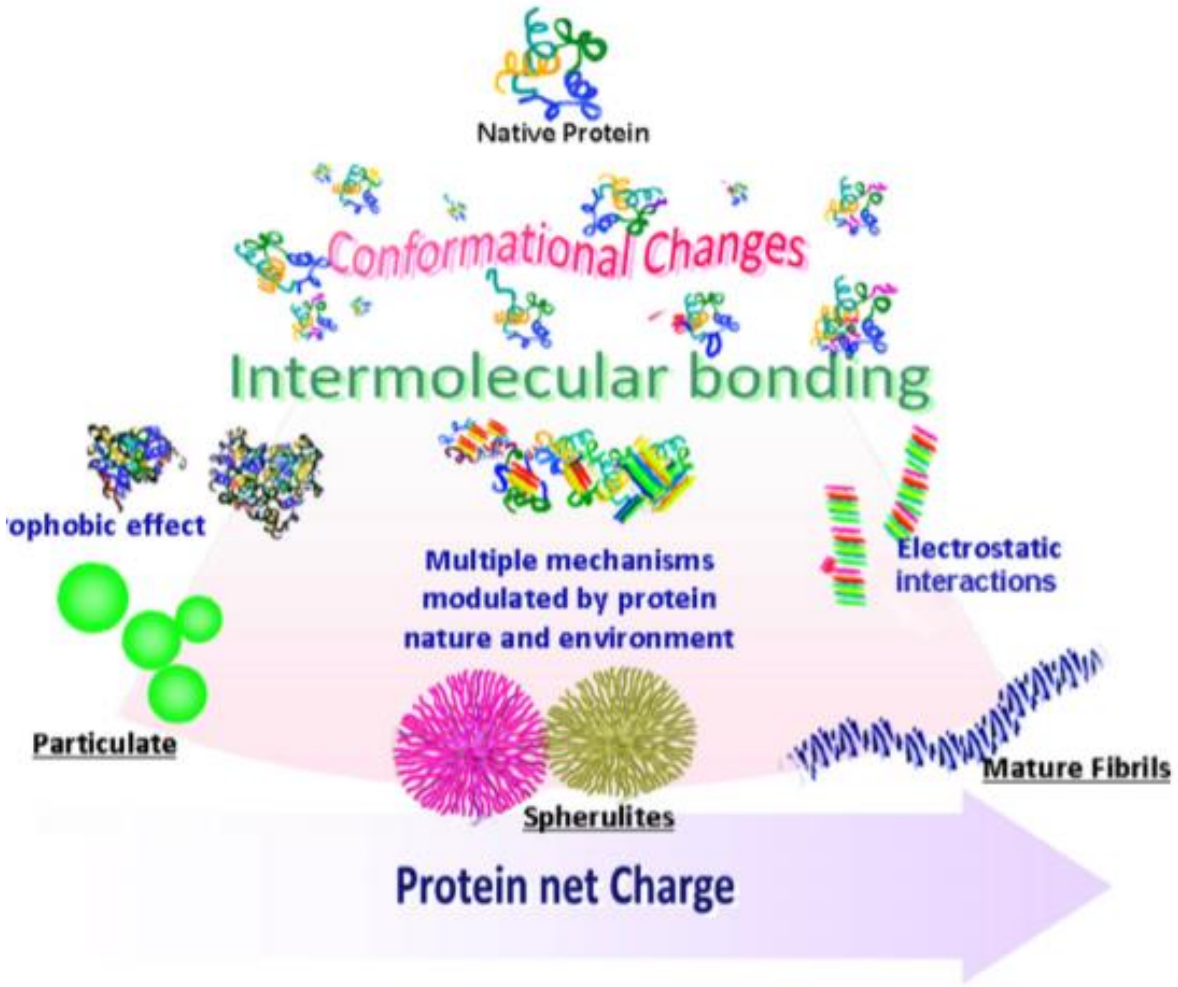
Dirk Fennema Galparsoro

Università degli studi di Palermo

Department of Physics and Chemistry-Emilio Segrè

UNIVERSITÀ DEGLI STUDI DI PALERMO

Protein Aggregation



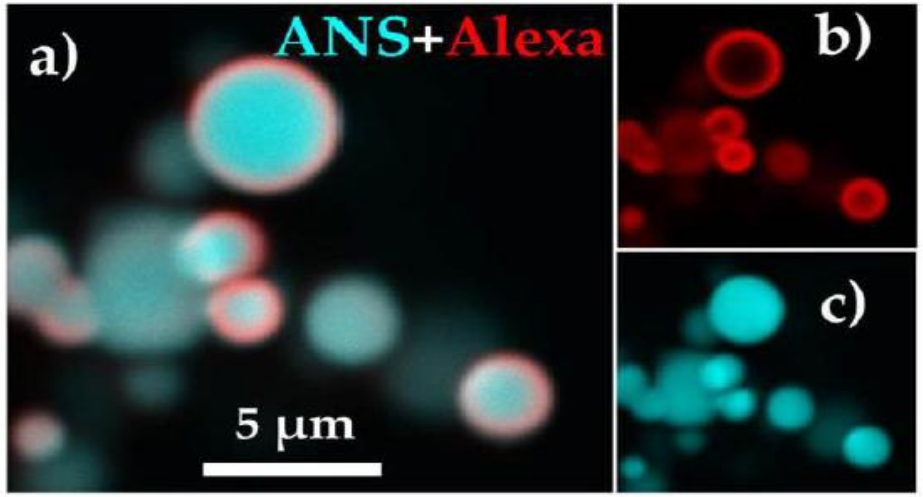
- Different protein aggregates
  - Generic state of proteins
  - Susceptible to the environment
- Understand the mechanism(PPI)
- Tunable properties

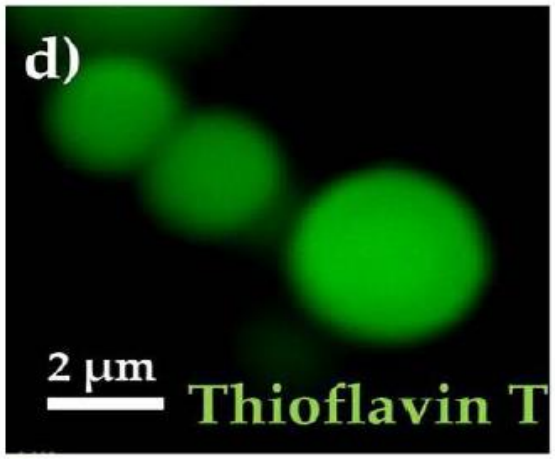
Vetri V., Foderà V. FEBS Letters 2015

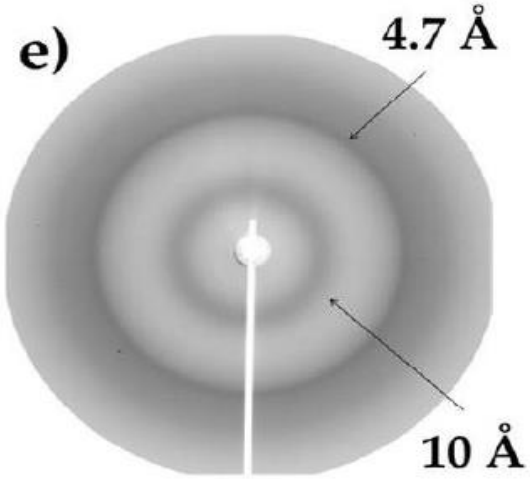
Dobson, C. M. Trends Biochem. 1999

Dobson C.M. Nature 2003

Protein particulates





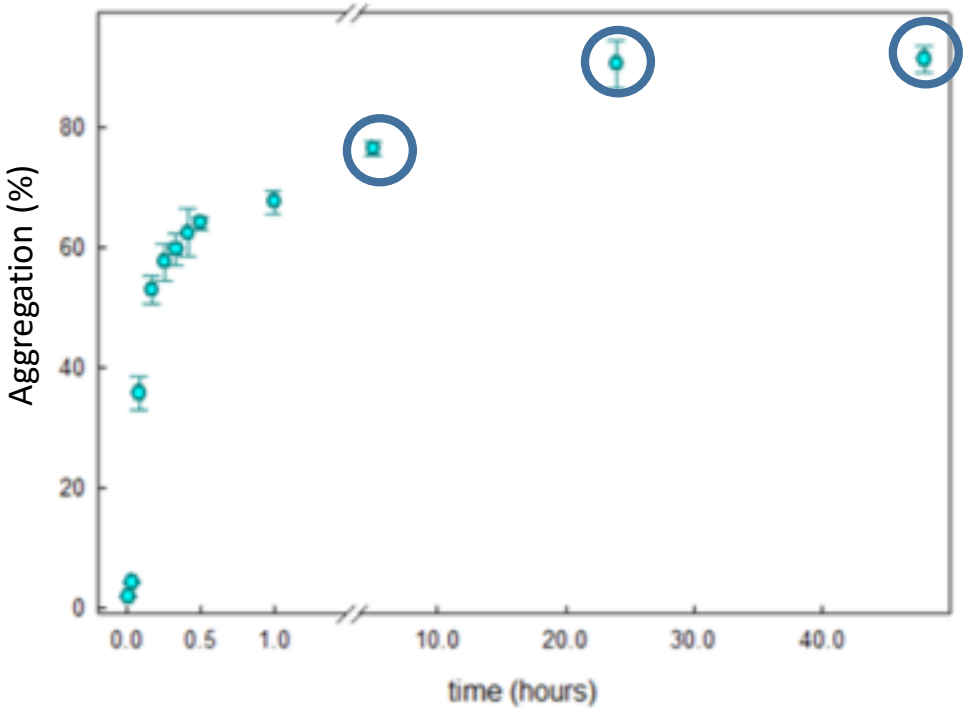


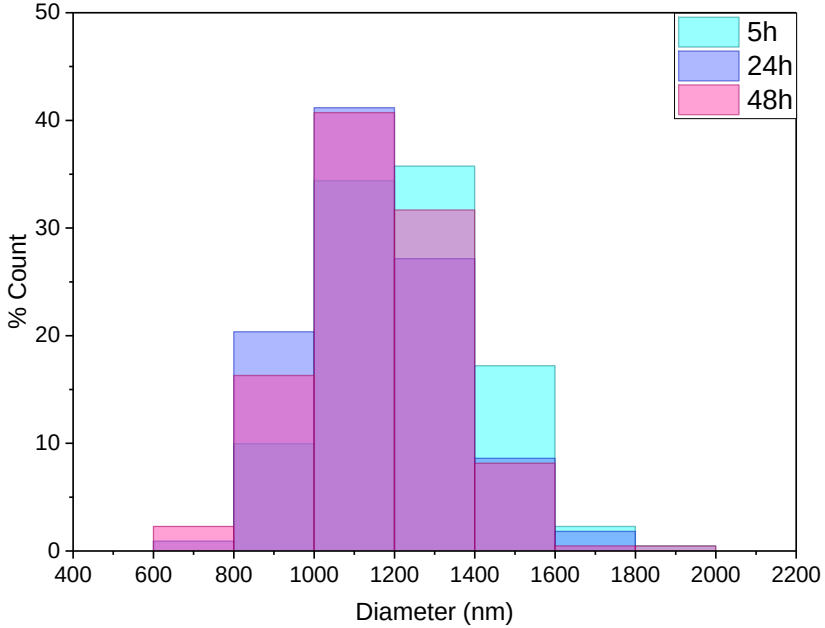
- Generic state of globular proteins
- Spherical
- pH≈pI of the protein and high T
- Hydrophobic properties
- They are stained by Thioflavin T
- Beta sheets spherical symmetry

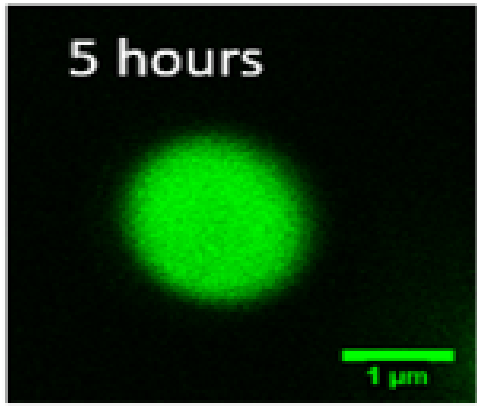
Vetri V., Foderà V. FEBS Letters 2015

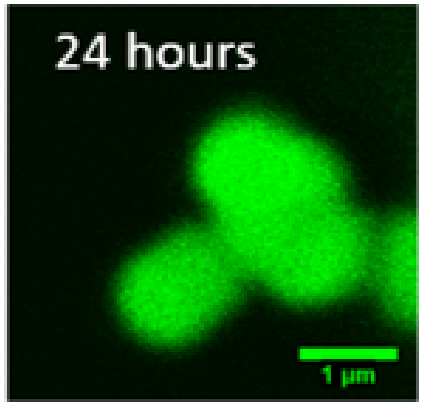
Krebs M.R.H. Biophysical Journal 2007

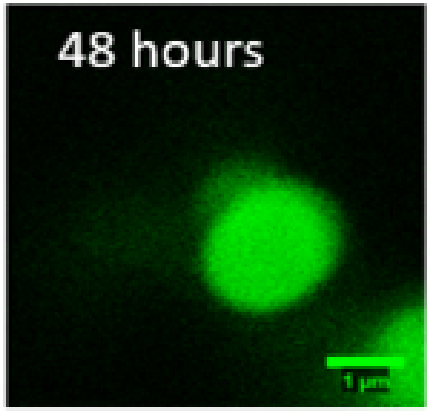
Maturation of ALA protein particulates



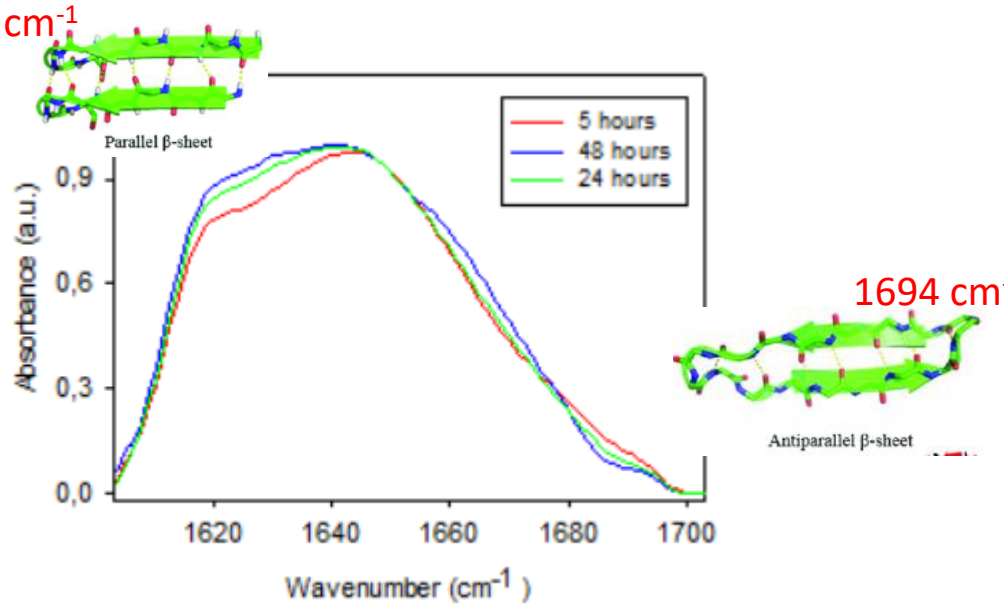






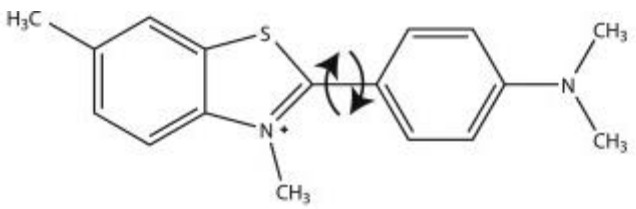


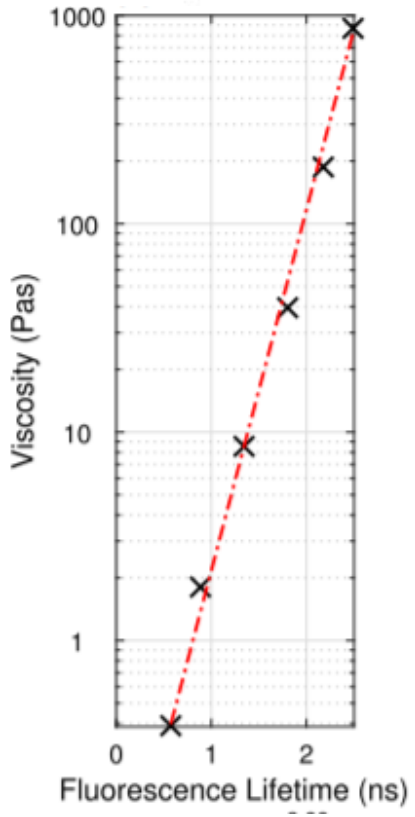
1620 cm^{-1}



1694 cm^{-1}

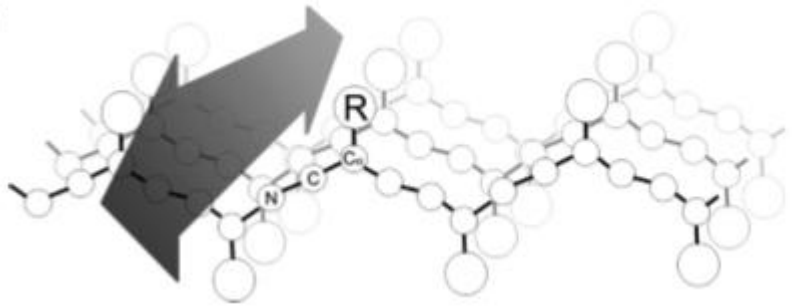
Thioflavin T fluorescence lifetime studies





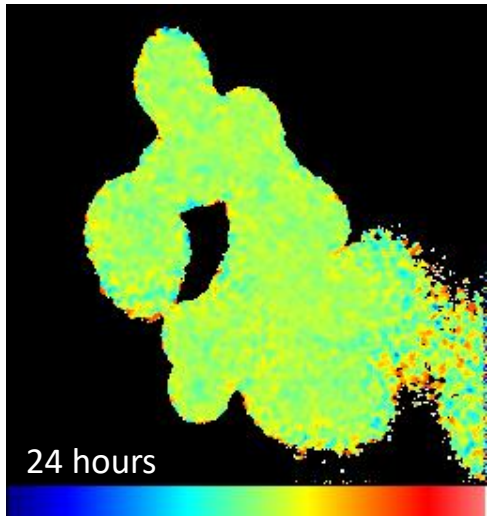
Dench J. Tribol Lett 2017

- **Specific fluorophore** to amyloid structures
- Binds parallel to the long axis of the fibril
- Molecular rotor



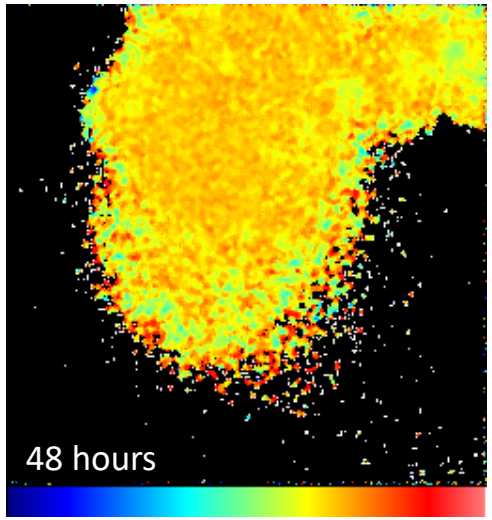
Krebs M.R.H. Journal of Structural Biology 2005

FLIM on aged paticulates stained with ThT



100% t=0.6ns

Ratio of lifetimes



100% t=0.6ns

Ratio of lifetimes

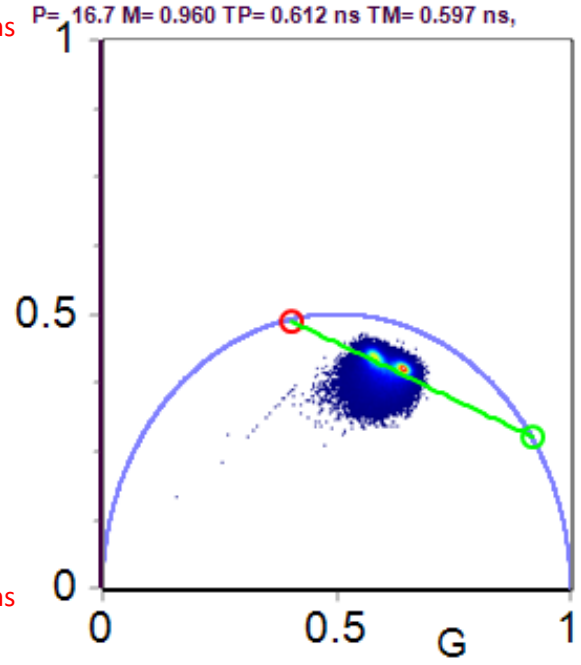
Analysis by means Phasor approach (model using 2 components)

- Assignation:

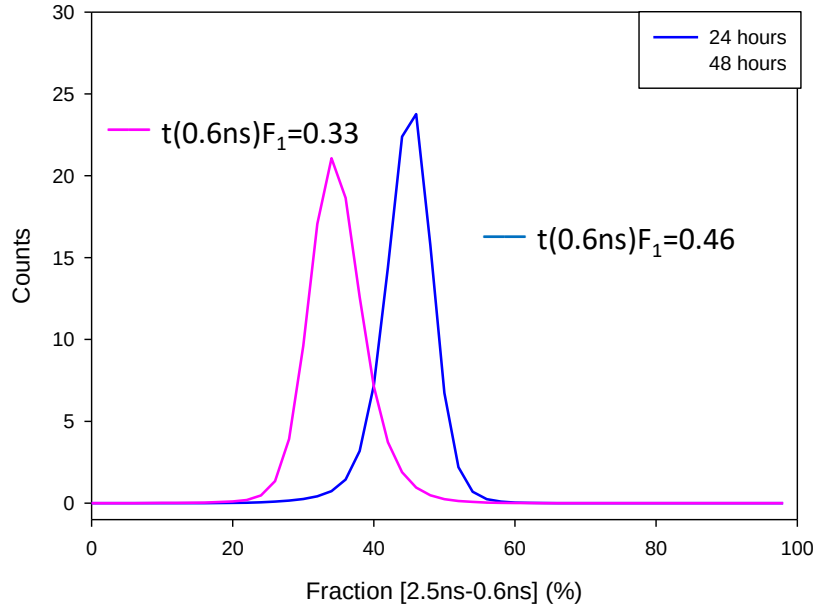
  1- Steric impediments for rotation due to β–aggregates (2.5ns)

  2- More density/ Viscosity (0.6 ns)

100% t=2.5ns



100% t=2.5ns

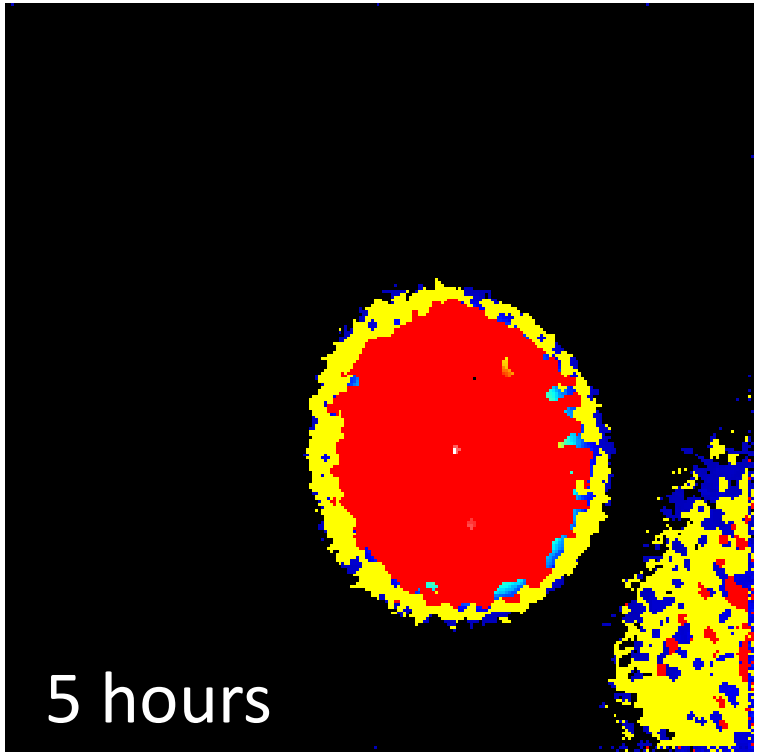


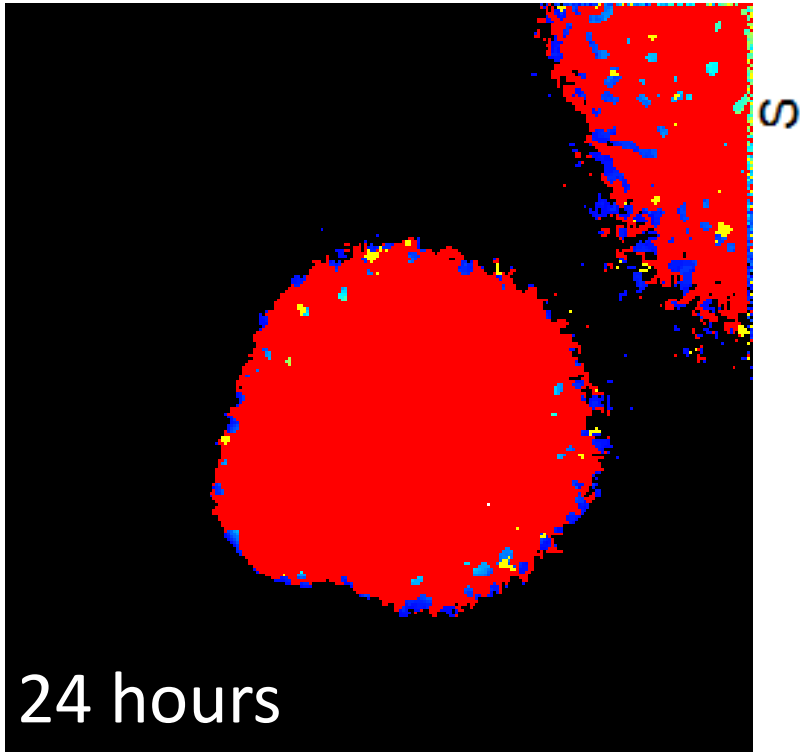
Stsiapura V.I. J. Phys. Chem. B 2008)

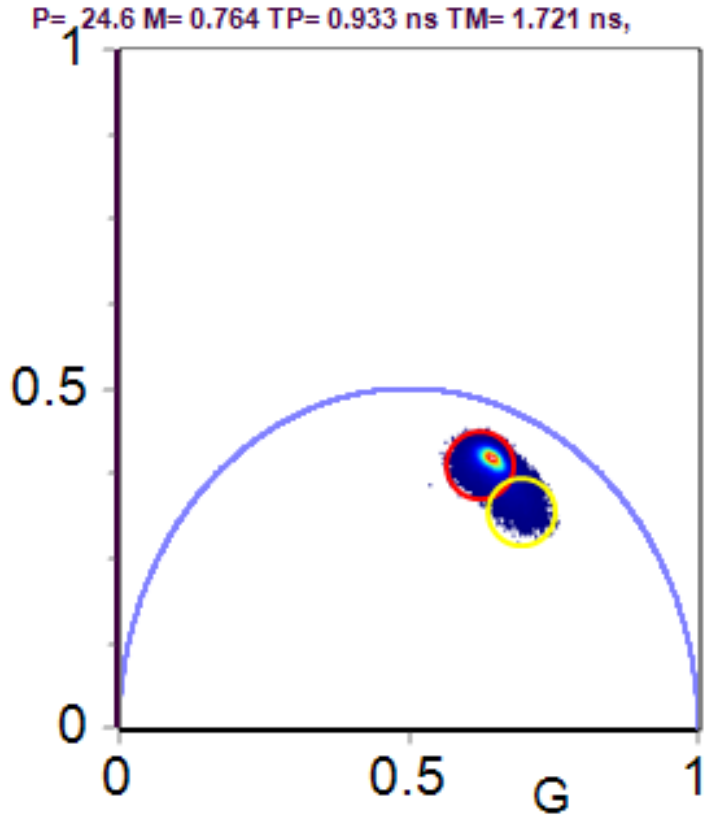
Digman M.A. Biophys. J 2007

Lindberg D.J. Biochemical and Biophysical Research Communications 2015

FLIM on particulates stained with ThT: 5 hours





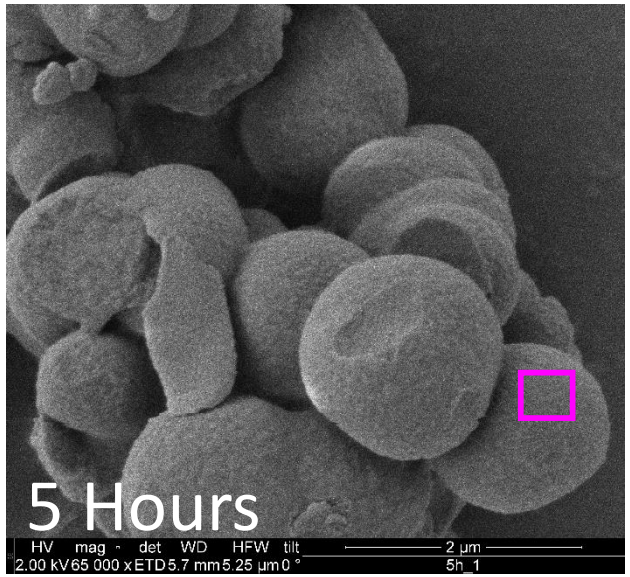


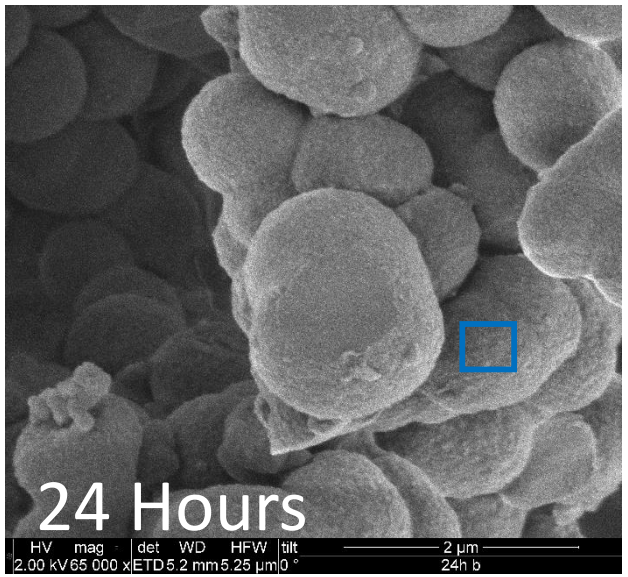
Phasor plot reveals ThT quenching mechanisms at 5 hours

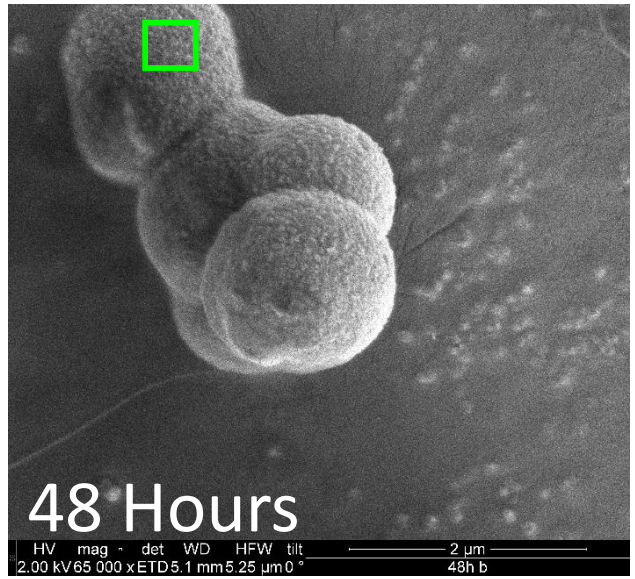
- Different surface?
- Water exposure?

Digman M.A. Biophys. J 2007

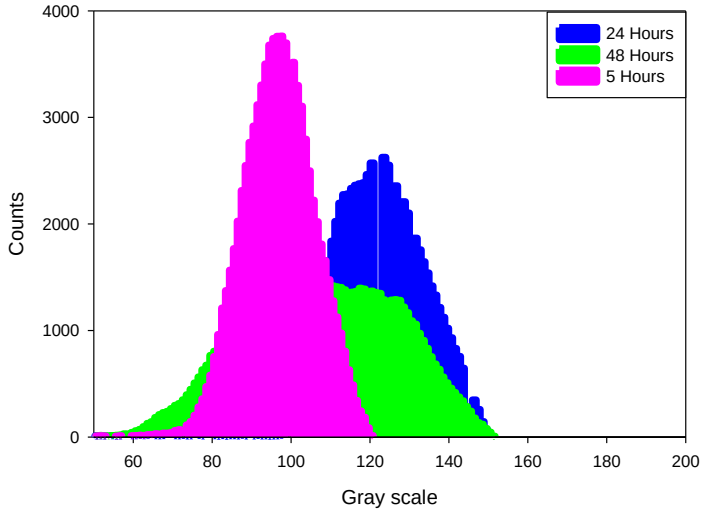
SEM measurement







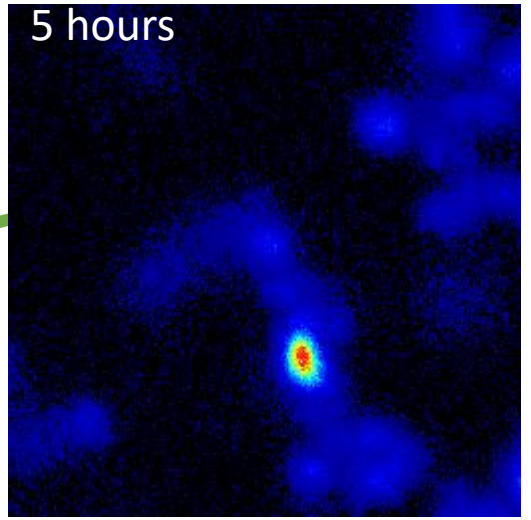
Roughness plot



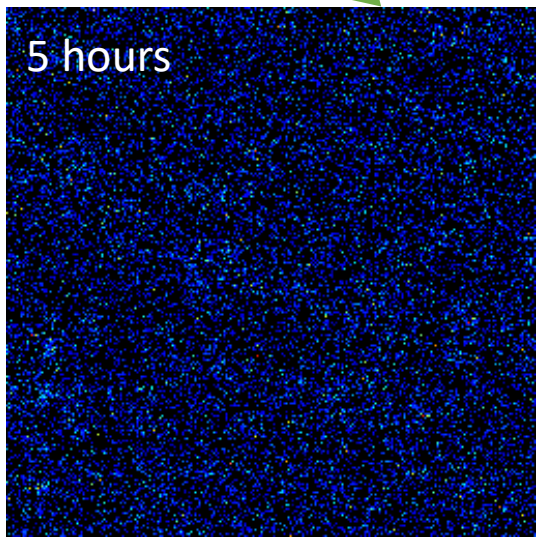
- Same shape
- Increasing roughness of the surface

Stability of particulates against pH : 5 hours

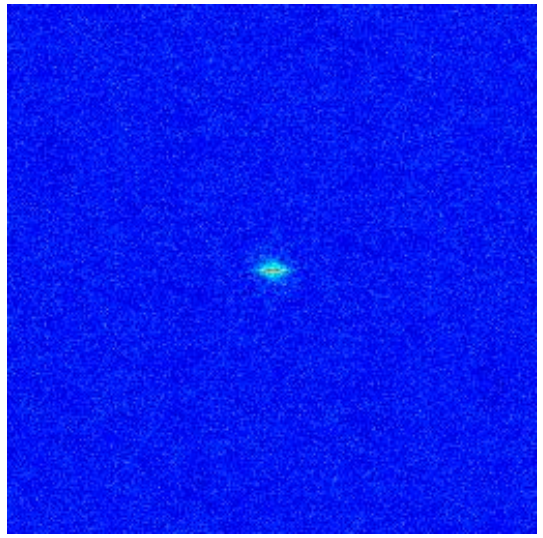
Raster Imaging Correlation Spectroscopy arrow Diffusion coefficient



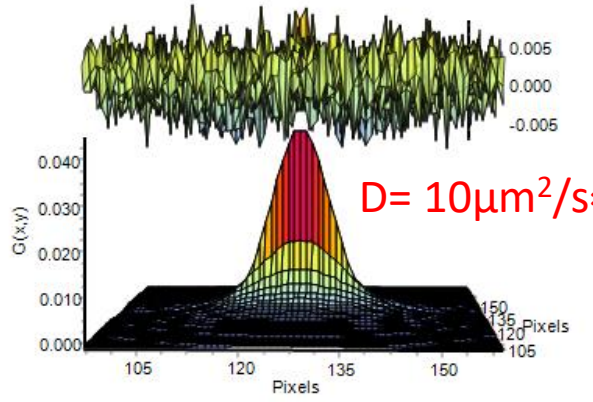
pH 2



RICS



Fitting



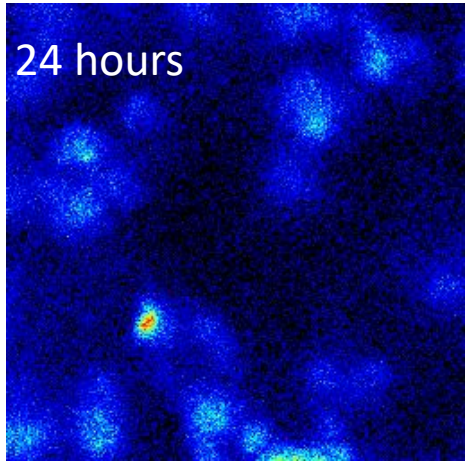
D= 10µm^2/s≈100 nm

Digman M.A. Biophys. J 2005

Spatial correlation function

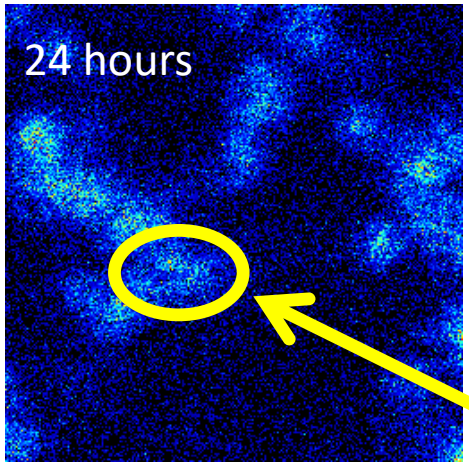
Stability of protein particulates

Confocal intensity image



pH 2

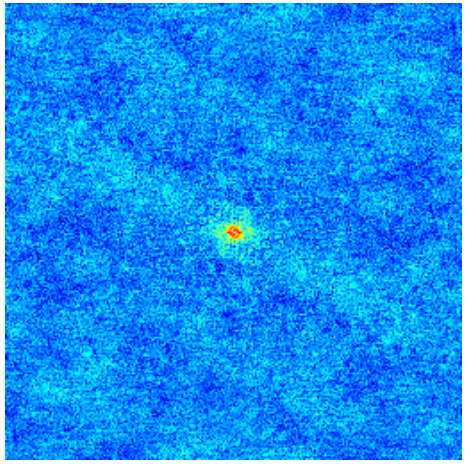
Confocal intensity image



Particulates resistant to pH 2

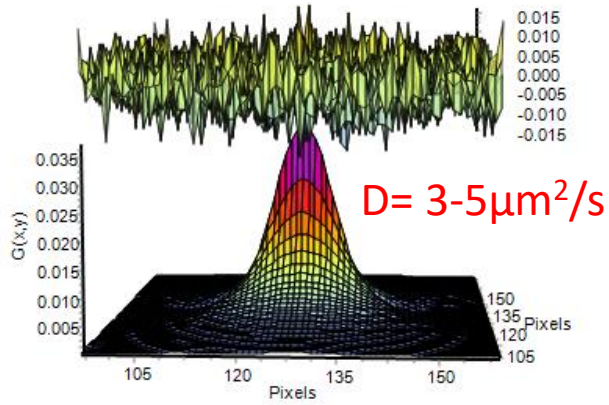
Subtraction of the immobile fraction

Spatial correlation function



Release of fragments

Fitting



D= 3-5µm^2/s

Small fragments among part.

Main result

5 Hours

Maturation

24 Hours

pH 2

pH 2

Maturation confers higher resistance to acidic pH

Digman M.A. Biophys. J 2005

Conclusions

- We characterized the final states of aggregation kinetics revealing maturation effects on microsphere structures
  - 1.2 µm diameters sphere are formed with increasing content of β-aggregates as a function of maturation
  - Maturation increases surface roughness
  - Maturation increases particulates stability against pH changes

Acknowledgements

Department of Physics and Chemistry-Emilio Segrè, Università degli studi di Palermo

Valeria Vetri

Maurizio Leone

Valeria Millitelo

Giuseppe Sancataldo

Sara Anselmo (poster)

Department of Pharmacy, University of Copenhagen

Vito Foderá

Xin Zhou

Camilla Dyngbo Thorlaksen

Hussein Chaaban

VILLUM FONDEN

UNIVERSITÀ DEGLI STUDI DI PALERMO

UNIVERSITY OF COPENHAGEN

UNIVERSITY OF COPENHAGEN

Highly tunable protein microspheres for drug delivery

Dirk Fennema Galparsoro

Università degli studi di Palermo

Department of Physics and Chemistry-Emilio Segrè

UNIVERSITÀ DEGLI STUDI DI PALERMO