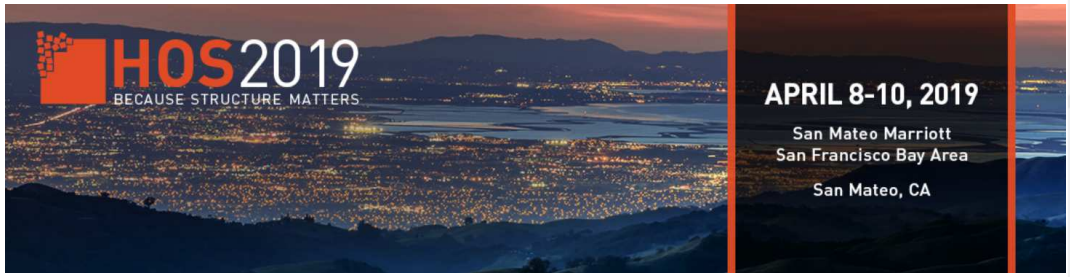
Structural and Morphological Variability of Protein Aggregates



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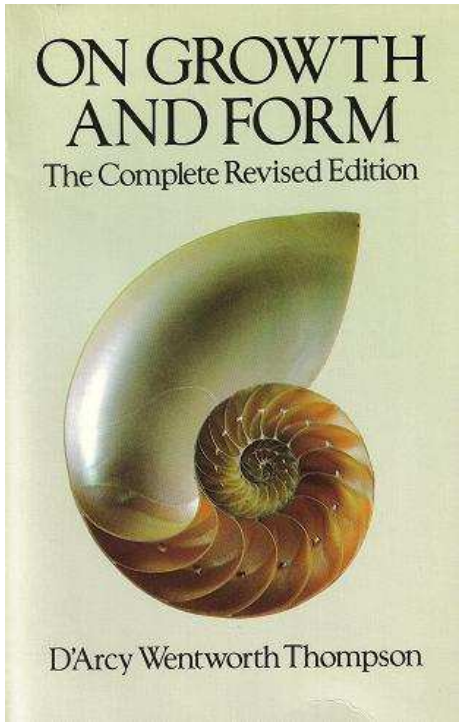
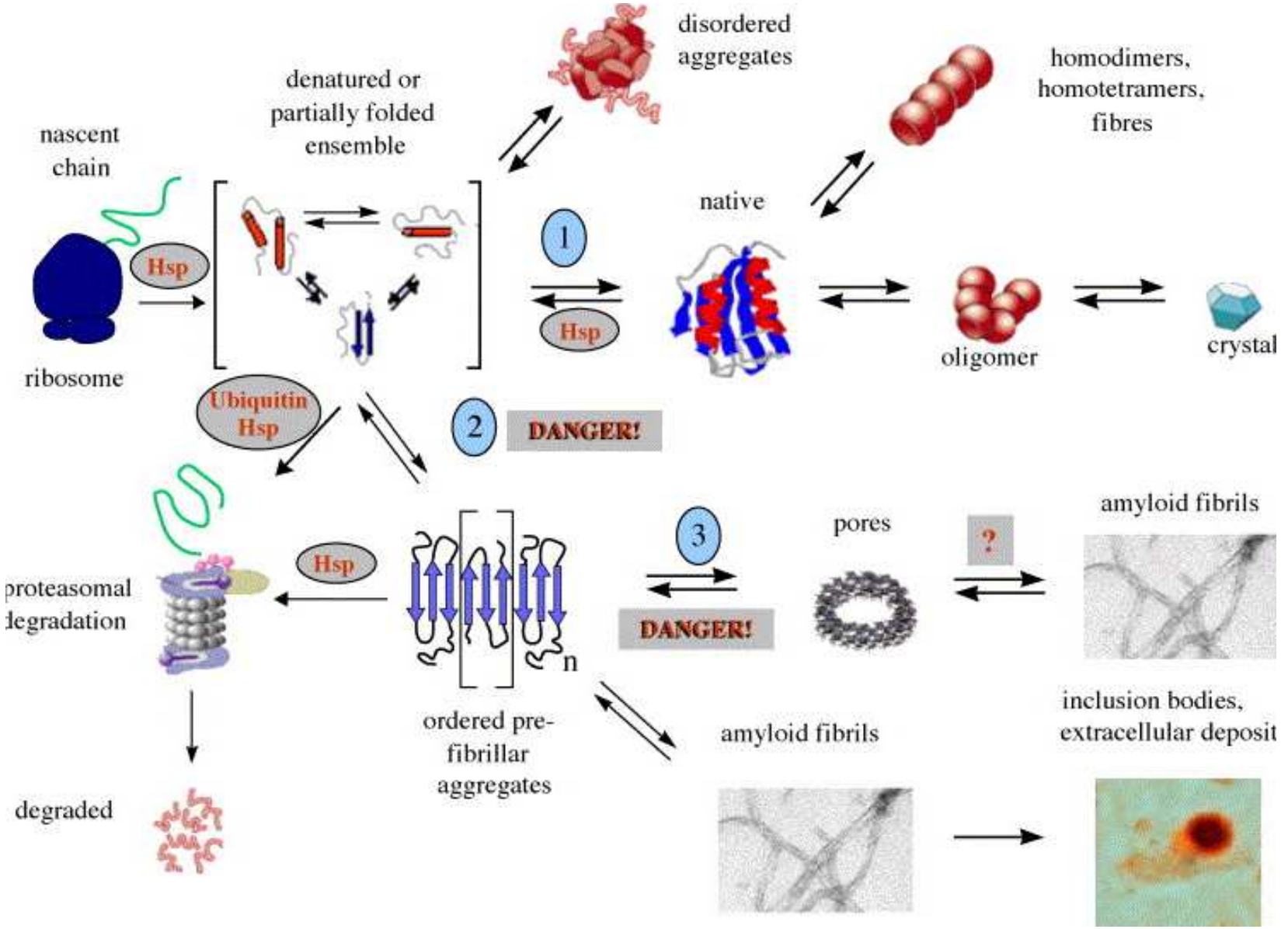
UNIVERSITY OF COPENHAGEN

A very inspiring book



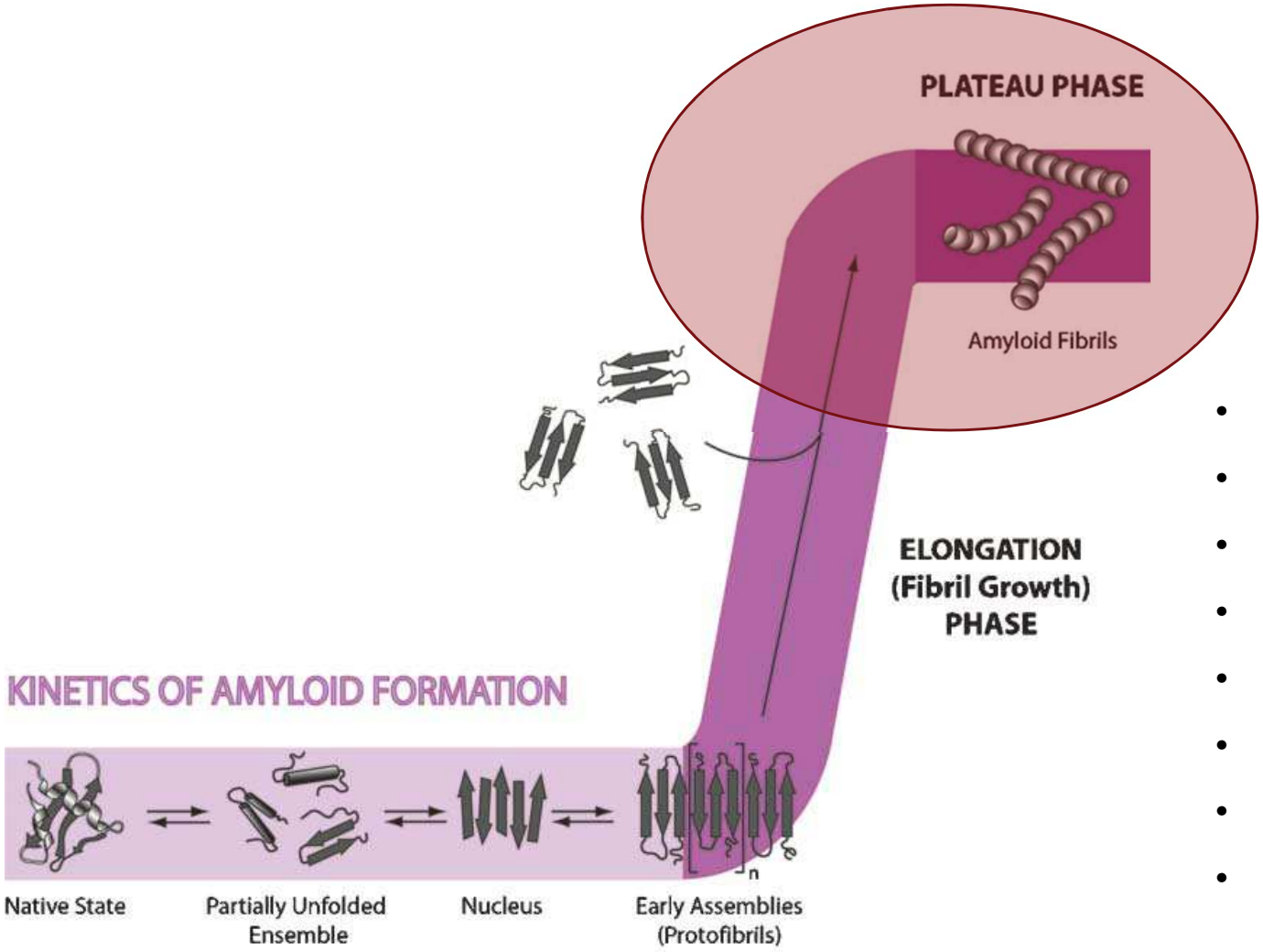
"An organism is so complex a thing, and growth so complex a phenomenon, that for growth to be so uniform and constant in all the parts as to keep the whole shape unchanged would indeed be an unlikely and an unusual circumstance. Rates vary, proportions change, and the whole configuration alters accordingly."

Pathways in Protein Aggregation

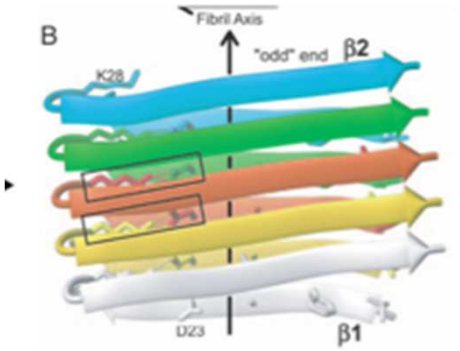


Chiti et al Annu. Rev. Biochem. 75:333–66, 2006

Amyloid Aggregation Process



cross-beta structure

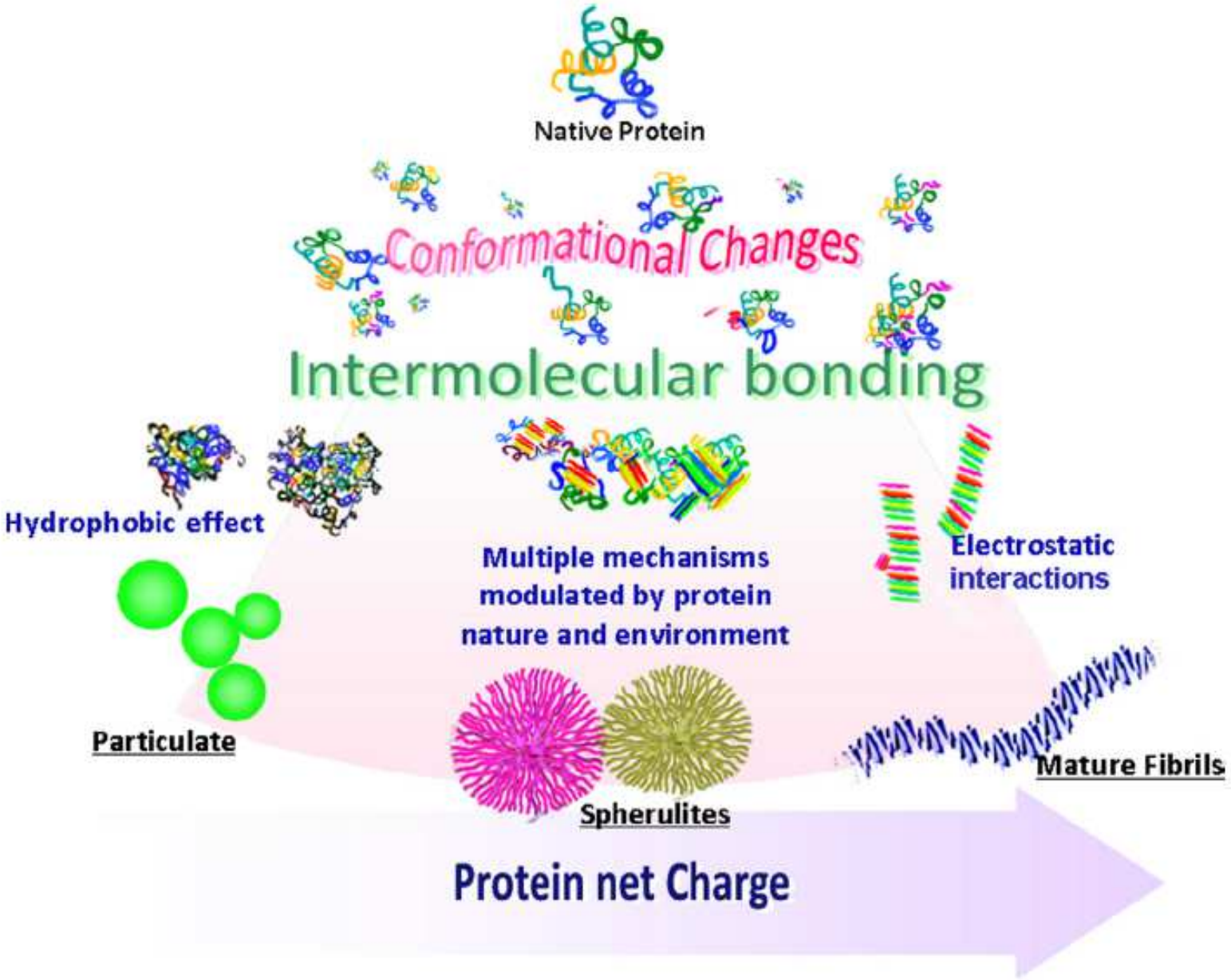


- Thioflavin T and Congo Red
- Electron Microscopy
- Circular Dichroism
- Fluorescence
- Scattering
- Confocal microscopy
- AFM
- …

- Polymorphism
- Globular proteins
- Intrinsically disordered protein

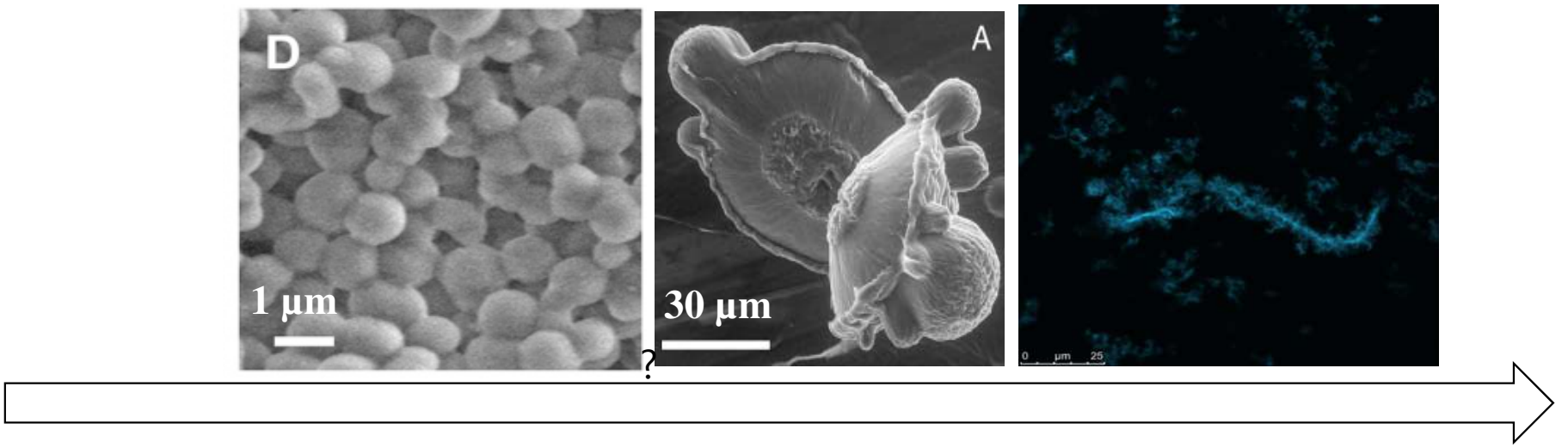
Wilson et al. ***Mol. BioSyst.*** *, 2008, 4 , 42–52*

A broader perspective in the amyloid world



Vetri V and Foderà V. ***FEBS Letters*** *2015, 589, 2446–2463*

Amyloid-like Superstructures



Insulin

Lysozyme

β-lactalbumin

A-β peptide

…

pH

Salinity

Shear

Protein Concentration

…

Mechanisms

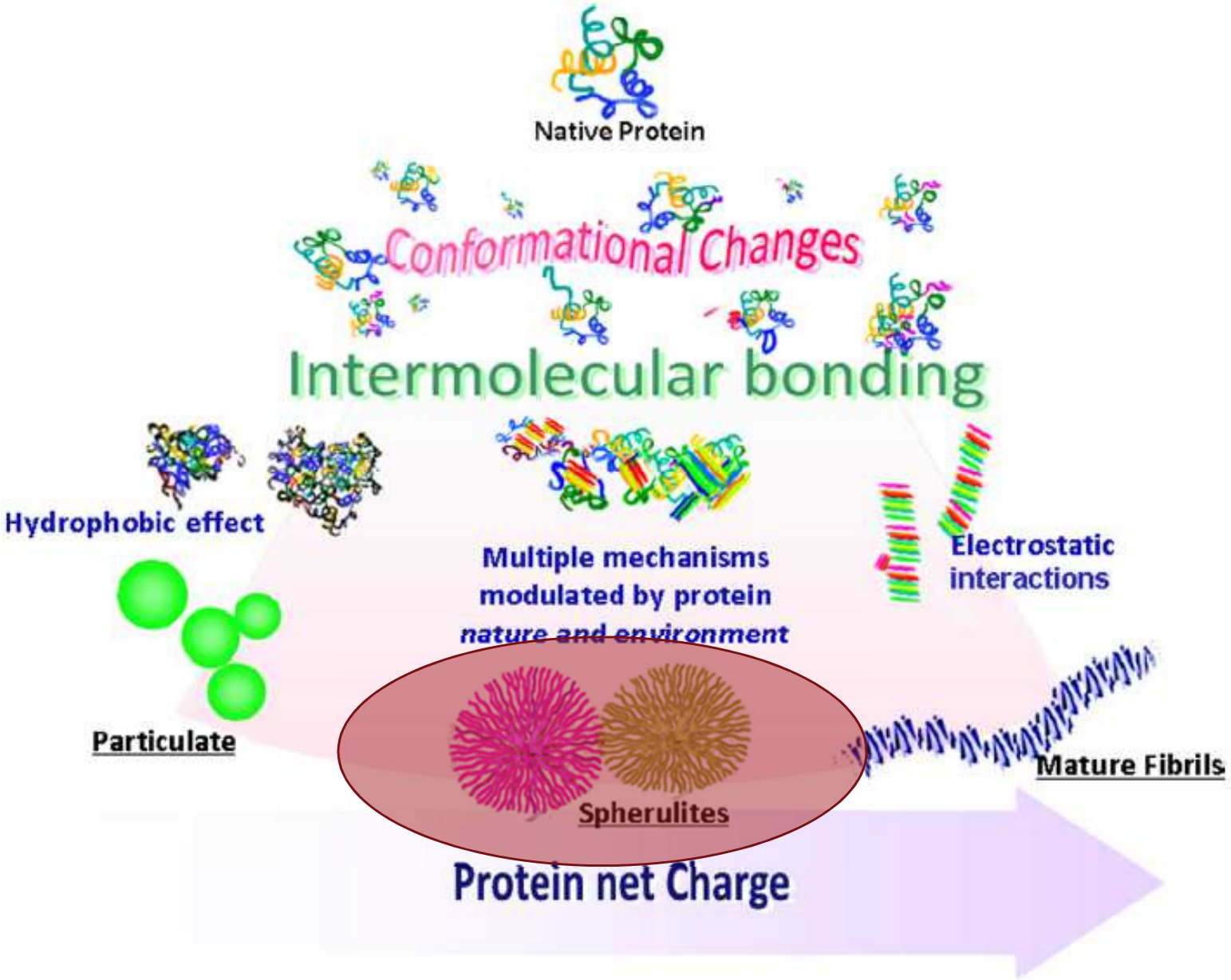
Shape

Bio-effects

…

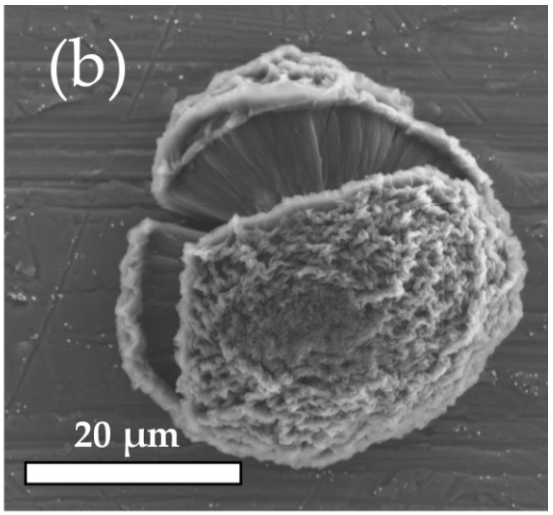
*Krebs at al **Biophys J**. 2005, 88, 2013; Jansen et al. **Biophys J**, 2005, 88, 1344–1353*

A broader perspective in the amyloid world

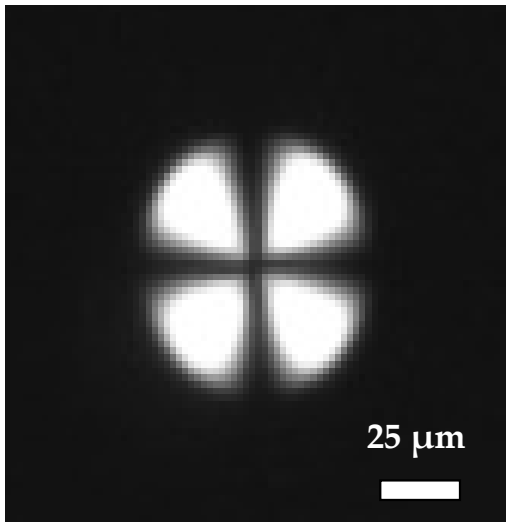


Vetri V and Foderà V. ***FEBS Letters*** *2015, 589, 2446–2463*

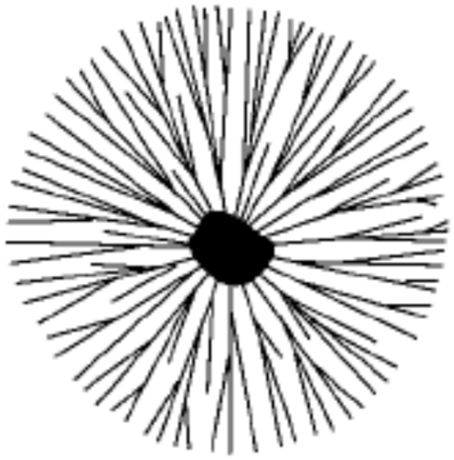
An "ID" for Spherulites



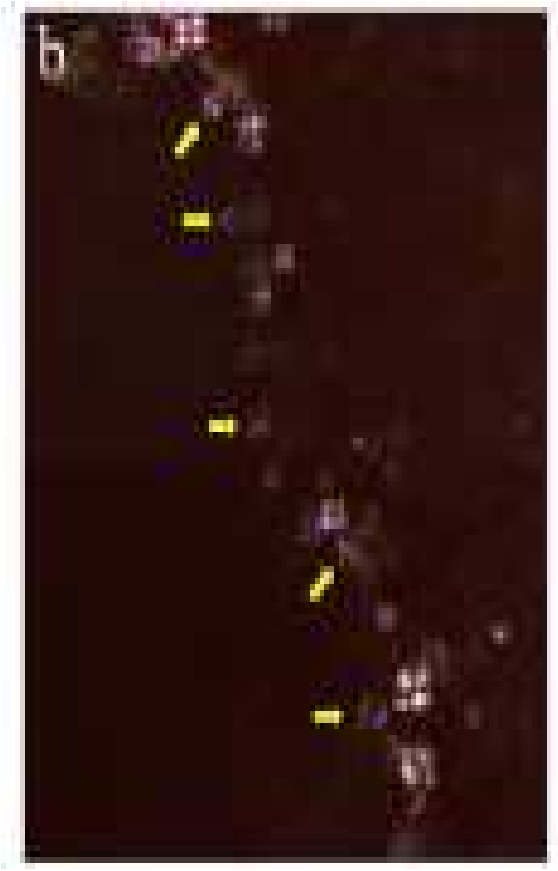
Environmental Scanning Electron Microscopy (ESEM)



Cross-polarized optical microscopy



Formation of a dense core and then radially oriented elongated structures



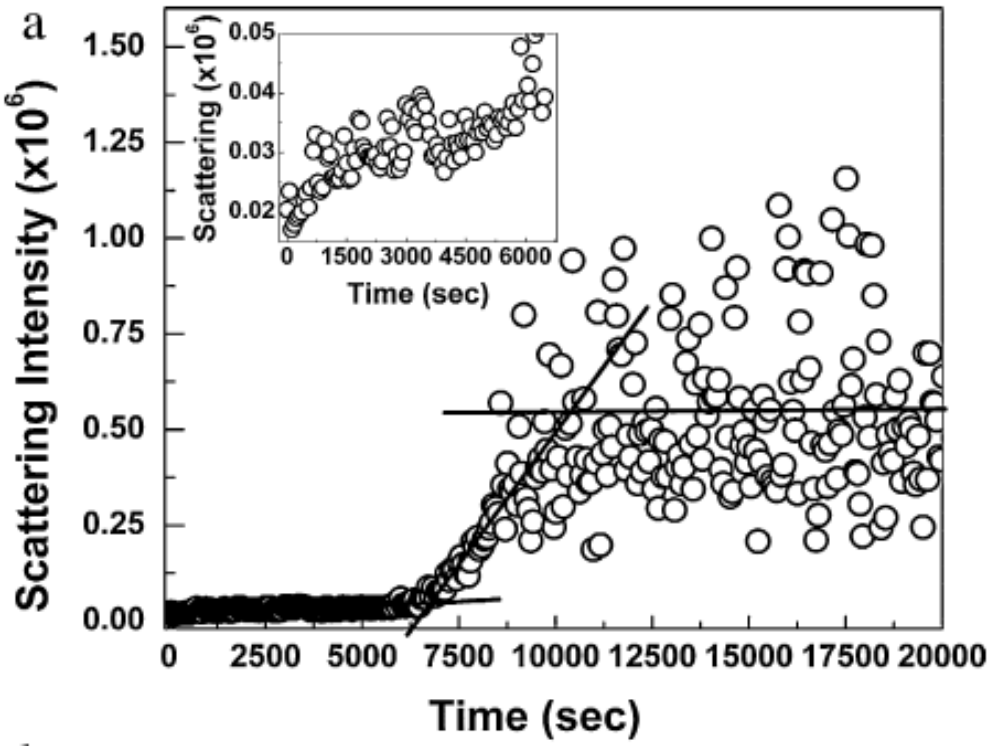
Alzheimer's hippocampus

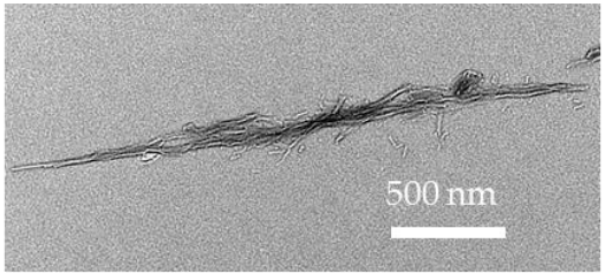
House et al. ***Journal of Alzheimer's Disease*** *6 25 2011 43–46*

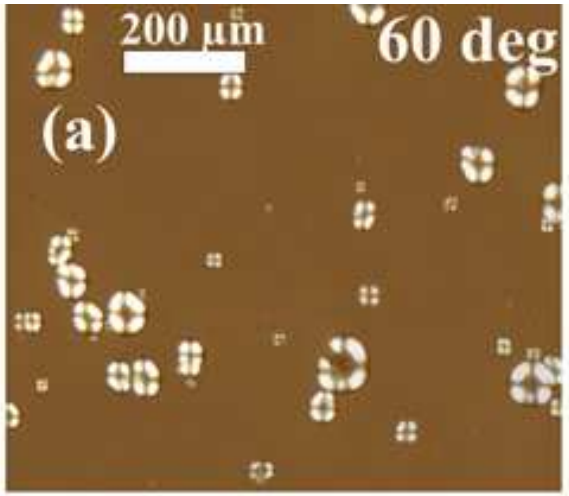
Foderà et al. ***J. Phys. Chem. Lett.*** *2012, 3, 2803–2807*

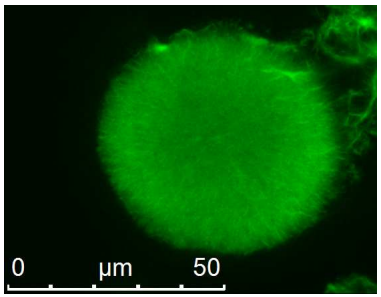
Factors affecting spherulites formation

Protein system: Bovine insulin, 4mg/ml
Conditions: pH=1.75, [NaCl]= 25mM, T=60 degrees C









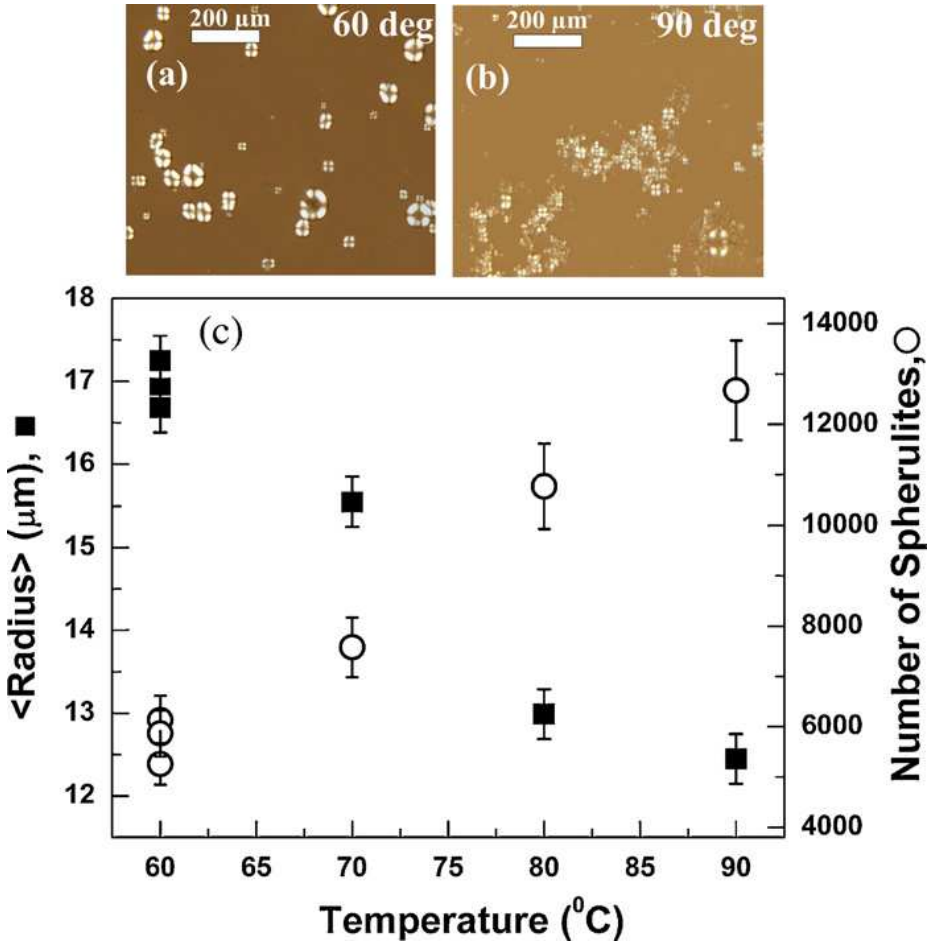
ThT

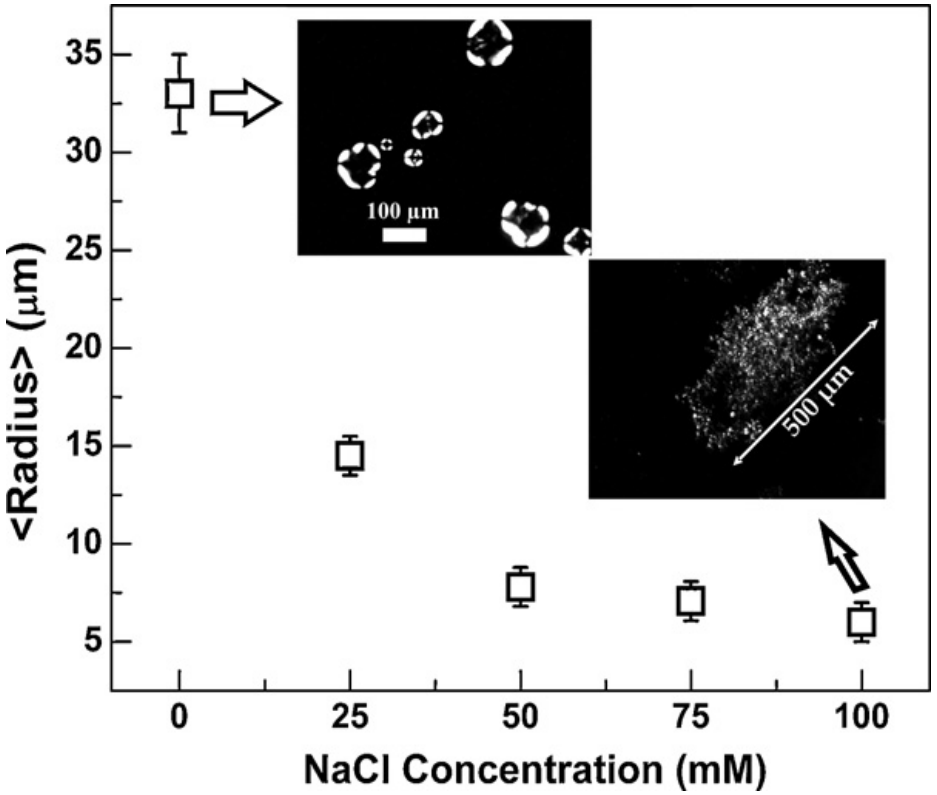
Growth kinetics of spherulites and fibrils are indistinguishable

Smith IM, Foderà V. et al. ***Coll Surf B*** *2012, 89, 216–222*

Factors affecting spherulites formation

Protein system: Bovine insulin, 4mg/ml
Conditions: 1<pH<2.6, 0mM <[NaCl]< 100mM, 60<T<90 degrees

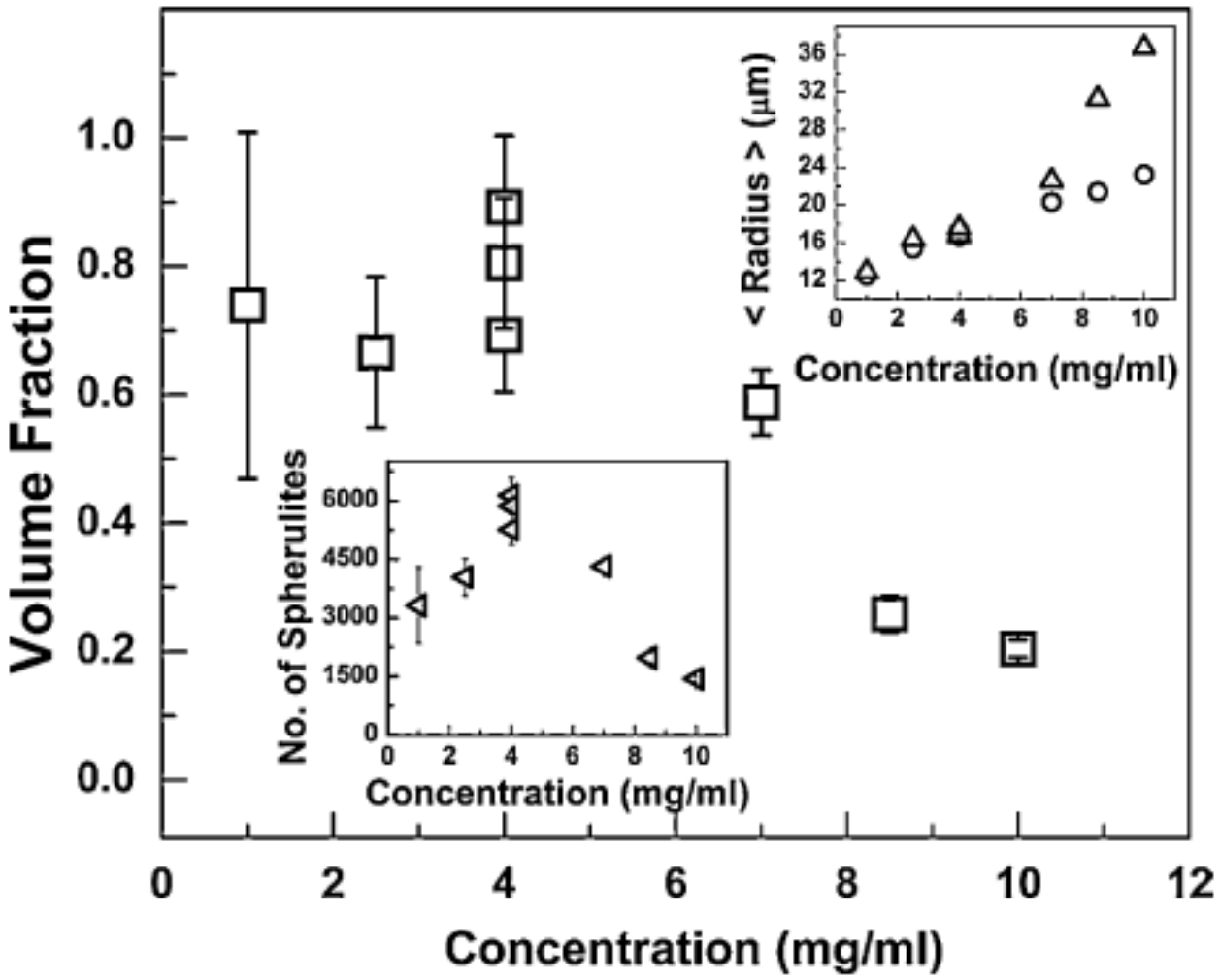




Smith IM, Foderà V. et al. ***Coll Surf B*** *2012, 89, 216–222*

Factors affecting spherulites formation

Protein system: Bovine insulin, 1mg/ml<[BI]<12mg/ml
Conditions: pH=1.75, [NaCl]= 25mM, T=60 degrees

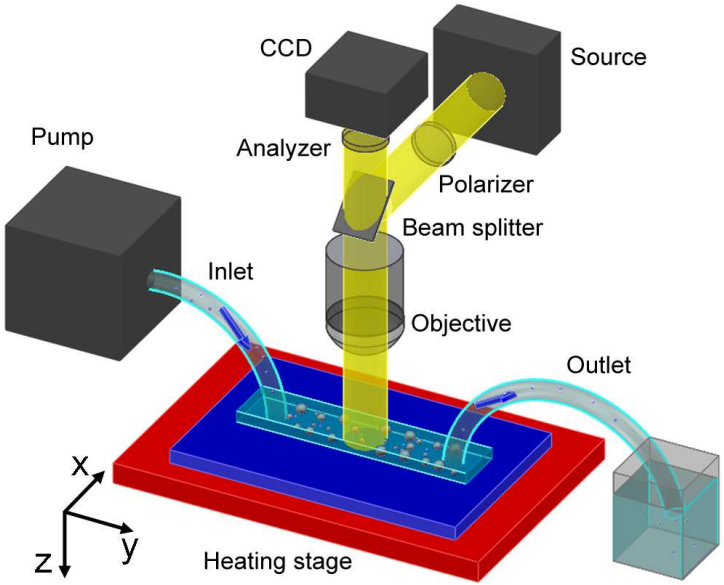


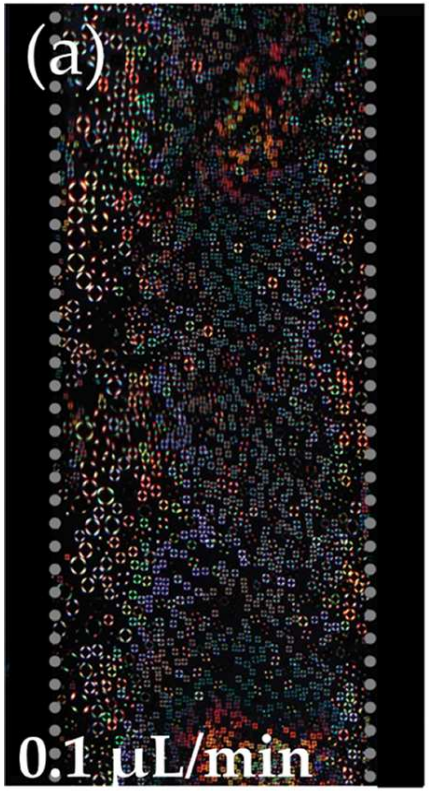
Smith IM, Foderà V. et al. ***Coll Surf B*** *2012, 89, 216–222*

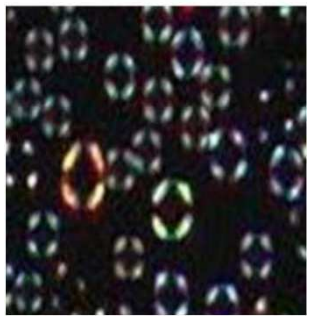
Flow Regime and Confinement

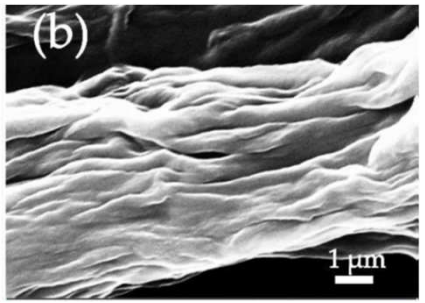
In vitro aggregation:
temperature
pH
ionic strength
denaturants
organic solvents

In vivo aggregation:
Fluid flows in micro-enviroments







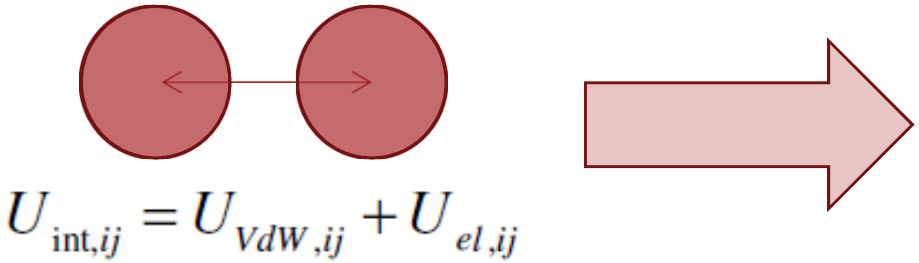


Foderà et al. ***J. Phys. Chem. Lett.*** *2012, 3, 2803–2807*

How PPIs affect the spherulite properties

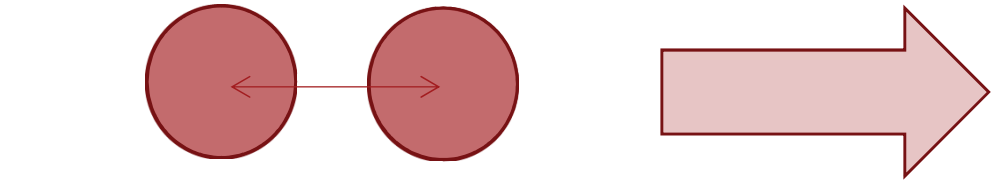
Selectively changing the physical intermolecular forces between proteins via co-solvents.

Protein system: Human insulin
Conditions: pH 1.8, 60 degrees (24 h) with 40% EtOH and without EtOH
Why: EtOH controls the protein hydration and dielectric constant of the medium

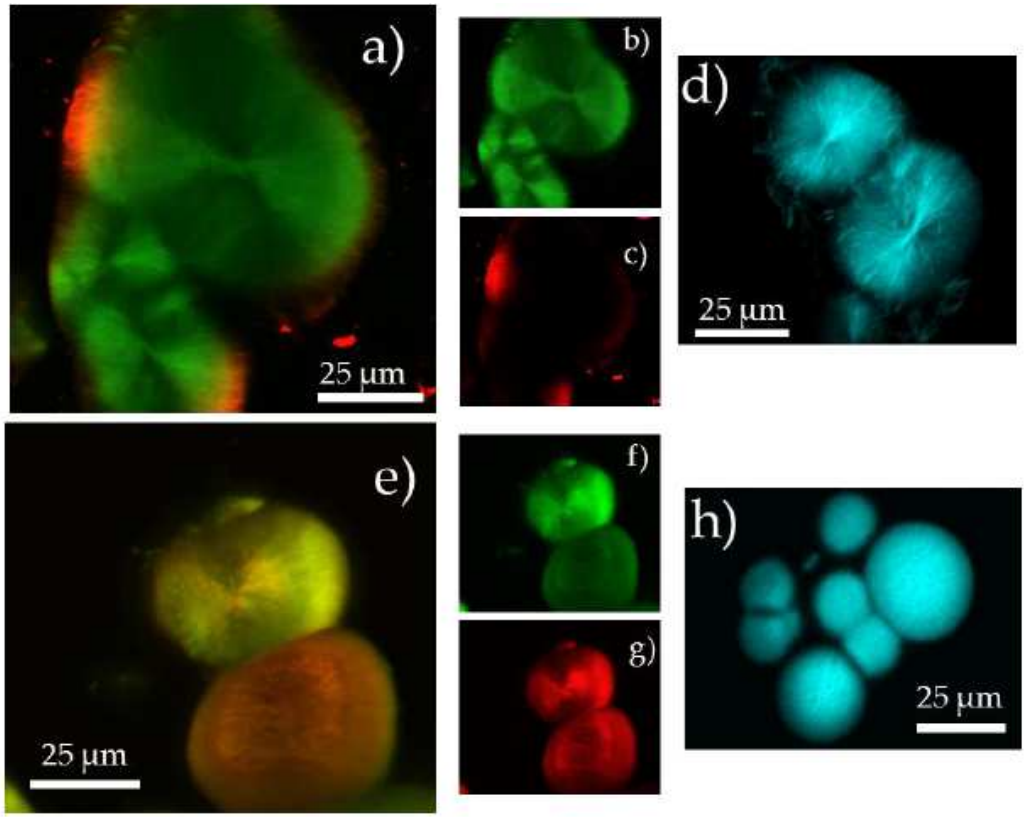


$$U_{\text{int},ij} = U_{VdW,ij} + U_{el,ij}$$

Red: *hydrophilic dye*
Cyan: *hydrophobic dye*
Green: *Amyloid –sensitive dye*



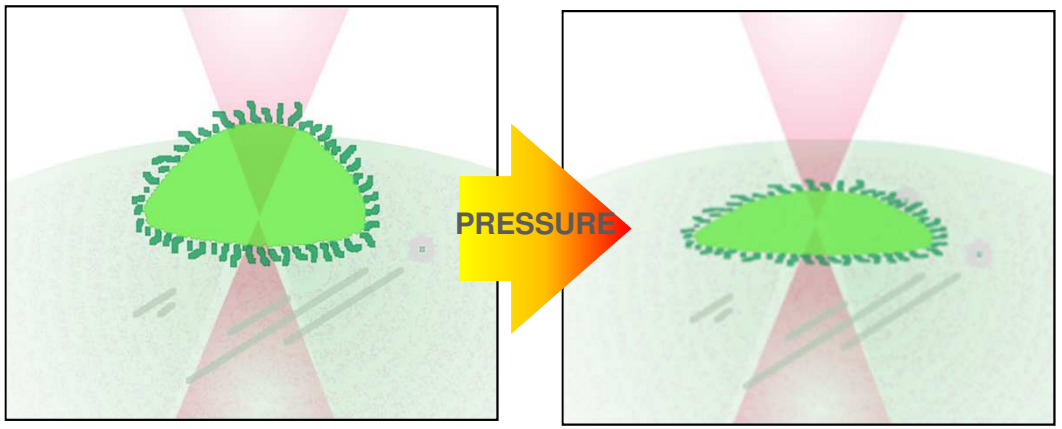
$$U_{\text{int},ij} = U_{VdW,ij} + U_{el,ij} + U_{hydr}$$

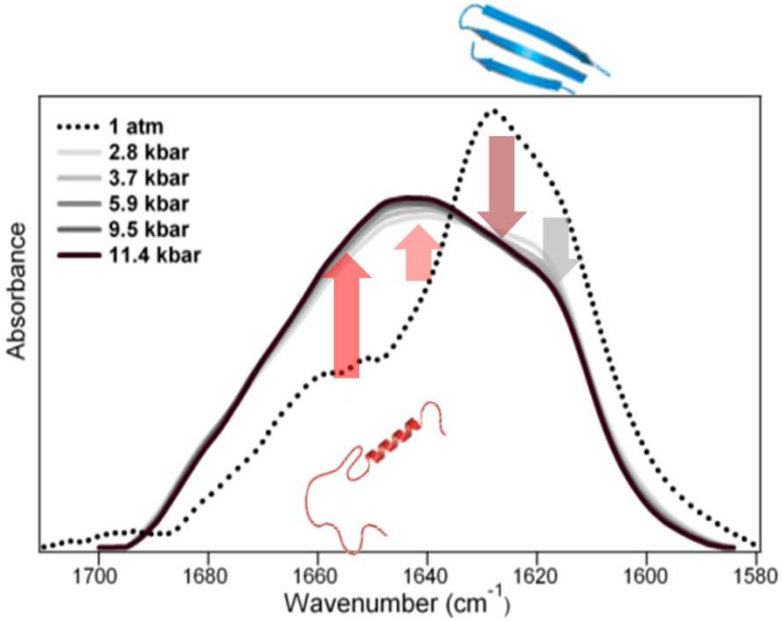


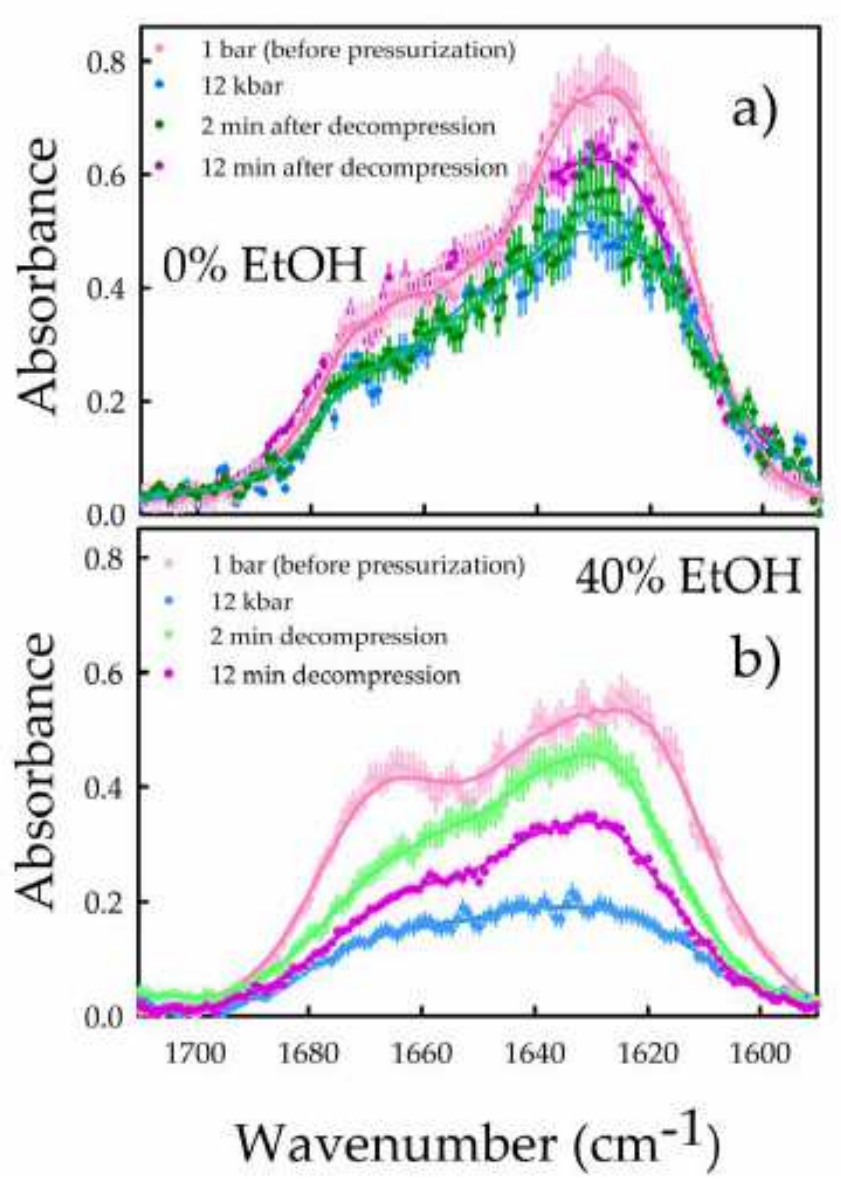
Foderà V, et al 2013, ***Phys Rev Lett,*** *111, 108105*

Javid, et al. ***PhysRevLett*** *2007 99, 028101 Dzwolak et al.* ***Biochemistry*** *2005, 44, 8948-8958 Vetri V., et al 2018* ***J. Phys Chem B*** *122, 3101–3112*

Mechanical Properties: high pressure FTIR

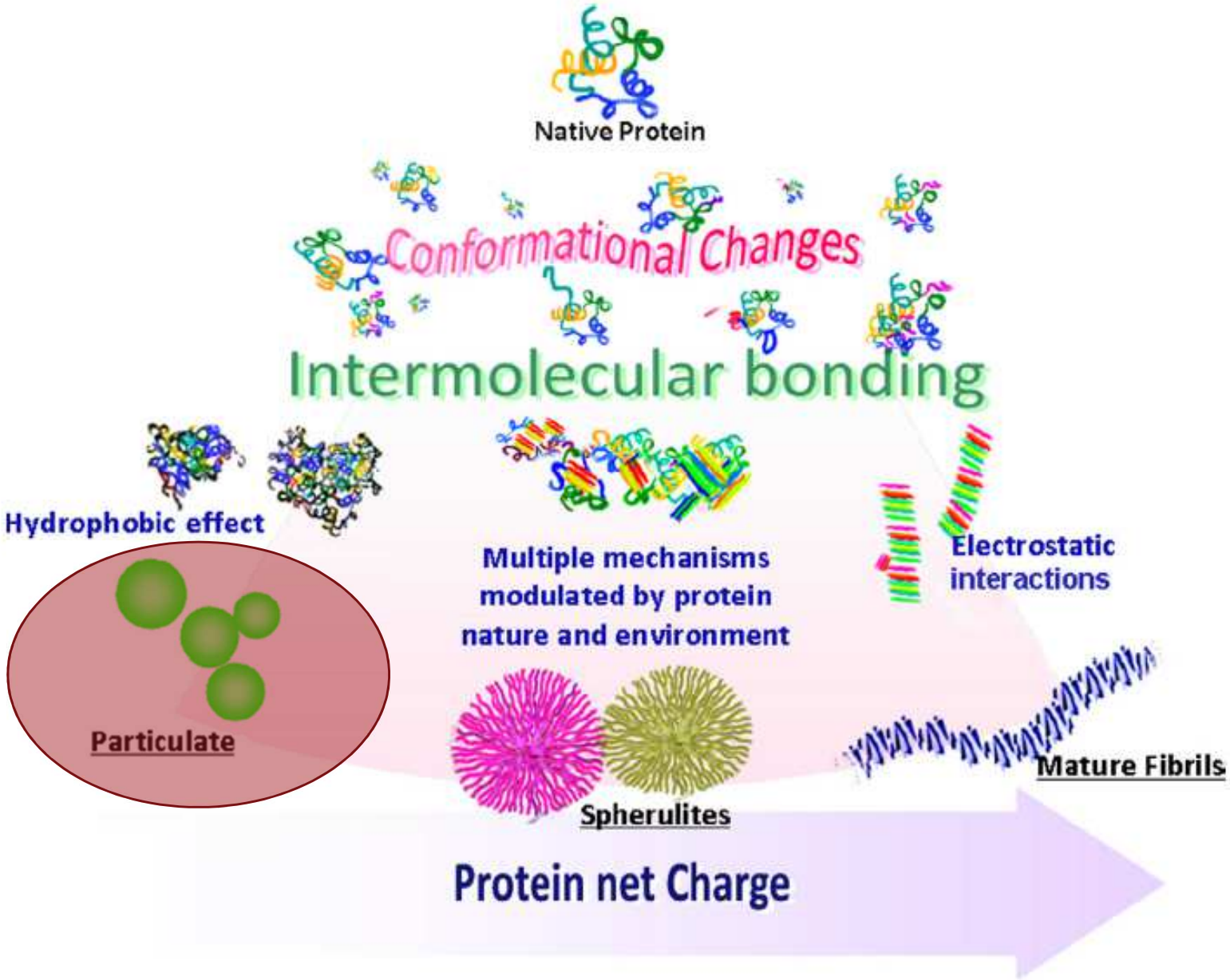






Piccirilli et al. 2012 ***Soft Matter*** *8, 11863-11870* *Vetri V., et al 2018* ***J. Phys Chem B*** *122, 3101−3112*

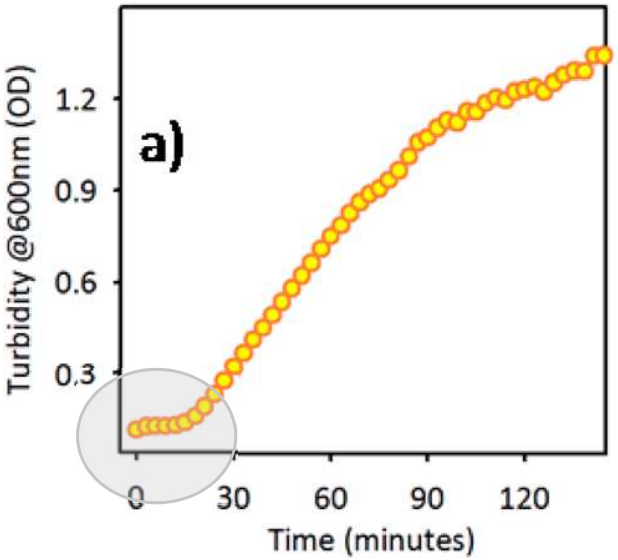
A broader perspective in the amyloid world



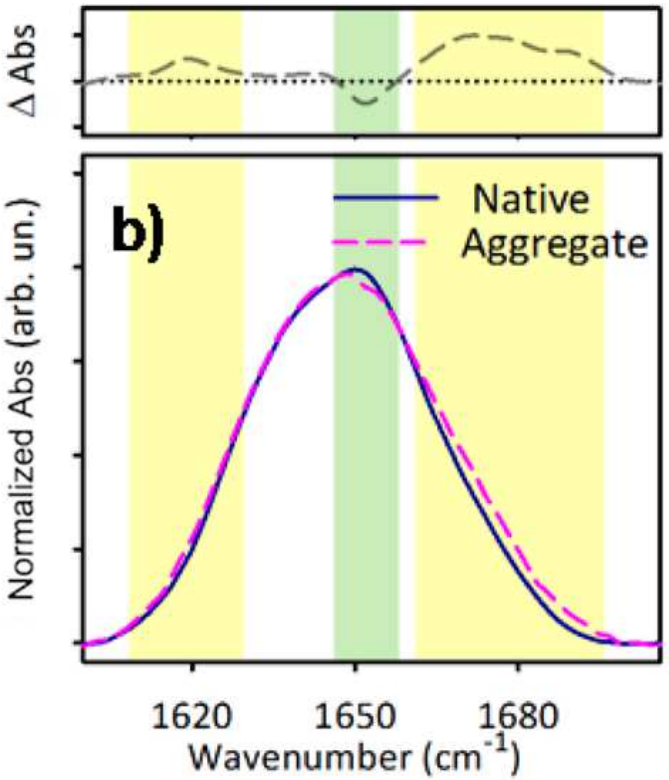
Vetri V and Foderà V. ***FEBS Letters*** *2015, 589, 2446–2463*

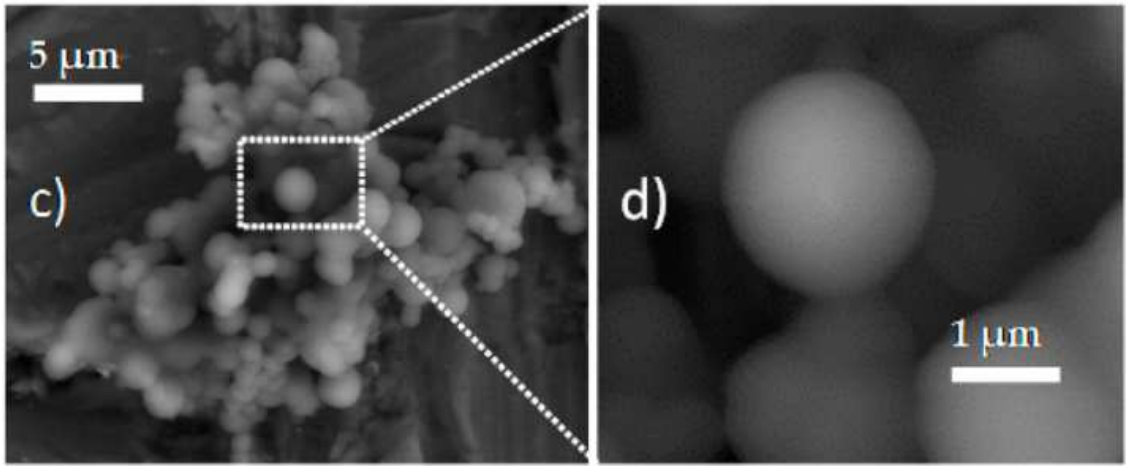
Protein Particulates

Protein system: Equine Lysozyme
Conditions: pH 8.5 (close to the pI), 20mM sodium acetate 57 degrees



-Secondary structure almost conserved
- Shoulders at 1620, 1670, and 1690 cm–1 indicates a fraction of intermolecular β-sheets and β-turns

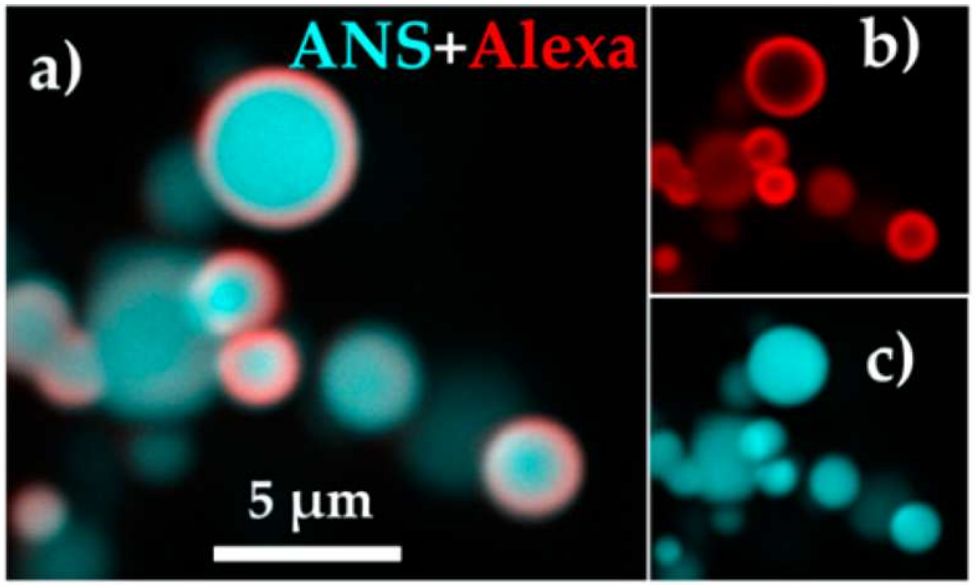


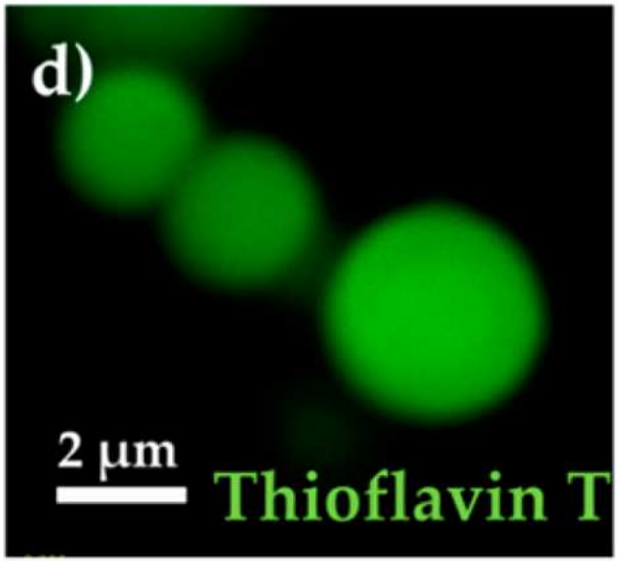


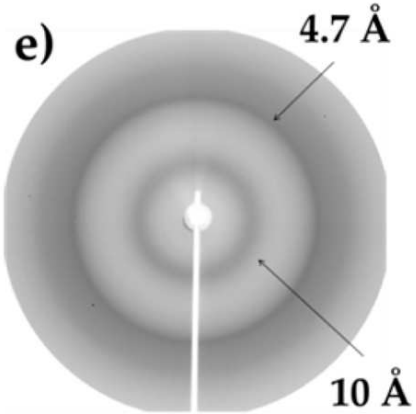
Foderà et al. ***J Phys Chem Lett.*** *2014, 5, 3254-3258*

Protein Particulates

Physico-chemical features → **Two-photon excitation fluorescence microscopy**







Fibre Diffraction confirms the β-sheet arrangement in a spherical simmetry for particulates

Foderà et al. ***J Phys Chem Lett.*** *2014, 5, 3254-3258*

Summary

- Formulation determines the occurrence of specific superstructures
- Spherulites: β-rich structures in competition with fibrils
- Particulates: minor changes of the native structure
- Shear controls the morphology of protein aggregates

Perspectives

- Immunogenicity
- More structural investigations
- Polymorphic nature of the superstructures
- Balance between different superstructures within the same protein sample
- Large screening for further identification of different particles

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Aleksandra Bartosik
Edres Sayedi
Anas Jaaloul
Michele Corezzi
Adrian Dario Juncos Bombin
Gretha Di Donato
Jeff Jin Rong Quach
Gloria Singla
Ivana Files

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Federica Piccirilli
Gianpiero Buscarino

Alessio Zaccone
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Urszula Łapińska

Erin Attril
Stefano Pagliara

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Graduate Programme in Pharmaceutical Sciences

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Ministero dell'Istruzione dell'Università e della Ricerca

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