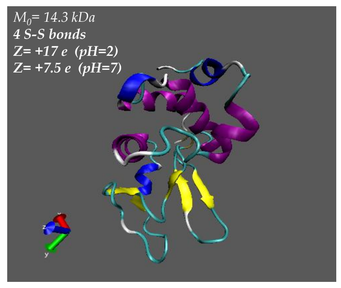
Role of electrostatic interactions in the fibrillogenesis of lysozyme

Samuele Raccosta[1,2], Vincenzo Martorana[1] and Mauro Manno[1]

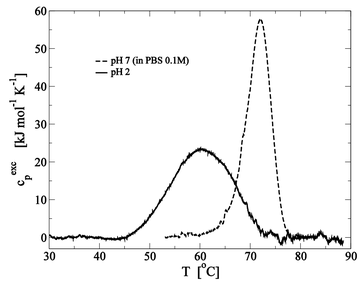
1. Institute of Biophysics at Palermo, National Research Council of Italy, Via U. La Malfa 153, 90146 Palermo, Italy.
2. Department of Physical and Astronomical Sciences, University of Palermo, Via Archirafi 36, 90123 Palermo, Italy.

AIM: *Highlighting the role of repulsive* ***electrostatic interactions*** *in* ***protein unfolding and self-assembly,*** *with a focus on the formation of elongated fibrillar aggregates.*

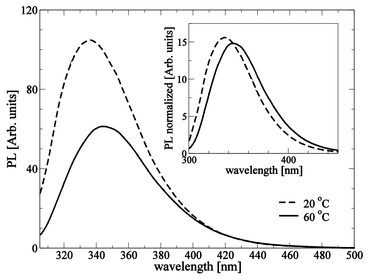
Model system:
Hen Egg-White Lysozyme



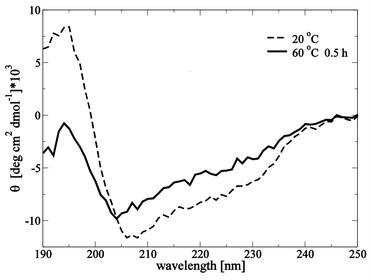
Thermal stability at acidic pH



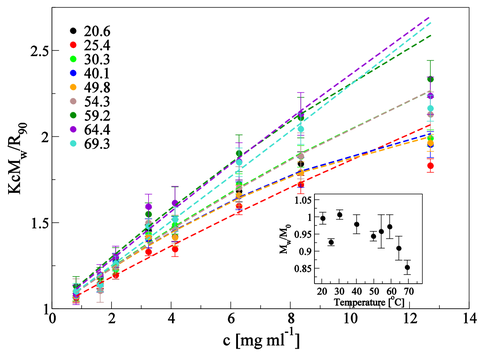
DSC
pH=7: lysozyme is stabilized by surface salt bridges.
pH=2: all protonable residues are positive, salt bridges are broken, intramolecular repulsive interactions destabilize lysozyme.
Above 45 °C lysozyme changes conformation reversibly .



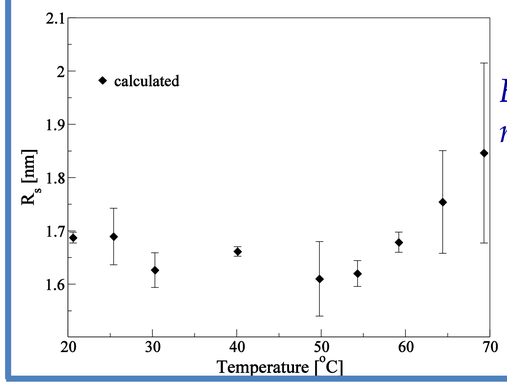
CD *and trp-***PL** *spectra show respectively the decrease of α-structure [Arnaudov et al. 2005] and the concomitant exposure of trp to the solvent above 60 °C.*



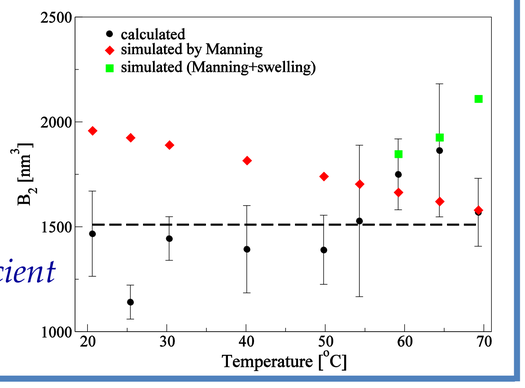
Intermolecular interactions at high temperature



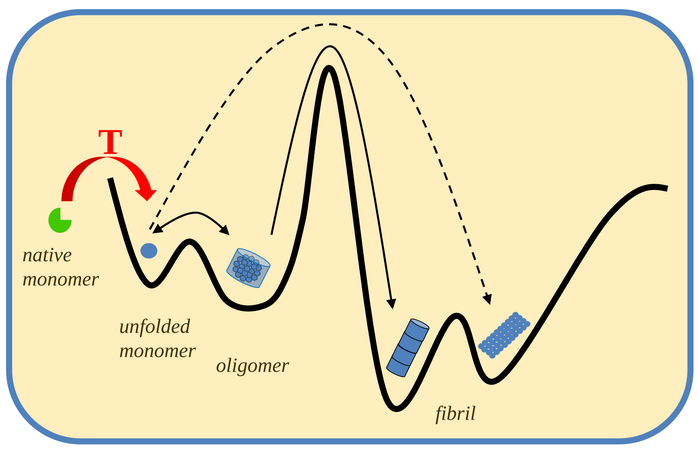
a) Experimental data: compressibility curves at different temperatures.
b) 1st result: Swelling of lysozyme at high temperature.
c) 2nd result: Second virial coefficient (B_2) has no dependence upon temperature.
Lysozyme as a charged sphere with counterions collapsed on its surface (Manning condensation).
Order of magnitude of B_2 is explained by a screened intermolecular electrostatic repulsion.



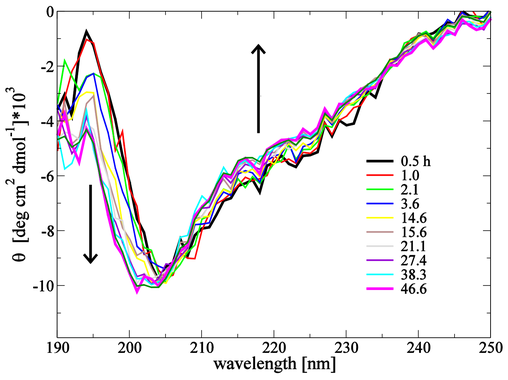
Effective hydrodynamic radius



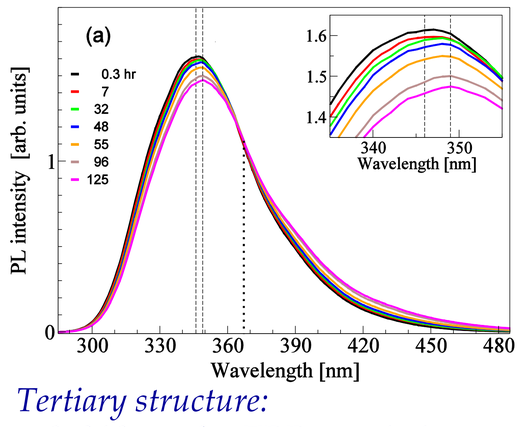
Second virial coefficient



Slow conformational changes (60–65 °C): two-state transition

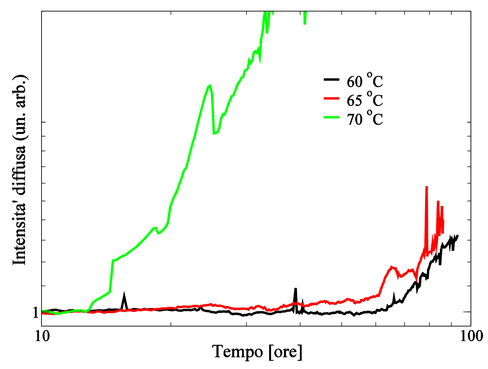


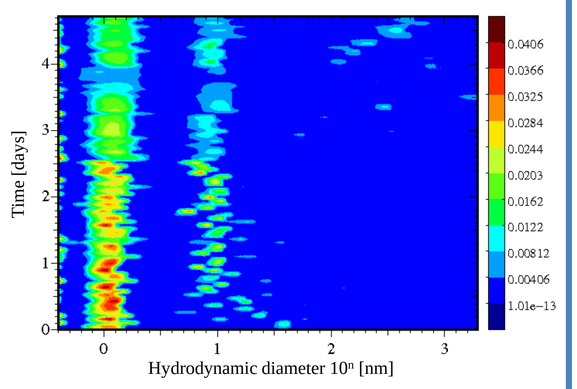
Secondary structure:
loss of α-structrure (isodichroic point at 204 nm)



Tertiary structure:
red-shidt of TRP emission (isosbestic point at 368 nm)

Fibrillation kinetics by Static and Dynamic Light Scattering



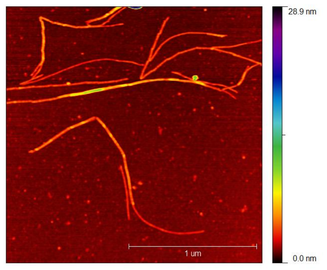


Intensity autocorrelation functions g2(t)

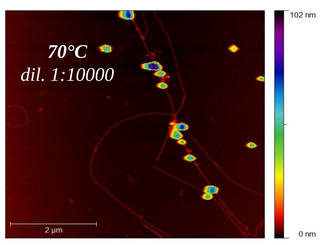
$$g_2(D) = 1 + \beta \cdot \left| \int P(D) e^{-Dq^2 t} dt \right|^2$$

diffusion coefficient distribution P(D)

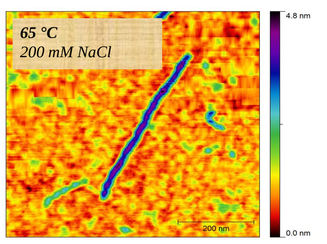
-LAG PHASE:
Coexistence of Monomers *($R_h \approx 1$nm) and* ***Oligomers*** *($R_h \approx 10$nm)*
AFTER A FEW DAYS
Appearence of ***Fibrils*** *and other big aggregates (R_h > 80 nm)*



AFM. *Typical size of fibrils is 20 nm in width, few microns in length, 50 nm in axial periodicity.*



At 70 °C amorphous aggregates are in competition with fibrils.



Amorphous aggregation is enhanced if some salt (20-200 mM) is added in solution. If incubation temperature is lower fibrils are formed even if salt is added [Hill et al. 2009]

Results

- *High protein charge reduces protein stability towards unfolding.*
- *Self-assembly at high T due to the exposure of hydrophobic residues is slowed down by the strong electrostatic repulsive interaction ➡ more stable solution and organized aggregation.*
- *The coexistence of monomers and oligomers suggests a competing effect of hydrophobic and electrostatic interaction*
- *The kinetics of fibril formation, their morphology and recent FTIR results [Freire et al. 2009] suggest that oligomers may be on-pathway fibril precursor.*
- *No relevant secondary mechanisms of fibrillation*

Work in progress

1) *Study more deeply of the protein-protein interactions at different temperatures and incubation times by SAXS*
2) *Characterization of structure by SAXS*

Contact: samuele.raccosta@pa.ibf.cnr.it