Role of electrostatic interactions in the fibrillogenesis of lysozyme

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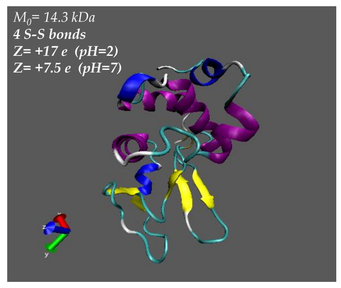
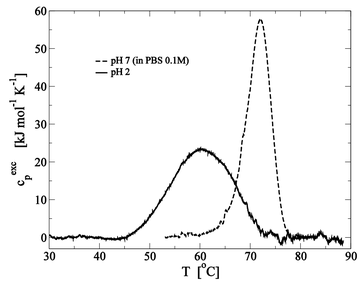
AIM: *Highlighting the role of repulsive* ***electrostatic interactions*** *in* ***protein unfolding and self-assembly,*** *with a focus on the formation of elongated fibrillar aggregates.*

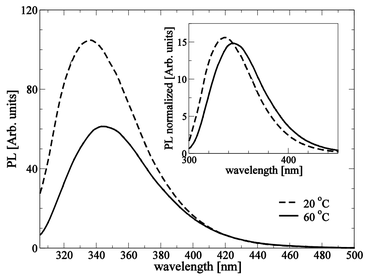
Model system:
Hen Egg-White Lysozyme



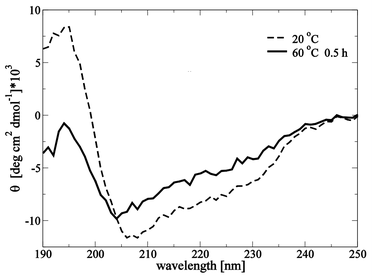
Thermal stability at acidic pH



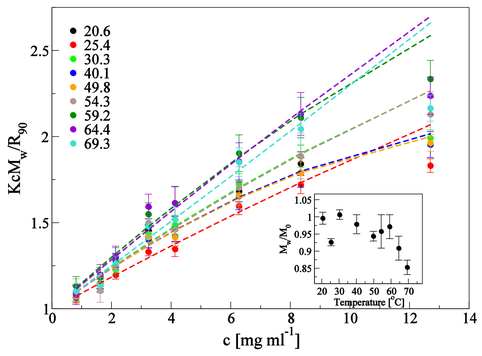
DSC
pH=7: lysozyme is stabilized by surface salt bridges.
pH=2: all protonable residues are positive, salt bridges are broken, intramolecular repulsive interactions destabilize lysozyme.
Above 45 °C lysozyme changes conformation reversibly .



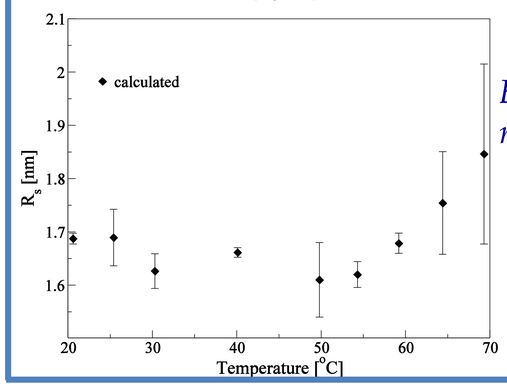
CD *and trp-***PL** *spectra show respectively the decrease of α-structure [Arnaudov et al. 2005] and the concomitant exposure of trp to the solvent above 60 °C.*



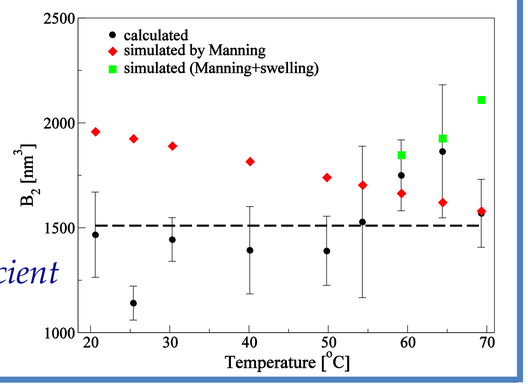
Intermolecular interactions at high temperature



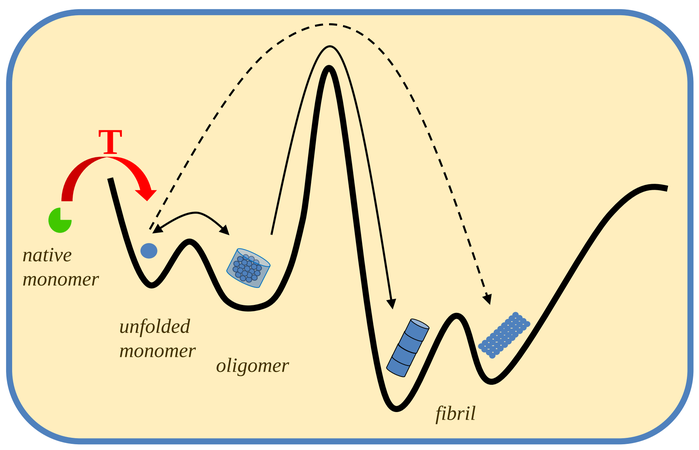
a) Experimental data: compressibility curves at different temperatures.
b) 1st result: Swelling of lysozyme at high temperature.
c) 2nd result: Second virial coefficient (B_2) has no dependence upon temperature.
Lysozyme as a charged sphere with counterions collapsed on its surface (Manning condensation).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



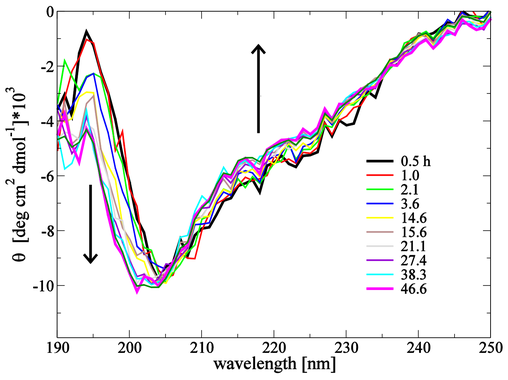
Effective hydrodynamic radius



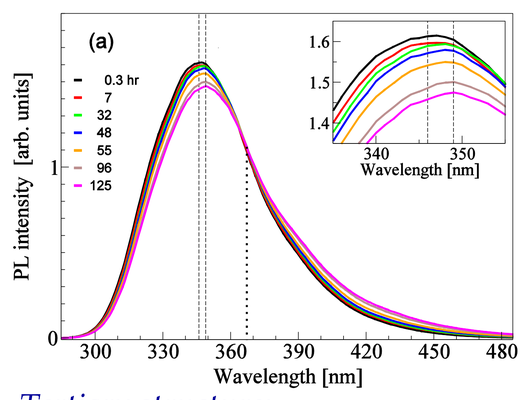
Second virial coefficient



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

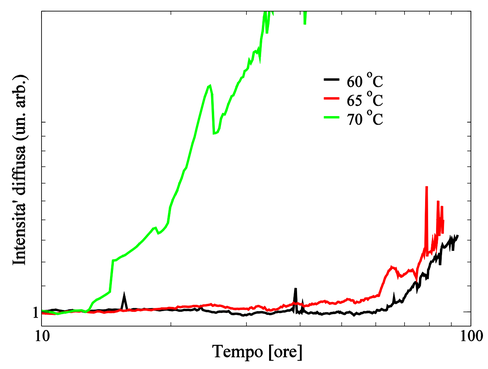


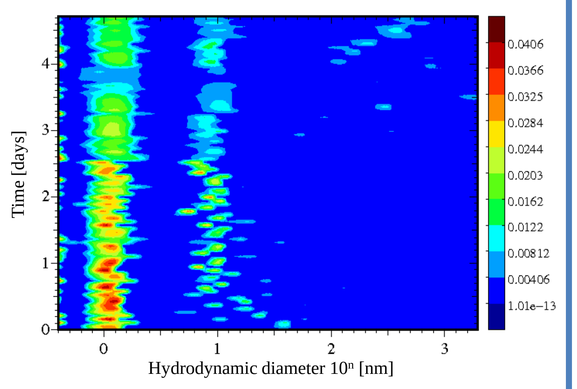
Secondary structure:
loss of α-structrure (isodichroic point at 204 nm)



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

Fibrillation kinetics by Static and Dynamic Light Scattering



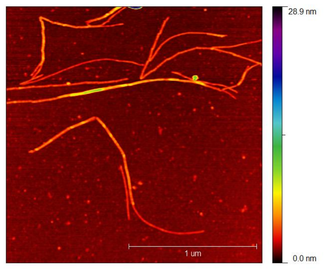


Intensity autocorrelation functions g2(t)

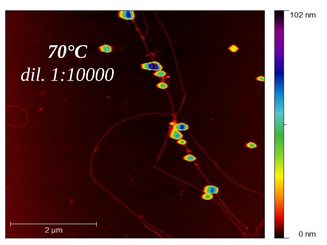
$$g_2(D) = 1 + \beta \cdot \left| \int P(D) e^{-Dq^2 t} dt \right|^2$$

diffusion coefficient distribution P(D)

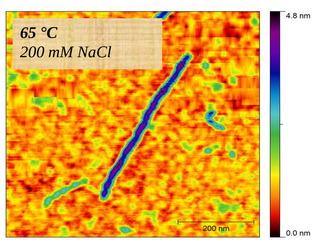
-LAG PHASE:
Coexistence of Monomers *(R_h≈1nm) and* ***Oligomers*** *(R_h≈10nm)*
AFTER A FEW DAYS
Appearence of ***Fibrils*** *and other big aggregates (R_h > 80 nm)*



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*

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