# Vibrational study of a self-assembling globular protein

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Natural bio-macromolecules are potentially good candidates for tissue engineering and drug delivery as they meet the majority of the design criteria for biomaterials. An easy way to form biocompatible hydrogels is to exploit the self-assembly of macromolecules, a certain percentage of unfolded proteins is required to promote this process [1]. During protein's denaturation, there is a change of its native structure, which is the result of interplay among different types of interactions, strongly affected by the solvent. The balance between intramolecular and protein-solvent attractions determines the equilibrium between folded and unfolded state [2, 3]. Depending on solvating conditions, the stabilization driving force can favor the folding of the protein system toward its native structure or can lead to aggregation of protein molecules [4]. The aggregation processes are characterized by different steps in which proteins undergoes conformational rearrangements and intermolecular associations to form stable structures of increasing complexity. The b-sheet motif is the precursors of amyloid fibrils, but in certain conditions, they forms small oligomers that can clusterize and originate a 3D network (hydrogel) which can retain a large amount of water. Changing the self-assembling conditions it is possible to modulate the proprieties of such biomaterials [5]. It has been found that for acid solutions of lysozyme through thermal treatments it is possible to produce transparent thermoreverisible gels. The uniqueness of this protein is that lysozyme based hydrogels are cytocompatible to living fibroblast cells, suggesting that globular protein-based hydrogels may be useful as scaffolds for tissue engineering. In this work, we have investigated the unfolding, aggregation and gelation processes of highly concentrated solution of lysozyme in denaturing conditions at different temperatures. The selected concentrated conditions have the double benefit of favoring the gelation process and mimicking the crowding condition of the cytoplasm in living cells. The use of highly concentrated solutions excludes the use of traditional spectroscopies such as UV-Vis absorption and circular dichroism. Structural properties of macromolecules and their environment can be probed in concentrated samples during the phase transformations by mean of Infrared (IR) and UV Raman Resonant (UVRR) spectroscopies. The goal of the study is to develop and standardize a methodology aimed to preparation and characterization of these systems.

**References**

[1] C. Yan, et al. Chem. Soc. Rev. **39**, 3528, (2010)

[2] P. H. J. Yancey *Exp. Biol.* **208**, 2819 (2005)

[3] G. Merlini, et al. *N. Engl. J. Med.* **349**, 585 (2003)

[4] P. Csermely *Cell. Mol. Biol. (Noisy-le-grand)* **47**, 791 (2001)

[5] A. Giugliarelli, et al. J. Phis. Chem.,**119**, 13009 (2015)