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## **ACCEPTED MANUSCRIPT**

# Combination of acoustic levitation with small angle scattering techniques and synchrotron radiation circular dichroism. Application to the study of protein solutions

Viviana Cristiglio<sup>a</sup>, Isabelle Grillo<sup>a</sup>, Margarita Fomina<sup>b</sup>, Frank Wien<sup>d</sup>, Evgenyi Shalaev<sup>c</sup>, Alexey Novikov<sup>e</sup>, Séverine Brassamin<sup>e</sup>, Matthieu Réfrégiers<sup>d</sup>, Javier Perez<sup>d</sup>, Louis Hennet<sup>e,\*</sup>

#### **Abstract**

Background: The acoustic levitation technique is a useful sample handling method for small solid and liquids samples, suspended in air by means of an ultrasonic field. This method was previously used at synchrotron sources for studying pharmaceutical liquids and protein solutions using x-ray diffraction and small angle x-ray scattering (SAXS).

Methods: In this work we combined for the first time this containerless method with small angle neutron scattering (SANS) and synchrotron radiation circular dichroism (SRCD) to study the structural behavior of proteins in solutions during the water evaporation. SANS results are also compared with SAXS experiments.

Results: The aggregation behavior of 45  $\mu$ l droplets of lysozyme protein diluted in water was followed during the continuous increase of the sample concentration by evaporating the solvent. The evaporation kinetics was followed at different drying stage by SANS and SAXS with a good data quality. In a prospective work using SRCD, we also studied the evolution of the secondary structure of the myoglobin protein in water solution in the same evaporation conditions.

Conclusions: Acoustic levitation was applied for the first time with SANS and the high performances of the used neutron instruments made it possible to monitor fast container-less reactions in situ. A preliminary work using SRCD shows the potentiality of its combination with acoustic levitation for studying the evolution of the protein structure with time.

General Significance: This multi-techniques approach could give novel insights into crystallization and self-assembly phenomena of biological compound with promising potential applications in pharmaceutical, food and cosmetics industry.

#### **Highlights**

- First measurements made by small angle neutron scattering of protein solutions using acoustic levitation
- In-situ fast kinetics of drying protein solutions
- First results from circular dichroism spectroscopy of myoglobin protein using acoustic levitation.

**Keywords:** Acoustic levitation, Small angle scattering, Circular dichroism spectroscopy, Lysozyme, Myoglobin, Aggregation

<sup>&</sup>lt;sup>a</sup> Institut Laue Langevin, 71 avenue des Martyrs, 38000 Grenoble cedex 2, France

<sup>&</sup>lt;sup>b</sup>Department of Physics and Chemistry, University of Palermo, via Archirafi 36, 90123 Palermo, Italy

<sup>&</sup>lt;sup>c</sup> Allergan Inc., 2525 Dupont Drive. Irvine, CA 92612, USA

<sup>&</sup>lt;sup>d</sup> Synchrotron Soleil, L'Orme des Merisiers, BP 48 91192 Gif sur Yvette Cedex, France

<sup>&</sup>lt;sup>e</sup> CNRS-CEMHTI, 1D, avenue de la Recherche Scientifique, 45071, Orléans cedex 2

<sup>\*</sup>Corresponding author: louis.hennet@cnrs-orleans.fr

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