Research project (PhD)

Tracking the first steps of protein aggregation

Capturer les premiers stades de l'agrégation des protéines

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Protein aggregation in organized mesoscopic structures, like fibrils, is ubiquitous in neurodegenerative diseases. However, the precise role of the fibrils, the way they grow, and what triggers their formation is still largely misunderstood. One key step in aggregation processes consists in forming the nucleus which triggers the growth. The medium in which nucleation takes place is thus inherently a mixture of multiple oligomers co-existing in a dynamic equilibrium. The complexity of this medium makes usual structural probes (NMR, crystallography, etc.) difficult, or impossible to apply. In this context, early diagnosis of the associated diseases is challenging. A popular approach to the early detection of aggregation seeds consists in exploiting their capacity to induce aggregation through intermolecular interactions. In particular, real-time quaking-induced conversion (RT-QuIC) consists in crowding the medium with high concentrations of monomeric substrate proteins. In these conditions, even very small amounts of bioactive seeds can trigger fast aggregation. Such amplification in the concentration of protein aggregates is then readily monitored by fluorescence measurements, based on the specificity of the response of chromophores, like thioflavin T (ThT), to the presence of fibrils. The specificity of such amplification approaches is nevertheless questionable. Indeed, as for natural aggregation, the triggering process is not understood. Moreover, the specificity of the fluorescent probes is debated.

We propose to investigate the early steps of protein aggregation using a combination of mass spectrometry (MS) and ion mobility spectrometry (IMS). MS is able to separate and isolate the different oligomers co-existing in the aggregation medium as a function of their mass, and IMS provides structural information on these oligomers.[1] The combination of those techniques will allow to monitor the evolution of the composition and structural diversity of the aggregation medium as a function of time and temperature. This bottom-up approach to aggregation will then provide information on the thermodynamics of aggregation at the molecular level.

As a first step, we will focus on the formation of fibrils from α-Lactalbumin, a 14 kDa protein. The aggregation process is long enough (hours) to allow for time-resolved measurements by IMS-MS, and then monitor the evolution of the size and shape distribution of the oligomers. Measurements will be done in different media, leading or not to the formation of fibrils. The observations at the microscopic level will be correlated to the structure and shape of the fully grown fibrils. The same procedure will be applied in a second stage to the characterization of fibrils obtained by the RT-QUiC technique in collaboration with the Neuroscience Research Center of Lyon.[3] A second part of the project will consist in investigating the interaction of ThT with the oligomers, in particular by correlating the time evolution of the fluorescence to the microscopic observations. Complexes of ThT with small oligomers will also be possible to investigate by IMS-MS coupled to laser excitation.

For this project, the candidate will have access to the instrumental platform developed by the specrtrobio team: high resolution IMS and MS instruments coupled to laser exitation, MALDI and ESI ion sources, and possibly charge detection mass spectrometry to investigate high mass (MegaDalton) species. Moreover, this project will benefit from the different tools available at the iLMTech platform, including high level microscopic and spectroscopic methods, for fibrils characterization.

The candidate is expected to have a minimum background in mass spectrometry and physical chemistry, and an interest for experimental works would be appreciated.

1. Bernstein, S. L. *et al.* Amyloid β-Protein: Monomer Structure and Early Aggregation States of Aβ42 and Its Pro 19 Alloform. *J. Am. Chem. Soc.* **127**, 2075–2084 (2005).
2. Le Fèvre, A., Dugourd, P. & Chirot, F. Exploring Conformational Landscapes Using Trap and Release Tandem Ion Mobility Spectrometry. *Anal. Chem.* **93**, 4183–4190 (2021).
3. Tsirkou, A, Kaczorowski, F., Verdurand, M., Raffoul, R., Pansieri, J., Quadrio, I., Chauveau, F. & Antoine, R. Charge Detection Mass Spectrometry On Human-Amplified Fibrils From Different Synucleinopathies, *Chem. Commun.* **58**, 7192-7195, (2022).