Investigation at the molecular level of the interaction of fluorescent probes with early protein aggregates

Protein aggregation is ubiquitous in many neurodegenerative diseases. Different types of fibrils may be formed in relation with each pathology. However, fibril formation mechanisms are overall still poorly understood. The first steps of aggregation involves the emergence of ill-folded proteins and the formation of the early oligomers. These early steps are particularly difficult to investigate. Optical methods involving fluorescent probes like thioflavin T or congo red A are broadly used to detect and monitor the appearance of protein aggregates: the optical properties of these fluorophores are dramatically affected upon binding to β-sheet rich structures such as fibrils. The increase of fluorescence is generally interpreted as an increase of the fibrils concentration, thus allowing to monitor the aggregation kinetics. Promising methods for the early diagnosis of neurodegenerative diseases build on this very principle.[1]

Several issues nevertheless limit the specificity and reproducibility of fluorophore-based methods. In particular, the connexion between the fibrils morphology and their chromophore binding affinity remains an open question.[2] Moreover, it is not clear whether the fluorophore properties depend on the fibril development stage (early oligomers vs. advanced fibrils).

We propose to investigate the interaction between fluorophores and small oligomers at the molecular level through an original approach based on ion mobility and mass spectrometry (IMS-MS) coupled to action spectroscopy. The interest of IMS-MS in the present context is to isolate the different oligomers present in the heterogeneous aggregation medium in order to study them separately. Namely, MS allows precise determination of the stoichiometry of the oligomers and the exact number of chromophores attached to it. In addition, IMS provides information in the conformational properties of the oligomers.[3] As fluorescence is difficult to measure in a mass spectrometer, the optical properties of the oligomer-fluorophore complexes will be probed by action-spectroscopy: **the originality of our approach consists in performing photofragmentation measurements on the oligomer-chromophore complexes in the mass spectrometer in order to characterize their optical properties in relation with their size (MS) and shape (IMS).**

As a case study, model peptide sequences displaying well-known aggregation behaviour will be investigated. Beyond the stoichiometry (from MS), and the geometry (from IMS) of the oligomer-chromophore complexes, their stability will also be investigated from collision-induced activation in the gas phase. Variable-temperature electrospray experiments are also planned in order to investigate their stability in solution. The PhD candidate will have access to high-level IMS-MS and MS instruments coupled to different laser sources available at iLM.

Hiring profile – Master degree in Physics/Chemistry with strong background in mass spectrometry and/or spectroscopy, skills for team working and a taste for experimental works! Basic knowledge in mass spectrometry and minimal programming skills would be appreciated. Fluency in English is mandatory.

Conditions and benefits – Duration of the PhD program – **36 months full time work contract** (37.5h/week)**, starting October 2024**. **Attractive salary. Funding includes travel to secondments.** Additional benefits: 47 days paid holiday leave, sick leave, parental leave, unemployment insurance.

(1) Atarashi, R. *et al.* Ultrasensitive Human Prion Detection in Cerebrospinal Fluid by Real-Time Quaking-Induced

Conversion. *Nat. Med.* **2011**, *17* (2), 175–178. https://doi.org/10.1038/nm.2294.
(2) De Giorgi, F. *et al.* Novel Self-Replicating α-Synuclein Polymorphs That Escape ThT Monitoring Can Spontaneously Emerge and Acutely Spread in Neurons. *Sci. Adv.* **2020**, *6* (40). https://doi.org/10.1126/sciadv.abc4364.
(3) Bleiholder, C. *et al.* T. Ion Mobility Spectrometry Reveals the Mechanism of Amyloid Formation of Aβ(25-35) and Its Modulation by Inhibitors at the Molecular Level: Epigallocatechin Gallate and Scyllo-Inositol. *J. Am. Chem. Soc.* **2013**, *135* (45), 16926–16937. https://doi.org/10.1021/ja406197f.

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Possible secondments within the ArchiFun consortium include complementary characterization of the oligomers by mass photometry at Institut Pasteur.

Main ArchiFun theme involved:

- ☐ Host-pathogen interactions;
- ☐ Mechanisms of bacterial resistance and cancer onsets;
- ☑ Neurodegenerative and autoimmune diseases;
- ☐ Translational research in prevalent diseases;
- ☐ Physiology and ecology;
- ☐ Neurosciences and cognition.