PI: Rita Grandori

Project title: Depicting the conformational ensemble of a-synuclein and its response to ligands by native mass spectrometry and single-molecule spectroscopy.

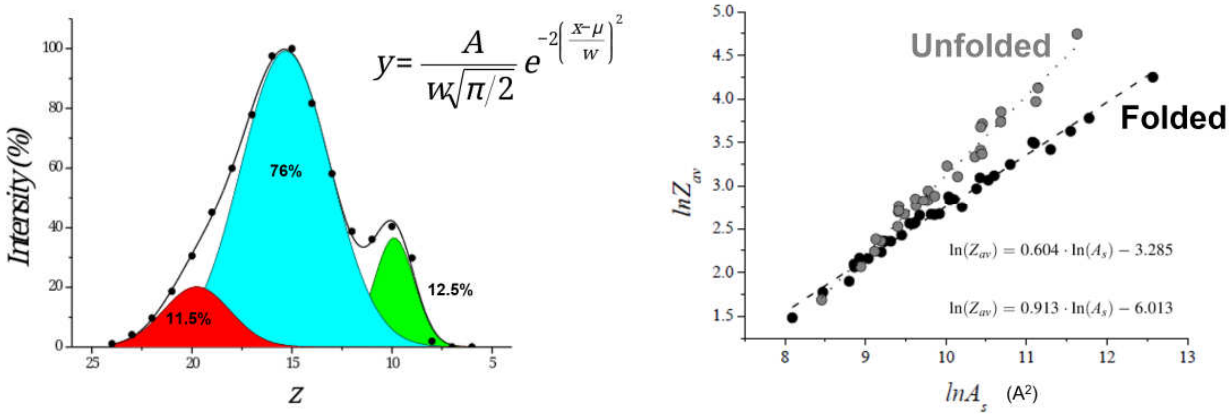
Abstract

Recently developed approaches to describe conformational ensembles by native mass spectrometry (MS) will be applied to an intrinsically disordered protein (IDP) of biomedical relevance, a-synuclein (AS). Qualitative and quantitative parameters will be extracted, such as number and relative abundance of conformers, solvent accessible surface area (SASA), compaction index (CI), and collisional cross-section (CCS) of each conformer. The response of the conformational ensembles to the presence of ligands will be assessed by titrations under controlled conditions. Metal ions and small molecules known to act as modulators of AS aggregation. The wild-type and mutant AS in their oxidized and non-oxidized forms. Binding affinity and stoichiometry will be assessed by data analysis and by testing alternative binding models. Binding sites will be mapped by top-down approaches.

Background, aims and significance of the proposed work

IDPs play important biological roles and are key targets for innovative therapeutic strategies against cancer, neurodegenerative and infectious diseases. Their structural investigation is particularly challenging, since they exist as highly dynamic conformational ensembles. Their structural and functional properties are governed by interactions with ligands, proteins, protein complexes, membranes and other biological structures, in an often unknown manner.

While conventional structural biology methods face major limitations characterizing dynamic and heterogeneous conformational ensembles, native MS has proven a powerful approach, thanks to its inherent ion-sorting mechanism, which makes it more comparable to single-molecule techniques than to bulk spectroscopic methods. In particular, AS species distributions by native-MS and atomic-force microscopy (AFM) have been compared by our group, in collaboration with Francesco Mantegazza (Milano-Bicocca), showing similar trends in the reshaping of structural disorder by ligands.



Scheme 1. *Left, Identification of distinct conformers by gaussian fitting of CSDs. Right, relation between average charge state (Z_{av}) and SASA (A_s)*

AS aggregation has been recognized as a defining feature of Parkinson's disease and several point mutations have been linked to early onset and familial forms of the disease. Mechanistic models of the action of this amyloidogenic protein in health and pathology require structural characterization of the conformational ensembles in the presence and absence of ligands, mutations and post-translational modifications.

The results will provide a combined view of the conformational and binding equilibria of these IDPs, revealing mode of binding and induced-folding processes. It will be possible to interpret the effects of ligands, mutations and post-translational modifications in terms of structural features of the conformational

ensemble. Structural parameters useful for computational modelling by experimental constraints will be obtained. Finally, the feasibility of high-throughput screening of potential ligands for a given target protein will be explored.

Experimental plan

- **WP1 (months 1-6):** *Protein in the absence of ligands.*
  The conformational components of the proteins in solution will be described by charge-state distribution (CSD) analysis. Recombinant, human AS will be provided by the collaborator Giuseppe Legname (SISSA, Trieste). The effects of solvent conditions and denaturants will be tested. SASA and CI will be assessed. Ion-mobility experiments to measure CCS will be performed in collaboration with Frank Sobott (Leeds, UK).
- **WP2 (months 7-12):** *Interactions with ligands.*
  Reported AS aggregation inhibitors will be tested. The same tools used in WP1 will be applied to monitor changes in the conformational ensemble in response to ligands, comparing mutants and wild-type. The most interesting conditions will be used to compare species distributions obtained by MS and AFM in collaboration with Francesco Mantegazza. The same approach will be followed to monitor the effects of metal ions. Binding site will be mapped by top-down structural proteomics approaches.
- **WP3 (months 13-18):** *Post-translational modifications.*
  The effect of post-translational modifications on conformational and binding properties will be investigated, comparing oxidized with non-oxidized AS, obtained by incubation with dopamine or hydrogen peroxide. Their position and identity will be mapped by bottom-up and top-down proteomics approaches.
- **WP4 (months 19-24):** *AS mutations in familial forms of Parkinson's disease.*
  Mutant AS will be provided by Giuseppe Legname. The effect of mutations on AS the conformational ensemble and binding properties will be evaluated. The most interesting systems will be considered for comparative analysis by AFM (Francesco Mantegazza).

Feasibility and financial support

- yes

Literature references

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PI recent papers related to the topic

1. Corti R. et al. Int J Mol Sci. 2019 Oct 19;20(20):5181.
2. Ponzini E. et al. J Biol Chem. 2019 Apr 5;294(14):5657-5665.
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