Dawit Negasi
Supervisor- Dr Dinesh Kumar Chinthapalli

Investigating the Role of Alpha-Synuclein Charge in Synaptic Vesicle Fusion and Neurotransmitter Release Using Mass Spectrometry

Background

α-Synuclein is a key protein in the pathogenesis of Parkinson's disease and related synucleinopathies. Its intrinsically disordered nature allows dynamic conformational changes that influence both its physiological functions and pathological aggregation. The pathological aggregation of α-synuclein are known to be modulated by lipid interactions, as these occur frequently during its normal roles in synaptic vesicle trafficking and neurotransmitter release. Such interactions can dictate whether the protein remains stabilised or misfolds into a pathogenic conformation. Electrostatic interactions are the driving forces for these interactions and are central to its biology: α-synuclein carries a net positive charge at physiological pH, while phosphoinositides (PIP_2/PIP_3) are membrane lipids that carry negative charge. We hypothesise that this opposite charge might enable a strong interaction that influences α-synuclein's aggregation, membrane association and conformational states. In addition, natural compounds such as epigallocatechin gallate (EGCG) have been reported to modulate α-synuclein aggregation. To understand these effects at the molecular level, we further investigated EGCG binding to wild-type and compared it with its mutant (H50A) α-synuclein, assessing whether the mutation alters conformational ensembles and oligomeric states.

Why is This Study Relevant

Research like this provides new insights into how α-synuclein interacts with signalling lipids such as PIP_2 and PIP_3 by capturing binding and structural dynamics in a near-native state. This has potential relevance, as disrupting or modulating lipid-protein interactions may represent a novel strategy to slow or prevent α-synuclein aggregation and disease progression. Additionally, examining α-synuclein mutants with EGCG shows how genetic variations alter binding compared to the wild type and whether mutations influence conformational states, offering deeper understanding and potential avenues for drug development while guiding future research

Aims and Objectives

- To investigate how negatively charged **PIP_2 and PIP_3** interacts with α-synuclein and alter its conformational landscape
- To understand the effect of genetic variations of α-synuclein (WT and **H50A** mutant) interactions and conformational analysis with the natural product **EGCG** under physiological conditions.

Methodology

- Recombinant **wild-type** and **H50A** α-synuclein were incubated with **EGCG, PIP_2** and **PIP_3** at desired molar ratios.
- Native mass spectrometry (nMS) was performed to detect ligand binding, and ion mobility (IM-MS) was used to resolve conformational states across charge states (10+ to 7+).
- Binding intensities were quantified by calculating **bound/apo ratios** within the same charge state
- Drift times were compared to evaluate conformational ensemble changes.

Key Findings

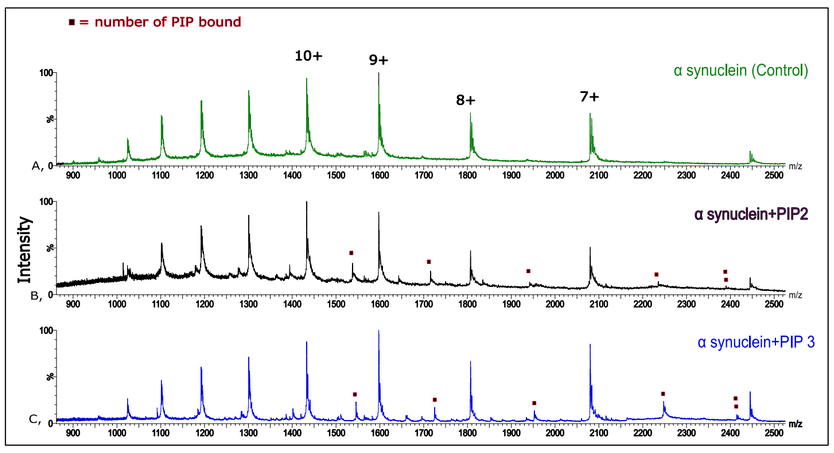

Fig 1a. Native Mass spectra of α-synuclein in absence of lipids (control and lipid presence at 1:20 (protein: lipid) molar ratio.

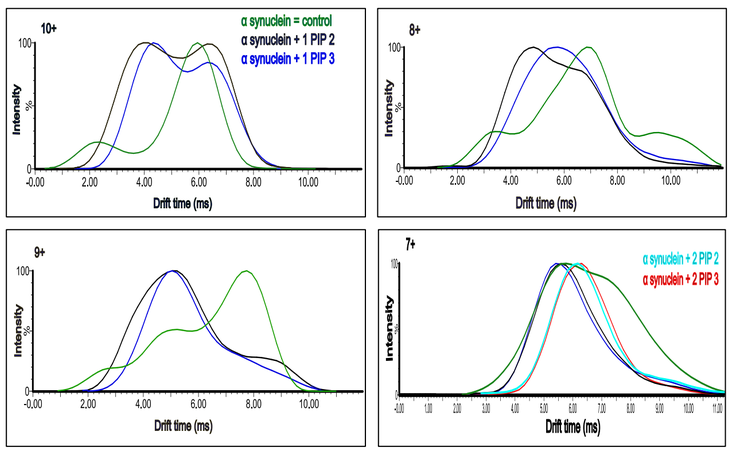

Fig 1b. Drift time plot extracted from nMS spectra of Figure1a, showing conformational effect of alpha synuclein as a result of PIP interaction across +10 to +7 charge states.

- Equal numbers of PIP_2 and PIP_3 bound states were observed across the charge state (10+ to 7+) (Fig. 1a), suggesting that the protein has the same potential binding sites for either of the lipids. However, PIP_3 showed slightly higher binding capacity, in line with its additional negative charge.
- Ion mobility analysis (Figure 1b) further revealed conformational changes upon PIP binding, indicating that in some charge states the protein acquired more extended conformations, whereas in others, the conformational ensemble remained compact or only slightly shifted relative to the apo form. This implies that PIP_2 and PIP_3 binding remodel α-synuclein's conformational landscape rather than enforcing a single structural state.

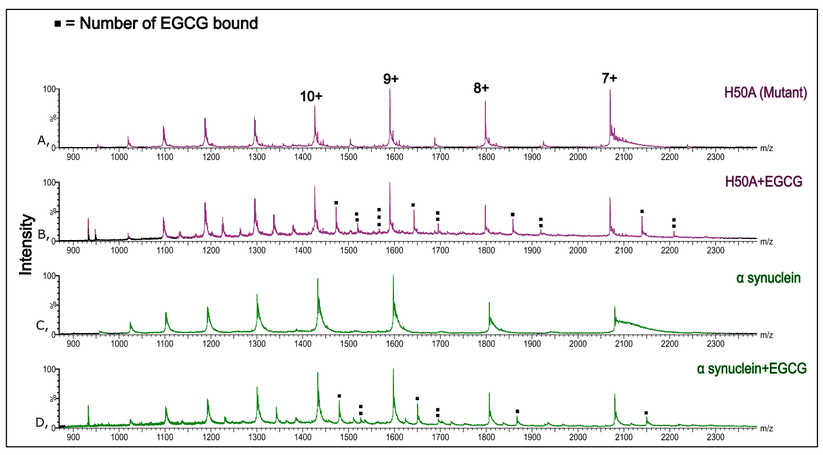

Figure 2a. Native mass spectra of both H50A and wild type alpha synuclein, recorded with and without the presence of EGCG at 1:20 (protein: lipid) molar ratio.

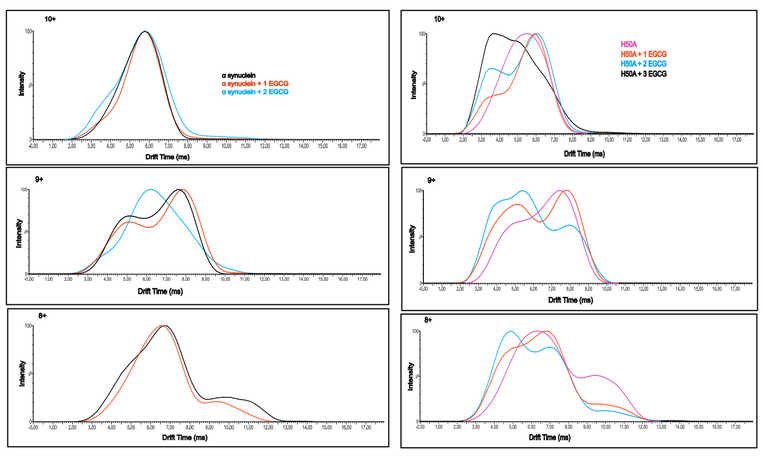

Figure 2b. Drift time plots extracted from Figure 2a, showing the conformational states acquired by wild-type α-synuclein (right) and the H50A mutant (left) upon binding to EGCG across the +10 to +8 charge states

- Wild-type, EGCG-bound peaks are detected (Fig. 2a), but they are relatively low in intensity, with the apo species remaining predominant. In contrast, the mutant showed markedly increased EGCG-bound peak intensity and, notably, multiple bound states (up to 2-3 EGCG molecules per monomer) were more prevalent in the mutant than the wild-type, indicating stronger ligand association. This suggests that mutation introduces or exposes additional high-affinity binding sites or increases the affinity of EGCG binding regions.
- In the drift time distribution (Fig. 2b), Wild-type α-synuclein displayed a modest change at 9+ charge state, introducing a compact conformational state. In contrast, the H50A mutant shows pronounced heterogeneity and formation of very compact species compared to the wild type.

Conclusion

This study highlights the importance of PIPs interactions with α-synuclein, which are likely to be driven by electrostatic interactions between the positively charged termini of α-synuclein and the negatively charged PIPs. The interaction of PIPs remodels the conformational ensembles of α-synuclein and influences its oligomerisation and aggregation pathways. Similarly, EGCG demonstrated binding to both mutant and wild-type α-synuclein, with the mutant showing stronger binding intensity, a higher number of bound EGCG species, and a greater conformational effect, indicating the potential for designing therapeutic strategies based on the specific structural changes introduced by different mutations.