

High-Resolution NMR Study of Ligand and Ca²⁺ binding to the C-Terminal Region of α -Synuclein

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Keywords: solution NMR, small molecules and biomolecules.

Nuclear Magnetic Resonance (NMR) spectroscopy is a powerful technique for characterizing the interaction between intrinsically disordered proteins (IDPs) and various binding partners at atomic resolution even in conditions approaching the physiological ones. Human α -synuclein (α -syn), an IDP implicated in several neurodegenerative diseases including Parkinson’s Disease, is composed of 140 amino acids organized into three regions: a positively charged N-terminal tail, a hydrophobic central region, and a negatively charged C-terminal region. The latter has been shown to interact with

both small molecules and metal ions.^{1–4}

In this study, we investigate the interaction between α -syn and Fasudil, a compound known to interact with the C-terminal part of α -syn and to delay the formation of its toxic aggregates.¹ We also examine the modulatory role of calcium ions in this interaction. For our study we use a construct comprising α -syn residues 112–140 (C- α -syn). High-resolution NMR spectroscopy exploring ¹³C and ¹H detection, supported by molecular dynamics simulations, enables us to characterize these interactions at the side chains level. Our study focuses on the side chains of aspartic acid, glutamic acid, and tyrosine residues, which play a key role in Fasudil and calcium ions binding. We analyze their behavior in the presence and absence of calcium ions to elucidate calcium’s influence in this process (Figure 1)

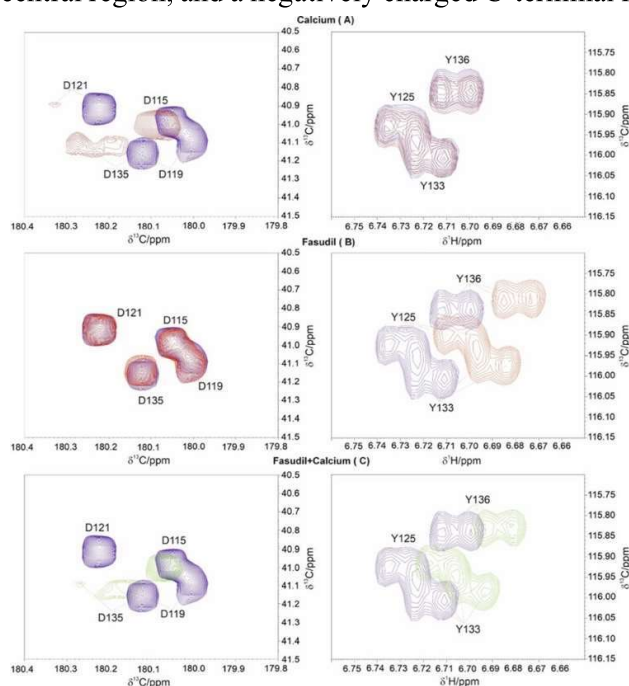


Fig. 1. Zoomed-in CACO spectra (left) highlight aspartate side chain resonances ($C^{\gamma}-C^{\beta}$); HC-TROSY spectra (right) show the $H^{\epsilon}-C^{\epsilon}$ region. The free form of C- α -syn is always shown in blue. Panel A, B and C show the spectra upon addition of calcium, Fasudil and Fasudil + calcium in red,

References

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Acknowledgements

We thank #NextGenerationEU (NGEU) and the Italian Ministry of University and Research (MUR) for funding under the National Recovery and Resilience Plan (NRRP) projects “A Multiscale integrated approach to the study of the nervous system in health and disease” (MNESYS Grant PE0000006), “Potentiating the Italian Capacity for Structural Biology Services in Instruct-ERIC” (ITACA.SB, Grant IR0000009), and “Tuscany Health Ecosystem” (THE, Grant ECS0000017). We also acknowledge financial support from MUR through the DM 118/2023 PhD program.