STRUCTURAL ANALYSIS OF CHALLENGING TARGETS: HYBRID METHODS AND MORE

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Machado Joseph Disease (MJD) is the autosomal dominantly-inherited ataxia with the highest prevalence among the Portuguese population, and it was originally described in people of Portuguese Azorean descent. The disease is caused by an expansion of a repetitive (CAG) tract within the ATX3 gene, which is translated into a polyglutamine (PolyQ) repeat in the Ataxin-3 protein. Ataxin-3 (Atx3) is a modular protein with an N-terminal globular Josephin domain (JD), followed by an extended tail composed of two ubiquitin interacting motifs (UIMs), an expandable polyglutamine (polyQ) tract, and a variable C-terminal region, which in some variants includes an additional UIM [1]. In result of its modular multidomain architecture, Atx3 is known to engage in multiple intra- and intermolecular interactions [2,3], which might be unbalanced when the polyQ tract is expanded, culminating in aggregation and formation of intracellular inclusions, a unifying fingerprint of this group of neurodegenerative disorders.

Structural characterization of ataxin-3 at atomic resolution is critical to further understand the physiological function of this protein and to develop novel compounds that can prevent aggregation and pathogenesis occurring upon pathogenic polyQ expansion. The tendency of the protein to self-assemble, independently of the polyQ segment [2], has so far constituted a major bottleneck that has largely prevented the growth of diffraction-quality crystals. Recently, we have identified a native ataxin-3 macromolecular interactor that drastically reduces its propensity to form amyloid fibers. This study highlighted the relevance of identifying native and non-native ataxin-3 interacting proteins/compounds with the ability to compete with ataxin-3 self-assembly, thereby acting as chaperone-like molecules. With this purpose, the Instruct platform “Nanobodies4Instruct” was critical for the design and development of specific binding molecules with the ability to selectively recognize different ataxin-3 domains. The thorough biophysical characterization of the anti-aggregation properties of ataxin-3 binding molecules requires a transdisciplinary approach and the utilization of hybrid methods, some of which are only accessible through the Instruct platforms. We expect that the identified modulators of Atx3 aggregation can be used not solely as chaperone-like molecules for ataxin-3 structural studies, but also as the basis for future therapeutic compounds.

References:

[1] Almeida, B., Fernandes, S., Abreu, I.A., Macedo-Ribeiro, S. (2013). Trinucleotide repeats: a structural perspective. Front Neurol.4:76.

[2] Gales, L. et al., and Macedo-Ribeiro, S. (2005). Towards a structural understanding of the fibrillization pathway in Machado-Joseph's disease: trapping early oligomers of non-expanded ataxin-3. J Mol Biol. 353:642-54.

[3] Almeida, B., Abreu, I.A., Matos, C.A., Fraga, J., Fernandes, S., Macedo, M.G., Gutiérrez-Gallego. R., Pereira, P.J., Carvalho, A.L., Macedo-Ribeiro, S. (2015). SUMOylation of the brain predominant Ataxin-3 isoform modulates its interaction with p97. Biochim Biophys Acta. 1852(9): 1950–59

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