α-synuclein/hDAT interaction: a new target against Parkinson's Disease

Parkinson's disease (PD) is a neurodegenerative disorder characterized by a loss of dopamine (DA) neurons. Currently symptomatic management exists using the dopamine precursor Levodopa, however, in the long-term this treatment induces levodopa-induced dyskinesias (LIDs). Thus new strategies of molecular intervention are critically needed to identify new therapies against PD.

DA neurons of PD patients feature insoluble deposits, Lewy bodies, primarily formed by aggregates of a small neuronal protein named α-synuclein (αS). It is now established that the aggregation of αS generates neurotoxic species impairing the cellular viability [1].

While the function of αS is unknown, recent *in vivo* evidences indicate that it may be a regulator of the human dopamine active transporter (dDAT) [2-4]. Moreover, alterations of this interaction promote early and late PD-related symptoms.

Using an interdisciplinary approach across structural and cellular biology this proposal aims at characterising the mechanisms of regulation of hDAT by αS, under normal and pathological conditions.

The project will start from the development and optimisation of a biological platform to study hDAT /αS interaction. hDAT over-expression will be obtained in hek293T cells using both transiently and stably hDAT overexpressing clones [7,8]. These clones will also contribute to relate hDAT intracellular levels to the overall cytotoxic effect of αS. αS-hDAT interactions in the cell and in reconstituted membranes will be studied at high resolution using state-of-the-art methods of biomolecular NMR pioneered in the De Simone lab [1,5,6]. NMR will also inform the design of hDAT mutants with reduced or impaired binding to αS, which will be tested in hek293T cells to refine the mechanism of interaction [9]. Our ultimate target is to use this platform to identify new molecular strategies to suppress the aberrant αS-hDAT interaction under pathological conditions.

We have preliminary tested the expression of hDAT in hek293T cells, whereas monomers, oligomers and fibrils of αS are regularly studied in the De Simone lab [1]. These preliminary data indicate that, while highly ambitious, the project has significant chances to obtain transformative results from the PhD work.

References

1. Fusco et al De Simone, ***Science***, 2017, 358:1440-3
2. Giordano et al, ***Brain***, 2018, 141:505-20
3. Durante et al,***Brain***, 2019,142:1365-85
4. Tozzi et al, ***Brain***, 2021, 144:3477-91
5. Fusco et al De Simone, ***Nature Communications***, 2016, 7:15623
6. Sanz-Hernandez et al De Simone, ***PNAS***, 2021, 118:e2019631118
7. Radner et al. Stornaiuolo ***Journal of Structural Biology***, 2012 vol. 179, p. 46-55, ISSN:
8. Stornaiuolo et al., ***Nature Communications***, 2013 vol. 4, ISSN: 2041-1723
9. Generoso et al Stornaiuolo ***Nature Chemical Biology***, 2015 vol. 11, p. 280-6-286