A high throughput direct-to-biology screening platform for LRRK2 degraders

Ayomipo Adegeye[1], Francesca Cavallo[1], Roland Hjerpe[1], Benoit Gourdet[1]

[1] Sygnature Discovery, Bio City, Pennyfoot St., Nottingham NG1 1GR, UK
adegeye_ayomipo@yahoo.com

Parkinson's disease (PD) is the second most common neurodegenerative disease affecting about 10 million people globally and characterized by the progressive degeneration of dopaminergic neurons in the midbrain resulting in chronic motor disabilities such as tremor, rigidity and bradykinesia. With PD being heavily linked to genetic alterations in about 10 % of cases, mutations in the leucine-rich repeat kinase 2 (LRRK2) gene, especially G2019S variant, represents the most common monogenic cause of PD. The resulting gain of kinase function mutation strongly proposes LRRK2 as a promising kinase drug target for treatment of PD, and LRRK2 degradation by PROTACs as an interesting therapeutic strategy, since protein degradation essentially circumvents accumulation of inhibited LRRK2, thus reducing undesirable side effects caused by standard kinase inhibitors. Recently, the BBB-penetrant VHL-based LRRK2 degrader - XL01126, developed by Ciulli lab, was described as demonstrating potent degradation in multiple cell lines and the selective and potent CRBN-based LRRK2 degrader - ARV-102, developed by Arvinas, was progressed into Phase 1 clinical trials. In this study, we have developed a high throughput compound screening cell-based platform to assess, identify and characterize hit compounds from a PROTAC library of new compounds synthesized at Sygnature Discovery using plate-based chemistry. This screening approach, via Sygnature's proprietary CHARMED® platform, with reference VHL-based tool compound XL01126 as control, afforded two promising CRBN-based LRRK2 degraders (3994-85, 10 nM and 3994-86, 29 nM) using both HTRF and AlphaLISA immunoassays to measure the effect of compounds on LRRK2 degradation in A549 cells. Furthermore, we are currently optimizing and validating key hit compounds in cell-based and neurologically relevant assay models using a plate-based direct-to-biology approach with HTRF/AlphaLISA immunoassays to measure LRRK2 readouts.

[1] X. Liu, A.F. Kalogeropulou, A. Ciulli *et al., J. Am. Chem. Soc.,* **2022**, 144(*37)*, 16930-16952.
[2] C. Bouvier, R. Hjerpe, F. Cavallo *et al., Cells,* **2024,** 13(*7*), 578.

My current research theme in invitro neuroscience focuses on identifying and characterizing hit compounds capable of degrading LRRK2, a protein implicated in Parkinson's disease. This entails developing and optimizing a neuronally relevant cell-based compound screening assay to identify, validate and characterize potent compounds from a compound library of synthesized PROTACs. This requires rational selection of the cellular assay model based on significant LRRK2 expression, preservation of the disease signalling pathway and assay format optimization to generate robust biological data that demonstrates a compound-specific degradative effect on LRRK2 with the design and development of secondary, counter and orthogonal screens. The copious data generated from these experiments by myself and other collaborative lab scientists are combined and carefully evaluated to determine the most active hits based on a cut-off criteria that demonstrates reproducible pharmacological effect on LRRK2. Inferential analysis is employed to suggest improvements in both the assay protocol and the rationally designed compounds to generate more potent hits with improved physicochemical, pharmacokinetic and toxicity profiles. This iterative cycle aims to optimize the structure-activity relationship of tested compounds to fit set compound desirable criteria.

Knowledge gained from this workshop in AI/ML models will be instrumental to my project and career path as ML algorithms will make it easier to analyse volumes of generated data from my experiments and the collaborative lot to quickly identify trends/patterns in choosing and optimizing cellular models for performant assays that best suits the disease and target of interest. The aggregation of pharmacological (selectivity, specificity, potency, binding affinity and stabilization of PROTAC ternary complexes) as well as pharmacokinetic and toxicology data will be better harnessed by a machine learning model to predict which compounds will be potentially good hits at the early stage of drug discovery to minimize drug attrition, thereby speeding up the drug discovery process.