Poster

Protein Targeting to Glycogen (PTG), a validated target in Lafora disease

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The Protein Targeting to Glycogen (PTG) facilitates the dephosphorylation and activation of glycogen synthase (GYS) by directing the protein phosphatase (PP1) to glycogen. In the brain, PTG undergoes ubiquitination via the Laforin/Malin complex, leading to its degradation and resulting in minimal glycogen levels in neurons and reduced levels in astrocytes [1]. Mutations in either Laforin or Malin protein are linked to Lafora disease (LD), a rare and fatal neurodegenerative disorder typically emerging during adolescence and progressing over years, resulting in dementia and a vegetative state due to the accumulation of insoluble glycogen in the brain [2].

Studies in mice have demonstrated that PTG knockout results in a considerable reduction in insoluble polyglucosans and resolution of neurodegeneration and myoclonic epilepsy symptoms [3]. The elucidated structure of PTG's carbohydrate-binding domain (CBM) and its complex with PP1 [4] have advanced research, allowing for drug discovery studies. Identifying the most effective interacting molecule and iteratively enhancing it into larger and more potent binders could indeed lead to the development of a PTG degrader aimed at restoring normal PTG proteostasis and glycogen homeostasis.

The CBM's site displays a low micromolar affinity (KD) and is considered non-druggable due to its polar nature. However, the performed molecular dynamics simulations and pocket detection analyses revealed two additional pockets with suboptimal druggability. Given PTG's role as a scaffold protein, these sites are likely expected to be within transient protein-protein interacting regions. Moreover, one site transiently exposes a cysteine residue, indicating potential targeting with covalent fragments. Taking advantage of our optimized production and crystallization protocol, which yielded two well-diffracting PTG crystal forms, we applied the reverse chemogenomic strategy and conducted a crystallographic fragment screening. The XChem screen [5] allowed us to identify first hits for further optimization, leading to considerations for developing functional PROTACs by coupling the PTG-binding moiety with E3 ligase recruiters. Protein crystals were exposed to high concentrations of small molecules from two sub-libraries to analyse chemical-biological interactions, observed through electron density maps. Further refinement of promising fragments is ongoing, with plans for a full screen involving approximately 1000 fragments and subsequent follow-up.

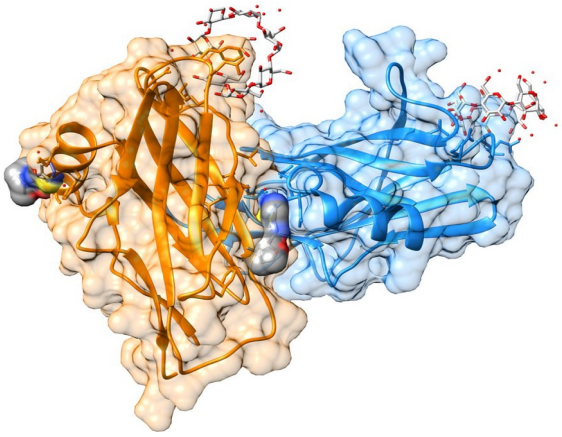

Figure 1. PTG's CBM crystallographic structure with a first positive hit.

[1] Worby, C.A., et al. (2008). Malin decreases glycogen accumulation by promoting the degradation of protein targeting to glycogen (PTG). *J Biol Chem*. **283**(7), 4069-76.
[2] Turnbull, J., et al. (2016). Lafora disease. *Epileptic Disord.* **18**(S2), 38-62.
[3] Turnbull, J., et al. (2014). PTG protein depletion rescues malin-deficinet Lafora disease in mouse. *Ann. Neurol.* **75**, 442-6.
[4] Semrau, M.S., et al. (2022). Molecular architecture of the glycogen-committed PP1/PTG holoenzyme. *Nat. Comm.* **13**, 6199.
[5] Douangamath, A., et al. (2021). Achieving Efficient Fragment Screening at XChem Facility at Diamond Light Source. *J Vis Exp*. **29**, 171.