Toward understanding the molecular architecture of a glycogen-committed PP1/PTG holoenzyme: a putative target for Lafora Disease

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Lafora disease (LD), a fatal incurable neurodegenerative disorder characterized by teenage-onset progressive epilepsy, due to mutations in the EPM2A and EPM2B genes encoding laforin and malin, respectively. The pathophysiological status is correlated to the accumulation of insoluble polyglucosans in brain, determined by an increased glycogen production. In healthy brain cells, the malin/laforin E3-ligase complex regulates, among others, the degradation of PTG (Protein Targeting to Glycogen), a scaffolding protein involved in the activation of glycogen synthesis. PTG carries the type 1 protein phosphatase (PP1) to its substrates: glycogen synthase (GYS) and glycogen phosphorylase (PYG). At the molecular level, loss-of-function mutations in laforin or malin cause PTG accumulation, leading to abnormal glycogen aggregation and formation of neurotoxic Lafora bodies (LB) that drive neurodegeneration.

Based on knock-out experiments in LD mice models, PTG is a validated target for LD [2]. Using a reverse chemogenomic approach we underwent in identifying the molecular structure of PTG and its interaction with PP1 in order to select hit compounds to indirectly block glycogen accumulation in neuron. Moreover, we are interested in defining the molecular architecture of the macromolecular complexes involved in LD insurgence and the effects of pathogenetic mutations on such architecture.

Here, we will present the x-ray crystal structures of the PTG carbohydrate binding motif 21 (CBM21) and of PTG/PP1 in complex with carbohydrates and the structural and biochemical analyses performed to investigate the PTG-mediated PP1 recruitment to glycogen. These studies resulted in identifying: (i) an unusual combination of recruitment sites, (ii) their contributions to the overall binding affinity, and (iii) the conformational heterogeneity of this complex through in-solution SAXS analyses [3].

Starting from these initial achievements we are now pursuing with different strategies aiming at identifying small molecules halting and possibily reversing the course of Lafora disease. A detailed report of the methods and strategies applied to progress toward our task, will be presented together with a perspective of the ongoing efforts to solve the criticalities encountered.

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