

Combining Machine Learning and *in vitro* approaches to identify potential chaperones for lysosomal storage diseases

Ana C. Puhl, Jennifer J. Klein, Daniel Foil, Kimberley M. Zorn and Sean Ekins

Collaborations Pharmaceuticals, Inc. 840 Main Campus Drive, Lab 3510, Raleigh, NC 27606, USA.

Lysosomal storage diseases (LSD) cause severe disability and have a devastating effect on quality of life. The current standard of care for the majority of LSD is enzyme replacement therapy (ERT) while gene therapies are under development. Neurodegenerative changes in the central nervous system are a major problem in several LSDs and cause severe disability and behavioral disturbances. The future of LSD therapy may lie in small molecules acting as agents for enzyme-enhancement therapy (EET). EET employs small molecules as 'pharmacological chaperones' to rescue misfolded and/or unstable mutant enzymes or proteins that have residual function. EET also offers the possibility of treating neurodegenerative lysosomal disorders since these small therapeutic molecules may cross the blood-brain barrier. We have developed a platform approach for small molecule drug discovery for LSD targets including CLN1 and CLN10 Batten Disease, Sialidosis, Fucosidosis and Multiple Sulfatase Deficiency (MSD). Our approach involves employing machine learning techniques to build models using data publicly available (ChEMBL, PubChem) and our Assay Central software to find new molecules that can bind to these targets. The molecules identified computationally are subsequently tested *in vitro* using enzymatic assays, isothermal titration calorimetry, differential scanning fluorimetry (DSF) and microscale thermophoresis (MST). After validation, we evaluate the effect of small molecule therapy in primary cells derived from patients with the respective LSD in terms of measurement of enzyme activity and the level of substrate accumulation. In cases where data is not available for the disease of interest, we screen a library of compounds to generate data to feed into Assay Central to build the models to discover molecules with improved activity. Using this approach, we have identified several small molecules for CLN1, MSD and Sialidosis disease which are currently being tested in patient cell lines.