Correcting protein misfolding with Structurally Targeted Allosteric Regulators: Applications in rare diseases and brain therapeutics

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ABSTRACT

Insufficient levels of a particular protein's activity - due to deleterious mutations or to more systemic effects -are the cause of a number of genetic rare diseases with CNS manifestations and other severe brain pathologies. Direct, in-situ, recovery of protein function with brain-penetrant drugs represents an ideal therapeutic strategy, as it attacks the root of the disease with all the advantages of small-molecule drugs (e.g. oral administration, distribution to all tissues). However, discovery of molecules that cause a gain-of-function effect is riddled with difficulties, and existing examples were mainly discovered using random approaches. At Gain Therapeutics, we have developed a comprehensive platform to rationally discover Structurally Targeted Allosteric Regulators (STAR). STAR molecules bind to a protein of interest, preventing misfolding and, thus, increasing its total amount and activity levels in the cell. Discovery starts with a proprietary structure-based computational approach that allows us to identify druggable allosteric sites and characterize their binding preferences. Then, we perform in silico screening of multi-million compound collections, leading to the selection of a few tens of compounds that will be experimentally tested. Here I will outline our computational approach and showcase successful applications, including the discovery of STAR molecules for the treatment of GBA-associated Parkinson's Disease. Our molecules combine excellent pharmacokinetic and toxicological profile with increase of GBA activity, substrate deaccumulation and reduction in the levels of phosphorylated α-synuclein.

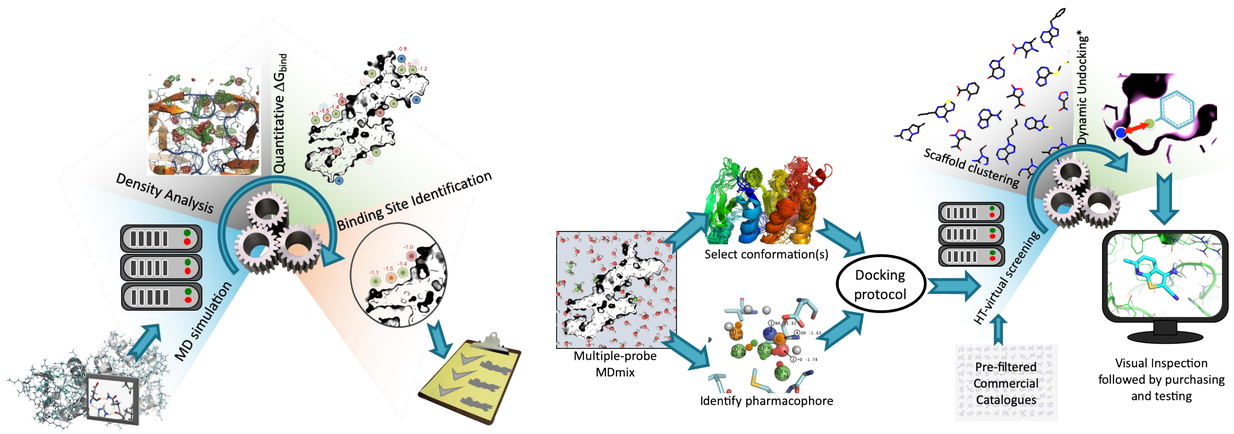
THE TECHNOLOGY

1) Identification of a new allosteric binding sites

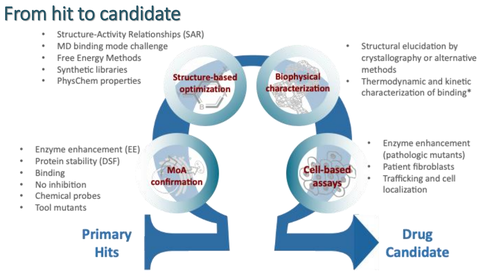
- A good quality 3D structure of a protein target is the only required input
- Molecular dynamics simulations of the protein in organic-aqueous solvent mixtures (MDmix) reveal druggable cavities.
- MDmix also identifies key interaction sites (binding hot spots), which are used as pharmacophoric restraints to guide docking
- MDmix is also used to explore the conformational flexibility of the binding site, and can identify cryptic pockets.

2) Hit ID by Virtual Screening

- A virtual collection of >6 million commercially-available compounds are evaluated computationally with the docking program rDock using the standard scoring function, pharmacophoric restraints and a high-throughput protocol.
- Best scoring compounds are subjected to Dynamic Undocking (DUck), to remove false positives
- Visual inspection and clustering methods are used to select a final set of 50-100 diverse compounds.



3) From hit to candidate

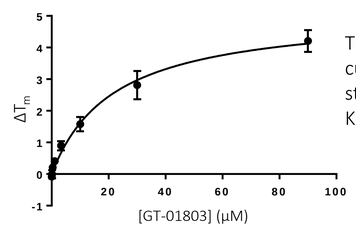


- Compounds are purchased an tested in a DSF assay. Typical hit rates are >10%
- Initial hits are validated by SAR exploration (DSF) + orthogonal assays
- Secondary assay: enzyme enhancement in patient-derived cell lines
- Hits should not inhibit at concentrations showing enzyme enhancement
- Medicinal chemistry: Optimizable and patentable series
- BBB penetrant (MW <400 Da, lipophilicity logP =3-5, rotatable bonds <5)

STAR MOLECULES - IDUA (MPS1)

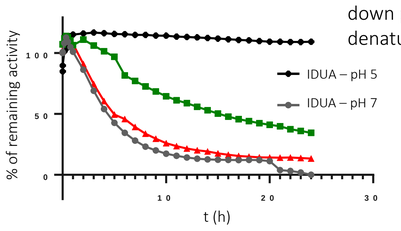
1) Allosteric stabilization of the purified enzyme

GT-01803 increases IDUA thermal stability



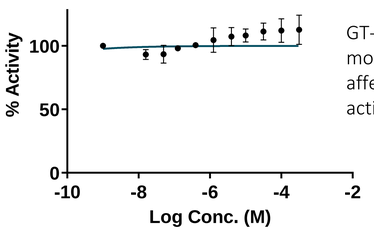
Thermal shift dose-response curve. Compound GT-01803 stabilizes rhIDUA
K_D = 22μM

GT-01803 prevents IDUA denaturation



GT-01803 (30μM) slows down pH-induced denaturation of rhIDUA

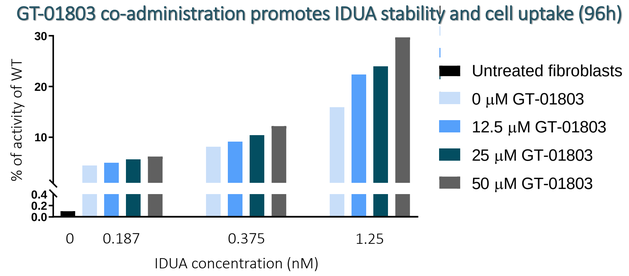
GT-01803 does not inhibit IDUA



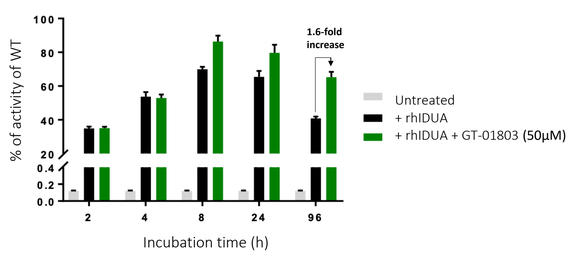
GT-01803 is a silent allosteric modulator that does not affect the intrinsic enzymatic activity of rhDUA.

2) Enhanced cellular activity in combination therapy

GT-01803 co-administration promotes IDUA stability and cell uptake (96h)



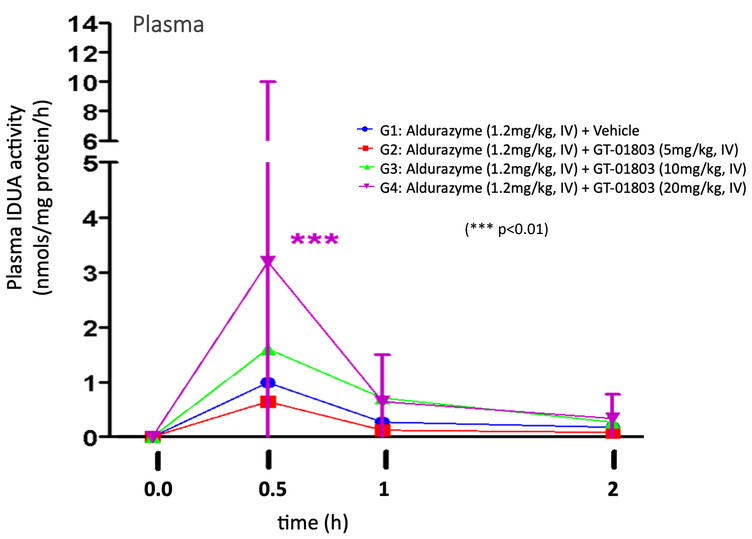
GT-01803 beneficial effect increases at longer incubation times



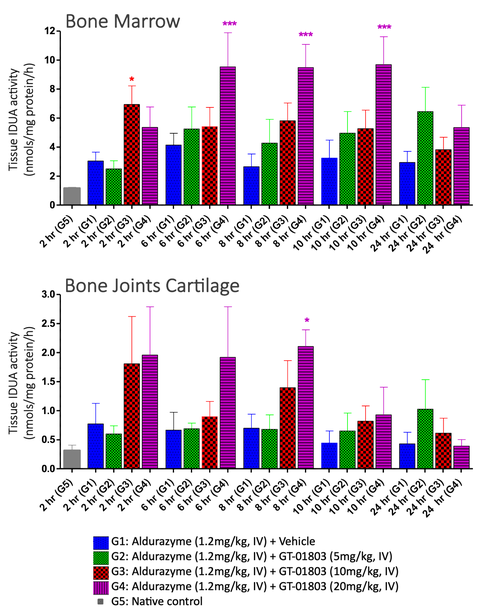
IDUA activity increases in a dose-dependent manner after the addition of rhIDUA to the culture medium in Hurler-Scheie fibroblasts (Basal activity: 0.23) Co-administration with GT-01803 shows a marked increase of IDUA cell activity (vs. single agent) at three different concentrations and in dose-dependent manner. The cell-based EC_{50} (13 μM) is in good agreement with the K_D by DSF (22 μM).

3) In vivo: combination therapy improves PK and tissue activity of Aldurazyme

GT-01803 co-administration improves the PK profile of Laronidase



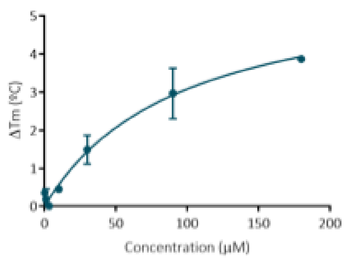
GT-01803 co-administration increases Laronidase tissue exposure



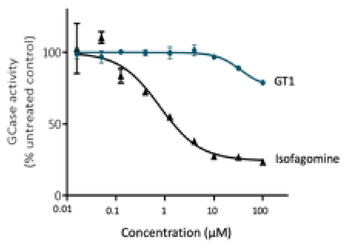
STAR MOLECULES - GBA (Gaucher / Parkinson)

1) Biophysical, biochemical and cell-based activity

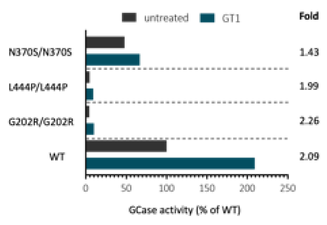
GT1 increases GBA thermal stability



GT1 is a non-inhibitory ligand

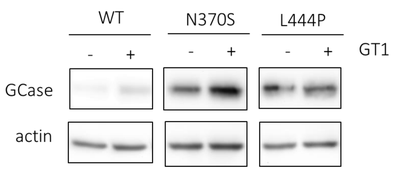


GT1 induces Enzyme Enhancement in fibroblasts

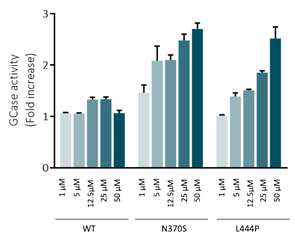


2) Activity on dopaminergic neurons (WT and PD-associated mutations)

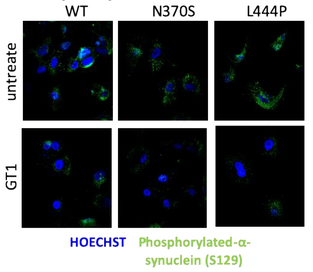
GT1 increases GBA levels



GT1 increases GBA activity



GT1 prevents P-alpha-synuclein accumulation



CONCLUSIONS

- Our structure-based computational methods make it possible to discover small drug-like molecules that bind to novel allosteric sites.
- These allosteric binders present excellent phys-chem properties for drug development.
- We have validated the technology on multiple rare disease programs with an important neurological component.