

Use of the SEE-Tx[®] Drug Discovery Platform to Identify Pharmacological Chaperones for Glutaric Acidemia Type 1

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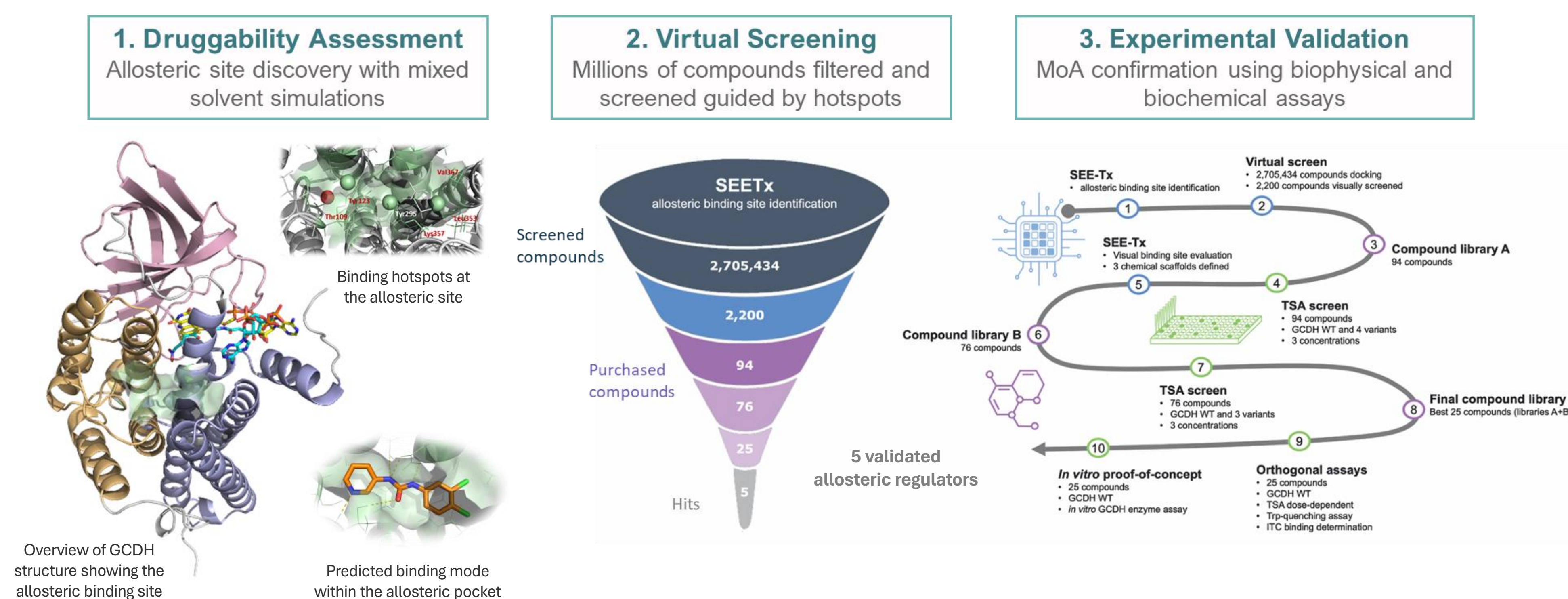
Background

Glutaric acidemia type 1 (GA1) is a rare inherited metabolic disorder caused by a deficiency of glutaryl-CoA dehydrogenase (GCDH), affecting 1 in 125,000 to 1 in 250,000 newborns. Current treatment challenges include strict dietary management, emergency interventions during metabolic crises, and limited efficacy in preventing neurological complications in about 15% of patients.

Pharmacological chaperones are small molecules that can bind to and stabilize misfolded proteins, potentially rescuing their function. In GA1, these chaperones could stabilize mutant GCDH enzymes, increasing their activity and reducing toxic metabolite accumulation. This approach offers a targeted therapy that may complement existing treatments and improve long-term outcomes for GA1 patients.

AIM: Identify allosteric regulators for the enzyme glutaryl-CoA dehydrogenase (GCDH) using SEE-Tx technology.

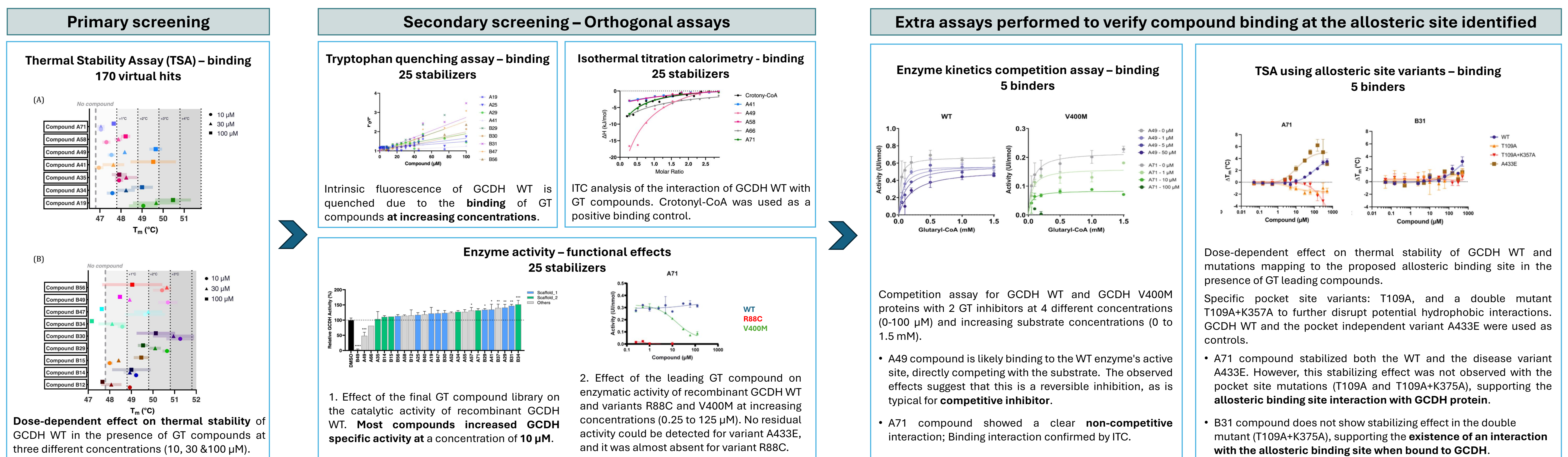
SEE-Tx[®] Drug Discovery Platform



SEE-Tx's physics-based technology utilized the published 3D structure of GCDH to identify an allosteric binding site and predict its druggability.

This guided a high-throughput virtual screening, using a unique automated methodology to explore commercial compound libraries. Through this computational approach, we identified and purchased structurally diverse small molecules as virtual hits.

This was followed by a step-by-step experimental validation approach using thermal shift assays and additional orthogonal biophysical and biochemical assays, which allowed the identification of 5 validated allosteric regulators of GCDH.



A two-step biophysical screening approach was employed, using a thermal shift assay (TSA) as the primary screen to monitor the thermal denaturation of GCDH WT and four variants in the absence and presence of 170 compounds. To further characterize the top 25 stabilizing hit compounds, a series of orthogonal assays, including tryptophan quenching, isothermal titration calorimetry (ITC), and enzyme activity assays, were conducted. These assays led to the identification of 5 novel binders for the GCDH protein. To confirm the effect of these five lead compounds and validate the allosteric pocket, two additional approaches were implemented: enzyme-substrate competition assays and compound stability assays after pocket mutation.

Conclusions

- SEE-Tx computational platform successfully identified a novel druggable allosteric site in GCDH, the enzyme deficient in GA1.
- Through virtual screening and experimental validation, five lead compounds were discovered that bind to and stabilize GCDH, demonstrating concurrent effects on enzyme activity, protein stabilization, and binding.
- These identified allosteric regulators show potential as pharmacological chaperones for GA1, offering a promising starting point for developing new therapies that address protein misfolding without competing with the natural substrate.

Bibliography: *J. Med. Chem.* 2024, 67, 19, 17087-17100

