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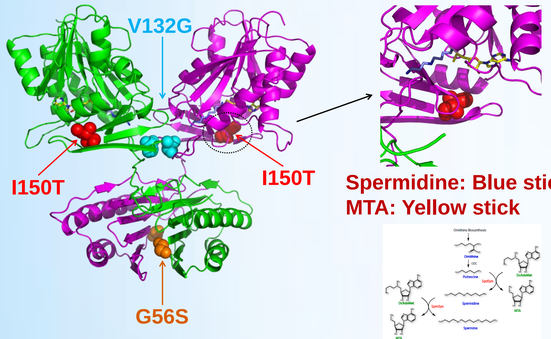
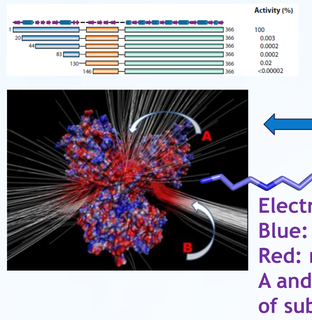
Abstract

Snyder-Robinson Syndrome (SRS) is an X-linked mental retardation disorder. Three missense mutations (G56S, V132G and I150T) on human spermine synthase (SMS) were reported to cause SRS. SMS is an important enzyme which converts spermindine into spermine, both of which are two polyamines controlling the normal cell growth and development. *In vitro* experiments showed that the dimer conformation played a crucial role on the SMS function. Our *in silico* studies including energy calculation, pKa calculation and molecular dynamics (MD) simulation based on the available 3D structure of SMS revealed that these mutations affected SMS function by affecting the dimer affinity, monomer stability or hydrogen bond network. One of the above sites, G56S, is accessible from the water phase, thus it provides the opportunity to rescue the disease-causing effect by binding an appropriate small molecule to the vicinity of the mutation site. Currently we run MD simulation to generate multiple receptor conformations and identified two potent binding pockets. Then two programs, Surflex and Autodock Vina, were applied for structure-based virtual screening (SBVS) and a consensus list of about 200 common compounds selected by both of the programs was created, and these compounds were tested experimentally by our collaborators.

Spermine Synthase





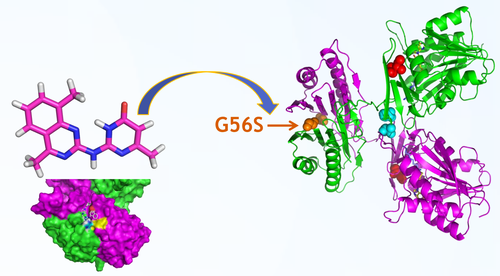
In vitro experiments

In silico explanation

Spermidine: +3e

Electrostatic field map:
Blue: positive charged region;
Red: negative charged region;
A and B are two possible pathways of substrate spermidine.

How to Rescue the Effect



MD simulation to identify the conformations having good cavities

↓

Asinex; ChemBridge; ChemDiv; LifeChemicals;
Total: ~ 300,000 molecules

↓

Consensus list from Surflex and Autodock Vina

↓

MD simulation again and free energy calculation

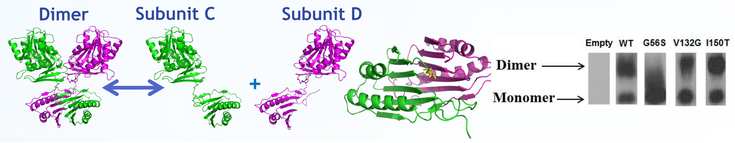
↓

In vitro experiments to verify our selections

Binding Free Energy

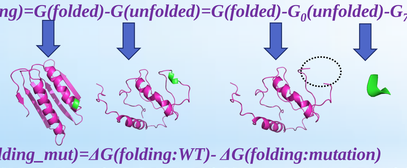
$$\Delta\Delta G(binding) = \Delta G(dimer) - \Delta G(C) - \Delta G(D);$$
$$\Delta\Delta\Delta G(mut) = \Delta\Delta G(binding:WT) - \Delta G(binding:mutant);$$

where ΔG(dimer) is the potential energy of the dimer;
ΔG(C) and ΔG(D) are that of the monomers;
ΔΔG(binding) is the binding free energy of the dimer;

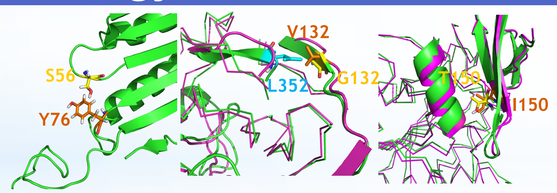


Folding Free Energy

$$\Delta G(folding)=G(folded)-G(unfolded)=G(folded)-G_0(unfolded)-G_7(unfolded)$$

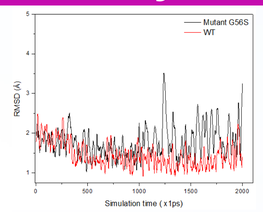


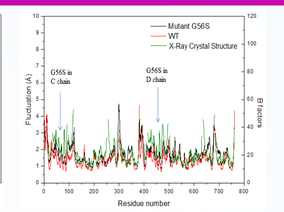
$$\Delta\Delta G(folding\_mut)=\Delta G(folding:WT)-\Delta G(folding:mutation)$$
$$G(folded:WT)-G7(unfolded:WT)-G(folded:mutation)+G7(unfolded:mutation)$$



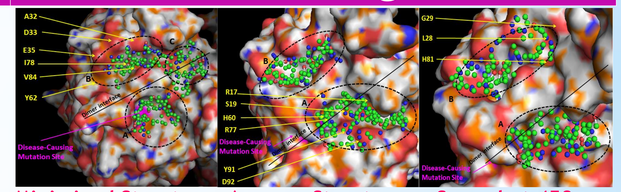
Mutant G56S Mutant V132G Mutant I150T

Reliability of MD Simulation





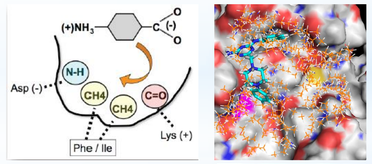
Potential Binding Pockets



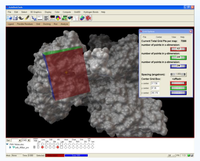
Minimized Structure Average Structure Snap shot 458ps

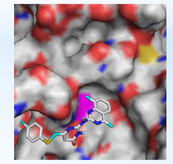
Surflex vs. Autodock Vina

Surflex

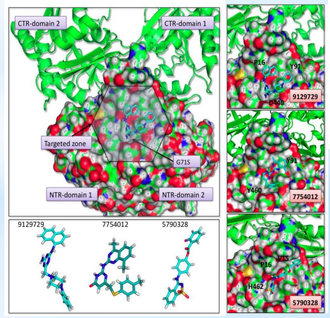


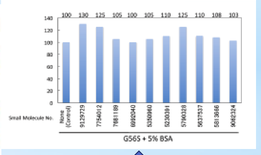
Autodock Vina





Selected Residues

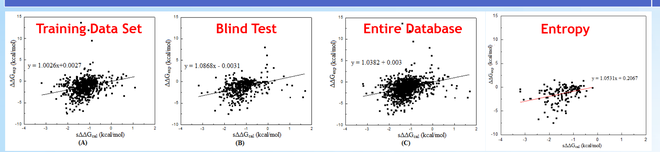




Activity of the mutant G56S with small molecules binding

Best poses of the three active molecules docked on the mutant G56S dimer

sMMGB method



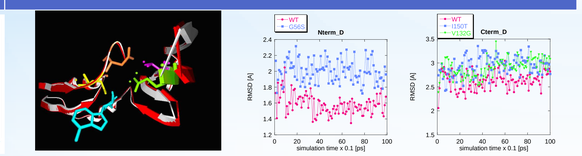
Training Database:
RMSD = 1.79 kcal/mol;
SD = 0.51 kcal/mol;

Blind Test:
RMSD = 1.76 kcal/mol;
SD = 0.58 kcal/mol;

Entire Database:
RMSD = 1.78 kcal/mol;
SD = 0.54 kcal/mol;

Entropy:
RMSD = 1.6 kcal/mol;
SD = 0.56 kcal/mol;

Ionized States and MD



Left Panel: Zoomed C-terminal domain of the p.I150T mutant (white) superimposed onto the wild type structure (red). The mutant residue, Thr150 is shown in magenta, the titratable residue affected by the mutation, Asp222, is shown in orange, the coordinating residues Gln148 in green and Glu220 in yellow. The MTA in cyan. The hydrogen of Thr150 making H-bond with Asp222 and the hydrogens of Gln148 are also shown in the figure; Middle Panel: RMSD of N-terminal domain; Right Panel: RMSD of C-terminal domain.

Acknowledgement

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