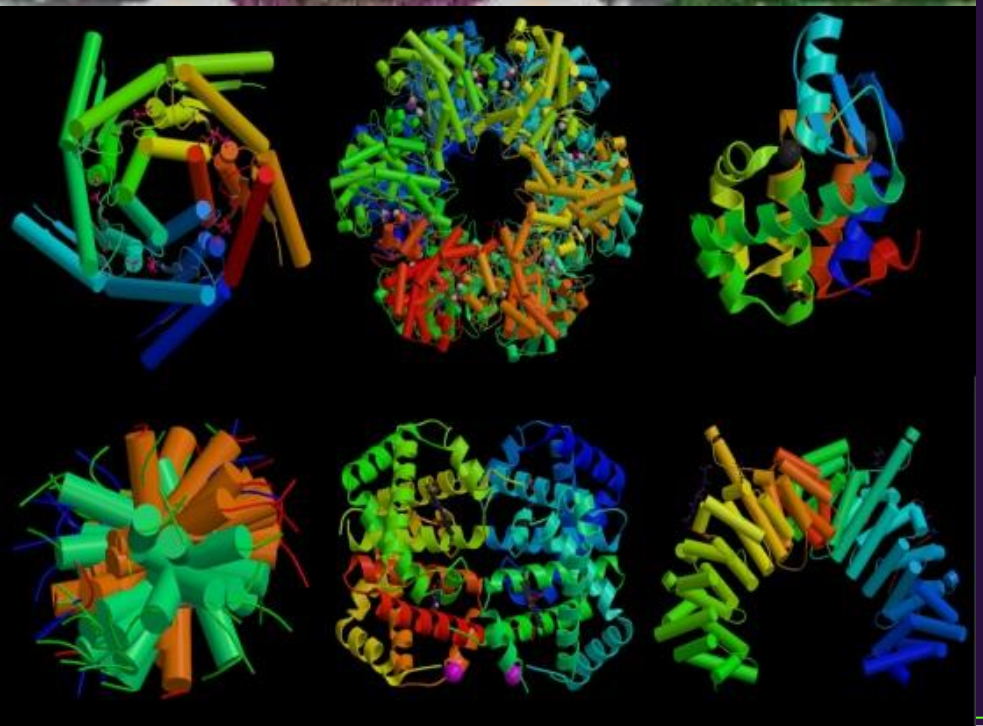




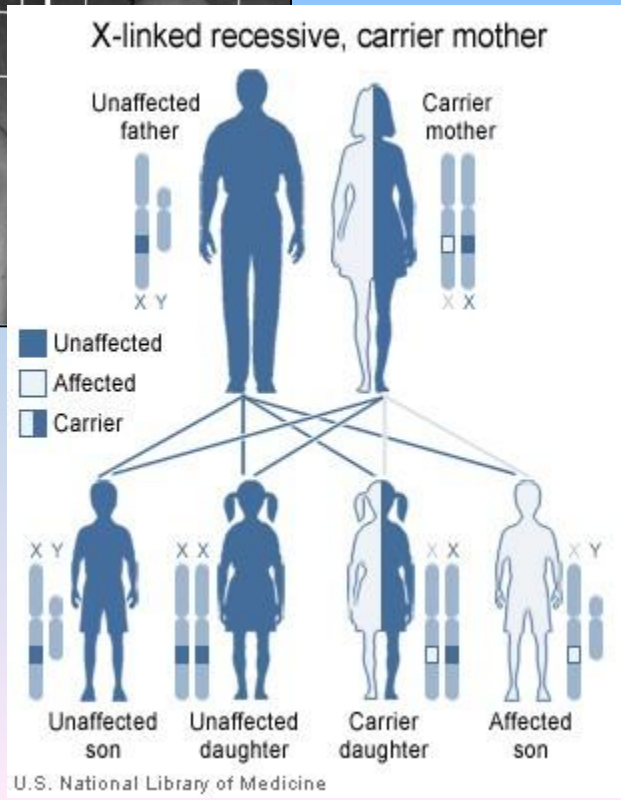
Snyder-Robinson syndrome: molecular mechanism and rescuing the effect with small molecules

Emil Alexov
Clemson University

2014

Snyder-Robinson Syndrome





An X-linked mental retardation disorder

Symptoms: Thin Habitus; Facial Asymmetry; Hypotonia; Osteoporosis.

Reported mutations

Background: It was shown clinically that the disease is caused by a single mutation in spermine synthase. Currently several missense mutations in spermine synthase are shown to be disease-causing:

G56S: Reported by G. de Alencastro et al.
V132G: Reported by L.E. Becerra-Solano et al.
I150T: Reported by Z. Zhang et al.
Y328C: Reported by Z. Zhang et al.
C112L: under investigation and several more

The goal is to investigate the effects of these missense mutations on stability, dynamics and interactions of spermine synthase.

G. de Alencastro, D.E. McCloskey, S.E. Kliemann, C.M. Maranduba, A.E. Pegg et al, J Med Genet 2008, 45(8), pp. 539-543.

L.E. Becerra-Solano, J. Butler, G. Castaneda-Cisneros, D.E. McCloskey, X. Wang, A.E. Pegg, C.E. Schwartz, J. Sanchez-Corona and J.E. Garcia-Ortiz, Am J Med Genet A 2009, 149A(3), pp. 328-335.

Zhang Z, Teng S, Wang L, Schwartz CE, Alexov E. Hum Mutat. 2010 Sep;31(9):1043-9.

Zhang Z, Norris J, Kalscheuer V, Wood T, Wang L, Schwartz C, Alexov E, Van Esch H. Hum Mol Genet. 2013 Sep 15;22(18):3789-97

Function of SMS

SPD+DcAdoMet $\xrightleftharpoons{\text{SMS}}$ SPM+MTA

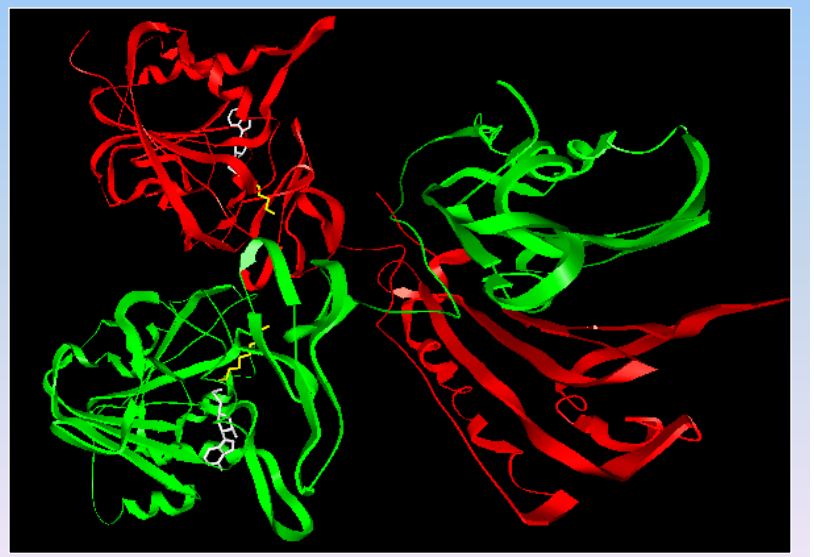
Where:

SPD: Spermidine;

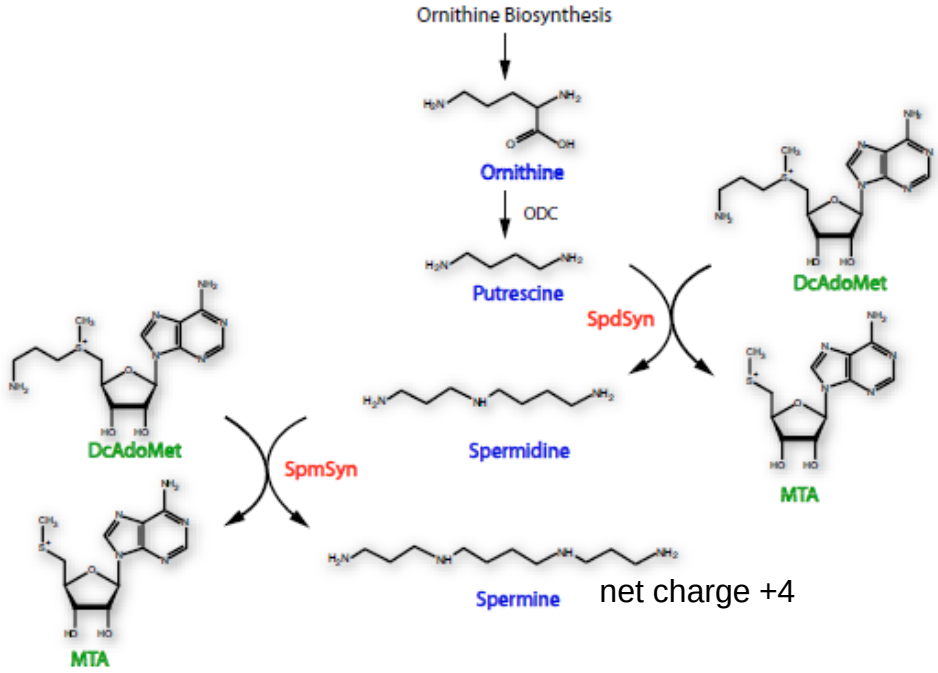
SPM: Spermine;

DcAdomet: Decarboxylase S-adenosylmethionine;

MTA: S-methyl-5'-thioadenosine;



Wu et al. 2008. Crystal structure of human spermine synthase: implications of substra

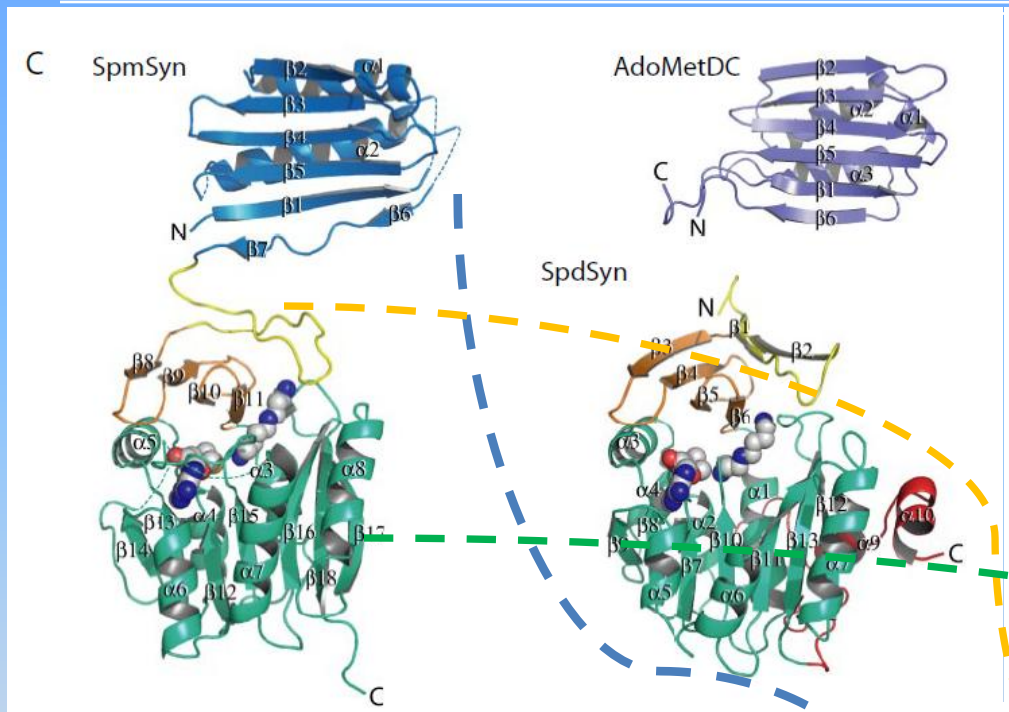


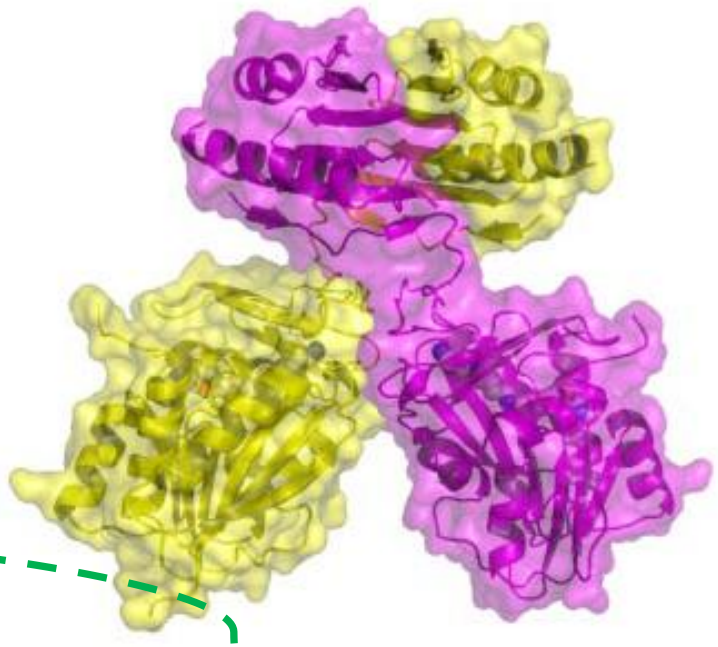
Crystal Structure of Human Spermine Synthase: IMPLICATIONS OF SUBSTRATE BINDING AND CATALYTIC MECHANISM

Hong Wu, Jinrong Min, Hong Zeng, Diane E. McCloskey, Yoshihiko Ikeguchi, Peter Loppnau, Anthony J. Michael, Anthony E. Pegg and Alexander N. Plotnikov

J. Biol. Chem. 2008, 283:16135-16146.

Structure-function relations



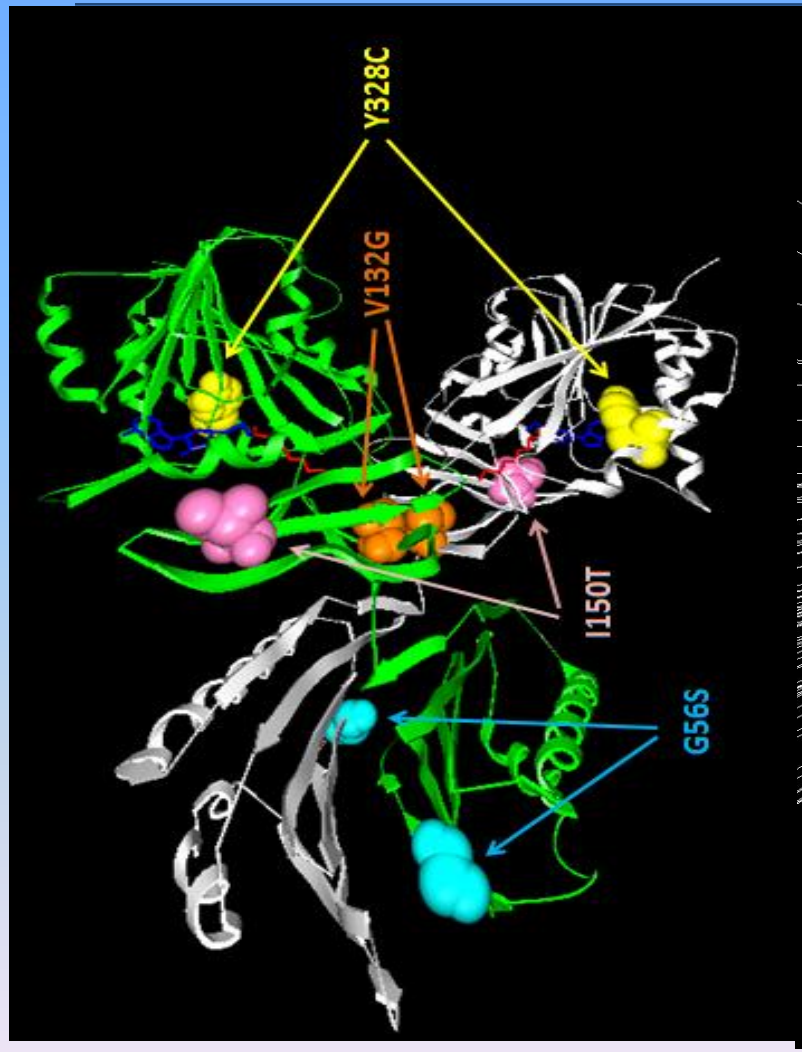


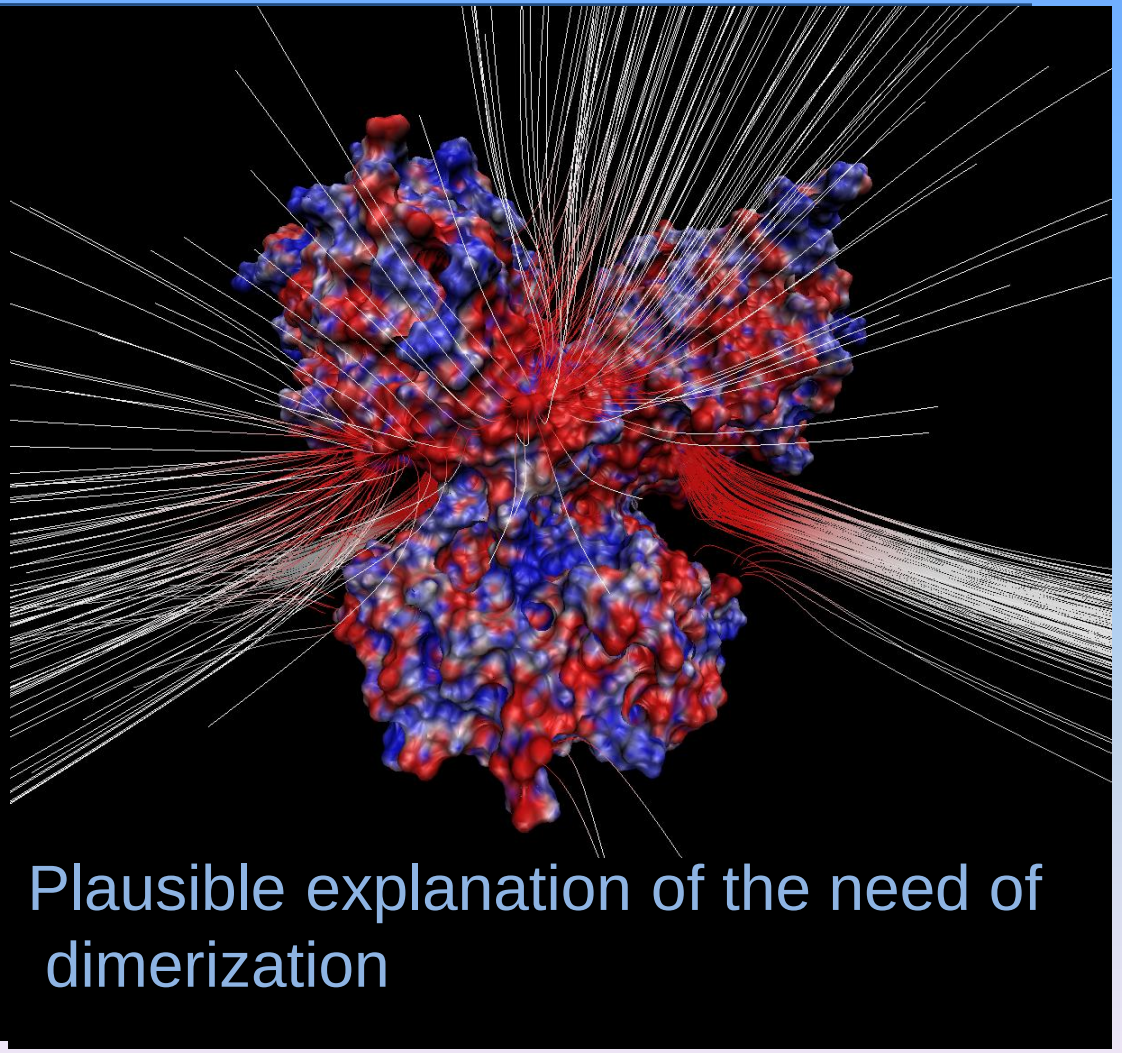
Crystal Structure of Human Spermine Synthase: IMPLICATIONS OF SUBSTRATE BINDING AND CATALYTIC MECHANISM

Hong Wu, Jinrong Min, Hong Zeng, Diane E. McCloskey, Yoshihiko Ikeguchi, Peter Loppnau, Anthony J. Michael, Anthony E. Pegg and Alexander N. Plotnikov
J. Biol. Chem. 2008, 283:16135-16146.



3D structure of SMS





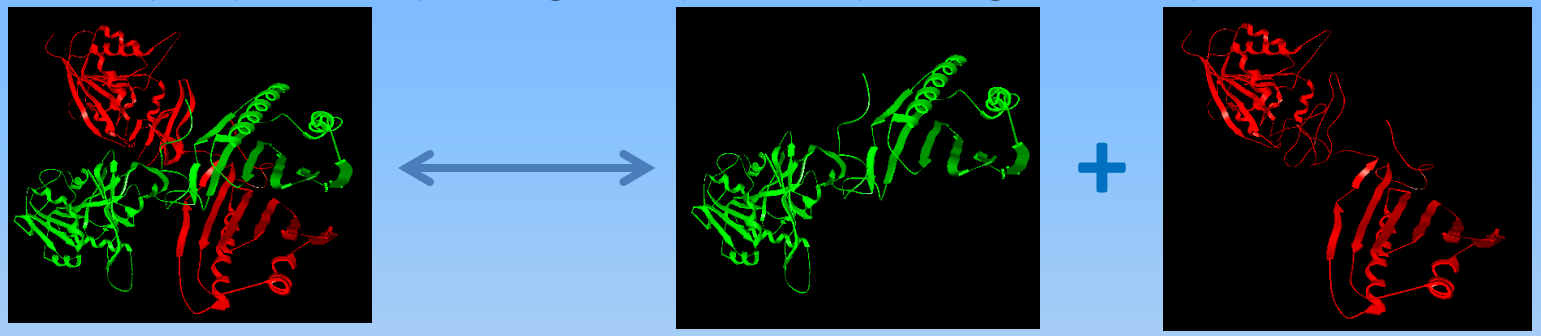
Plausible explanation of the need of dimerization

Calculating energy changes

BINDING FREE ENERGY CHANGE

$$\Delta\Delta G(binding) = \Delta G(\text{dimer}) - \Delta G(C) - \Delta G(D),$$

$$\Delta\Delta\Delta G(mut) = \Delta\Delta G(binding : WT) - \Delta\Delta G(binding : \text{mutant})$$

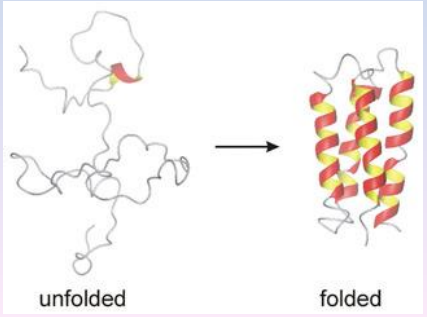


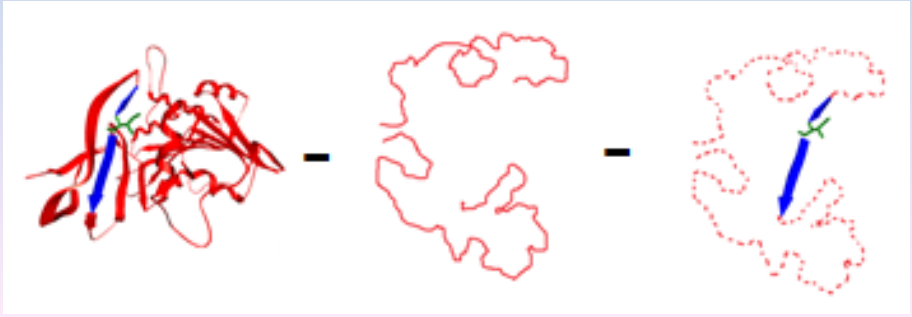
FOLDING FREE ENERGY CHANGE

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

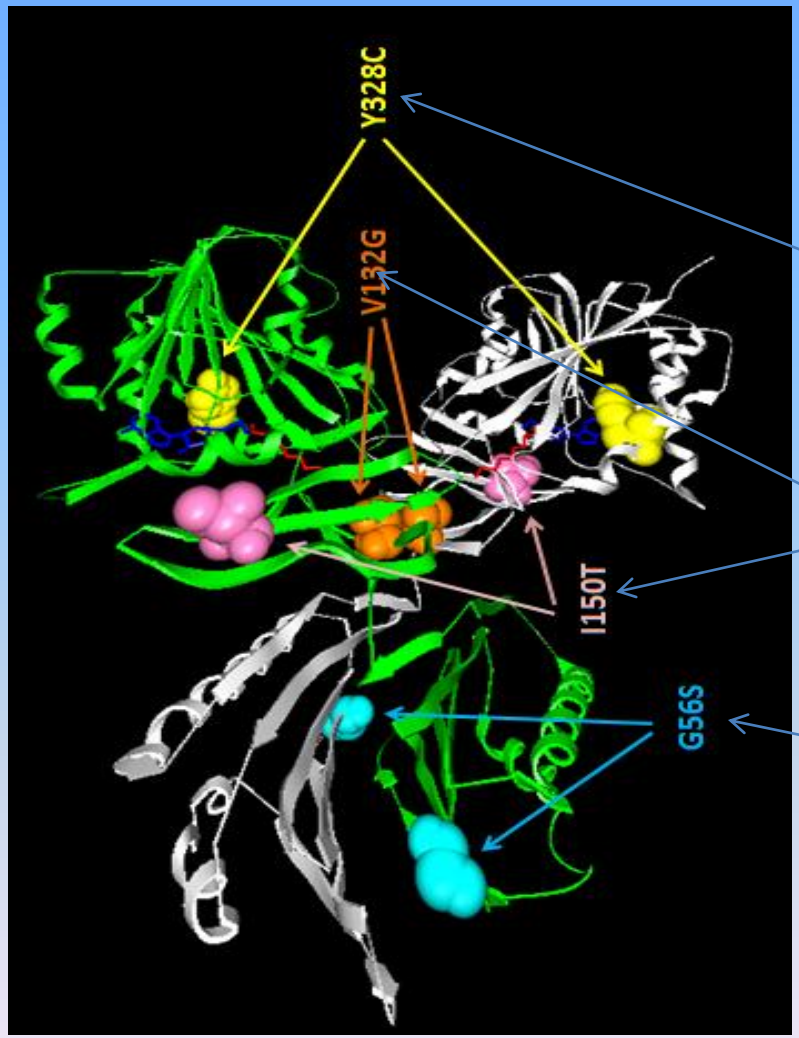
$$\Delta\Delta G(\ folding\ _mut) = \Delta G(\ folding\ : WT) - \Delta G(\ folding\ : mutation) =$$

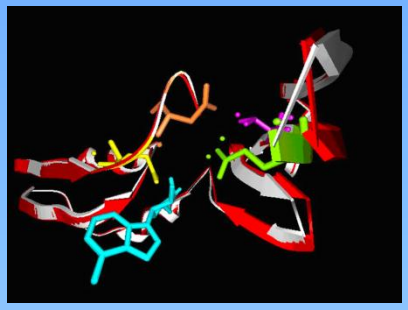
$$G(\ folded\ : WT) - G_7(unfolded\ : WT) - G(\ folded\ : mutation) + G_7(unfolded\ : mutation)$$





Predicted effects





Y328C and I150T: Strongly de-stabilize the monomer and affect the H-bond networks

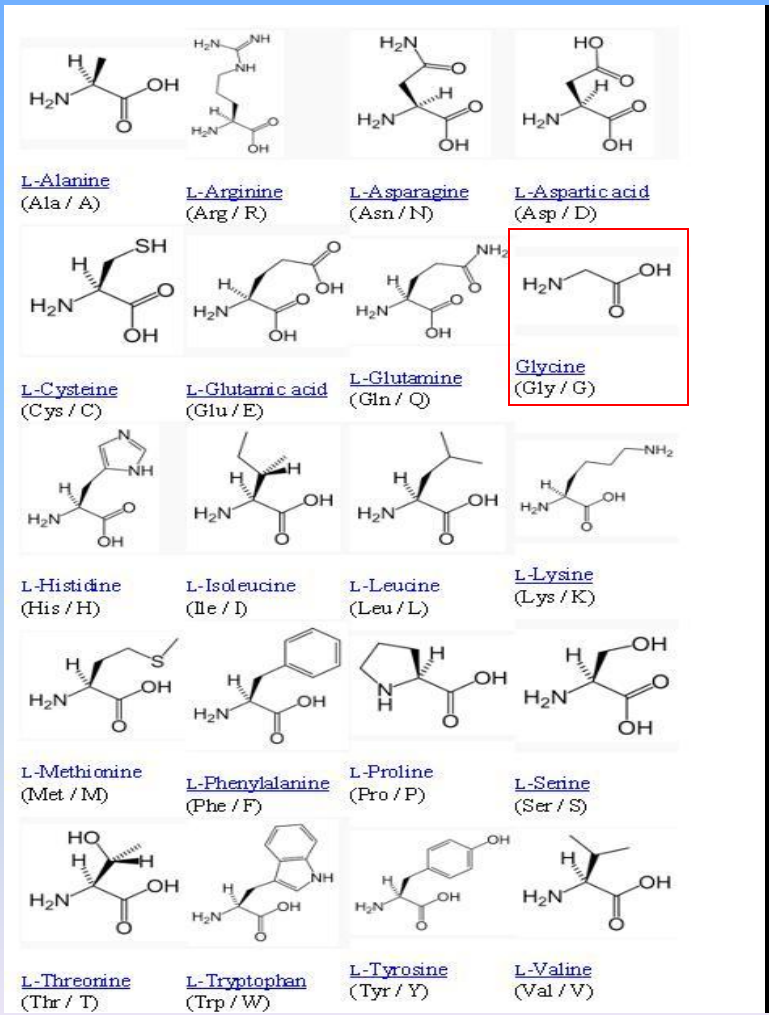
V132G: slightly de-stabilizes the dimer and monomer

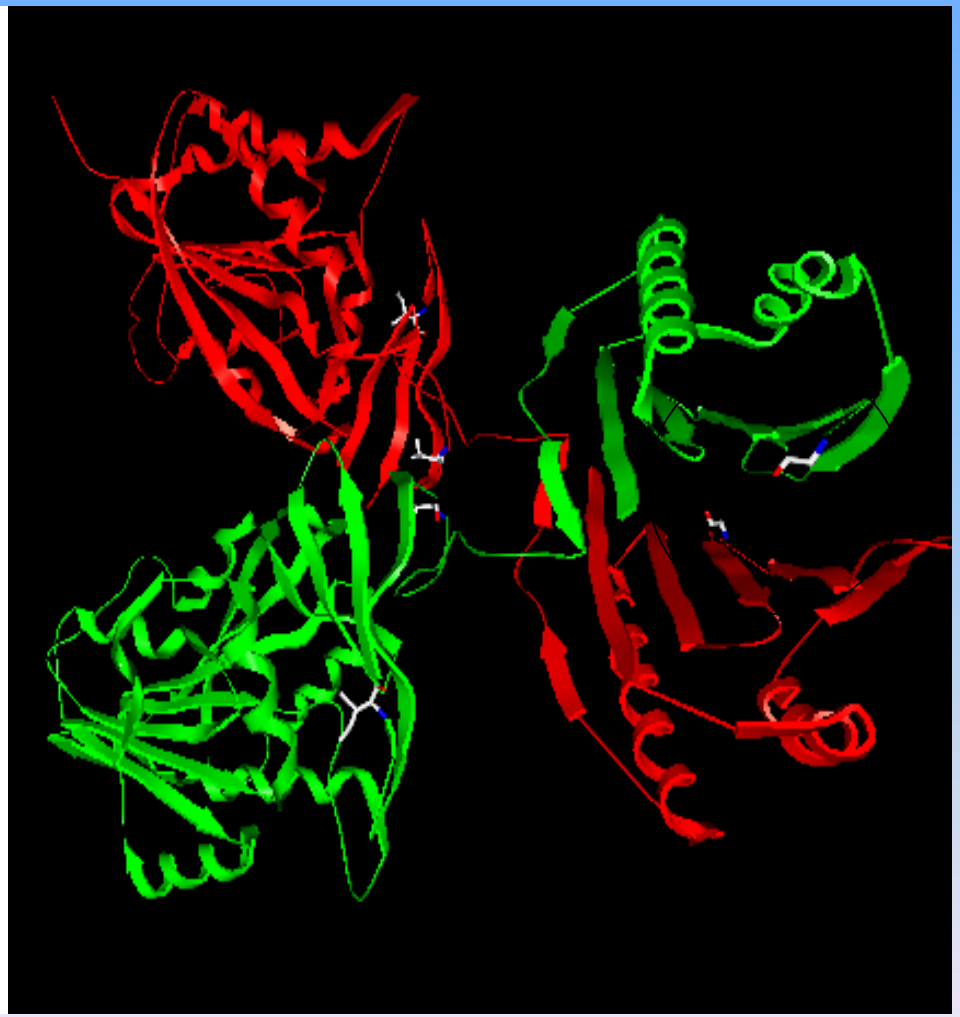
G56S: Strongly de-stabilizes the dimer

Zhang Z, Teng S, Wang L, Schwartz CE, Alexov E.
Hum Mut. 2010 Sep;31(9):1043-9.

Zhang Z, Norris J, Kalscheuer V, Wood T, Wang L, Schwartz C, Alexov E, Van Esch H.
Hum Mol Genet. 2013 Sep 15;22(18):3789-97

Can clinically observed disease-causing sites accommodate harmless mutations?





Zhang Z, Norris J, Schwartz C, Alexov E. PLoS One. 2011;6(5):e20373.

Z-Score and metrics

In order to see how many standard deviation away the missense mutations are from the mean, we calculate the Z-Score of these mutations.

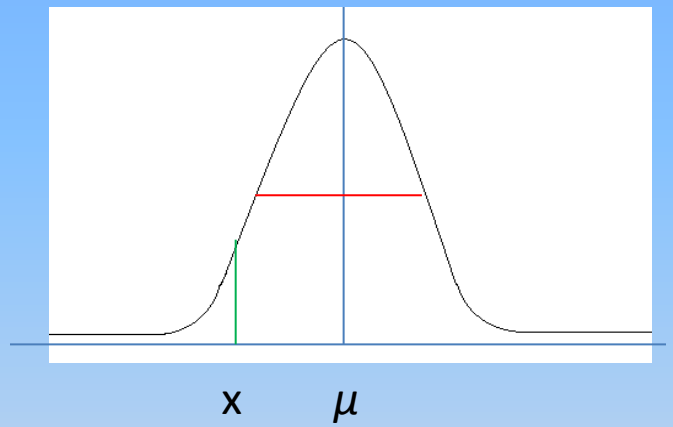
$$Z - score = \frac{x - \mu}{\sigma}$$

where:

x is a raw score to be standardized;

μ is the mean of the population;

σ is the standard deviation of the population;

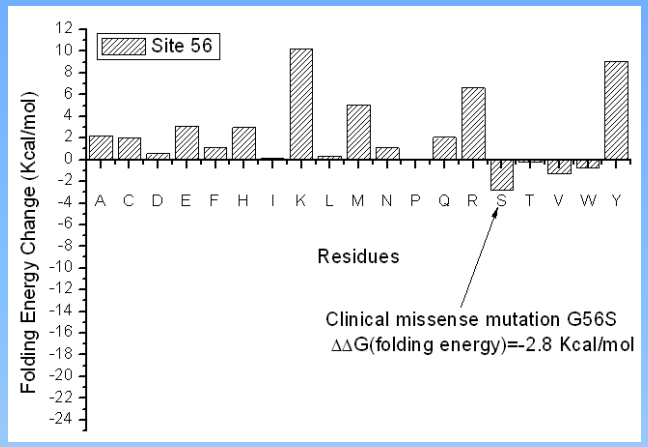


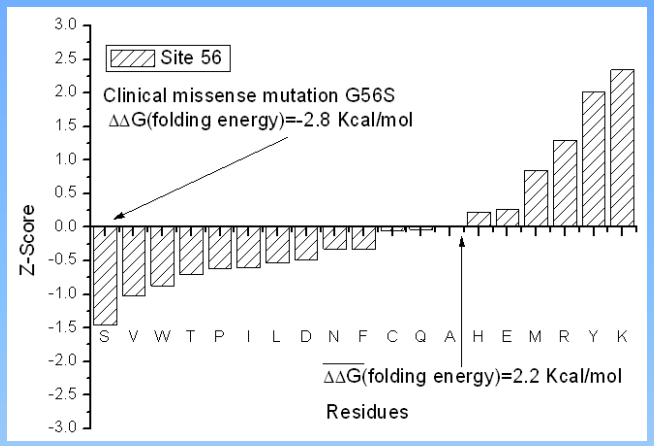
1 **"tolerance"** – if the mean of the distribution of the energy change upon amino acid substitutions at a given site is larger than particular threshold, the site is termed "**non-tolerable**".

2 **"specificity"** – a site is termed "**specific"** if more than 20% of amino acid substitutions are predicted to cause different effects from favorable to unfavorable energy change with a magnitude larger than the half of the standard deviation (HSTD) associated with the site. If the effects follow the same trend, then the site is termed "non-specific".

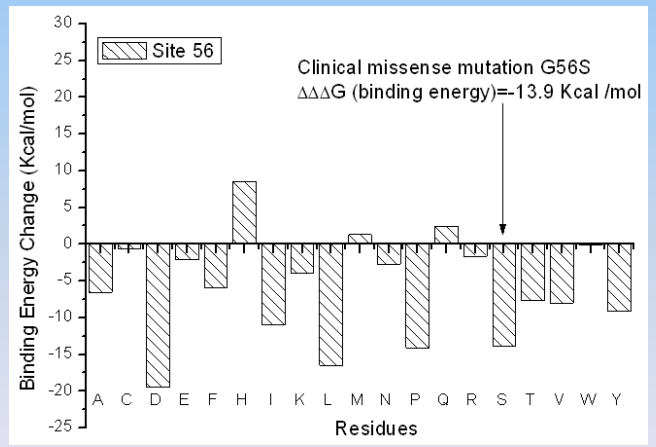
Zhang Z, Norris J, Schwartz C, Alexov E. PLoS One. 2011;6(5):e20373.

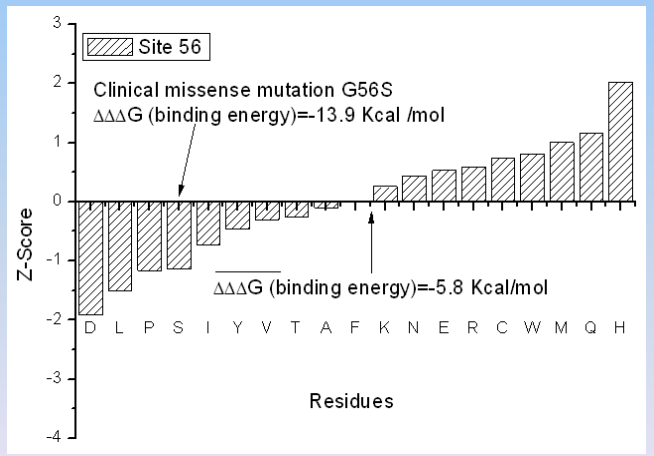
Site 56 stability and affinity (just for illustration)





Folding/stability changes

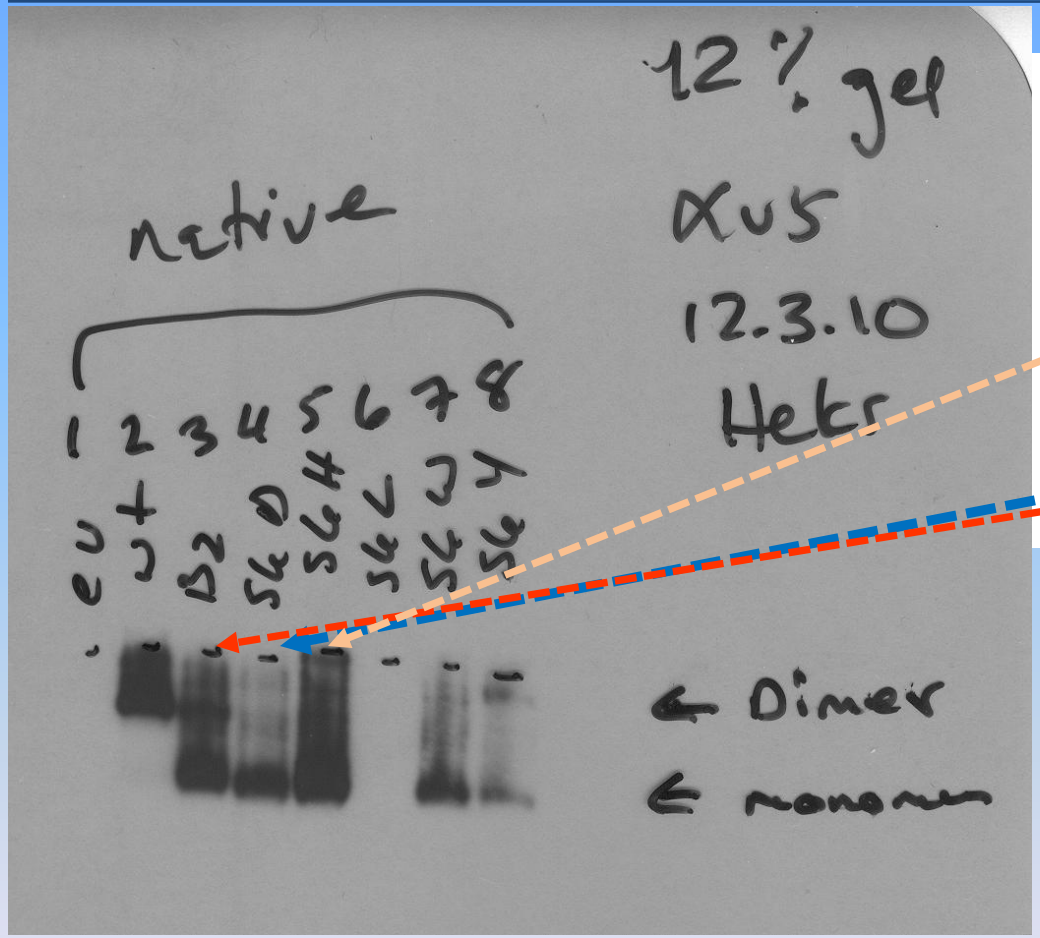


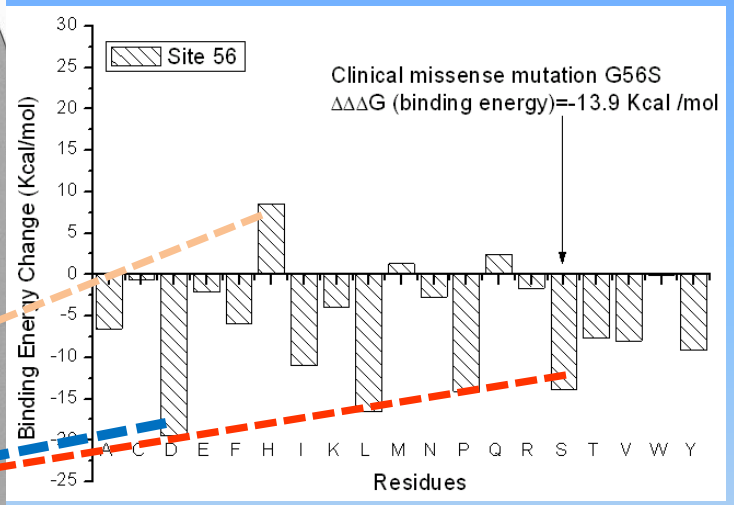


Binding/affinity changes

Zhang Z, Norris J, Schwartz C, Alexov E. PLoS One. 2011;6(5):e20373.

Experimental verification of dimer affinity





Charles Schwartz, Joy Norris, John Stowell, Greenwood Genetic Center, SC

Zhang Z, Norris J, Schwartz C, Alexov E.
PLoS One. 2011;6(5):e20373.

Designing better SMS

Transferring sequence information from termotoga maritima spermidine synthase to human spermine synthase

```
Homo Sapiens              1    MGSSHHHHHHSSGLVPRGSRHSTLDFMLGAKADGETILKGLQSIFQEQGMAESVHTWQDH   60
Thermotoga maritima            ------------------------------------------------------------

Homo Sapiens             61    GYLATYTNKNGSFANLRIYPHGLVLLDLQSYDGDAQGKEEIDSILNKVEERMKELSQDST  120
Thermotoga maritima       1    ------------------------------------------------------MKELEREL   8
                                                                                        ****

Homo Sapiens            121    GRVKRLPPIVRGGAIDRYWPTADGRLVEYDIDEVVYDEDSPYQNIKILHSKQFGNILILS  180
Thermotoga maritima      10    PRQHLW--------YFEYY-TGNNVGLFMKMNRVIYSGQSDIQRIDIFENPDLGVVFALD   60
                                *                 *  *            * *   *  * * * *      *    *

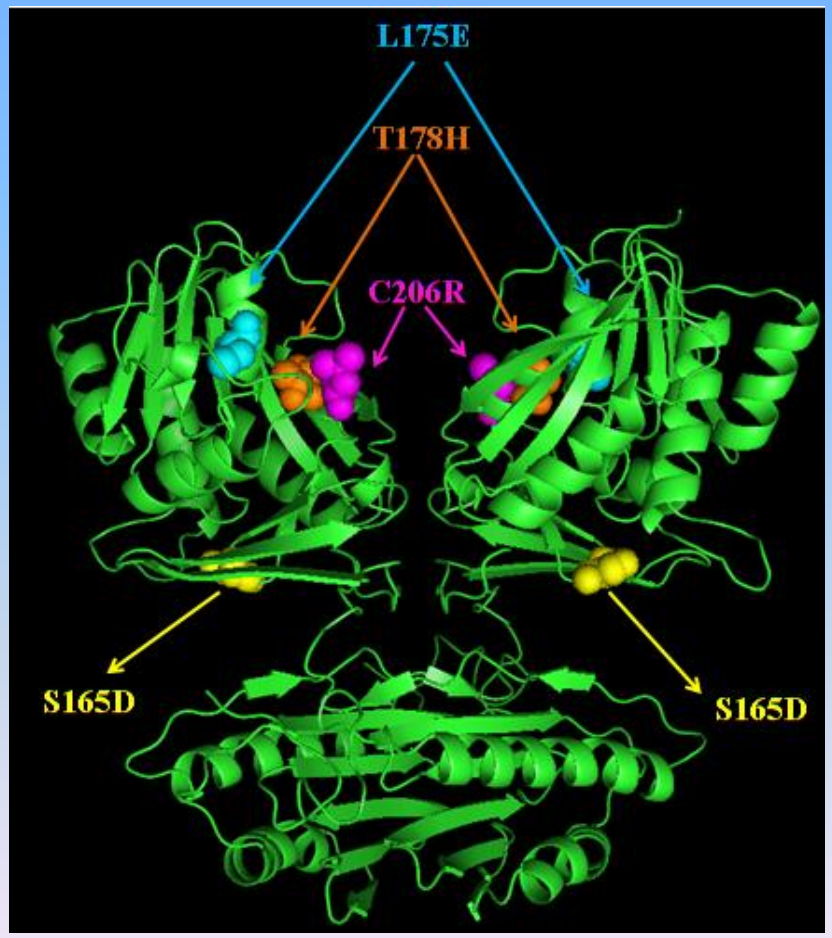
Homo Sapiens            181    GDVNLAESDL-AYTRAIMGSGKEDYT-GKDVLILGGGDGGILCEIVKLKP-KMVTMVEID  237
Thermotoga maritima      61    GITMTTEKDEFMYHEMLAHVPMFLHPNPKKVLIIGGGDGGTLREVLKHDSVEKAILCEVD  120
                               *     * *   *                 * *** ****** * *  *          * *

Homo Sapiens            238    QMVIDGCKKYMRKTCGDVLDNLKGDCYQVLIEDCIPVLKRYAKEGREFDYVINDLTAVPI  297
Thermotoga maritima      121   GLVIEAARKYLKQTSCG----FDDPRAEIVIANGAEYVRKFKN---EFDVIIIDSTDPTA  173
                                 **    **  *                *                *** * * *

Homo Sapiens            298    STSPEEDSTWEFLRLILDLSMKVLKQDGKYFTQGNCVNLTEALSLYEEQLGRLYCPVEFS  357
Thermotoga maritima      174   GQGGH-----LFTEEFYQACYDALKEDGVFSAETEDPFYDIGWFKLAYRRISKVFPI-TR  227
                                          *           ** **                      *

Homo Sapiens            358    KEIVCVPSYLELWVFYTVWKKAKP-----------------------------------  381
Thermotoga maritima      228   VYLGFMTTYPSGMWSYTFASKGIDPIKDFDPEKVRKFNKELKYYNEEVHVASFALPNFVK  287
                                     *      **   *

Homo Sapiens                   ------
Thermotoga maritima      288   KELGLM                                                        293
```

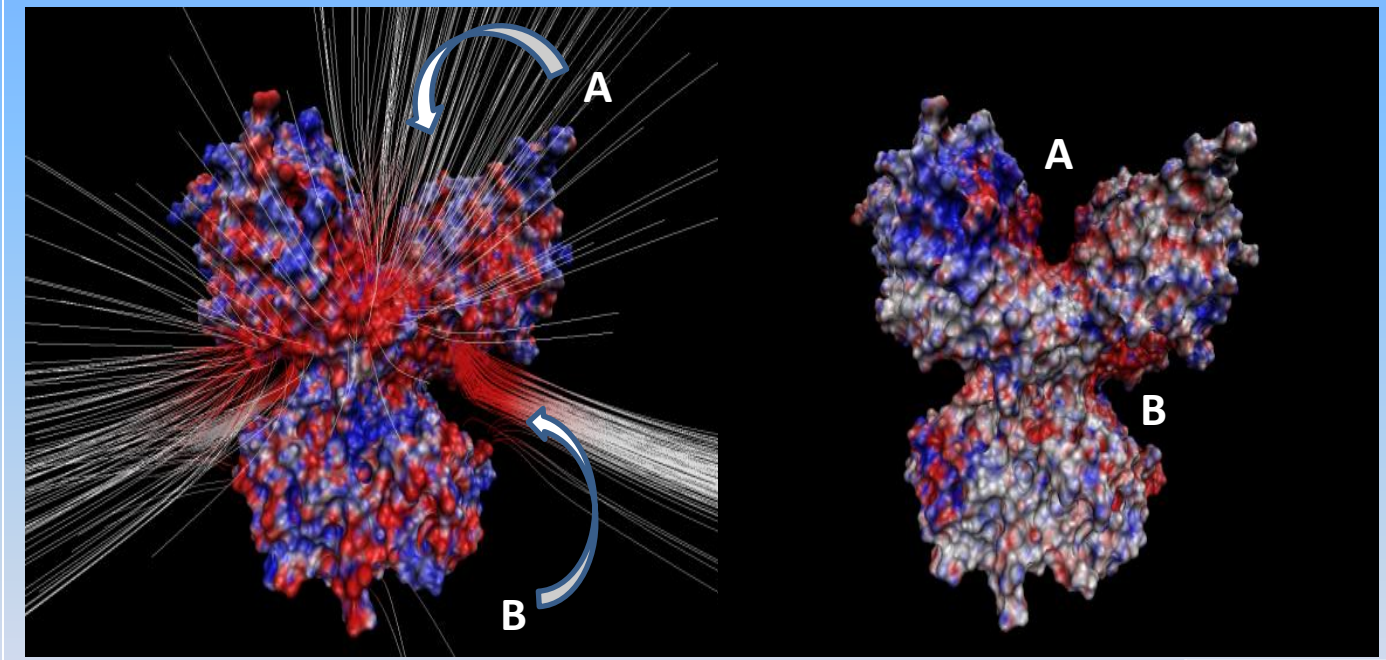


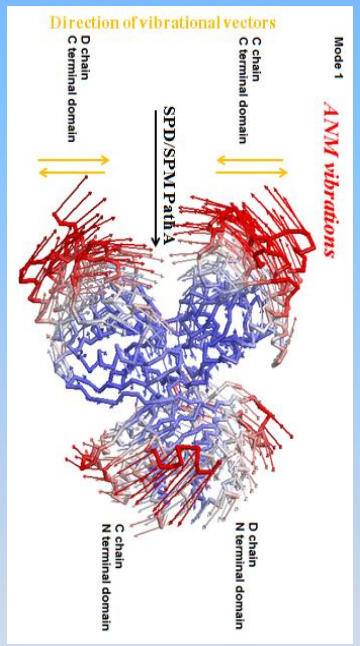
Zhang Z, Zheng Y, Petukh M, Pegg A, Ikeguchi Y, Alexov E.
PLoS Comp. Biol. 2013;9(2):e1002924

Some other puzzling results and facts

Facts: There are no harmless mutations in SMS

Results: Engineering 4 a.a. mutant with enhanced activity: S167D; L177E, T180H, C208R





Protein	Activity (nmol / h / mg)
WT	3776 ± 494
Fmut (S165D/ L175E/T178H/C206R)	40418 ± 3247

Zhang Z, Zheng Y, Petukh M, Pegg A, Ikeguchi Y, Alexov E.
PLoS Comp. Biol. 2013;9(2):e1002924

General conclusions

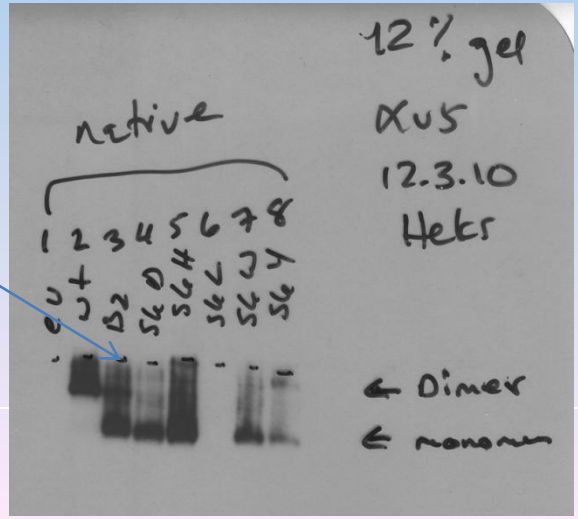
1) All cases have something in common but they are all **different**

2) None of the studied mutations involves catalytic residue

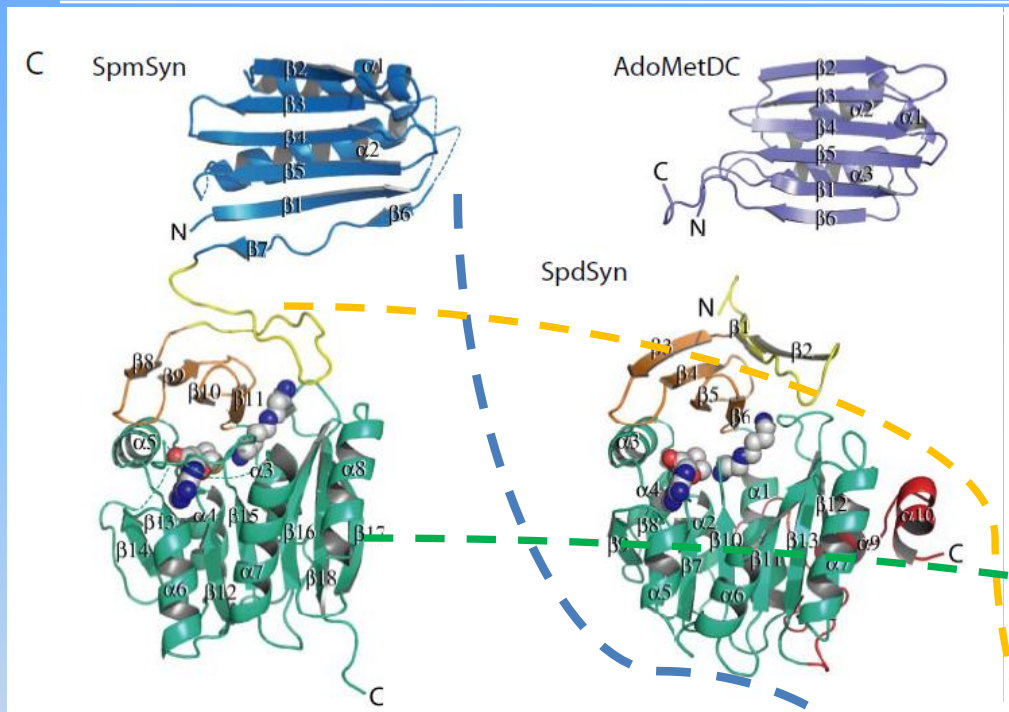
3) Most of the mutation sites are either surface exposed or partially surface exposed

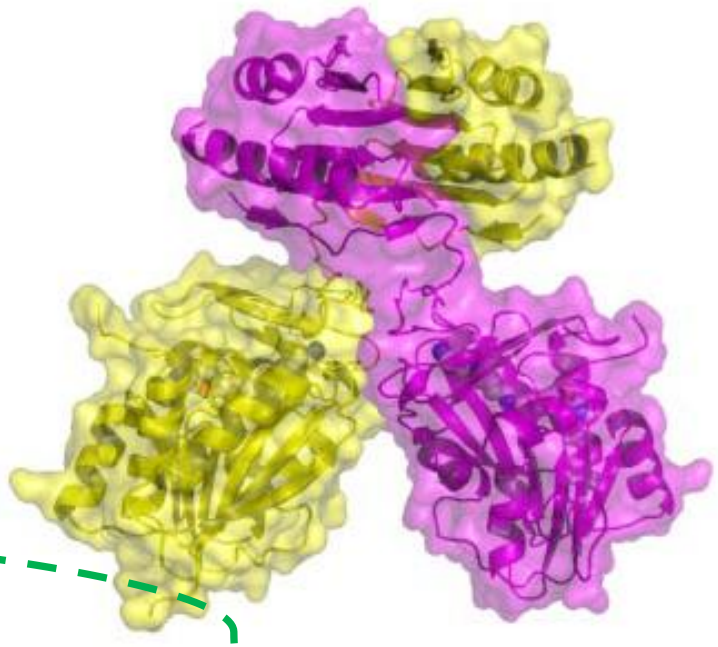
3) The calculated effects (on stability, affinity and hydrogen bond network) are relatively small. Some of the predicted effects were confirmed experimentally.

4) It is a monogenic disease



SMS: just to remain yourself





Crystal Structure of Human Spermine Synthase: IMPLICATIONS OF SUBSTRATE BINDING AND CATALYTIC MECHANISM

Hong Wu, Jinrong Min, Hong Zeng, Diane E. McCloskey, Yoshihiko Ikeguchi, Peter Loppnau, Anthony J. Michael, Anthony E. Pegg and Alexander N. Plotnikov
J. Biol. Chem. 2008, 283:16135-16146.



Binding pocket identification

Ensemble Conformation Generation of the G56S dimer
- MD simulations
- Hierarchical Ascendant Classification

Druggable Pocket Identification
- Surflex-Protomol
- DoGSiteScorer

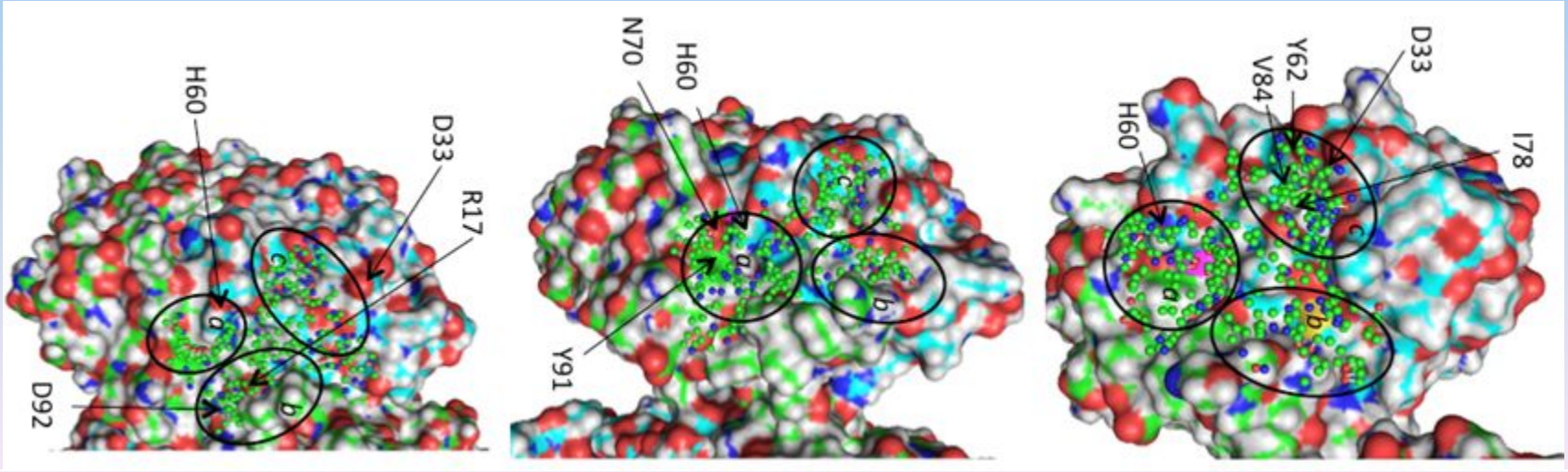
Virtual Screening
- Surflex
- Autodock Vina

Dimer Stabilization Estimation
- Free binding energy calculations

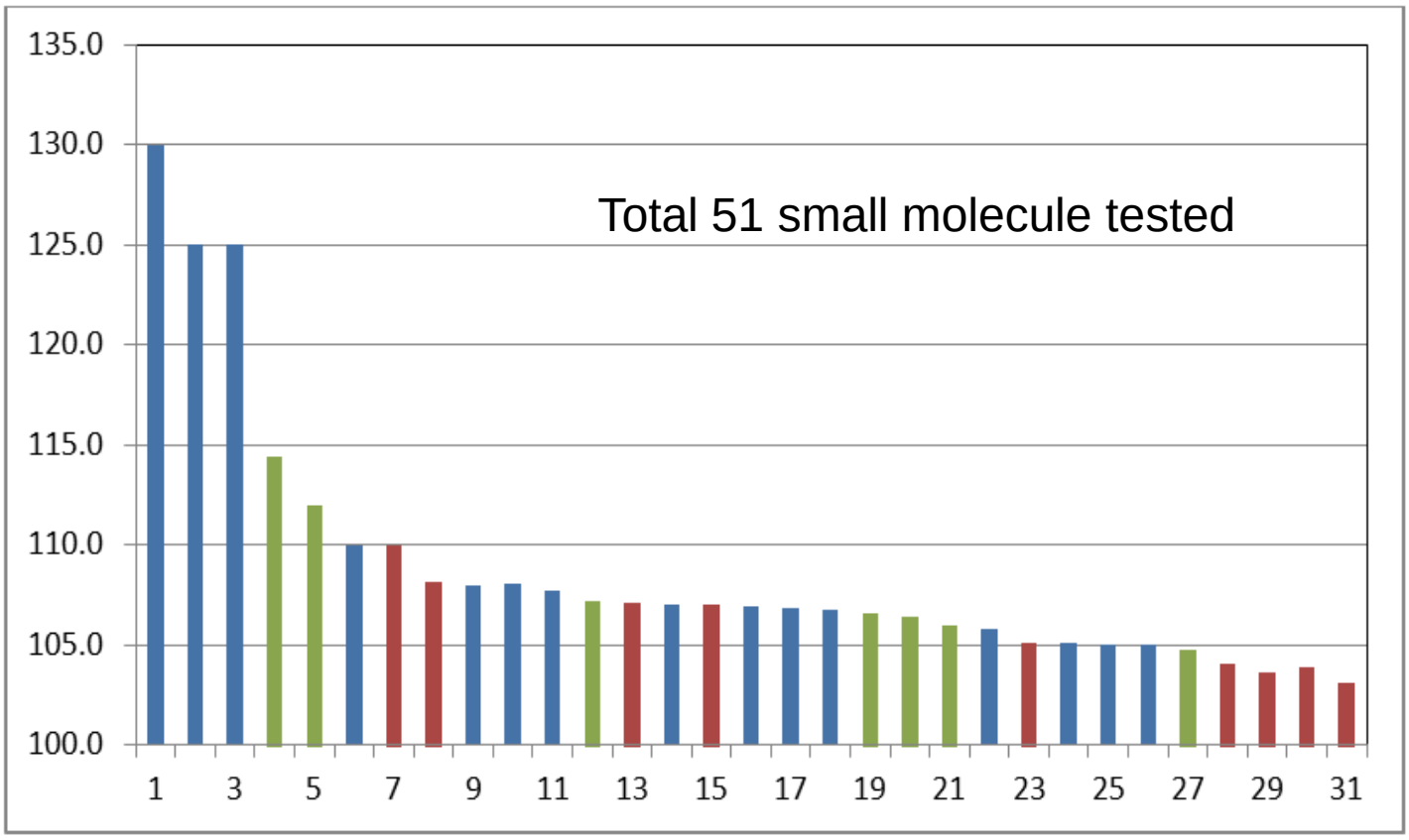
Experimental validation
- *In vitro* assay of G56S SMS activity

Five active chemical scaffolds
- Chemical similarity clustering

Maria Miteva,
University of Paris,
France



Rescuing the malfunctioning G56S SMS mutant (experimental results)



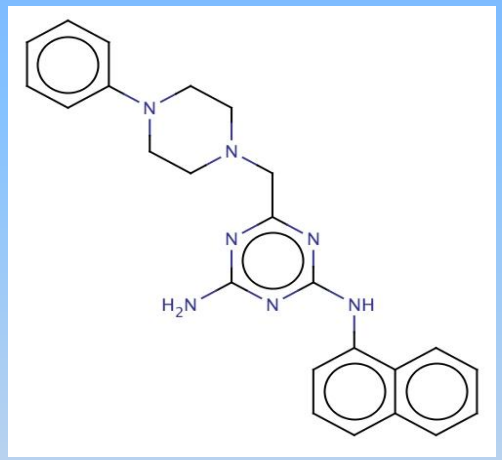
Activities (in %) of the 31 hit molecules identified by docking into the three receptor conformations: Charmm_mini (in blue bars), Charmm_ave the averaged structure (in red bars), Charmm_706ps (in green bars).

A rational free energy-based approach to understanding and targeting disease-causing missense mutations. Zhang Z, Witham S, Petukh M, Moroy G, Miteva M, Ikeguchi Y, Alexov E. J Am Med Inform Assoc. 2013 Jul-Aug;20(4):643-51.

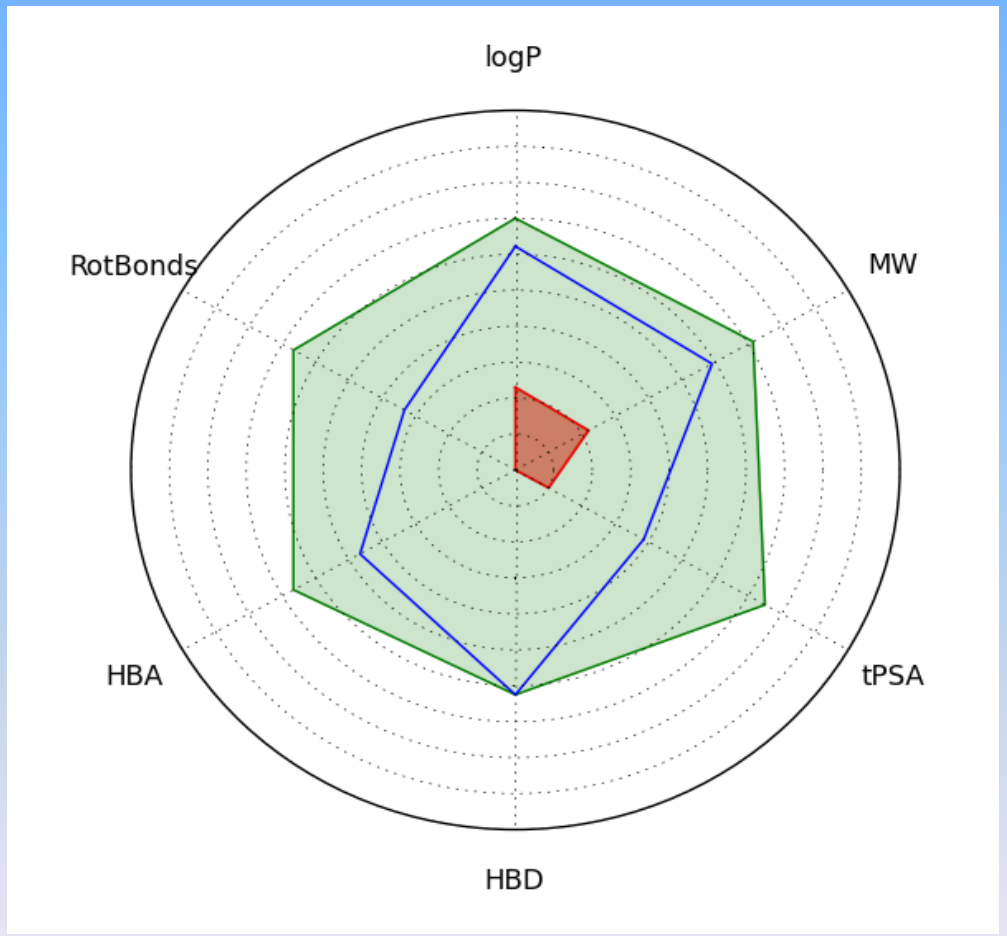
Properties of stabilizers

ChemBridge

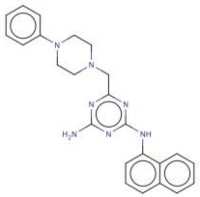
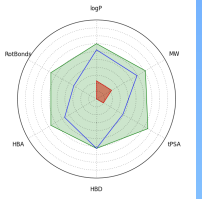
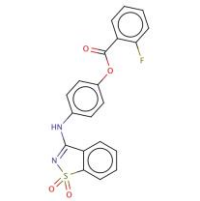
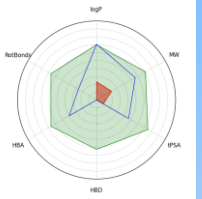
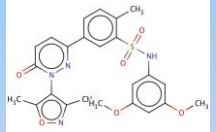
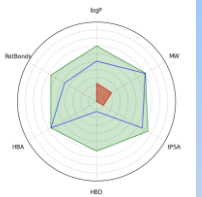
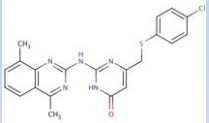
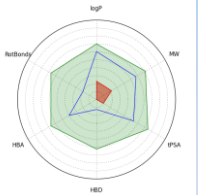
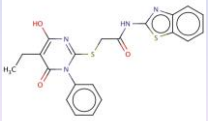
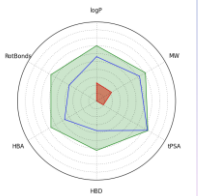
9129729

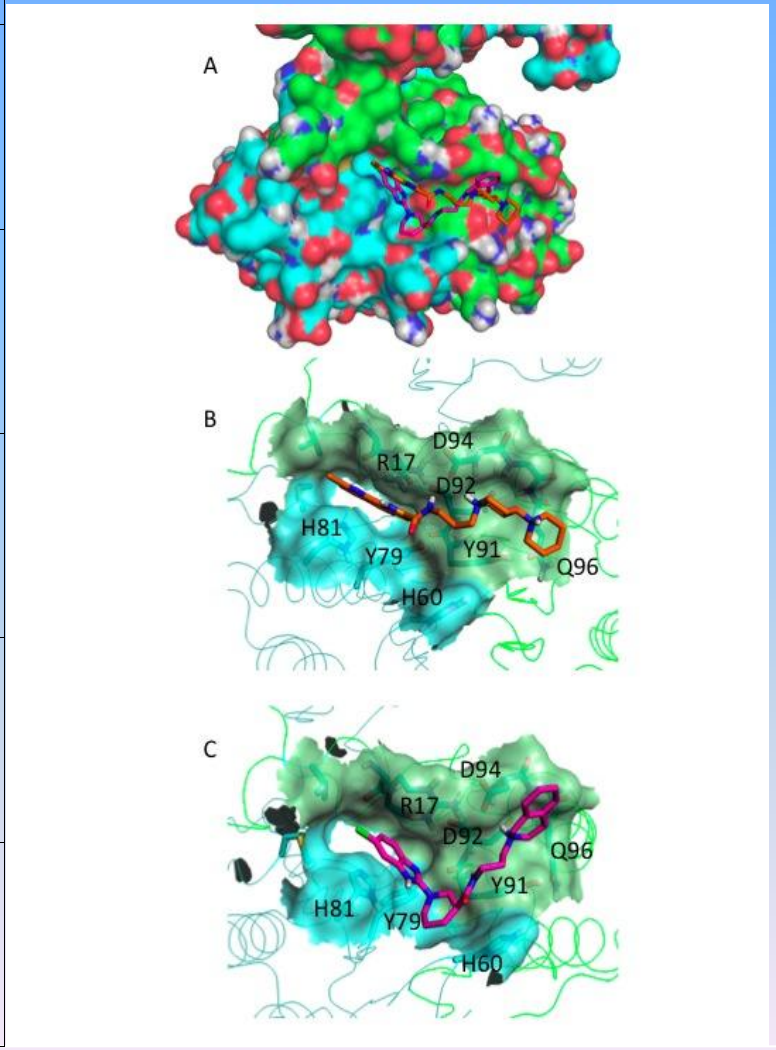


logP, molecular weight (MW), topological polar surface area (tPSA), rotatable bond (RotBonds), H-bonds acceptors and donors (HBA, HBD).



Properties of stabilizers

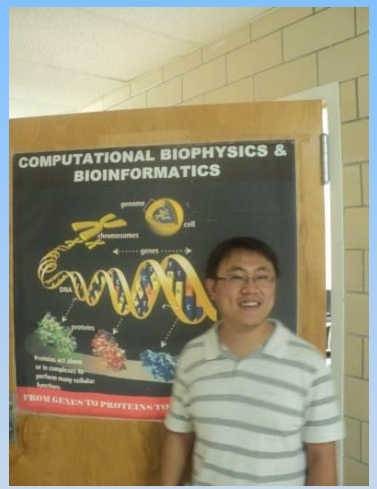
Cluster	ID	2D structure	Activity %	FAF-Drugs2 Oral bioavailability profile
I	**ChemBridge 9129729**		**130.0**	
II	**ChemBridge 5790328**		**125.0**	
	ChemDiv F946-0045		105.1	
III	**ChemBridge 7754012**		**125.0**	
	ChemBridge 5350960		105.0	



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