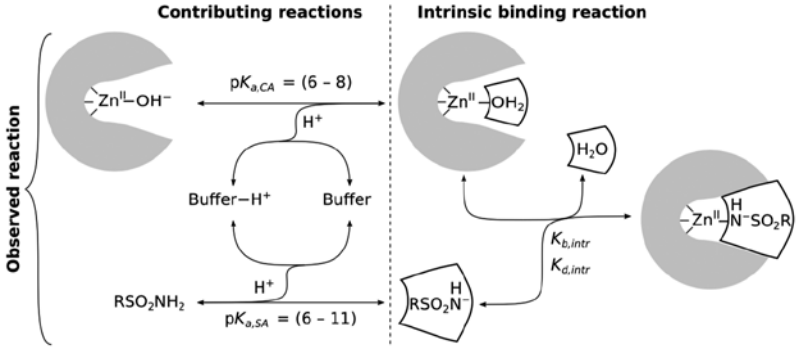


DAUMANTAS MATULIS
Research Professor
Head, Department of Biothermodynamics and Drug Design
Institute of Biotechnology
Email: daumantas.matulis@bti.vu.lt
Phone: +370 5 223 4364

Drug Design via Structure and Thermodynamics

Rational drug design should be able to make chemical compounds that bind to disease-associated target proteins with high affinity and selectivity over all the remaining proteins to avoid toxicity. Unfortunately, such a design is not possible and currently, pharmaceutical companies instead perform various high-throughput screenings of available chemical libraries and develop compounds that perform best in such highly random screens. The reason for such a non-rational approach is that the recognition phenomenon between chemical compounds and proteins is poorly understood. It is not possible to design compounds *in silico* and predict their affinity to target proteins. There is a lack of suitable, well-determined datasets, where chemical compounds binding to proteins would be characterized, including (a) the crystal structures of protein-ligand complexes, (b) the thermodynamics of interaction of the same protein-ligand complexes (including the enthalpy, entropy, Gibbs energy, volume, heat capacity and other thermodynamic parameter changes upon binding) and (c) the kinetics of the same protein-ligand binding. In order to make drug design truly rational and make their success rate much higher in clinical trials, it is important to solve the structure-energetics relationships and be able to predict the binding efficiency of the designed compounds.

Our scientists come from various backgrounds including molecular biologists, biochemists, organic chemists, biophysicists, physicists, computer modellers, biologists and pharmacists. Organic synthesis scientists design and perform the synthesis of novel compounds, molecular and cellular biologists perform the cloning, expression (both in bacterial and in human cell cultures) and purification of target proteins, primarily the family of human carbonic anhydrases and chaperones (Hsp90), biothermodynamicists determine the energetics of binding between the synthesized compounds and the target proteins by ITC or thermal shift and search for structure-energetics correlations, *in silico* modellers and crystallographers determine the X-ray crystallographic structures of protein-compound complexes, and the pharmaceutical scientists perform development studies of the effect of compounds in various biological systems including zebrafish and mice.

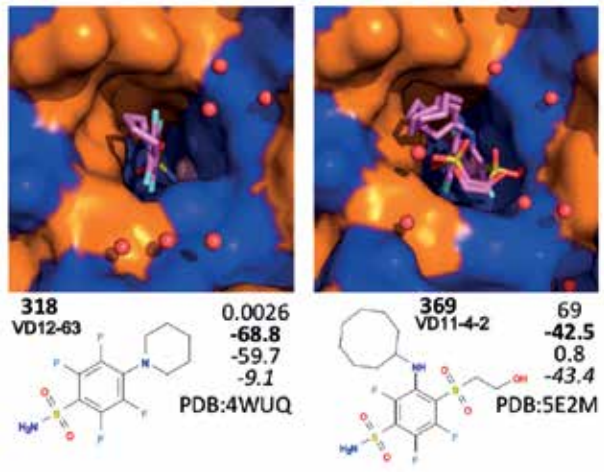
SELECTED PUBLICATIONS

1. Linkuvienė, V.; Zubrienė, A.; Manakova, E.; Petrauskas, V.; Baranauskienė, L.; Zakšauskas, A.; Smirnov, A.; Gražulis, S.; Ladbury, J.E.; Matulis, D. Thermodynamic, kinetic, and structural parameterization of human carbonic anhydrase interactions toward enhanced inhibitor design. Quarterly Reviews of Biophysics. 2018, 51. e10
2. Kazokaitė, J.; Niemans, R.; Dudutienė, V.; Becker, H.M.; Leitāns, J.; Zubrienė, A.; Baranauskienė, L.; Gondi, G.; Zeidler, R.; Matulienė, J.; Tārs, K.; Yaromina, A.; Lambin, P.; Dubois, L.J.; Matulis, D. Novel fluorinated carbonic anhydrase IX inhibitors reduce hypoxia-induced acidification and clonogenic survival of cancer cells. Oncotarget. 2018, 9(42): 26800–26816
3. Paketurytė, V.; Linkuvienė, V.; Krainer, G.; Chen, W.Y.; Matulis, D. Repeatability, precision, and accuracy of the enthalpies and Gibbs energies of a protein-ligand binding reaction measured by isothermal titration calorimetry. Eur Biophys J. 2018, in press.

Structure Thermodynamics Correlations of CA Inhibitors

Last year we published a major review (Linkuviene et al, *Quart. Rev. Biophys*. 2018) that included a collection of intrinsic thermodynamic data of 402 inhibitors binding to 12 human carbonic anhydrase protein isoforms. This dataset included not only the affinities (standard Gibbs energy changes upon binding), but also standard enthalpies and entropies of binding enabling to determine a more precise mechanism of each inhibitor interaction and recognition of the protein active site.

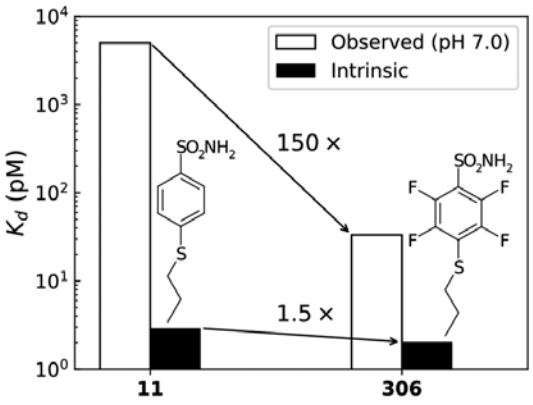
Together with a set of 85 crystal structures of compound complexes with 6 CA isoforms, the dataset allowed to evaluate and search for structure-thermodynamics correlations that are essential for rational drug design.



Intrinsic Thermodynamics of Inhibitor Binding to CAs

Most laboratories determine only the observed thermodynamic parameters of interaction between compounds and proteins. Such approach may lead to incomplete or even incorrect interpretation of the functional group contributions to the overall thermodynamics of compound binding thus leading to missed opportunities to understand the recognition phenomenon.

In this example, we see two compounds that both are very good inhibitors of CA I. Direct determination by any experimental technique, including enzymatic activity inhibition assay, isothermal titration calorimetry, or fluorescent thermal shift assay (that we consider advantageous in many instances), shows that the fluorinated compound on the right exhibits approximately 150-fold higher affinity for the protein than the non-fluorinated compound. However, dissection and accounting for the intrinsic parameters show that the fluorinated compound is only 1.5-fold stronger binder.

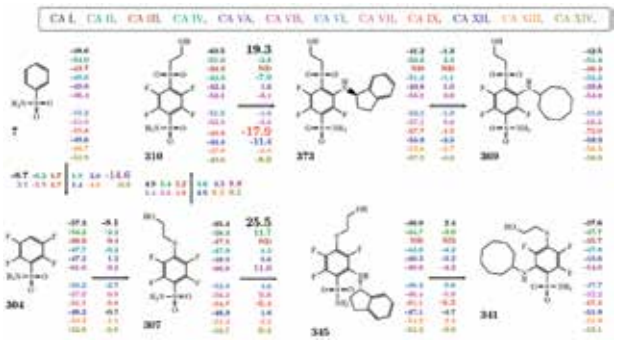


Fluorine atoms play a minor role in recognizing the protein surface, but instead they reduce the pKa of the sulfonamide group leading to stronger observed affinity.

Chemical Structure-Thermodynamics Maps in Search of Selective Inhibitors

The goal of rational drug design is to understand the structure-thermodynamics correlations to predict the chemical structure of the drug that would exhibit an excellent affinity and selectivity to a target protein. In this figure, we explore the contribution of the added functionalities of inhibitors to the intrinsic binding affinity. Interestingly, the binding enthalpies of the compounds possessing similar chemical structures and affinities were highly different, spanning a range from -90 to +10 kJ/mol and compensated by a similar opposing entropy contribution.

The numbers next to compound structures show the intrinsic standard Gibbs energies of binding to all 12 human CAs (shown in different colours, labelled above). Introduction of particular



functional groups has increased the affinity thousand fold and at the same time often affected the selectivity toward the isoform that is targeted as a disease-associated protein.