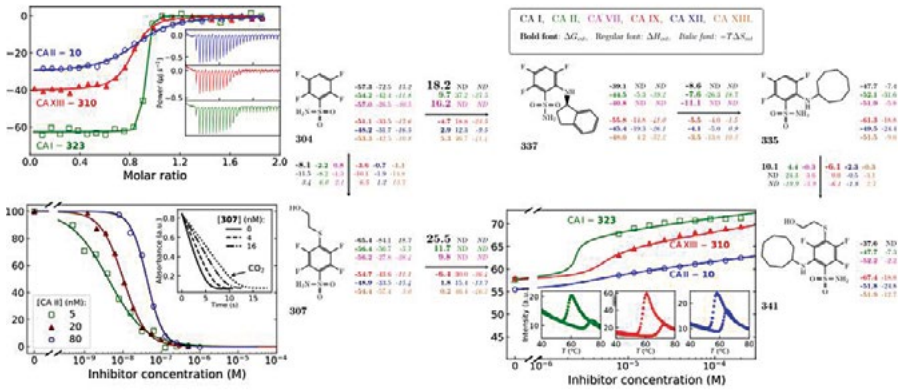


DAUMANTAS MATULIS
Research Professor
Head, Department of Biothermodynamics and Drug Design
Email: daumantas.matulis@bti.vu.lt
Phone: +370 5 223 4364

Drug Design via Thermodynamics

Rational drug design should be able to make chemical compounds that bind to disease-causing target proteins with high affinity and specificity over all remaining proteins to avoid toxicity. Unfortunately, such a design is possible only in theory 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 efficacy of the designed compounds.

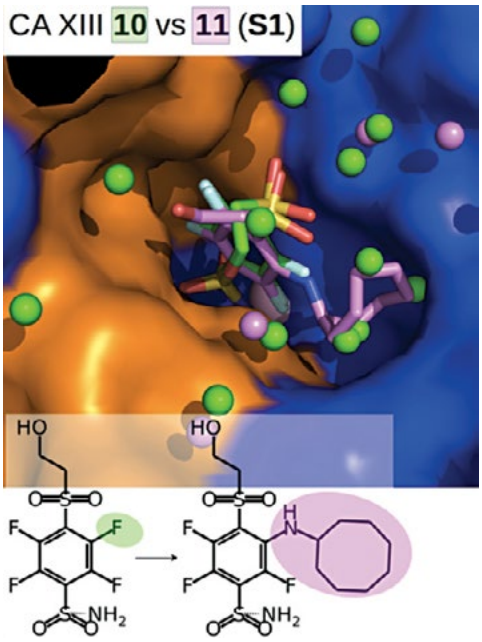
Our scientists come from various backgrounds including molecular biologists, biochemists, organic chemists, biophysicists, physicists, computer modelers, biologists and pharmacists. There are 6 teams in the department: 1) The team for organic synthesis performs the organic synthesis of novel compounds; 2) The team for molecular and cellular biology performs 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); 3) The team for biothermodynamics determines the energetics of binding between the synthesized compounds and the target proteins; 4) The team for *in silico* modeling and crystallography determines the X-ray crystallographic structures of protein-compound complexes, designs compounds, performs docking and searches for structure-energetics correlations; 5) The team for pharmaceutical development studies the effect of compounds in various biological systems including zebrafish and mice.

SELECTED PUBLICATIONS

1. Linkuvienė, V., Talibov, V. O., Danielson, U. H. and Matulis, D. 2018. Introduction of Intrinsic Kinetics of Protein – Ligand Interactions and Their Implications for Drug Design. J. Med. Chem. 61. 2292-2302.
2. Zubrienė, A., Smirnov, A., Dudutienė, V., Timm, D. D., Matulienė, J., Michailovienė, V., Zakšauskas, A., Manakova, E., Gražulis, S., Matulis, D. 2017. Intrinsic Thermodynamics and Structures of 2,4- and 3,4-Substituted Fluorinated Benzenesulfonamides Binding to Carbonic Anhydrases. ChemMedChem. 12. 161-176.
3. Smirnov, A., Zubrienė, A., Manakova, E., Gražulis, S., Matulis, D. 2018. Crystal structure correlations with the intrinsic thermodynamics of human carbonic anhydrase inhibitor binding. PeerJ. doi: 10.7717/peerj.4412.

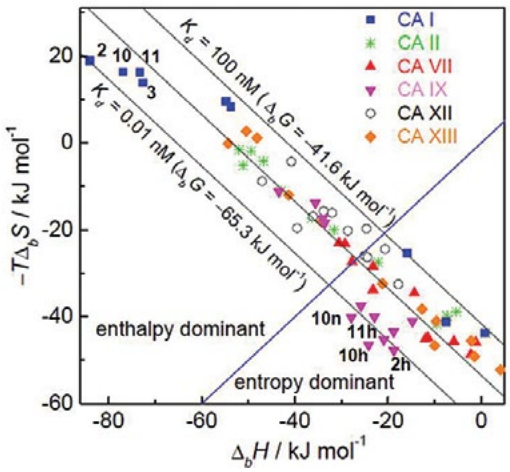
Crystal Structure – Thermodynamics Correlations of CA Inhibitors

The structure-thermodynamics correlation analysis was performed for a series of fluorine- and chlorine-substituted benzenesulfonamide inhibitor binding to several human carbonic anhydrase (CA) isoforms. The total of 24 crystal structures of 16 inhibitors bound to isoforms CA I, CA II, CA XII, and CA XIII provided the structural information of selective recognition between a compound and CA isoform. The binding thermodynamics of all structures were determined by the analysis of binding-linked protonation events, yielding the intrinsic parameters, i.e., the enthalpy, entropy and Gibbs energy of binding. Inhibitor binding was compared within the structurally similar pairs that differ by para- or metasubstituents enabling to obtain the contributing energies of ligand fragments. A deeper understanding of the energies contributing to the protein-ligand recognition should lead toward the eventual goal of rational drug design, where the chemical structures of ligands could be designed based on the target protein structure (Smirnov et al. PeerJ.4412).



The Intrinsic Thermodynamics of the Fluorinated Benzenesulfonamide Inhibitor Binding to CAs

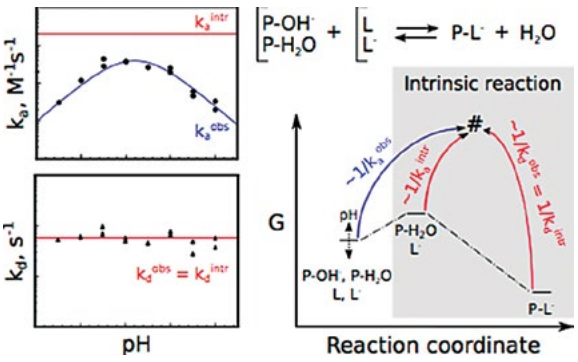
The goal of rational drug design is to understand the structure-thermodynamics correlations to predict the chemical structure of the drug that would exhibit an excellent affinity and selectivity to a target protein. Here we explore the contribution of the added functionalities of inhibitors to the intrinsic binding affinity, enthalpy and entropy. 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 intrinsic parameters of binding were determined by subtracting the linked protonation reactions. The development of meta- or ortho-substituted, fluorinated benzenesulfonamides toward a highly potent compound exhibiting the observed Kd_obs of 43 pM and intrinsic Kd of 1.1 pM toward CA IX, an anticancer target, is described by applying the



FTSA, ITC, and X-ray crystallography (Zubriene et al. ChemMedChem 2017, 12, 161-176).

The Intrinsic Kinetics of Protein–Ligand Interactions

Structure–kinetic relationship analyses and the identification of dominating interactions for the optimization of lead compounds should ideally be based on intrinsic rate constants instead of the more easily accessible observed kinetic constants. The intrinsic rate constants were determined by a surface plasmon resonance (SPR). The observed association rates were pH-dependent and correlated with the fraction of a deprotonated inhibitor and a protonated zinc-bound water molecule. The intrinsic association rate constants were pH independent. By contrast, the observed and intrinsic dissociation rate constants were identical and pH-independent, demonstrating that the observed association and dissociation mechanisms are inherently different. A model accounting for the



differences between intrinsic and observed rate constants was developed (Linkuviene et al. J.Med.Chem. 2018, 61, 2292-2302).