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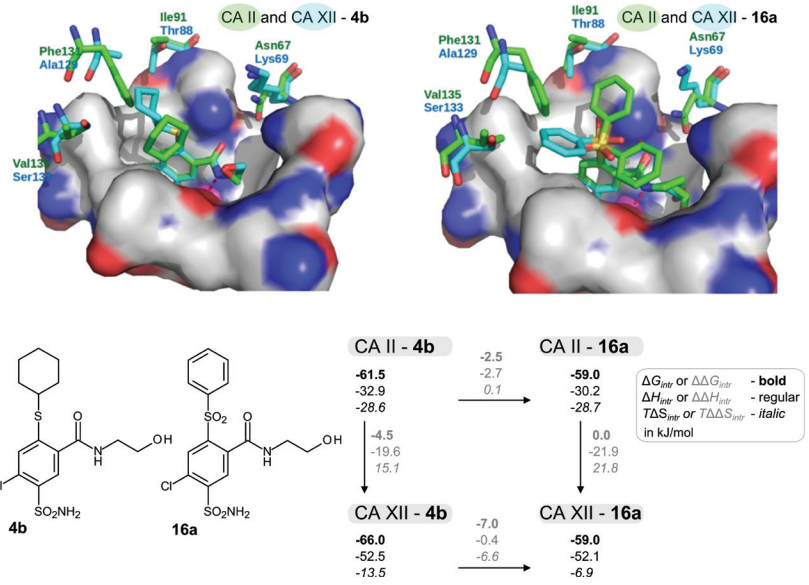


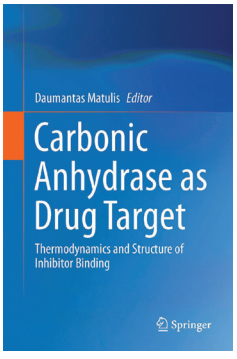
Drug Design via Structure and Thermodynamics

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. To make drug discovery process more rational, the recognition phenomenon between chemical compounds and proteins should be better understood. To help design compounds in silico and predict their affinity to target proteins, we are assembling 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 the 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. Matulis, D. Editor. Carbonic Anhydrase as Drug Target: Thermodynamics and Structure of Inhibitor Binding. Springer Nature, 2019.
2. Baranauskiene, L., Kuo, T.-C., Chen, W.-Y., Matulis, D. Isothermal titration calorimetry for characterization of recombinant proteins. *Current Opinion in Biotechnology*. 2019, 55, 9–15.
3. Zakšauskas, A., Čapkauskaitė, E., Jezepčikas, L., Linkuvienė, V., Paketurytė, V., Smirnov, A., Leitans, J., Kazaks, A., Dvinskis, E., Manakova, E. et al. Halogenated and di-substituted benzenesulfonamides as selective inhibitors of carbonic anhydrase isoforms. *European Journal of Medicinal Chemistry*. 2020, 185: 111825.



Isothermal Titration Calorimetry for Characterization of Recombinant Proteins and Ligand Binding

In our opinion, isothermal titration calorimetry (ITC) has wide applicability in the characterization of recombinant proteins (Baranauskiene et al. *Curr. Opin. Biotechnol.* 2019). ITC directly determines interactions of unaltered proteins with small molecules at physiological conditions and is thus highly useful to determine recombinant protein purity and ligand binding capability.

Figure 1 shows the application of ITC together with other methods to demonstrate the purity and activity of a recombinant protein preparation. (A) Titration of the enzymatic activity of a recombinant protein with a strong inhibitor. The enzyme concentration was 500 nM and the two lines cross at 500 nM showing that the protein preparation was highly pure and fully enzymatically active. (B) Dose-dependence curves of the inhibition of enzymatic activity with two inhibitors. Insets in A and B show a set of raw data curves at various inhibitor concentrations. (C) Application of ITC to demonstrate the purity of the protein preparation via determination of the fraction of binding-capable enzyme. Since the midpoint of the titration is at n=0.96, the protein preparation is 96% pure in its capability to bind the ligand. (D) The same interaction studied by the fluorescence-based thermal shift assay (FTSA), a high-throughput method to determine the affinities of protein-ligand interactions. Inset shows the raw protein thermal melting curves while the sigmoidal curve is a fit to the thermal melting datapoints confirming the affinity of the interaction as determined by ITC. Combination of these orthogonal methods is essential for good quality results

The principle of an ITC experiment (see Fig. 2). (A) Titration calorimeter contains 2 cells (sample cell that usually contains the protein and reference cell filled with water or solvent media) and a syringe used to inject the ligand solution to the sample cell. (B) Saturation of the protein binding sites by a ligand during titration. The experiment is planned so that most protein is saturated around the middle of the titration. The protein–ligand complex is colored red. (C) The power compensation between the sample and reference cells yields the raw ITC data peaks. Each peak represents a change in electric power needed to keep the temperature constant in the cell where a reaction has taken place. The area of the peak is transformed into the heat evolved upon one injection by appropriate calibration. (D) Binding isotherm, a set of datapoints obtained by integrating the raw ITC data peak areas. A single-binding-site model can be used to fit the data, yielding the affinity (Kb) and the change in enthalpy (ΔH) upon the interaction.

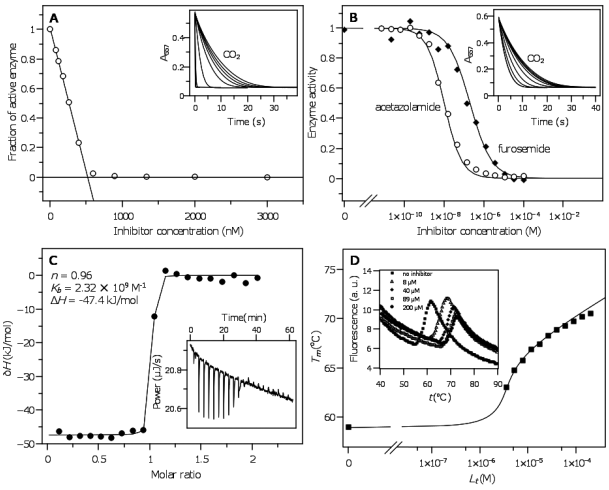


Fig. 1

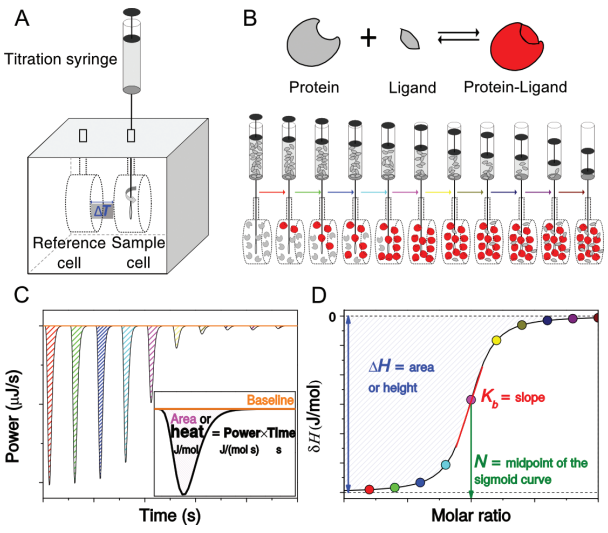


Fig. 2