Department of

Biothermodynamics and Drug Design

Chief Scientist and Head
Daumantas Matulis, PhD
phone: 370 5 2691884
fax: 370 5 2602116
e-mail: daumantas.matulis@bti.vu.lt
http://www.ibt.lt/en/laboratories/

Scientific staff
Virginija Dudutienė, PhD
Vaida Jogaitė, PhD
Jurgita Matulienė, PhD
Vytautas Petrauskas, PhD
Vilma Petrikaitė, PhD
Asta Zubrienė, PhD
Jelena Jachno, M.Sc.
Darius Lingė, M.Sc.
Vilma Michailovienė, M.Sc.

MoBiLi Project scientists
Vytautas Smirnovas, PhD

PhD students
Lina Baranauskienė, M.Sc.
Edita Čapkauskaitė, M.Sc.
Egidijus Kazlauskas, M.Sc.
David Daniel Timm, M.Sc.

Technical staff
Dalia Černiauskaitė, B.Sc.
Leokadija Davidian
Jurgita Revuckienė, B.Sc.

Associated scientists on leave
Prof Leonas Grinius, PhD, Dr. Habil

Undergraduate students
Sandra Bakšytė, Joana Gylytė, Eglė Ivanauskaitė, Aistė Kasiliauskaitė, Justina Kazokaitė, Eglė Maksimavičiūtė, Justė Mikučiauskaitė, Povilas Norvaišas, Miglė Kišonaitė, Aurelija Mickevičiūtė, Vaida Morkūnaitė, Donatas Ramaška, Gediminas Skvarnavičius, Alexey Smirnov, Darius Vagrys, Paulius Gibieža, Jorge David Bolanos Calvo, Akvilė Botyriūtė, Indrė Čižaitė, Martynas Grigaliūnas, Dovilė Janušaitė, Fausta Labanauskaitė, Ričardas Mališauskas, Arūnas Maisaitis, Ksenija Michailova, Katažyna Milto, Darius Šulskis, Tomas Šneideris, Aušra Želvytė

The Department of Biothermodynamics and Drug Design (DBDD) was established in 2006 in the place of the former Laboratory of Recombinant Proteins. The DBDD designs novel chemical compounds as anticancer agents. The efficiency of both naturally occurring and synthetic compounds is evaluated by structural biothermodynamics and molecular modelling methods. The laboratory's personnel consists of five teams according to their research activities:

The Team of Molecular and Cell Biology, headed by Dr. Jurgita Matulienė (Ph. D. in cell biology from the University of Minnesota, USA, 2003), produces drug target proteins by gene cloning, expression in *E.coli*, insect, or mammalian cells, and chromatografic purification of large quantities of active proteins sufficient for biothermodynamic measurements of compound binding. Several projects involve the design of protein domain constructs. Live human cancer cells are cultured for the evaluation of compound anticancer activity. Dr. Vilma Petrikaitė has a Ph. D. in pharmacy and performs compound testing in mice xenografts.

The Team of Organic Synthesis, headed by Dr. Virginija Dudutienė (Ph. D. in organic synthesis from the Vilnius University, 2005), synthesizes compounds that are designed to bind carbonic anhydrases and other drug target proteins. Compounds are designed by computer docking, molecular modelling, and comparison with naturally occurring or previously synthesized compounds. The special interests and capabilities of the group are in the field of synthesis of compounds with multiple conjugated aromatic heterocycles.

The Team of Biophysics, headed by Dr. Daumantas Matulis (Ph. D. in biochemistry, molecular biology and biophysics from the University of Minnesota, USA, 1998), measures compound binding to target proteins by isothermal titration calorimetry (ITC), thermal shift assay (ThermoFluor®), and pressure shift assay (PSA). The team performs the characterization of protein stability in the presence of various excipients and the measurements of target protein enzymatic activity.

The Team of Computer Modelling, headed by Vytautas Petrauskas (Ph. D. in physics from the Vilnius University, 2008), is responsible for the *in silico* docking of large compound libraries and the analysis of X-ray crystal structures of synthetic compound – protein complexes solved in collaboration with Dr. Saulius Gražulis' group in the Department of Protein – DNA interactions. Molecular modelling of candidate compounds often predicts novel compounds with improved binding capabilities. The group, together with several collaborating scientists is developing the software that estimates the energetics of compound binding to a protein when only the crystal structure of the free protein is available.

The Team of Amyloid Research

Recently a new team has started upon the return of Dr. Vytautas Smirnovas (Ph. D. from the Technical University of Dortmund, 2007) to Lithuania in 2011. The main interests and research of his team lie in the protein aggregation and amyloidogenesis that are involved in a number of diseases, including such neurodegenerative disorders as Alzheimer's and Parkinson's. His research is described in greater detail in the MoBiLi section of this issue.

MoBiLi project scientist Vytautas Smirnovas with students Akvilė Botyriūtė and Katažyna Milto

Research projects

Several protein targets have been selected for the investigation of protein – compound binding thermodynamics and the design of novel compounds with desired properties. A family of human carbonic anhydrases [1, 8], heat shock protein Hsp90 [2, 4, 5, 13, 14], and several epigenetically important proteins [3] were chosen as anticancer drug targets.

Novel methods and thermodynamic approaches are being used and developed in the laboratory. Detailed thermodynamic description of natural compound – protein interaction provides clues to improved compound affinity and specificity. In addition to the Gibbs free energy, enthalpy, entropy, and the heat capacity, the laboratory studies the volume and compressibility of the protein – compound interactions [9, 10].

The laboratory is interested in the fundamental thermodynamics of the hydrophobic effect [7] and the development of thermodynamic methodology for compound – protein interactions [11, 12].

Carbonic anhydrases as anticancer drug targets

Carbonic anhydrases (CAs), a group of zinc containing enzymes, are involved in numerous physiological and pathological processes, including gluconeogenesis, lipogenesis, ureagenesis, tumorigenicity and the growth and virulence of various pathogens. In addition to the established role of CA inhibitors as diuretics and antiglaucoma drugs, it has recently emerged that CA inhibitors could have potential as novel anti-obesity, anticancer, and anti-infective drugs (Supuran, 2008, 2012). CAs catalyze the conversion of CO_2 to the bicarbonate ion and protons. There are 12 catalytically active CA isoenzymes in humans. A number of CA inhibitors, mostly unsubstituted sulfonamides, have already been designed. However, most present inhibitors are insufficiently selective for targeting CA isozymes, such as hCAIX and hCAX-II, which are anticancer targets.

Here at the DBDD we have cloned and purified most cytoplasmic CAs and catalytic domains of transmembrane CAs. The organic synthesis team, together with collaborators, designed and synthesized over 500 novel compounds that bind CAs with submicromolar to subnanomolar affinity. Several novel series of CA inhibitors exhibited extremely tight affinity and an appreciable selectivity towards selected CA isozymes [1, 8].

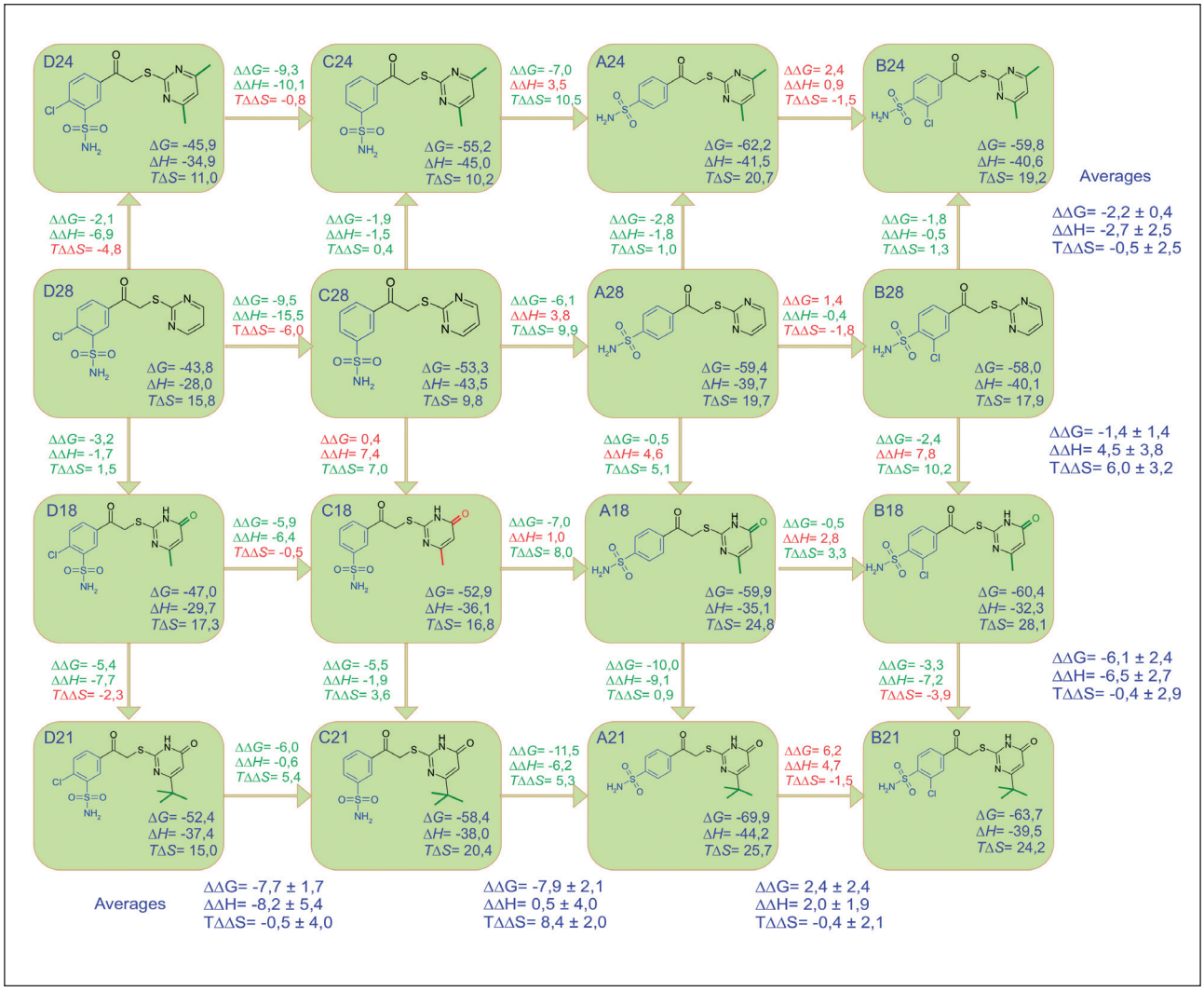


***Figure 1.** Additivity of the intrinsic thermodynamic parameters of compound binding to CA I (kJ/mol at 37 °C). Numbers at the compounds show the binding parameters while the numbers at the arrows show the differences. Averages of the differences are listed on the right and below the figure.*

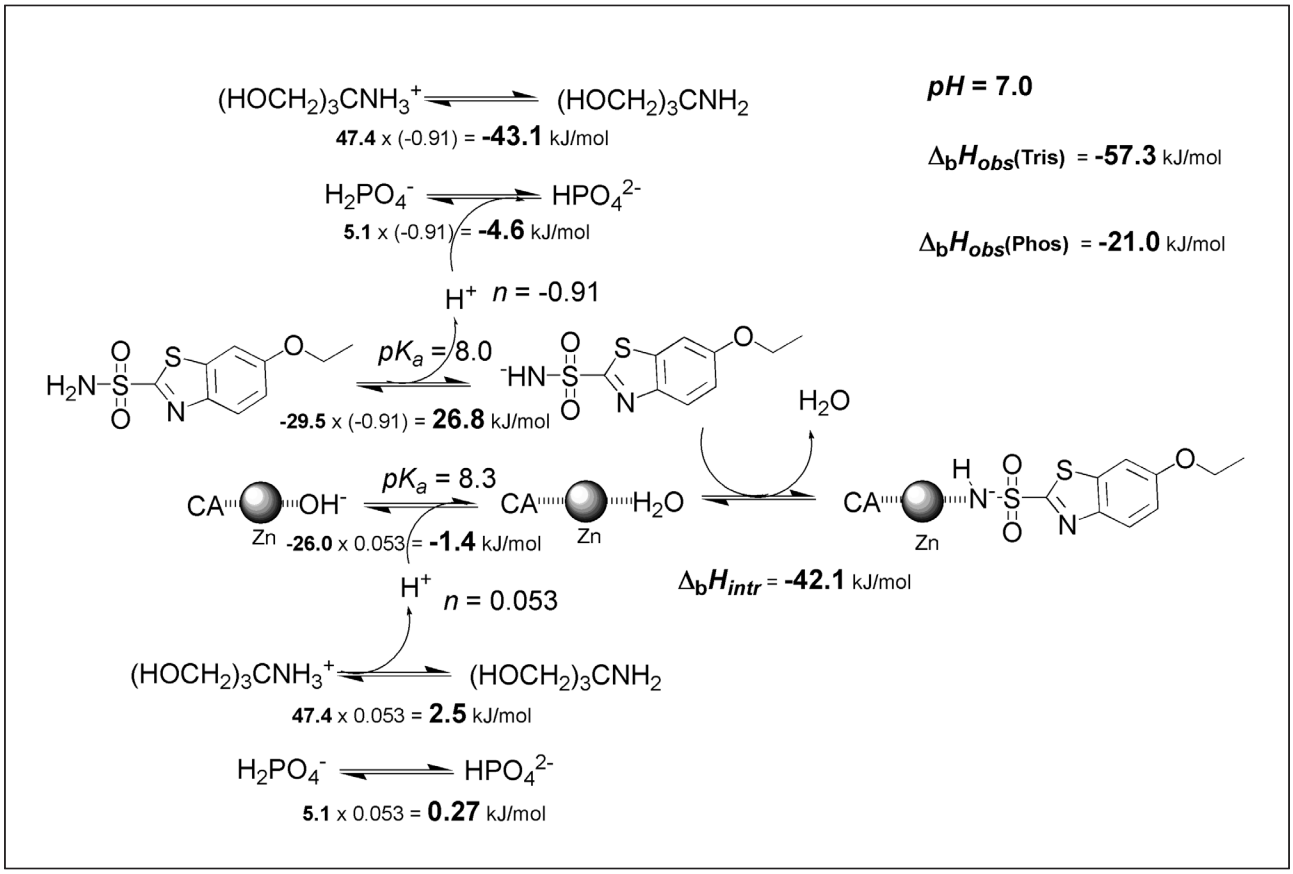


Figure 2. Contributions from linked reactions to the intrinsic binding enthalpy of ethoxzolamide to recombinant human CA XIII [8].

Ligand binding to proteins at high pressure

The volume changes accompanying ligand binding to proteins are thermodynamically important and potentially could be used in the design of compounds with specific binding properties. Measuring the volumetric properties could yield as much information as the enthalpic properties of binding. Pressure-based methods are significantly more laborious than temperature methods and are underused. The pressure shift assay (PressureFluor, analogous to the ThermoFluor, thermal shift assay) uses high pressure to denature proteins. The PressureFluor method was used to study the ligand binding thermodynamics of Hsp90 and human serum albumin. Ligands stabilize the protein against pressure denaturation, similar to the stabilization against temperature denaturation.

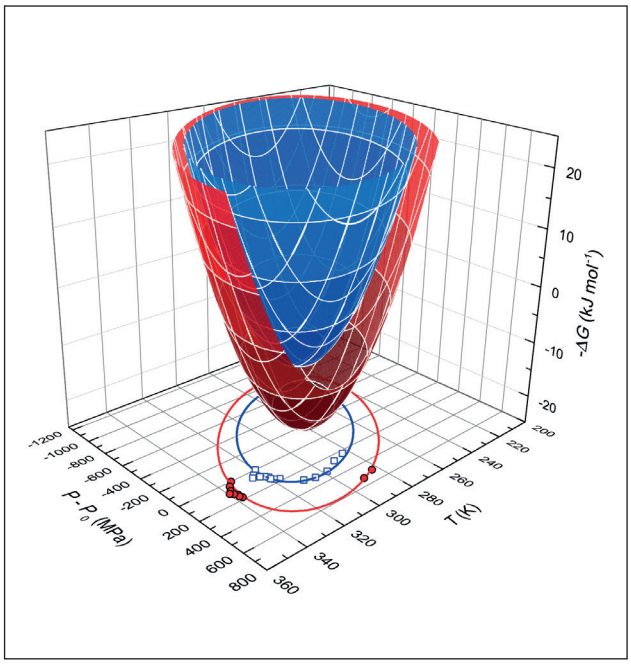


Figure 3. The Gibbs free energy dependence on pressure and temperature. Inner surface represents the ligand free Hsp90N stability region, while outer surface shows stability region of protein-ligand system with 200 μM of added ligand (Petrauskas et al 2013).

Inhibition of the Hsp90 chaperone

Heat shock protein 90 (Hsp90) is a molecular chaperone that is responsible for the correct folding of a large number of client proteins. The client proteins include many overexpressed oncogenes that are critical for the transformed phenotype observed in tumours.

Our laboratory is interested in the thermodynamics of inhibitor binding. A series of Hsp90 inhibitors were designed that exhibit extremely tight subnanomolar affinities. Intrinsic thermodynamics of their binding and the cocrystal structures were determined [2, 4]. Volumetric properties at high pressures were determined for the compound [10, and Petrauskas et al. 2013]. The EU and US patents have been obtained for the series.

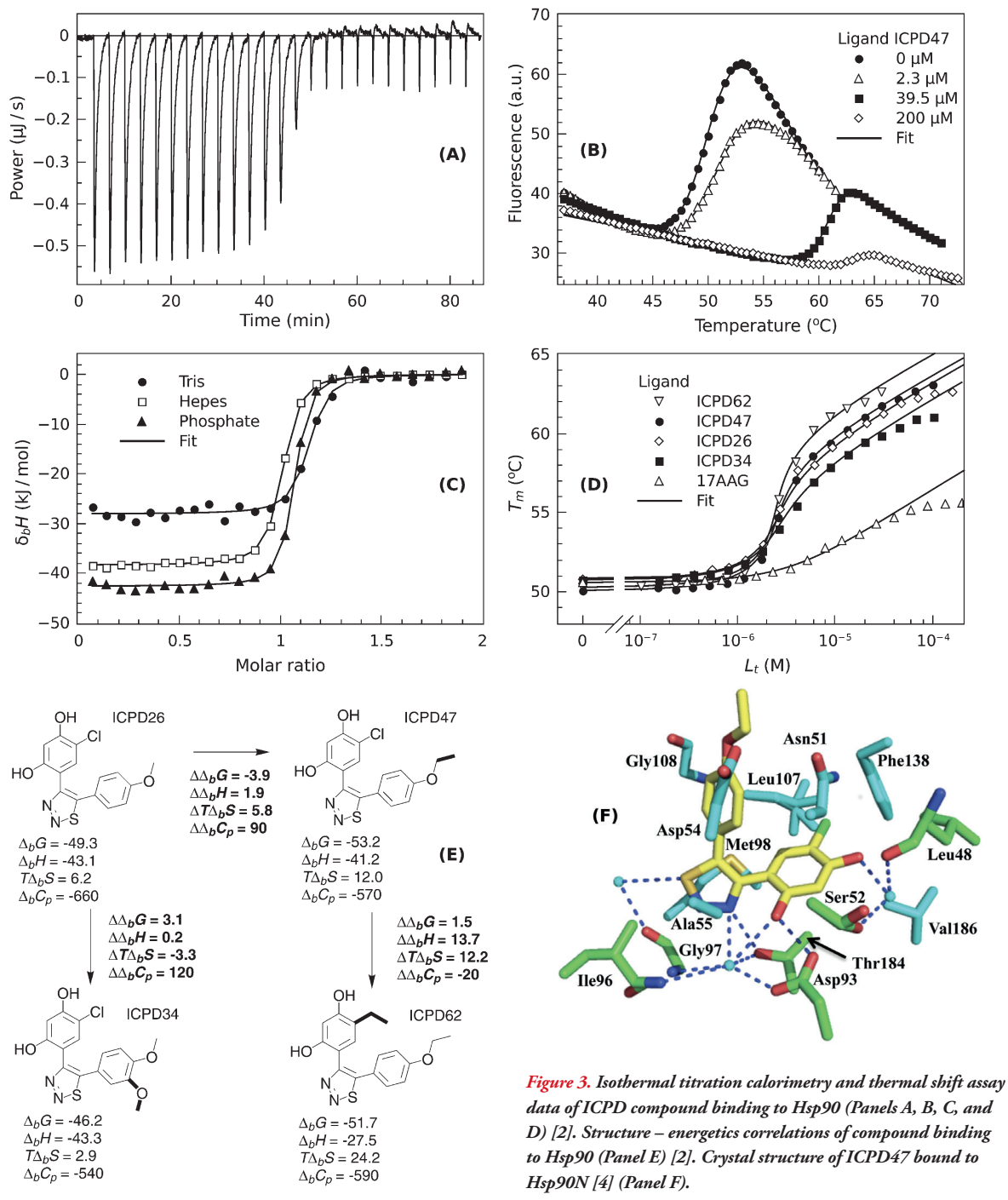
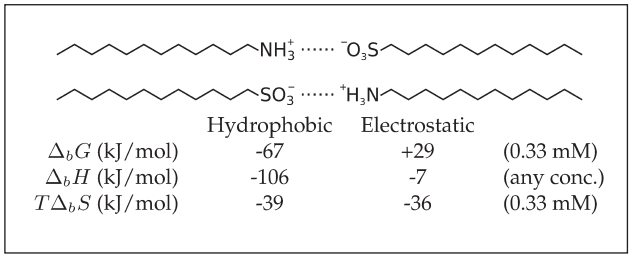


Figure 3. Isothermal titration calorimetry and thermal shift assay data of ICPD compound binding to Hsp90 (Panels A, B, C, and D) [2]. Structure – energetics correlations of compound binding to Hsp90 (Panel E) [2]. Crystal structure of ICPD47 bound to Hsp90N [4] (Panel F).

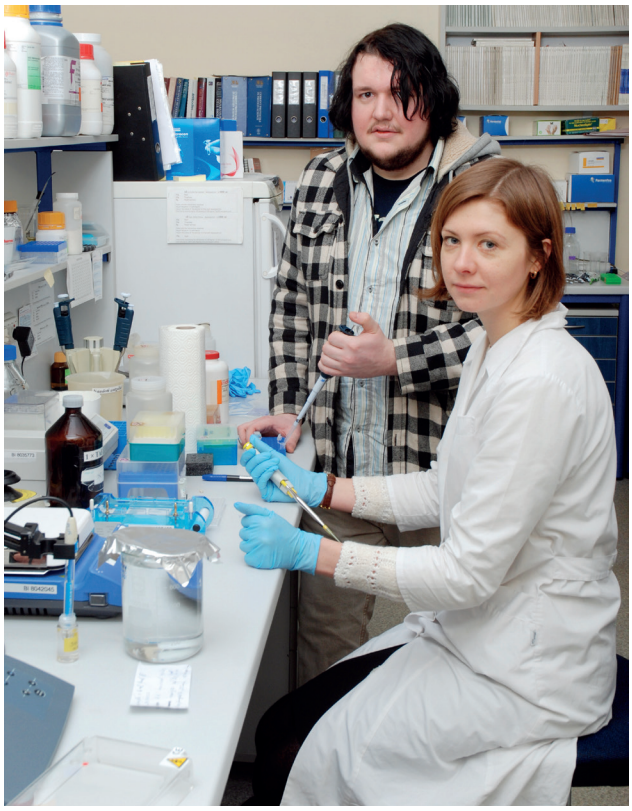
Thermodynamics of the Hydrophobic effect

The energetics of the hydrophobic effect is of fundamental importance to biophysics. It is important for the understanding of protein folding, ligand binding, and the formation of lipid membranes. The common view emphasizes entropic origins of the binding force of the hydrophobic effect. In our previous studies (Matulis and Bloomfield, 2001), we have shown the importance of enthalpy and phase changes in the system of long chain aliphatic compounds. The binding of oppositely charged detergents shows similar signatures of the hydrophobic forces.



	Hydrophobic	Electrostatic	
$\Delta_b G$ (kJ/mol)	-67	+29	(0.33 mM)
$\Delta_b H$ (kJ/mol)	-106	-7	(any conc.)
$T\Delta_b S$ (kJ/mol)	-39	-36	(0.33 mM)

Figure 4. Energetics of dodecylammonium binding to dodecane sulfonate forming solid aggregate [7].



Junior scientist Vaida Jogaitė and PhD student David Daniel Timm performing molecular biology experiments

Master student Sandra Bakšytė performing PCR experiment

Services

The DBDD is seeking to license out the compounds described in patents and patent applications. The DBDD is interested in collaborations where our expertise in recombinant protein production and the determination of compound – protein binding thermodynamics and recombinant protein stability characterization could be applied. Protein – ligand binding constants and protein thermal stability profiles at hundreds of conditions may be determined in a single experiment by consuming microgram quantities of protein.

Conferences

The DBDD regularly participates in many international conferences and symposiums, including:
International Conference on the Hsp90 chaperone machine
International Conference on the Carbonic Anhydrases
International Conference on High Pressure Bioscience and Biotechnology
Biothermodynamics Symposium
European Biophysics Congress
Biophysical Society Annual Meeting
Gibbs Conference on Biothermodynamics
International Conference of Lithuanian Biochemical Society
COST project CM0804 and TD0905 meetings

Collaboration

The DBDD has ongoing collaborations with a number of research laboratories and industry worldwide, including:
Institute of Medical Technology, University of Tampere, Finland
University of Florence, Italy
International Institute of Molecular and Cell Biology, Warsaw, Poland
Jensen Pharmaceuticals, Johnson&Johnson, USA
Centre for Structural Biochemistry, Montpellier, France
Institute of Organic Synthesis, Riga, Latvia
Institute of Organic Chemistry, University of Tubingen, Germany
Cancer Research Centre, University of Edinburgh, UK
St. Andrews University, UK
Institute of Chemistry, UMR CNRS 7272, Nice, France
Faculty of Chemistry, Vilnius University, Lithuania
Faculty of Natural Sciences, Vilnius University, Lithuania
Institute of Biochemistry, Vilnius, Lithuania
Lithuanian University of Agriculture, Kaunas, Lithuania
AB "Amilina", Panevėžys, Lithuania
Nature Research Centre, Institute of Botany, Vilnius, Lithuania

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Patents and Patent Applications

Matulis D., Dudutienė V., Matulienė J. and **Mištinaitė L.** Benzimidazo[1,2-C][1,2,3]Thiadiazol-7-Sulfonamides as Inhibitors of Carbonic Anhydrase and the Intermediates for Production Thereof. EP2054420

Matulis D., Čikotienė I., **Kazlauskas E.** and **Matulienė J.** 5-Aryl-4-(5-Substituted 2,4-Dihydroxyphenyl)- 1,2,3 Thiadiazoles as Inhibitors of Hsp90 Chaperone and the Intermediates for Production Thereof. EP2268626

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7. **Norvaišas P., Petrauskas V., Matulis D.** Thermodynamics of cationic and anionic surfactant interaction. J. Phys. Chem. B. 2012, 116(7):2138-44.
8. **Baranauskienė L., Matulis D.** Intrinsic thermodynamics of ethoxzolamide inhibitor binding to human carbonic anhydrase XIII. *BMC Biophys.* 2012, 5(1):12.
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12. **Petrikaitė V., Matulis D.** Natural and Synthetic Inhibitors Binding to Human Heat Shock Protein 90 and Their Clinical Application. *Medicina* (Kaunas) 2011, 47 (8):413-420.

Book Chapters

1. Cimmperman P. and **Matulis D.** Protein Thermal Denaturation Measurements via a Fluorescent Dye. // Biophysical Approaches Determining Ligand Binding to Biomolecular Targets. Detection, Measurement and Modeling. Editors: Podjarny A., Dejaegere A. and Kiefer B. RSC Publishing 2011, 247-274.
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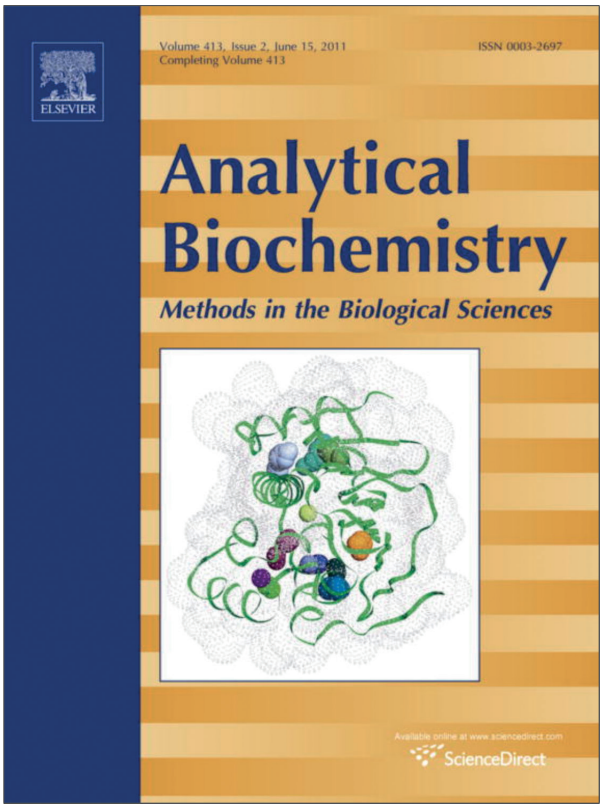

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