XXI CONFERENCE OF THE INTERNATIONAL SOCIETY FOR BIOLOGICAL CALORIMETRY ISBC 2022

June 8–10, 2022
Vilnius, Lithuania

ABSTRACT BOOK

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WELCOME

On behalf of the Conference Organizing Committee I would like to welcome you to the XXI Conference of the International Society for Biological Calorimetry.

We are delighted to welcome you in Vilnius, capital of Lithuania, and hope that you will enjoy both the scientific sessions and the cultural and social program.

Prof. Daumantas Matulis

Conference Chair
Director, Life Sciences Center
Vilnius University

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PROGRAM

JUNE 8, 2022 | Wednesday

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11:40-12:10	Differential Scanning Calorimetry - A Complimentary Approach For Biomedical Studies *Nichola Garbett* | University of Louisville, USA
12:10-12:40	Advances in Biofilm Detection and Monitoring Using Calorimetry, and The Push Towards Clinical Diagnostics Applications *Magnus Jansson* | Symcel AB, Sweden
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15:00-15:30	The Intrinsic Thermodynamic Parameters of Carbonic Anhydrase II and Acetazolamide Interaction *Eglė Vitkūnaitė* | Vilnius University, Lithuania
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PROGRAM

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10:00–10:30	Differences in Infectivity and Pathogenicity Between Delta and Omicron Strains of SARS-CoV-2 Can Be Explained by Gibbs Energies of Binding and Growth *Marko Popovic* | Technical University of Munich, Germany
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11:30–12:00	Biocalorimetry – An Early Warning Tool for the Detection of Legionella Pneumophila in Drinking Water *Christian Fricke* | University of Koblenz-Landau, Germany
12:00–12:30	Calorimetric Heat Waves to Study the Soil Sensitivity to Temperature *Nieves Barros* | University of Santiago de Compostela, Spain
12:30–14:00	Lunch
14:00–15:00	Poster session
15:00–16:00	ISBC meeting
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PROGRAM

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10:00-10:30	A Calorimetric Study of Interactions in the Systems of Charged Surfactants and Ionic Poly(amino acid)s *Vytautas Petrauskas* | Vilnius University, Lithuania
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11:30-12:00	Isothermal Calorimetry and Complementary Methods to Understand Nucleotide-Based Second Messenger Action on Proteins *Gert Bange* | Philipps University Marburg, Germany
12:30-12:40	Closing remarks *Daumantas Matulis* | Conference Chair

ORAL PRESENTATIONS

ISOTHERMAL CALORIMETRY AND COMPLEMENTARY METHODS TO UNDERSTAND NUCLEOTIDE-BASED SECOND MESSENGER ACTION ON PROTEINS

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Nucleotide-based second messengers, such as (p)ppGpp, c-di-GMP or di-adenosine-tetraphosphate (Ap4A) play important roles in adapting microorganisms to changing environmental conditions or the host (e.g., [1,2]). I will discuss how isothermal calorimetry provides the biochemical parameters enabling to determine biological significance of the interaction of a given second messenger for its target structure. I will also present complementary methods, which provide a further in-depth mechanistic understanding of nucleotide-based second messenger in action.

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[1] Bange G*, Brodersen D, Liuzzi A, Steinchen W (2021). Two P or not P: Understanding regulation by the bacterial second messengers (p)ppGpp. Annual Reviews in Microbiology, 75:383-406. PMID: 34343020 [2] Czech L, Mais CN, Kartzat H, Sarmah P, Giammarinaro P, Freibert S, Esser Esser H, Musial J, Berninghausen O, Steinchen W, Beckmann R, Koch HG, Bange G (2022). Inhibition of SRP-dependent protein secretion by the bacterial alarmone (p)ppGpp. *Nature Communications*, 13(1):1069 PMID: 35217658.

CALORIMETRIC HEAT WAVES TO STUDY THE SOIL SENSITIVITY TO TEMPERATURE

Nieves Barros[1], José Antonio Rodríguez-Añón[1], Jorge Proupín[1], César Pérez Cruzado[2]
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Heat conduction calorimeters can change the temperature along with a measurement of the heat rate in living systems, constituting an interesting option for studying, in situ and real-time, the response of living systems to temperature changes. In the case of soil, where reactions involving the carbon cycle take place, the role of temperature on soil bio decomposition is still under development. Those studies usually focus on temperatures at which enzymes are active, with little information about what happens when temperatures are higher than 45 ºC, something happening in our planet more and more often. There is no information about how living systems recover after extreme heat either.

We have designed a heatwave with a heat conduction calorimeter to study the sensitivity to extreme temperatures of different soil layers from Atlantic oak forests, and to monitor how they recover after being exposed at 60 ºC for about 15 hours. The observed sensitivity was attached to the soil organic matter nature by thermal analysis and differential scanning calorimetry. Calorimetry was sensitive enough to show the response of the different soil samples to the increasing and decreasing temperatures and allowed us to discern among the samples which resisted and did not resist the extreme heatwave.

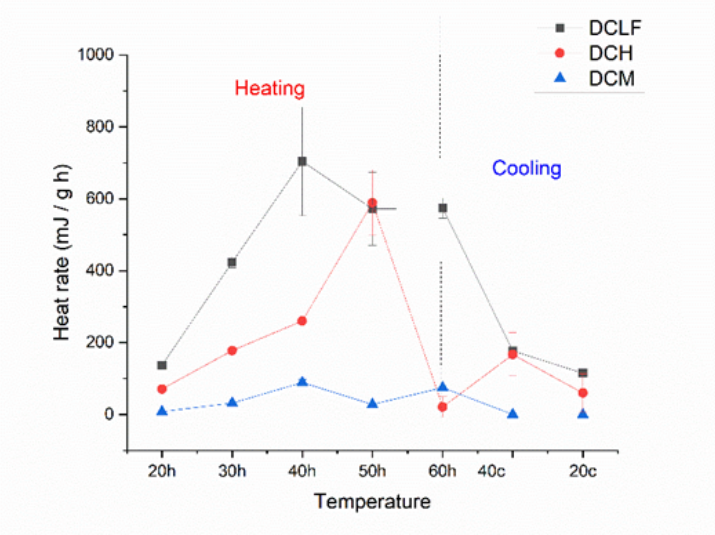

Figure 1. Evolution of microbial metabolism from three different soil layers to the increasing temperature from 20 to 60ºC and to cooling from 60ºC to 40 and 20 ºC. h indicates the heating phase of the experiment; c is the cooling period.

PHYSICAL BIOENERGETICS OF BIOLOGICAL SYSTEMS

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Cells are biological thermodynamic systems which function out of equilibrium. They rely on a continuous supply of energy to fuel cellular processes that control and maintain cellular homeostasis, growth and development. This energy is supplied by cellular metabolism, which converts energy-rich nutrients into biomass building blocks, ATP, and energy-poor metabolic waste molecules. While immense effort has been put into understanding the biochemistry of metabolism in the past decades, the general framework for the energetic principles and thermodynamic characterization of cellular metabolism has received little attention.

In the group of Energetics of Biological Systems, we aim to explore biological models using the prism of physical bioenergetics. Our goal is to gain quantitative understanding of how energy fluxes govern the behaviour of out-of-equilibrium systems (biochemical networks, cells, and developing organisms). We ask the following fundamental questions: How much energy is needed to keep a quiescent cell alive and out of equilibrium? What are the extra costs for transitioning to proliferation and how are they distributed throughout the cell cycle and among cellular subprocesses? Similarly, how is the embryonic development influenced by energetic metabolism and thermodynamic limitations?

To answer these questions, we use isothermal calorimetry to quantitatively measure the flow of energy between biological systemsand their surroundings in the form of heat. We combine these measurements with fluorescent and quantitative phase microscopy, biochemical perturbations, metabolome analysis and theoretical modelling. Here, we will share our recent advances of how these approaches can be used to obtain a quantitative understanding about the energetic flows and thermodynamic constraints in quiescent and growing biological systems. We will outline our prospective directions for exploring these traits in cultured mammalian cells and developing embryos.

ISOTHERMAL MICROCALORIMETRY AND THE DEVELOPMENT OF ANTIMICROBIAL ORTHOPEDIC IMPLANTS AND DENTAL ALIGNERS

Olivier Braissant[1], Sarrah Worreth[1], Tino Töpper[2], Elise Dard[2], Philipp Gruner[3], Walter Moser[4], Harald Holeczek[3], Michael de Wild[5], Monika Astasov-Frauenhoffer[1,6]
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Biofilms are prone to grow on surfaces and as a consequence they can cause serious issues. Medical devices and in particular implants are especially at risk as they are in contact with biological material. The consequences for the patients of contaminated medical devices or implants can be disastrous.

Therefore, medical device and implants manufacturers are trying to limit the contamination of such material by making their surface antimicrobial. For orthopedic implants providing a source of calcium allowing good osseointegration is of interest. Therefore, calcium hydroxide that is often used in dentistry during root canal treatment has been investigated for its antimicrobial properties on implants. A calcium hydroxide layer can be electrochemically deposited on implants thus making their surface very alkaline and antimicrobial. Still, human bone cells can handle such high pH under certain conditions [1]. As similar challenge, transparent dental aligners placed in the mouth are prone to colonization by oral bacterial. To reduce the risk of caries formation or early demineralization cellulose-based polymers can be loaded with natural antimicrobial agents. In this context, essential oils (such as cinnamaldehyde and limonene) can be adsorbed on the material of the aligner and later slowly released in the patient mouth [2].

In both cases it is rather difficult to investigate the antimicrobial properties of those material because of their nature and specific applications. Only the surface of opaque materials can be investigated by microscopy and porous materials poses problem in microbial recovery for plate counts for example. In this context, this study show how isothermal microcalorimetry can be used to assess antimicrobial properties of plastic and titanium coated with antimicrobials and demonstrate that valuable results on growth dynamics and inhibition can be obtained.

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CONSERVED PATTERNS OF HEAT RELEASE FROM CULTURED MICROORGANISMS REVEAL SIMPLE GROWTH-METABOLISM RELATIONS

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Quantitative analyses of cell replication address the connection between metabolism and growth. Various growth models approximate time-dependent cell numbers in culture media equally well, but physiological implications are vague. Isothermal microcalorimetry (IMC) measures with unprecedented sensitivity the heat (enthalpy) release from chemical turnover in metabolizing cells. Hence, the nutrient-dependence of metabolism can be studied independently of modelling the time-dependence of cell numbers. Surprisingly, IMC traces exhibit conserved patterns when expressed in the enthalpy-, rather than time-domain as exemplified for cultures of *Lactococcus lactis* (prokaryote), *Trypanosoma congolese* (protozoan) and non-growing *Brassica napus* (plant) cells. The data comply extraordinarily well with a dynamic adsorption-reaction model resembling a Michaelis-Menten equation generalized here to non-constancy of catalytic capacity. The proposed formalism reproduces the "life span" of cultured microorganisms from exponential growth to metabolic decline with fundamental consequences for the derivation of toxicity measures and for the transferability of metabolic activity data between laboratories.

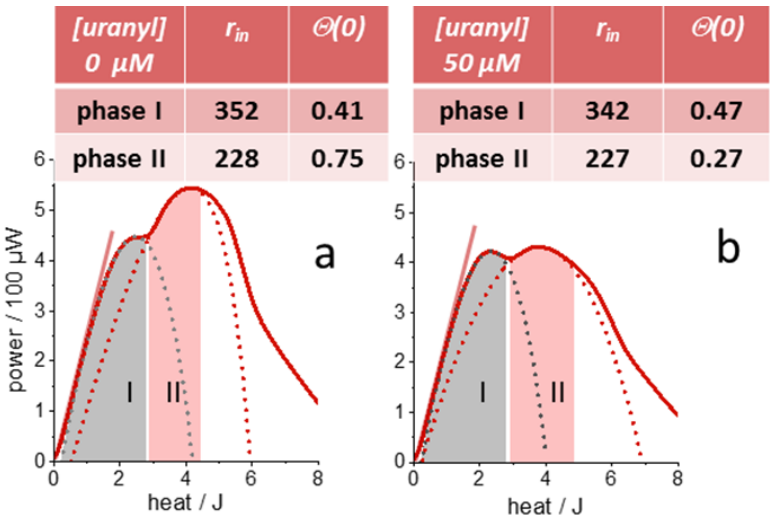

Figure 1. Uranyl toxicity is specific to the metabolic state during culture growth of *Lactococcus lactis* [1]. Traces were analyzed by „dynamic adsorption reaction thermogram simulation" (dAR-TS) revealing that the heavy metal affects the second growth phase by lowering the apparent substrate affinity Θ, rather than the initial growth rate r_{in}(in $10^{-3}h^{-1}$).

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BIOCALORIMETRY - AN EARLY WARNING TOOL FOR THE DETECTION OF LEGIONELLA PNEUMOPHILA IN DRINKING WATER

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Access to safe and clean drinking water is essential to all individuals on earth. Mismanagement and poor hygiene deteriorate the quality of drinking water. An immediate threat arises from pathogenic microorganism that can cause fatal diseases [1]. To detect microbial contaminations in drinking water, conventional microbiological analysis are performed based on visual inspection of culture plates. This procedure is easy to use, provides simple measures of the contamination level and allows follow-up analyses for further characterization. However, the procedure is time-consuming and error-prone due to subjective decisions [2].

Isothermal microcalorimetry (IMC) is regarded as a promising diagnostic tool for fast and reliable detection of bacterial contaminations in various matrices. Nevertheless, calorimetric detection of pathogens in drinking water analysis has scarcely been studied to date [3]. We selected *Legionella pneumophila*, a waterborne pathogen that frequently causes major outbreaks worldwide, as the target microorganism for our calorimetric investigations.

In this study, we demonstrated the rapid detection of *L. pneumophila* on conventional medium (BCYE agar) by IMC using two commercial (TAM III, MC-CAL / 100P) and a novel designed microcalorimeter. The detection time varied between 24 and 48 h, depending on the contamination level and the detection limit applied (conventional detection by plate counting takes up to 10 days) [4]. In addition, we also investigated the growth of commonly accompanied pathogens such as *E. coli*, *E. faecalis* and *P. aeruginosa* on selective (GVPC, BCYE-Ab) and non-selective (BCYE) medium. Preliminary results are promising, as no heat flow signals were observed for E. faecalis and P. aeruginosa on GVPC medium.

Our results show that a calorimetric approach includes all advantages of conventional Legionella analysis and can overcome the decisive drawback of slow detection.

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[4] C. Fricke, J. Xu, F-L. Jiang, Y. Liu, H. Harms, T. Maskow, *Microb. Biotechnol*., 13, 1262-72 (2020).

DIFFERENTIAL SCANNING CALORIMETRY - A COMPLEMENTARY APPROACH FOR BIOMEDICAL STUDIES

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The traditional application of differential scanning calorimetry (DSC) for the characterization of temperature-induced macromolecular transitions has been extended over the last ~15 years to characterize complex biofluid samples as a potential technology for clinical diagnostics.This presentation will discuss the use of DSC to provide a comprehensive assessment of the thermostability of biofluid proteins. There is a growing body of research which has demonstrated the utility of DSC in multiple disease settings with sensitivity to disease type, severity as well as response to treatment. The majority of reported studies have focused on the analysis of blood plasma, with others looking at urine, cerebrospinal fluid, and solid tumor samples. DSC is an interesting complementary approach to biomarker and clinical diagnostic studies in interrogating a different dimension of disease proteomics: characterizing the thermodynamic properties of high abundance proteins and the impact of biomarker modifications and interactions within disease proteomes.

ADVANCES IN BIOFILM DETECTION AND MONITORING USING CALORIMETRY, AND THE PUSH TOWARDS CLINICAL DIAGNOSTICS APPLICATIONS

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Antimicrobial resistance in biofilms is a growing problem during clinical care, and the study of antimicrobial resistance requires realistic models which are difficult to monitor in vitro. Isothermal microcalorimetry (ICM) has been used to study the complex microbial aggregates in biofilms and to deliver new insights into the response of biofilms to antimicrobials, which could impact the treatment of patients. Complex microbial communities can be built up and monitored in a realistic environment by using ICM. Recently published work has shown that ICM traces provide a detailed and unique fingerprint for microbial communities in a biofilm. This fingerprint can indicate how the microbial community will respond to antibiotics.

Further work has demonstrated that ICM can be used to trace the evolution of antimicrobial resistance and tolerance to antimicrobials in successive bacterial populations. With ICM as a tool for monitoring the metabolic response to antimicrobials, it was possible to screen for compounds that could reverse antimicrobial tolerance and treat biofilms in the clinic.

The possible case for using ICM as a tool for rapid In Vitro Diagnostic detection of biofilm-related infections as a standardized technology will be presented.

USING ISOTHERMAL MICROCALORIMETRY TO DETERMINE THE STABILITY OF WHEY PROTEIN BARS

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There is an increasing consumer demand for high protein product including whey protein bars. The shelf life of protein bars is limited by a defect in which whey protein bars go hard and become unpalatable by the consumer. Thus, when developing new formulations, it is critical to determine how quickly the bar gets hard. Currently, the process of bar hardening is measured by incubating formulations until hardening occurs which is often on the time scale of months. Isothermal microcalorimetry has previously been used to determine if the stability of micronutrients, pharmaceuticals, and batteries. In this study we are using microcalorimetry to measure whey protein bar hardening. We have made protein bars that are mixtures of protein (hydrolyzed whey protein isolate (HWPI), whey protein isolate (WPI)), lipid (vegetable shortening), and sugar (invert sugar, sucrose solution). We made 3 component mixtures of all possible combinations of protein, lipid, and sugar; and we made 2-component mixtures of each of the ingredients. Triplicates of each mixture at approximately 1-gram samples were sealed in ampules in the presence and absence of oxygen were placed in the TAM IV (TA instruments) microcalorimeter at 50^{0} C. The samples were then monitored for 3-12 days to determine both fast and slow reactions that occur in the samples. These samples demonstrated that there were at least two reactions that were occurring, one fast reaction and a slow reaction the reaction was still present at day 12. Each reaction occurred when the protein and sugar where present, but not in the absence. We therefore conclude that the reactions that limit protein bar stability occur between the protein and sugar.

ISOTHERMAL TITRATION CALORIMTERY FOR THE ANALYSIS OF THE KINETICS AND COMPLETNESS OF ENZYMATIC POLYETHYLENE TEREPHTHALATE (PET) NANOPLASTIC DEGRADATION

Thomas Maskow,[1] Kristina Vogel,[1,2] Hassan Al-Fathi,[1] Hans Fritsche,[1] Christian Ortmann,[3] Daniel Breite,[4] Irina Estrela-Lopis,[5] Tom Venus,[5] Uwe Bornscheuer,[6] Ren Wei[6]

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As a result of the increasing global production of plastics, which has reached 359 million metric tons p.a. excluding synthetic fibers in 2018, efficient waste disposal measures are urgently needed for the sustainable treatment of the equivalent amount of plastic waste. In 2015 only 9% of the plastic waste was recycled worldwide. A tremendous amount of improperly or untreated plastic waste, hence, enters and accumulates persistently in the natural environment including the oceans where larger plastic debris are fragmented into small particles called microplastics (<5 mm) and nanoplastics (NP, <100 nm). In comparison with their larger counterparts, NP are of higher concern due to their high permeation ability into human and animal tissues.

In case of nanoplastics from polyethylene terephthalate (PET-NP), enzymes [1], microorganisms (e.g. *Ideonella sakaiensis*) [2] or microbial communities able to hydrolyse PET are recently reported. In order to estimate the residence time of PET-NP in ecosystems or the hydrolysis for monomer recovery at technical scale, the depolymerization rate and the degree of final depolymerization as a function of environmental conditions should be known. Numerous different analytical techniques have been developed to answer these questions, but they can often only answer partial aspects, because the degradation products are very heterogeneous and the degradation takes place at the interface between the plastic particles and the aquous environment.

Calorimetry, however, in combination with appropriate thermokinetic models, quantifies in real time, unlike conventional methods, the rate of ester cleavage and the binding of enzymes to PET-NP, providing a holistic description of the process [3]. This thesis was tested using the recombinant cutinase TfCut2 from *Thermobifida fusca* KW3 and a not yet characterized depolymerase as examples. The thermokinetic model explains the observed heat production rates and provides plausible kinetic and thermodynamic parameters. Example parameters for TfCut2 are the enthalpy of the ester cleavage of – 58±1.9 kJ mol-1, the enthalpy of the enzyme adsorption on the PET-NP surface of 129±2 kJ mol-1, the apparent equilibrium constant of the enzyme substrate complex of 0.046±0.015 g L−1 and the degree of degradation between 10 and 16 %. The enzymatic cleavage is inhibited by the hydrolysis products. It could be determined that the heat production of PET NP degradation depends to 95% on the reaction heat and only to 5% on the adsorption heat. The results of the ITC experiments were confirmed by nanoparticle tracking analysis, XRD, SEM and FTIR.

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ISOTHERMAL TITRAION CALORIMETRY FOR THE DETERMINATION OF INTRINSIC ENTHALPIES OF PROTEIN-LIGAND BINDING

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Protein-ligand binding reactions are almost always accompanied by linked protonation-deprotonation reactions that occur simultaneously with the binding reactions. For example, when resorcinol-bearing compounds bind Hsp90, the hydroxyl group must protonate upon binding, while it may be deprotonated in solution depending on the pH. Such binding-linked reactions consume energy and diminish the observed binding constant. If not taking this effect into account, medicinal chemists may be mislead and assign the affinity to incorrect molecular features. Therefore, it is important to calculate the intrinsic thermodynamics that would account for the linked reactions.

We have designed over 1100 selective inhibitors of human carbonic anhydrase protein family and study their structure – thermodynamics correlations to better understand the protein-ligand binding recognition. The proteins are highly homologous and the compounds bear a sulfonamide group that makes them bind via a similar mechanism. This enabled us to study the effect of small chemical structural changes in the ligand on the intrinsic thermodynamics, primarily the change in the intrinsic enthalpy of binding. Determination of such parameters helped in the design of extremely-high affinity ligands that exceed 1 pM intrinsic affinity.

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A CALORIMETRIC STUDY OF INTERACTIONS IN THE SYSTEMS OF CHARGED SURFACTANTS AND IONIC POLY(AMINO ACID)S

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The complex nature of proteins makes it difficult to interpret the intermolecular interactions and dissect the overall interaction energy into electrostatic and hydrophobic contributions. Thus, it is essential to understand these interactions in the systems of interacting surfactants and poly(amino acid)s [1-3]. These systems resemble some properties of protein-small molecule interactions and are simpler than the real protein and ligand systems. In this work, we used isothermal titration calorimetry to determine the average standard values of thermodynamic parameters (the Gibbs energy, enthalpy, entropy, and the heat capacity) of interaction in various systems of charged amino acid homopolymers (polyarginine, polylysine, polyornithine, polyaspartic and polyglutamic acids) and both positively and negatively charged linear surfactants (Figure 1). The determined electrostatic and hydrophobic contributions to the standard thermodynamic parameters are important in the computational models of interacting proteins and small molecules.

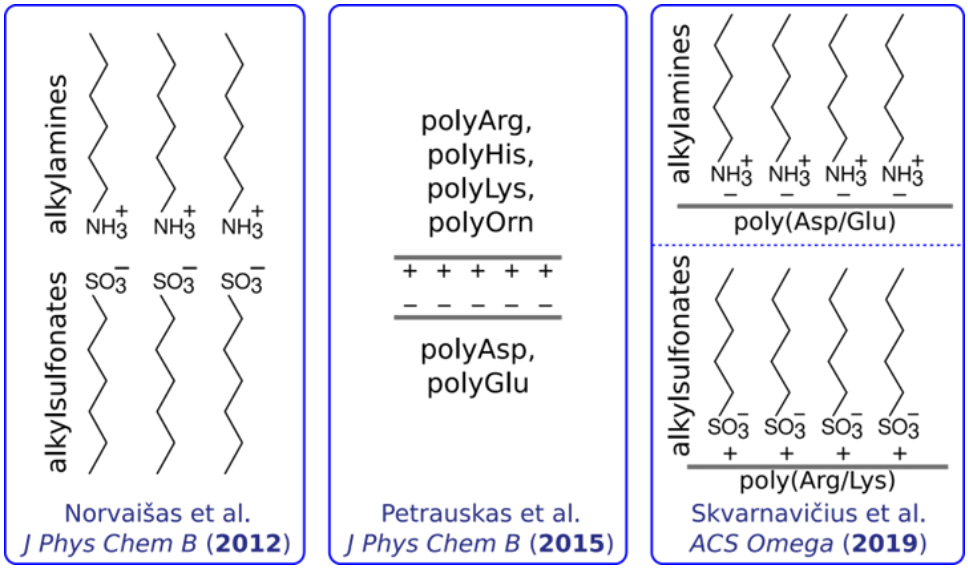

Figure 1. The systems of charged surfactants and ionic poly(amino acid)s used in this study.

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DIFFERENCES IN INFECTIVITY AND PATHOGENICITY BETWEEN DELTA AND OMICRON STRAINS OF SARS-COV-2 CAN BE EXPLAINED BY GIBBS ENERGIES OF BINDING AND GROWTH

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During the COVID-19 pandemic, biothermodynamics has given its contribution to characterization of viruses and research on energetics of processes performed by viruses [1-4]. Thermodynamic properties represent the driving force for processes performed by viruses and hence are an important element in predictive mechanistic models of virus-host interactions [2,3]. In this research, empirical formulas have been reported of the Delta and Omicron strains of SARS-CoV-2. The empirical formula of the Delta strain virion was found to be $CH_{1.6383}O_{0.2844}N_{0.2294}P_{0.0064}S_{0.0042}$. The empirical formula of the Omicron strain virion was found to be $CH_{1.6404}O_{0.2842}N_{0.2299}P_{0.0064}S_{0.0038}$. Based on the empirical formulas, standard thermodynamic properties of formation and growth have been calculated and reported for the Delta and Omicron strains. Moreover, standard thermodynamic properties of binding have been reported for Wild type (Hu-1), Alpha, Beta, Gamma, Delta and Omicron strains. For all the strains, binding phenomenological coefficients and antigen-receptor (SGP-ACE2) binding rates have been determined and compared, which are proportional to infectivity. The results show that infectivity of the Omicron strain is 50% greater than that of the Delta strain. The increased infectivity was explained in this paper using Gibbs energy of binding. However, no indications exist for decreased pathogenicity of the Omicron strain. Pathogenicity is proportional to the virus multiplication rate, while Gibbs energies of multiplication are very similar for the Delta and Omicron strains. Thus, multiplication rate and pathogenicity are similar for the Delta and Omicron strains. The lower number of severe cases caused by the Omicron strain can be explained by increased number of immunized people. Immunization does not influence the possibility of occurrence of infection, but influences the rate of immune response, which is much more efficient in immunized people. This leads to prevention of more severe Omicron infection cases.

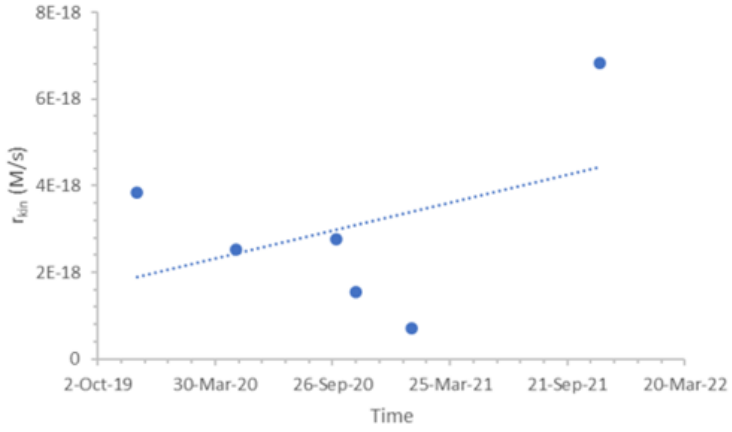

Figure 1. Binding rates of SARS-CoV-2 strains as a function of time.

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WHAT RELATIONSHIP BETWEEN HEAT GENERATION BY LIVING ORGANISMS, BY HEAT ENGINES AND BY THE HUMAN BRAIN?

A stroll through the concepts of entropy, driving forces and irreversibility in technical and living systems

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The International Society of Biological Calorimetry studies one of the most general and typical features of all living things: the continuous generation of heat. One often hears that this is a telltale characteristic of life: People distinguish sometimes "the cold inanimate world as opposed to the warmth of life". Yet heat is also produced by many other systems such as heat engines. It is generally accepted that entropy is part of the fundamental reason of this phenomenon in both systems, and that entropy has to do with the lack of order and structure of a system. Therefore, entropy also is a pertinent state function in describing growth of living organisms, which gives rise to highly organized forms of matter. But what is the exact relationship between the organization of living matter and the heat produced in heat engines? How can a state function defined by $dS = dQ_{rev}/T$ characterize the absence or presence of order in living matter? Why is growth such a vigorously spontaneous and irreversible process when the increase of entropy, i.e. of disorder, is the ultimate driving force for all spontaneous processes?

The proposed contribution is an attempt to put such questions into perspective and in relation to each other. We will start with the historical experiences with heat engines that led to the definition of the concept of entropy [1]. A simple entropy balance reveals clearly why even reversibly operating heat engines cannot transform all the energy transferred to them into useful work. An extension to living organisms shows that the heat generated by them serves in fact the same purpose as the heat "wasted" by heat engines: namely to export excess entropy. In both real, irreversibly operating heat engines and growing organisms most of the entropy that must be evacuated stems from its production by irreversible processes. This production is needed in order to compensate the reduction of entropy resulting from, respectively, the production of useful work from heat and organizing matter in growth. It is inexact to say that in growing organisms this heat is wasted, because entropy generation is also the driving force for growth. In contrast to heat engines, growing microbial cells may perform the exportation of this entropy not only in the form of heat but also by excreting small high-entropy molecules. There are chemotrophic strains that export so much entropy by the evacuation of small molecules that they do not need to generate any heat, but can even take it up and thus grow endothermically. As part of several speculative questions, we will finally also explore the possible role of heat generation in the human brain in creating the impression of an "arrow of time".

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THE INTRINSIC THERMODYNAMIC PARAMETERS OF CARBONIC ANHYDRASE II AND ACETAZOLAMIDE INTERACTION

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Rational drug design focuses on the inventive process of finding new medicaments. This process requires a good understanding of how the protein target and inhibitor interact. One way to identify the mechanisms of interactions between biomolecules is to study their thermodynamic properties. This type of analysis provides insight into the driving forces driving the binding of molecules or the determination of the binding constants of these molecules [1]. In addition, a complete thermodynamic profile (free energy, enthalpy, entropy) can be obtained, which contributes to a better understanding of molecular bonding [2].

This study determined the thermodynamic profile of human carbonic anhydrase II and acetazolamide interaction. Carbonic anhydrases (CA) are proteins of the lyase family that catalyse the reversible reaction of carbon dioxide hydration to a bicarbonate ion and a proton *in vivo* [3]. There are twelve catalytically active α-CA isoforms in humans. Some of them are associated with human diseases such as glaucoma, oedema, epilepsy, and cancer. CA is a good model protein for biophysical studies, particularly for the study of protein-ligand interactions. CA is a well-characterised protein whose mechanism of inhibition by ligands binding to the zinc ion is simple and well described. The CA protein is stable, easy to purify and cheap to obtain. As an enzyme, CA is involved not only as an anhydrase/hydratase but also as an esterase, the catalysed reaction of which is easy to study experimentally. The α-CA isoforms are highly homologous, and their active centre is structurally conserved. The homology suggests that the findings of one carbonic anhydrase study may be valid for other isoforms of the α class [4].

This study focuses on intrinsic thermodynamic parameters of CA II – acetazolamide interaction. We used two biophysical methods: isothermal titration calorimetry and fluorescence thermal shift assay and analysed this reaction by performing experiments at various pH, buffers and temperatures. We calculated the intrinsic binding parameters (K_d, ΔH, ΔCp) based on the dependence of observed binding parameters on reaction conditions.

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THE ACTIVITY OF WOOD DECAYING FUNGI MEASURED BY ISOTHERMAL CALORIMETRY

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Wood decaying fungi („rot fungi“) are a diverse group of fungi that have the ability to degrade cellulose and (in some cases) lignin. They are the main decomposers of wood and other plant material in nature and they are also a significant problem in wood constructions. To increase the understanding of how such fungi colonize and degrade wood, we have developed a method based on isothermal calorimetry to study this1. Calorimetry is in many respects a perfect method for such laboratory studies as it has a high sensitivity, and makes it possible to do long-term continuous measurements. The measured thermal power also has a strong connection to the fungal activity (metabolism, respiration).

I will present the method and the latest results. Possibly the most interesting result is that nearly all measurements show oscillating thermal powers with a period of about 1 day. Whether this has a circadian origin is not known, but it has in several studies been shown that fungi have such rhythms.

A central problem with fungal decay in buildings is that of what moisture content that is needed. This is not well known, and widely differing values are given in the literature. The present method may be able to shed some light on this problem, even if it is not trivial to control the level of humidity during a long term experiment; for example because the fungal metabolism produces water.

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POSTER PRESENTATIONS

ISOTHERMAL MICROCALORIMETRY TO TEST PHAGE PRODUTS EFFICACY AGAINST URINARY TRACT INFECTIONS

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Urinary tract infections are commonly encountered and their treatment mostly rely on antibiotics. However, there are several alternatives such as bacteriophages. Those viruses are specific to their host bacteria and do cannot infect human cells therefore they could be used to decrease the inappropriate use of antimicrobials and limit the appearance of resistant bacterial strains. Although they are only seldomly used in occidental countries there are many commercial products existing in Eastern Europe (mostly Georgia and Russia). However, to treat a patient, one need to be able top rapidly decipher which product might be appropriate. In this context isothermal microcalorimetry can provide a rapid and sensitive assessment of bacterial lysis when the workload required to perform bacterial and phage counts would be much higher. Indeed, plaque assays (the current gold standard) requires making double layer agar and incubate for at least 18 hours before being able to observe lysis area. Isothermal microcalorimetry on the contrary only require to mix the phages products with a sample containing growing bacteria. Such sample could come from a culture or directly from human urine collected from a patient.

In this study the use of calorimetry was investigated to rapidly determine if a phage cocktail could be effective against 2 commonly encountered pathogens and if decrease in bacterial metabolism indicating lysis could be observed in the thermogram. Although there are many studies performed using phages in rich conventional microbiological media, we focused on the use of sterilized human urine and artificial urine where no data could be found. In those bacterial growth is usually slower and phages might behave slightly differently. Monitoring the heatflow as proxy for the metabolic activity of 2 pathogens exposed to a commercial phage cocktail, we could see that both *E.coli* and *P.mirabilis* were strongly inhibited within 5 to 8 hours. Still, full inhibition was not reached as some regrowth was observed for *E.coli* in artificial urine. In addition, we also showed that combination of the product tested and conventional trimethoprim / sulfamethoxazole treatment at 50% MIC (minimum inhibitory concentration) was also effective thus emphasizing that the use of antimicrobial could be reduced.

Overall isothermal microcalorimetry proved to be a valuable asset when measuring the efficacy of phage cocktails against commonly encountered uropathogens. Still, there is a strong need to test more pathogens and improve the current test routine until this approach can be implemented in a clinical setting.

TOWARDS A DSC CLINICAL TEST

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Differential scanning calorimetry (DSC) is a highly sensitive technique used to study the thermodynamic properties of macromolecules with a rich history in the life sciences. Recently, the clinical application of DSC has been validated by multiple research groups across many diverse diseases [1-15]. Published research over the last 15 years have shown that DSC yields reproducible results for healthy patients and is sensitive to proteomic changes associated with disease due to detectable differences in the thermodynamic properties of biofluid proteins. Despite many years of promising results, the translation of DSC into a clinical technology requires the development and rigorous assessment of all aspects of the DSC measurement. This presentation describes the major factors associated with clinical DSC measurements, including assessing the impact of differences in biospecimen collection protocol, sample storage, sample preparation and parameters of DSC data collection, as well as potential biases in data post-processing and applied data analysis approaches.

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THERMOTT: ONLINE TOOL FOR DSF DATA ANALYSIS

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Differential scanning fluorimetry (DSF) (also known as thermal shift assay) is a widely applicable method for determining protein stability in various conditions and protein-ligand binding affinities.

DSF experiments have been performed for almost two decades. However, protein-ligand binding affinities were rarely determined due to the required nonlinear regression analysis of complex thermodynamic equations. DSF can also be used for simple protein stability screening experiments, such as determining the best storage buffer for a protein.

Our developed online tool – *Thermott* – is capable of performing such analyses (fig. 1) and is easily accessible as a web application [1]. With the help of this tool, we believe that DSF might become a much more widely used technique in the field.

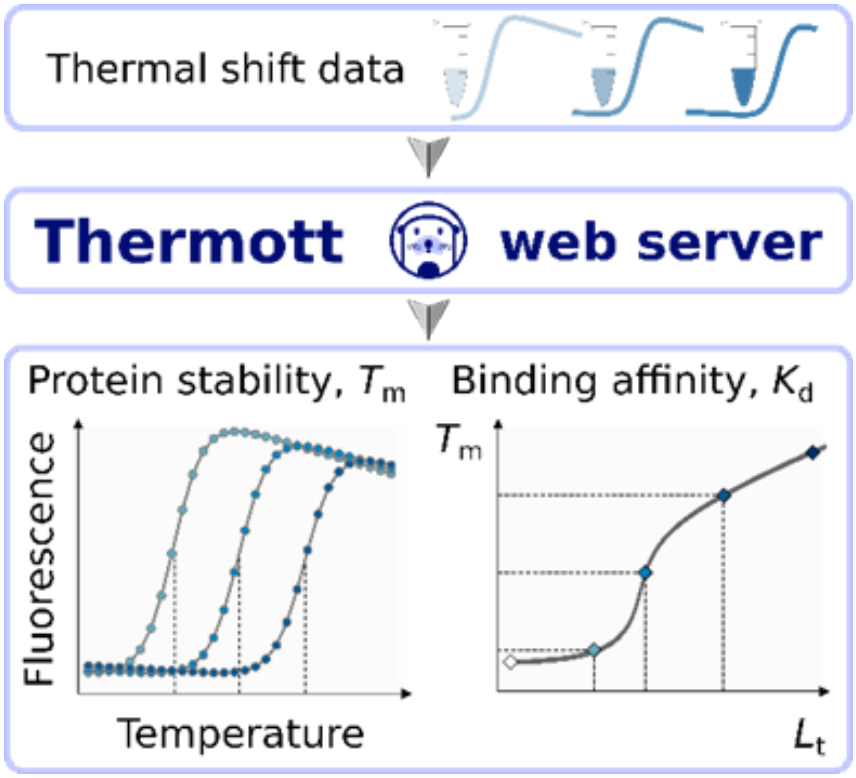

Figure 1. Graphical abstract. Adapted from [1].

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CONSTANT UNCERTAINTY IN PROTEIN-LIGAND BINDING: ASYMMETRIC CONFIDENCE INTERVALS VS STANDARD ERRORS

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The determination of equilibrium binding constants, K_b, or the equivalent dissociation constants, $K_d = 1/K_b$, and the corresponding Gibbs energy changes ($\Delta G_b = -RT\ln K_b$) between chemical compounds and target proteins *in vitro* is important for the understanding of biological interaction mechanisms. Uncertainty of the data is crucial for decision-making in many scientific fields. The K_b, $K_d$and ΔG_b are commonly assumed to follow a symmetric normal, Gaussian, distribution, and are often reported as experimental value plus/minus standard deviation. However, to varied degrees, the distributions of these values are all asymmetric. We have illustrated the effect of asymmetric distributions with a simulation approach [1], but it can be applied to any experimental technique. The Gibbs energy change and binding or dissociation constants cannot both follow a normal distribution. We propose that a more appropriate way of expressing the uncertainties of K_b, K_d, and ΔG_b is to consistently report 95% Confidence Intervals (CIs). The CIs associated with the asymmetric F statistics are easier to apply when converting between different expressions for the affinity and better reflect the real uncertainties in the data. [2] The concepts presented for determining the precise uncertainties are applicable to any technique used to determine binding affinities or other equilibrium-related quantities.

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REPRODUCIBILITY AND INTERPRETABILITY OF CALORESPIROMETRIC MEASUREMENTS OF GLUCOSE SPIKED SOIL SAMPLES

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Soil is an important key element of the global earth system. It contains unique biotopes and biocenosis governing the flux of matter and energy from the photoautotrophic primary production to final mineralization. These fluxes are largely influenced by the microbial soil community, that converts the soil organic carbon (OC) into CO_2 through catabolic reactions (exothermic) and incorporates the carbon (C) in the biomass through anabolic reactions (endothermic). Recently, systems understanding of soil function is rather poor due to fragmented knowledge about the determinants of microbe-driven processes of soil organic matter (SOM). The situation is aggravated by the fact that the boundary conditions are constantly changing due to for instance climate change. As part of a priority program funded by the DFG (German Research Council; https://soilsystems.net/), an attempt is now being made to develop a new understanding of complex soil systems by taking a holistic view of soil systems, in particular using the predictive power of thermodynamics. A prerequisite for this is the reliable measurement or calculation of thermodynamic state variables from soil analysis such as enthalpy, entropy and Gibbs energy of the total soil reaction progress. The reaction enthalpy was measured calorimetrically using three different calorimeters (TAM Air, TAM III and MC-Cal 100P), spiking soil samples with glucose as an example substrate. The calorimetric data were applied to calculate the energy usage efficiency (EUE). The combination of calorimetry and respirometry (using sodium hydroxide (NaOH) as a CO_2 trap or gas chromatography for the quantitative analysis of the CO_2) providing the calorespirometric ratio (CR) was applied to estimate the carbon use efficiency (CUE) following the theory of Hansen and its further developments [1-3]. Here we show the first results on the reproducibility of the CR measurements and its interpretability related to EUE and CUE. We derive statements on the suitability of the different calorimeter types for soil experiments, on expected error ranges for the resulting thermodynamic state variables.

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DOSE DEPENDENT EFFECT OF CYCLOPHOSPHAMIDE TREATMENT ON ACTIN

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The actin is the essential unit protein of cytoskeleton and muscle sarcomeres. The continuous management of filaments is the key machinery of eukaryotic cytoskeletal plasticity which based on the different complexes with divalent cations (Ca^{2+}or Mg^{2+}) and nucleotides (ATP, ADP). Any structural modification of nucleotide binding sites in G actin can bind ATP or ADP under different cation conditions and can initialize the remodeling of the cleft and change the stiffness of two main domains. We carried out DSC scans to investigate the direct effect of cyclophosphamide (CP) on the structural thermodynamics of actin. It has been demonstrated that run of DSC curves, melting temperatures (T_ms) together with the calorimetric enthalpy change (ΔH_{cal}) exhibit clear CP effect. In case of Ca^{2+}G-actin it is manifested in a well separated second high denaturing temperature as a consequence of CP binding into the cleft. This way the nucleotide binding cleft with subdomain 1 and 3 seems less flexible, indicating clear sensitivity to CP treatment. In F-actin samples the main peak represents the thermal denaturation of subdomain 1 and 3, and the increased calorimetric enthalpy administrating Ca^{2+}as well as CP refers to a more rigid structure. These alterations can be the molecular background in the malfunction of muscle in case of polyneuropathy after CP treatment. The other interesting point of our study is the evolutionary importance of physical links between domains to understand the multi domain development of protein functions. We have analysed the thermal stability modifier act of inter domain links in proteins, monitored by DSC, with the concept of that how did the nucleotide binding cleft between the two main domains of actin affect the activation energy of domains if it was blocked by CP binding. We have investigated the importance of inter domain linkers on the thermodynamic properties of actin. $Ca^{2+}$and Mg^{2+} bound G-actin can be stabilized by CP binding or by polymerization. CP treatment of Ca^{2+}F-actin lacks the structural integrity of a more flexible polymer and shows same stability as CP bound monomers. However, Mg^{2+}F-actin did not show any kinetic response to the CP treatment. We can assume that the CP binding blocks the inter domain linkers of actin which originally reduced the stability of domains thus resulted a more reactive and variable structure with the thermodynamic advantage in the development of multi domain proteins. Of interest, even a single dose of CP modifies the whole structural dynamics of each subdomain in actin monomers.

INFLUENCE OF CYTOSOLIC CONDITIONS ON THERMODYNAMICS AND KINETICS OF GLYCOLYTIC REACTIONS ANALYSED BY IRREVERSIBLE THERMODYNAMICS AND CALORIMTERY

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Systems biology attempts to describe quantitatively life processes. The second law of thermodynamics is applied in this regard to reduce the solution space given by the genomic and proteomic potential and thus to make predictions more reliable. Unfortunately, the influence of intracellular conditions (e.g. pH, ionic strength and macromolecular crowding) on thermodynamic state variables and on kinetic models has usually been neglected in these approaches. Instead, standard conditions were chosen for investigation. However, in the last years it has been shown that cellular conditions (especially crowding [1-3]) have a considerable influence on the kinetics and thermodynamics of metabolic reactions and reaction sequences. The volume of cells is occupied by a wide variety of macromolecules for instance 300 – 400 g L^{-1}in *E. coli* or up to 40 % of the cell volume.

In our work we focused on reactions of glycolysis. Reaction enthalpies were determined by direct (titration calorimetry) and indirect (van 't Hoff) methods. We found out that pH and crowding (simulated with PEG 20,000) have the greatest influence on the reaction enthalpy. Furthermore, we could show that identical reaction enthalpies are obtained with both methods if identical measuring conditions are chosen. In addition, kinetic parameters of the reactions mimicking cytosolic conditions were determined and their influence evaluated. A new thermokinetic approach based on irreversible thermodynamics was used for the investigation of reversible reactions, to overcome the weaknesses of the conventional Michaelis-Menten kinetics. Our results show that the biggest influence originates from the crowding effect that dramatically slows the reaction due to the size exclusion effect, while all other parameters also have an impact [4, 5].

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A NOVEL USE OF ISOTHERMAL TITRATION CALORIMETRY FOR CANCER RESEARCH

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It is estimated that over 19 million new cancer cases emerged in 2020. Cancer cells exhibit altered glucose metabolic pathways, a phenomenon known as the Warburg effect. The Warburg effect confers cancer cells with drug resistance, making it a desirable target of cancer treatment. Isothermal titration calorimetry (ITC) measures the heat absorbed or released during a reaction.Cell metabolism is a series of exothermic reactions that can be measured using ITC. Jurkat cells, a leukemia cell line, and PC-3 cells, a prostate cancer cell line, were suspended in the ITC to determine ideal cellular concentration ranges for accurate heat output measurement. Next, the cell lines were individually treated with the pro-apoptotic drug, staurosporine. Heat output was measured in the ITC 24, 48, or 72 hours after drug treatment and compared to the heat output of live cells. Cells were also injected with staurosporine to confirm that the ITC can accurately detect a change in heat while cell death occurs, and therefore be used as a real-time method for assessing cell viability. The ITC can be accurately used in whole cancer cell heat analysis and may be employed in research of drugs that target the Warburg effect.

LOW TEMPEATURE CALORIMETRIC STUDY OF MICROORGANISMS: STANDARD HEAT CAPACITY AND ENTROPY FROM 2 TO 300 K

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Biotechnology and life sciences have benefited significantly from biothermodynamics, which contributed to fields ranging from bioreactor design and monitoring [1,2], through quantitative approach to virus-host interactions [3,4], to explaining fundamental properties of life [5]. Thus, thermodynamic properties have been published for some microorganism species [6,7]. However, many more species remain uncharacterized.

Here we report determination of thermodynamic properties of 5 microorganism species, using experimental and computational methods. The experimental methods involve low-temperature calorimetry. The experiments were performed on 5 microorganism species using a PPMS (Quantum Design), from 2 to 300 K. The results were divided and analyzed in three regions: low temperature, mid-temperature and high temperature. In the low temperature region, heat capacity of microorganisms was found to be represented the best by an expanded Debye-T^3 function. No magnetic, electronic, lattice vacancy and similar terms were observed. In the mid-temperature region, heat capacity was represented using a polynomial, for better connection of the high and low regions. In the high-temperature region, a combination of Debye and Einstein functions were used. The integration of fitted heat capacity data gave standard specific entropies. Moreover, phase transitions were observed between 265 and 305 K, which were explained by transition of membrane lipids from liquid-like to crystalized form.

Standard specific entropies were determined computationally using the Battley and Roels methods. The Battley method was found to be more accurate, by comparison with experimental data. Similarly, heat capacities were found using the Kopp's rule and Hurst-Harrison equation, which were found to have similar accuracy.

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A NOVEL THERMODYNAMIC ASSAY FOR PREDICTING AND MONITORING BIOMOLECULAR STRUCTURE STABILITY

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Perturbations in the structure of large biomolecules (DNA, mRNA, proteins, and antibodies) results in concomitant changes in biomolecular function. Biological molecules require stabilization for in vitro analysis, as well as extended storage, manufacturing, and processing. In the case of biotherapeutic molecules, changes in structure could unexpectedly reduce or enhance drug product quality and safety. The primary stabilizing forces for biomolecules in solution are the non-covalent interactions between the biomolecule and its immediate solution environment. In living organisms, biomolecules are stabilized through a network of active cellular processes, but in solution, biomolecules rely on excipients (salts, detergents, sugars, buffers, etc.) for stabilization. Analytical instruments that can quantify the chemical influence of excipients on the structural stability of a biomolecule include: Spectroscopic, scattering, fluorescence, and thermodynamic techniques. Thermodynamic techniques are sensitive, reproducible, and extremely easy to setup because samples do not require labeling or modification. We performed a comparability study of a liquid protein reference formulation using a nano differential scanning calorimeter (Nano DSC) to reveal that this thermodynamic technology detects the smallest changes in biomolecular structure in response to subtle manipulations in the reference formulation.

Lysozyme reference samples in glycine buffer were pH adjusted to 5 different pH values or 6 different concentrations of sorbitol. Each reference sample formulation condition was run in triplicate on a Nano DSC (TA Instruments). The structure stability changes in the protein sample were measured. Determination of the peak thermodynamic stability temperature (Tmax) for each sample was calculated and visualized. Comparative analysis and modeling were performed via plot of Tmax versus pH, or Tmax versus sorbitol concentration, and the average Tmax and percent relative standard deviations were calculated.

Information from Nano DSC experiments enable scientists to make the best formulations, as well as monitor changes in their product over time. For the lysozyme reference sample, changes in pH elicit large differences in biomolecular stability, and changes in solubilizing agent revealed miniscule changes in biomolecular stability. The small changes measured during the sorbitol studies highlight the sensitivity of the Nano DSC. When used as part of a DOE (Design of Experiment), Nano DSC could determine ideal formulation conditions, identify changes that occur in drug manufacturing, or storage. Nano DSC also offers significant ease of use because samples do not require fluorescent labeling, fusion-tagging, or chemical digestion. While it is uncommon for these types of modifications to perturb the biomolecule's structure-function relationship, there are well known cases where such sample modifications have impeded the progress of science. When automated, the Nano DSC is an efficiency-increasing tool that provides peace of mind in decision making.

CALORIMETRIC AND SPECTROSCOPIC ANALYSIS OF ACETYLATION INFLUENCE ON LIGAND-HSA INTERACTION AS WELL AS HSA ANTIOXIDANT ACTIVITY. COMPARATIVE STUDY

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Human serum albumin (HSA) is one of the most important protein in human body. Due to the presence of Cys-34 amino acid residue [1], HSA antioxidant properties, that can be modulate by drug chemical modification [2] as well as binding of certain ligands, such as ketoprofen or α-tocopherol [3,4], were determined.

The main aim of this study was a calorimetric and spectroscopic new look at the effect of acetylation on ligand-HSA interaction and HSA antioxidant activity using salicylic acid (SA) as well as acetylsalicylic acid (ASA). To achieve the research goal, nano ITC (nano isothermal titration calorimetry), CD (circular dichroism) and UV-vis spectroscopy were applied.

Nearly the same HSA affinity towards SA and ASA has been conducted. Both SA and ASA binding with HSA, accompanied by hydrogen bonds and van der Waals interaction (SA-HSA complex) as well as ionic interaction (ASA-HSA), are spontaneous and exothermic [3,5]. The effect of ligands-HSA binding is synergistic. Changes below 1% in α-helix and β-sheet structure confirm no effect of SA and ASA binding on HSA secondary conformation. Using model free radicals, i.e. DPPH slight SA and ASA influence on antioxidant activity has been observed.

Based on the comparative study it can be concluded that the process of SA acetylation does not affect the interaction with HSA and SA slightly more effective than ASA modulates HSA antioxidant activity.

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PICOMOLAR INHIBITORS OF CARBONIC ANHYDRASE: IMPORTANCE OF INHIBITION AND BINDING ASSAYS

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Human carbonic anhydrases (CAs) are targets for drug design due to their role in numerous diseases including glaucoma, epilepsy, altitude sickness, and cancer. Clinically used CA inhibitors as drugs are rather weak and non-selectively bind all twelve CA isozymes exhibiting toxic side effects. There is a huge demand for novel high-affinity and high-selectivity CA inhibitors. However, the task is challenging due to the high sequence homology between CA isozymes. Additionally, the enzymatic activity inhibition methods have limitations for the determination of the tight-binding inhibitor's affinity.

Currently, the IC_{50} of CA inhibitors is usually determined by the stopped-flow CO_2 hydration assay. This method directly follows the inhibition of CA enzymatic activity [1]. However, the assay is limited by CA activity and concentration in the sample. Analysis of dose-response curves fitting with Hill's and Morrison's equations showed that only the Morrison's model is applicable for the determination of tight-binding inhibitor's K_d. The widely used isothermal titration calorimetry directly measures thermodynamic parameters of binding but is limited by the Wiseman c-factor. Both methods do not determine the K_d below several nM. We have designed and synthesized a series of picomolar inhibitors [2] that selectively target human CA IX, an isoform that is nearly absent in healthy humans but highly overexpressed in numerous cancers. Only the fluorescent thermal shift assay, based on measuring protein thermal stability, allowed us to accurately determine picomolar affinities of a tight-binding anticancer drug candidates. A combination of enzymatic activity inhibition and binding techniques was necessary for the precise characterization of CA–inhibitor interactions.

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DO I NEED TO AERATE MY TERRESTRIAL BIOLOGICAL SAMPLES?

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When making measurements on aerobic terrestrial biological samples – respiring samples that are not in an aqueous environment – in an isothermal calorimetry, it is important to know how if the samples consumption of oxygen and production of carbon dioxide can lead to low oxygen levels and high carbon dioxide levels that interfere with the activity of the sample. With isothermal calorimetry it is possible to calculate the approximate gas concentrations from the calorimetric result, as the thermal power (heat production rate) is strongly connected to the oxygen consumption rate through the so called Thornton‘s constant of about -455 kJ/mol(oxygen)1. As most of the heat produced in aerobic metabolism comes from the oxidation of carbon substrate, this value can be used to calculate how much the oxygen concentration in a calorimetric vial decreases during a measurement. Carbon dioxide concentrations can also be calculated if the respiratory quotient is known. Such calculations can be made both for closed vials and for aerated vials, and methods for such calculations will be presented.

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