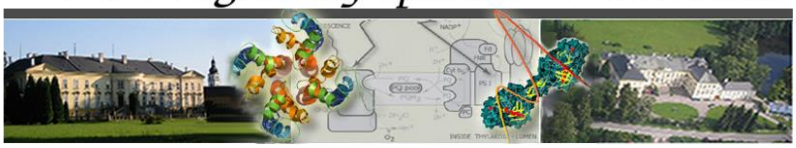
7th Visegrad Symposium on Structural Systems Biology



7th VISEGRAD SYMPOSIUM ON STRUCTURAL SYSTEMS BIOLOGY

PROGRAM & ABSTRACTS

21st – 24th June 2017

Nové Hrady

Czech Republic

Organization

Centre for Nanobiology and Structural Biology
Institute of Microbiology
Academy of Sciences of the Czech Republic

Přírodovědecká fakulta
Faculty of Science

University of South Bohemia in České Budějovice

Academy and University Center of Nove Hrady

C4Sys *Center for Systems Biology in the Czech Republic*

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Rüdiger Ettrich, Academy of Sciences, Nove Hrady, Czech Republic
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Rüdiger Ettrich
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Martin Novák
Daniela Hambergrová

Symposium program

Wednesday, June 21

15.00-17.15	**Registration**
17.00-17.30	**Conference opening**

Chairperson: Giorgia Brancolini

17.30-18.00	**Victor Guallar (Barcelona)**: PELE Studio: the next generation interactive and smart molecular design software
18:00-18:30	**Vojtěch Spiwok (Prague)**: Flying Gaussian Method
18:30-19:00	**Sergio Marques (Brno)**: Computational Tools to Aid Protein Design and Modelling

19.30-	**Poster session, welcome party**

Thursday, June 22

Chairperson: Rüdiger Ettrich

09:00-09:30	**K. V. Venkatachalam (Ft. Lauderdale, FL)**: Substrate Promiscuity of Methionine Gamma Lyase Deaminase from Porphyromonas gingivalis: Characterization by UV-VIS Spectroscopy
09:30-10.00	**Magdalena Kowacz (Krakow)**: Infrared spectroscopy study on the effect of light on protein structure and interactions. Possible role of nanobubbles.
10:00-10:30	**Nacer Idrissi (Lille)**: Local Structure of Dilute Aqueous DMSO Solutions, as Seen from Molecular Dynamics Simulations.

10:30-11:00	Coffee break

Chairperson: Christian Schröder

11:00-11:30	**Giorgia Brancolini (Modena)**: Exploring Protein-Nanoparticle Interactions by Modelling and Simulations
11:30-12:00	**Christopher Reynolds (Colchester)**: Addressing biased signalling in class B G protein-coupled receptors
12:00-12:30	**Pavel Izák (Prague)**: Purification of mixture of gasses with supported liquid membranes

12:30-14:00	**Lunch**

Chairperson: Jan Urban

14:00-14:30	**Pal Jedlovszky (Eger)**: Lateral pressure profile and free volume properties in phospholipid membranes containing anesthetics
14:30-15:00	**Mario Vazdar (Zagreb)**: Model Biological Membranes During Oxidative Stress
15:00-15:20	**Milan Melicherčík (Bratislava)**: Computational study of AMP-membrane interactions
16:00-21:30	**Trip to Třeboň, brewary excursion, dinner**

Friday, June 23

Chairperson: Jannette Carey

09:00-09:30 **Christian Schröder (Vienna)**: Computational Solvation Dynamics Spectroscopy

09:30-09:50 **James Valdes (Brno)**: "Water, water, everywhere…" for an antiviral resistance

09:50-10:10 **Vasilina Zayats (Warsaw)**: Role of slipknot topology in Alkaline phosphatase-like superfamily

10:10-10:30 **Oliver Brylski (Bochum):** Effects of the cellular environment on PAPS-Synthase stability

10:30-11:00 Cofee break

Chairperson: Magdalena Kowacz

11:00-11:30 **Jannette Carey (Princeton)**: Allostery: Monod's Second Secret

11:30-12:00 **Roman Tuma (Leeds)**: Allosteric mechanism of protein translocation though the bacterial SecYEG-SecA translocon

12:00-12.30 **Andrew Jackson (Lund)**: Structural Studies of Biomolecules in Deep Eutectic Solvents

12:30-14:00 **Lunch**

Chairperson: Béla Viskolcz

14:00-14:20 **Béla Fiser (Miskolc)**: Theoretical Investigations of Glutathione and Glutathione Analogues

14:20-14:40 **Franco A. Cimino Prado (Valparaíso)**: Formation Mechanism of the Simplest Carbohydrate in Interstellar Medium

14:40-15:00 **Andrea Guljas (Toronto)**: Theoretical Analysis of TRPV1 Ligands: Preliminary Results

15:00-15:20 **Filip Šebesta (Prague)**: Reduction of Pt(IV) complexes by small

15:20-15:40 **Denys Biriukov (Č. Budějovice)**: Adsorption of oxalic acid on rutile surface

15:40-16:10 Coffee break

Chairperson: Victor Guallar

16:10-16:30 **Vladimír Sychrovský (Prague)**: The Excision of OxoG Base with hOGG1 BER Enzyme; Towards Validation of the Catalytic Pathway

16:30-16:50 **Jakub Šebera (Prague)**: The mechanism of the glycosylase reaction with hOGG1: concerted effect of Lys249 and Asp268 during excision of oxoG

16:50-17:10 **Robert Welch (Leeds)**: FFEA: Continuum Simulations for Obnoxiously Large Biological Systems

17:10-17:30 **John J. Villar (Diliman)**: Computational Modeling and Analysis of Backbone-Sidechain Interactions in Selected Amino Acid Diamide 5D Conformational Potential Energy Surfacebiomolecules

17:20-17:40 **Imre Jákli (Budapest)**: Quantum chemical study of hexopyranose configuration induced conformer distributions

17:40-18:00 **Closing remarks**

19:00- **Conference diner, farwall party**

Saturday, June 24: Departure

List of posters

1. **Veronika Zeindlhofer (Vienna):** Extraction of coffee ingredients from aqueous Ionic Liquid mixtures
2. **Esther Heid (Vienna)**: Influence of disaccharides on water dynamics revealed by computational solvation dynamics
3. **Dmitri Dormeshkin (Minsk)**: Molecular dynamics study of anti-CD19 Chimeric Antigen Receptor (CAR).
4. **Yaraslau Dzichenka (Minsk)**: Structural peculiarities of cytochromes P450 in complex with antifungal azoles
5. **Tatsiana Siarchenia (Minsk)**: A fluorescence spectroscopy, circular dichroism and molecular docking study of human alpha-1-microglobulin interactions with xenobiotics
6. **Sara Matić (Zagreb)**: Combination of SAXS and molecular modeling in structure-function characterization of the plant DPP III orthologue
7. **Adrian Sanchez-Fernandez (Bath**): Structure of proteins and phospholipid monolayers in deep eutectic solvents
8. **Veronika Szentirmai (Szeged)**: Adsorption of Methylamine at the Surface of Ice. A Grand Canonical Monte Carlo Simulation Study
9. **Olga Dvořáčková (Č. Budějovice)**: The effect of substituents on the kinetics of the hydration reactions of trans-Platinum com
10. **Ján Urban (Bratislava)**: Redshift of the wavelength in the Spinach aptamer complex, suggestions from QM:MM calculations
11. **Zsófia Borbála Rózsa (Szeged)**: A molecular dynamics study on the effects of 1,4-dioxane on model membranes
12. **Attila Surányi (Miskolc)**: Fourier type potential energy functions for conformational change of selected organic functional groups
13. **Bálint Kiss (Miskolc)**: Thermodynamics of Mixing of Formamide and Water in Computer Simulation
14. **Zsanett Boros (Miskolc)**: Study on Reactivity of Methylene Diphenyl Diisocyanate (MDI) with Biomolecule Motifs
15. **Anett Juhász (Miskolc)**: Aniline metabolism in hepatocytes and erythrocytes
16. **Ádám Prekob (Miskolc)**: Development of carbon nanotube-coated core-shell structured nanocomposite catalysts
17. **Wafaa Cheikh (Miskolc)**: Computational study of the phosgenation reaction mechanisms of the MDA

18. **Rachid Hadjadj (Miskolc)**: Systematic theoretical investigation for high energy $C_2H_8O_4$ molecules
19. **Tünde Szamák (Miskolc)**: Mechanistic Study of Formaldehyde Synthesis from Methanol
20. **Lois Foo (Toronto)**: Formation Mechanism of C_2H_5NO Isomers in Interstellar Medium
21. **Zhuoyuan Li (Toronto)**: Formation Mechanisms of Three-Carbon Sulfur-Containing Organic Compounds in ISM
22. **Lubabah Ahmed (Toronto)**: Rational Design of Odorants – A Case Study of $C_2H_4O_2$
23. **Ziqi Li (Toronto)**: Computational Modelling and Analysis of 4D Conformational Potential Energy Surface of N-acetyl-valine-N-methylamide
24. **Logine Negm (Toronto)**: Computational Modeling and Analysis of 5D Conformational Potential Energy Surface of N-Acetyl Glycylglycine N-Methylamide
25. **Yinan Liu (Toronto)**: Model Aided Biofuel Design: Thermodynamic Properties of the $C_5H_{10}O$ Constitutional Isomers
26. **Yilei Xue (Toronto)**: Model Aided Biofuel Design: A Case Study of $C_6H_{12}O$
27. **Min-Yen Lu (Miskolc)**: Theoretical Investigation of Green Polyurethane in Industrial Applications
28. **Daniel Bonhenry (N. Hrady)**: Designing voltage-sensitive probes for neuronal imaging
29. **Deepika Kale (N. Hrady)**: Biochemical approaches to determine the role of long hydrophilic loop and C, N tail of Saccharomyces cerevisiae potassium translocating proteins
30. **Saurabh Pandey (N.Hrady)**: Mechanism and Energetics of L-arginine Binding to Arginine Repressor Protein in *E. Coli*
31. **Lydie Plačková (N.Hrady)**: Computational Modeling of Phosphokinase Activity of Human 3'-Phosphoadenosine 5'- Phosphosulfate Synthase
32. **Xichong Liu (Princeton)**: Computational Modeling of Sulfurylase Activity of Human 3'-Phosphoadenosine 5'- Phosphosulfate Synthase
33. **Alison Salamatian (Storrs, CT), Chris Soha (Ft. Lauderdale, FL)**: Human 3'-Phosphoadenosine 5'- Phosphosulfate Synthase: Protein Expression, Purification, Crystallization and Activity
34. **Brielle Tilson (Upland, IN**): Ammonium Ligand Binding to dibenzo-18-crown-6 via Density Functional Theory

Abstracts of oral presentations

The authors of the abstracts bear the full responsibility for the scientific and linguistic content.

PELE Studio: the next generation interactive and smart molecular design software

Victor Guallar

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We are clearly wistnesing a rise of computational predictions in industrial drug design. This, has been made possible by the significant improvement in software (algorithms such FEP, etc.), but also by the rise in computational power, (multicore platforms, cloud computing and GPUs). Next generation drug design software, however, will embrace additional technological developments. In this line we are combining PELE, our Monte Carlo sampling technique highlighted as an outstunding achievement in the latest CSAR blind test, with machine learning algorithms, high performance computing and improved 2D/3D visualization techniques. Our aim is to provide a drug design software capable of:

i) Interactive: instantaneous answers;
ii) Accurate: quantitative answers;
iii) Smart: self-learining capabilities;
iv) Connecting: providing a virtual working space.

With this combination, for example, we are capable of finding the active site and correct pose for vary complex systeems: nuclear hormone receptors, GPCR, etc. in less than half an hour. Our efforts and initial results in this line will be provided in this talk, along with recent success stories and our future vision.

Biography

Dr Guallar completed his PhD in collaboration between UAB (Spain) and UCBerkeley (USA) and postdoctoral studies from Columbia University. After an assistant professor position at Washington University School of Medicine (USA), he was awarded an ICREA professor position at the Barcelona Supercomputing Center (BSC). He is also founder of Nostrum Biodiscovery, the first spin off from BSC. He has published more than 100 papers in reputed journals and has been the recipient of presitioug grants like an Advanced ERC from the European Union.

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Flying Gaussian Method

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Molecular simulations are widely applied in many fields including drug discovery and material design. Unfortunately, these calculations are very computationally expensive. It is possible to simulate nanoseconds on personal and microseconds on high-performance computers, however, chemical reactions, protein folding, protein-ligand interactions usually take place in longer time scales.

Poor sampling of molecular simulations can be improved by enhanced sampling methods, such as metadynamics [1]. This method enhances sampling by energetically disfavoring states of the simulated system that have been already sampled by the simulation. Its parallel variant – multiple walker metadynamics [2] – simulates multiple replicas of the studied system in parallel and energetically disfavors states that have been already sampled by any walker. Inspired by these two methods we developed Flying Gaussian method [3]. Similarly to metadynamics it simulates multiple replicas of the system and it disfavors sampling of a same state by two or more replicas. We will present the results of its application on various model systems and we will discuss its advantages and disadvantages compared to metadynamics and other methods.

Acknowledgements*: We gratefully acknowledge funding by the Czech Science Foundation (15-17269S). We also gratefully acknowledge computational time provided by Metacentrum (LM2015042), CERIT-SC (LM2015085) and IT4I (LM2015070).*

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Computational Tools to Aid Protein Design and Modelling

Sergio M. Marques[1,2], David Bednar[1,2], Jiri Filipovic[3], Ondrej Vavra[1,2], Barbora Kozlikova[4], Adam Jurcik[4], Jan Byska[5], Jiri Damborsky[1,2]

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Proteins have been used in an ever-expanding number of biotechnological applications, ranging from household products to fine chemicals and biopharmaceuticals. However, their application can be limited by low stability or activity. Protein engineering techniques can thus be used to improve the properties of natural proteins via directed evolution or rational design. Many enzymes have their active sites buried in their protein cores rather than exposed to the bulk solvent. In these cases molecular tunnels serve as communication pathways for the exchange of substances. CAVER software [1] was developed to aid a deep insight on the structural features of proteins with functional tunnels. This widely used tool was designed to calculate the protein tunnels in static or ensembles of 3D structures and provide a multitude of information that may help identifying important hotspots for mutagenesis. CAVER Analyst provides a graphical interface to CAVER calculations and can perform many other useful analyses [2]. CaverDock [3] is a new software tool under development that combines CAVER and AutoDock. It is aimed at quickly predicting the trajectory and energy profile of a substrate, product or inhibitor travelling through a molecular tunnel, and will provide information on the physical and energetic bottlenecks of the ligand transport. The applicability of these tools in enzyme engineering will be demonstrated and discussed.

Acknowledgements*: We gratefully acknowledge funding from the Czech Ministry of Education (LQ1605, LO1214, LM2015051, LM2015047) and the Grant Agency of Masaryk University (MUNI/M/1888/2014).*

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Substrate Promiscuity of Methionine Gamma Lyase Deaminase from *Porphyromonas gingivalis:* Characterization by UV-VIS Spectroscopy

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Methionine Gamma Lyase Deaminase (Mgld) is a pyridoxal phosphate (PLP) dependent enzyme that cleaves L-methionine into methylthiol and forms the deaminated product alpha-ketobutyrate. Cloned, Overexpressed, Mgld was purified by affinity, gel filtration and DEAE ion exchange chromatography [1]. Purified Mgld was used for characterization of therapeutic use [2] and for enzyme kinetic studies [3]. D-methionine is slow in reactivity relative to L-isomer. Thus, the stereo selectivity is not perfected by Mgld. The thio ether bond is not a mandatory requirement for cleavage, since cysteine and homocysteine that has thiol group, can be cleaved into hydrogen sulfide and forms the corresponding deaminated, keto acid products pyruvate and alpha-ketobutyrate. Alpha-methyl DL-methionine is not a substrate due to steric hindrance that prohibits external aldimine formation with the Mgld-PLP. N-formyl and N-acetyl methionine are not substrate for Mgld, which makes sense, since the alpha-amino group is mandatory for binding as well as external aldimine Schiff's base formation. Thus, F-met or protein modified N-acetyl-met formation is a mechanism by which organisms have managed to protect methionine from degradation and keeps the modified methionine strictly for anabolic purposes. Serine and homoserine are weak substrates for Mgld which means sulfur to oxy substitution can be tolerated to certain degree for cleavage. L-methionine sulfone and L-sulfoxy methionine are sulfur modifications of the methionine, and Mgld exhibits higher Km and a change in Kcat making the overall catalytic efficiency (Kcat/Km) lower compared to L-methionine. Mgld is a good model system for structure/function studies of coenzyme mediated tight activesite catalysis.

Acknowledgements: I thank all the students who worked on this project especially Mr. D. Morcos, B. Schmier, N. Ledra, and T. Foo for their valuable time on Mgld expression, purification, UV-VIS spectroscopy etc.

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Infrared spectroscopy study on the effect of light on protein structure and interactions. Possible role of nanobubbles

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Motion is an essence of protein biological activity. In order to perform their action proteins undergo certain conformational fluctuations. These collective motions are driven not only by direct interactions between atoms in protein but they are also affected by the solvent.

We show that remote physical trigger – a non-ionizing infrared (IR) radiation - can induce protein structural fluctuations oscillating between partially disordered and folded state on exposure to IR light. These structural motions are associated with i) interfacial water release (entropy gain) and transition to lower energy state on folding and ii) polarization and immobilization of surface waters by exposed unfolded compartments in low entropy, high energy state. Furthermore, we show that IR-induced hydration changes can modulate protein-protein and protein-surface interactions. In this context, polarization of surface waters and formation of adhesive water bridges supports protein self-assembly in solution. Hydration state determines also protein propensity to separate from solution and adsorb to the surface.

We can explain these results assuming that nanobubbles are the main factor mediating response of biomolecules to IR light. Such assumption is consistent with previous findings showing that weak electromagnetic radiation can trigger oscillations of bubbles in solution and that this phenomena has certain consequences for biological systems [1]. Our studies suggest that IR can promote i) nucleation/adsorption of nanobubbles on protein hydrophobic compartments (thus displacement of hydration waters) and ii) further coalescence, growth and desorption of nanobubbles (and surface rehydration).

The ability to remotely induce protein structural motions and interactions can be of great importance from the perspective of bioengineering applications.

Acknowledgements*: We gratefully acknowledge funding by the National Science Centre trough grant FUGA number DEC-2015/16/S/ST4/00465.*

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Local Structure of Dilute Aqueous DMSO Solutions, as Seen from Molecular Dynamics Simulations

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The information about the structure of DMSO-water mixtures at relatively low DMSO mole fractions is an important step in order to understand their cryoprotective properties as well the solvation process of proteins and amino acids. Classical MD simulations, using the potential model combination that best reproduces the free energy of mixing of these compounds are used to analyze the local structure of DMSO-water mixtures at DMSO mole fractions below 0.2. Significant changes in the local structure of DMSO are observed around the DMSO mole fraction of 0.1. The array of evidences, based on the cluster and the metric and topological parameters of the Voronoi polyhedra distributions, indicates that these changes are associated with the simultaneous increase of the number of DMSO-water, and decrease of water-water hydrogen bonds with increasing DMSO concentration. The inversion between the dominance of these two types of H-bonds occurs around $X_{\mathrm{DMSO}} = 0.1$, above which the DMSO-DMSO interactions also start playing an important role. In other words, below the DMSO mole fraction of 0.1 DMSO molecules are mainly solvated by water molecules, while above it their solvation shell consists of a mixture of water and DMSO. The trigonal, tetrahedral and trigonal bipyramidal distributions of water shift to lower corresponding order parameter values indicating the loosening of these orientations. Adding DMSO doesn't affect the hydrogen bonding between a reference water molecule and its first neighbor hydrogen bonded water molecules, while it increases the bent hydrogen bond geometry involving the second ones. The close packed local structure of the third, fourth and fifth water neighbors also is reinforced. In accordance to previous theoretical and experimental data, the hydrogen bonding, between water and the first, the second and the third DMSO neighbors, is stronger than that with its corresponding water neighbors. At a given DMSO mole fraction, the behavior of the intensity of the high orientational order parameters values indicates that water molecules are more ordered in the vicinity of the hydrophilic group while their structure is close-packed near the hydrophobic group of DMSO.

Exploring Protein-Nanoparticle Interactions by Modelling and Simulations

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Understanding protein-inorganic nanoparticle interactions is central to the rational design of new tools in biomaterial sciences, nanobiotechnology and nanomedicine[1]. Theoretical modeling and simulations provide complementary approaches for experimental studies.

Experiments have reported that inorganic nanoparticles and surfaces can either accelerate or inhibit the fibrillation of amyloidogenic proteins, depending on the chemistry of the nanoparticle, their size, and the experimental conditions of the experiment. Some general mechanisms leading to acceleration and inhibition (such as enhanced nucleation or sequestration from the solution, respectively) have been identified; much less is understood on the role of the specific interactions between a given peptide and a given surface in affecting fibrillation propensity. In this contribution, our atomistic simulation results for ΔN6 and D76N, naturally occuring variants of β2-microglobulin protein, interacting with functionalized gold nanoparticles will be presented, and the possible mechanisms leading to inhibition or enhancement of fibrillation will be discussed[2-3].

Recent simulations of the interaction of Green Fluorescent Protein mutant (-30GFP) with AuNP-ARG and AuNP-COOH for the chemical nose sensing strategy[4], will be presented. Understanding the mechanisms of the interactions between the protein and the AuNP at the microscopic level, would pave the way to the fabrication of array-based sensors for robust detecting and identifying cancer, of broad interest in the community.

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Addressing biased signalling in class B G protein-coupled receptors

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The G protein coupled receptor (GPCR) superfamily is of huge interest as they are one of the four main privileged classes of drug targets. However, most of this pharmaceutical activity historically lies amongst a subset of the largest group, the class A GPCRs (e.g. the well-known β_2-adrenergic receptor). The class B peptide hormone receptors are in principle high-value drug targets as they are involved in numerous diseases, including heart disease, diabetes and cancer, but this therapeutic potential is very much under-exploited. Traditionally, it is understood that these GPCRs activate a particular G protein, e.g. for the CGRP receptor this is Gs, but Lefkowitz has shown that GPCRs can not only signal through multiple G proteins (e.g. Gi, Gq) but can also signal through β-arrestin. Thus, different ligands can give rise to biased signalling, giving rise to different pharmacological effects through activation of the same receptor. This is important, as signalling via the wrong pathway can lead to negative effects. Consequently, there is much interest in enhancing the current structural studies with computational methods with a view to gaining deeper insight into the structure and function and the origin of biased signalling. Our focus is primarily on the GLP-1 receptors, which is involved in diabetes and the CGRP receptor, which is involved in heart disease and migraine and that additionally requires an auxiliary protein (a RAMP) to function. Our approach was to combine modelling with site-directed mutagenesis. We will describe the development of a method for profile sequence alignment in or below the twilight zone that enabled generation of valuable initial models prior to suitable X-ray structural information [1-4]. We will outline methods for the generation of the complex homology models of GLP-1R[5] and discuss the insights gained into biased signalling from the modelling and molecular dynamics simulations[6, 7]. In addition, we discuss the role of photoaffinity labelling in the generation of these models[8], and the interesting twists presented by the RAMPs[9].

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Purification of mixture of gasses with supported liquid membranes

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Biogas, seems to be a very good candidate for the replacement of depleting fossil fuels. The raw biogas is created by the decomposition of waste through the process of anaerobic digestion and has to be purified in order to reach a fuel quality. Unwanted impurities such as CO2 or acidic gases have to be removed while the others impurities with concentrations lower than 1 vol. % are marginal for the biogas upgrading. The recent breakthrough in biogas upgrading represents the membrane separations with water-swollen thin polymer (skin) layer composite membranes when the presence of water vapor in raw biogas assists in the effective gas separation. For this reason, pre-treatment such as water vapor removal is avoided and a raw biogas feed is directly in contact with the composite membrane.

Two swollen polyamide thin-film composite membranes were tested for effective CO2/CH4 separation. It was found that the water wettability has a key role for the separation of binary mixture representing a raw biogas, i.e. containing CO2 and CH4. The transport properties were analyzed by means of a mathematical model simulating gas permeation. A new modification of the mass transport coefficient model provided the concentration profiles of individual components on both sides of the membrane (inaccessible in experiments). Furthermore, the model enabled the evaluation of the mass transport coefficients of the gases in the mixture under varying stream flow rates and arrangements with respect to the membrane separation cell size. Therefore, the possibility of scale-up was discussed for both membranes and flow cell arrangement. Although the mathematical model was developed for a flat sheet membrane configuration, the results can be applied for a real spiral wound module with a wider surface.

The Sterlitech membrane showed CO2 mass transfer coefficients of approximately one order of magnitude higher than the Koch membrane. On the other hand, the ratio of the CO2 and CH4 mass transfer coefficients was only fivefold in the case of the Sterlitech membrane, where as, in the Koch membrane, the difference was more than twentyfold. Based on our results, a real biogas upgrading plant can be designed. It should consist of the dual module arrangement with the first, highly permeable Sterlitech membrane module and the second selective Koch membrane module.

In our contribution we will demonstrate successful separation of mixture of gasses as CO2/H2, CO2/CH4 and SO2/N2 with supported liquid membranes. The originality of presented project consists in the synergic action of size sieving and solution-diffusion mechanisms of the tailor-made membranes with active surface or inner structure with a high affinity to CO2. Such approaches have a potential to eliminate or suppress common problems connected with known difficulties of only H2-selective or only CO2-selective membranes for H2/CO2 separation.

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Lateral pressure profile and free volume properties in phospholipid membranes containing anesthetics

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The effect of four general anesthetics, namely chloroform, halothane, diethyl ether, and enflurane on the free volume fraction and lateral pressure profiles in a fully hydrated DPPC membrane is investigated by means of computer simulation. In order to find changes that can be related to the molecular mechanism of anesthesia as well as its pressure reversal, the simulations are performed both at atmospheric and high (1000 bar) pressures. The obtained results show [1] that the additional free volume occurring in the membrane is localized around the anesthetic molecules themselves. Correspondingly, the fraction of the free volume is increased in the outer of the two membrane regions (i.e., at the outer edge of the hydrocarbon phase) where anesthetic molecules prefer to stay in every case. As a consequence, the presence of anesthetics decreases the lateral pressure in the nearby region of the lipid chain ester groups, in which the anesthetic molecules themselves do not penetrate. Both of these changes, occurring upon introducing anesthetics in the membrane are clearly reverted by the increase of the global pressure. These findings are in accordance both with the more than sixty years old "critical volume hypothesis" of Mullins, and with the more recent "lateral pressure hypothesis" of Cantor. Our results suggest that if anesthesia is indeed caused by conformational changes of certain membrane-bound proteins, induced by changes in the lateral pressure profile, as proposed by Cantor, the relevant conformational changes are expected to occur in the membrane region where the ester groups are located.

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Model Biological Membranes During Oxidative Stress

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Reactive aldehydes, such as 4-hydroxynonenal (4-HNE) and 4-oxononenal (4-ONE), are formed as a consequence of radical peroxidative reactions of polyunsaturated fatty acids in cellular membranes during oxidative stress. Due to the presence of several reactive sites, these compounds can readily react with membrane proteins [1] and phosphoethanolamine lipids [2] thus impairing the membrane protein function. As an efficient and a very useful tool in understanding how reactive aldehydes modify membranes at the molecular level, molecular dynamics (MD) simulations are used for monitoring and analyzing various changes in biophysical properties of model phospholipid bilayers [3].

In this work, we performed MD simulations of reactive aldehydes (4-HNE and 4-ONE) together with their covalent POPE adducts in different model biological bilayers. We offer an explanation of an interesting experimental observation why 4-HNE and 4-ONE change total conductivity in mixed phosphatidylcholine (PC) and phosphoethanolamine (PE) bilayers in contrast to neat PC bilayers. We also suggest why 4-HNE and 4-ONE have a different effect on total conductivity and how a small, but important difference in the chemical structure of 4-ONE as compared to 4-HNE, can lead to different properties of model biological membranes during oxidative stress.

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Computational study of AMP- membrane interactions

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Antimicrobial peptides (AMPs), small, positively charged, amphipathic molecules, keep antimicrobial activity and are rapidly mobilized to neutralize a broad range of microbes, including viruses, bacteria, protozoa, and fungi. The ability of these natural molecules to kill multidrug-resistant microorganisms can be used for the detection of variety of compounds. The antimicrobial peptide Polybia-MP1 belongs to this class of molecules. Extracted from the Brazilian wasp Polybia paulista has a broad spectrum of bactericidal activities against Gram-negative and Gram-positive bacteria without being hemolytic and cytotoxic.
Molecular dynamics simulations have been applied for the study of interactions of MP1 with membranes. We have studied protein behaviour in water as well as with different (charged and uncharged) membranes. Simulations were made with different number of protein molecules - 1, 8, 50, 100 and 200. Obtained results are discussed.

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Computational Solvation Dynamics Spectroscopy

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In Solvation Dynamics Spectroscopy the fluorescence band of a chromophore is followed after an electronic excitation by a laser pulse and yields information on the solvation behavior of various solvents. Since ultrafast dynamics of some solvents like water are at the limit of experimental resolution and the decomposition into individual components for the interpretation is complicated [1], computational approaches to monitor the time-dependent Stokes shift have gained interest in the past decades.

Although well-suited for modelling the excited state of the chromophore, ab initio MD simulations cannot cope well with the desired simulation period and simulation replicas for the required statistics. Furthermore, the explicit modelling of several hundred solvent molecules is a must to decompose the findings into various solvation shells [1,2]. Here, solvent polarizabilities play an important role [3,4].

Both experimental and computational Solvation Dynamics results can be converted to dielectric spectra [4]. However, the first technique probes the solvent by a local field whereas the second relies on a collective electric field.

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"Water, water, everywhere..." for an antiviral resistance

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Every year millions of humans are infected by Flaviviridae viruses worldwide. *Flaviviridae* are Group IV, positive-sense single stranded RNA viruses that encode a RNA-dependent RNA polymerase (RdRp), an enzyme crucial for viral replication in a host cell. The overall tertiary structure of RdRps is a heart-shaped, right-hand conformation divided into the thumb, fingers and palm domain. These domains are sub-divided into structural motifs A-G. Therapeutic 2'-C-methylated nucleoside analogues were found highly effective against *Flaviviridae* viruses, e.g., hepatitis C virus (HCV) [1] and tick-borne encephalitis virus (TBEV) [2]. These 2'-C-methylated nucleoside analogues target RdRps by incorporating into the synthesized genetic product causing premature termination during viral replication.

More than a decade ago Migliaccio *et al.* [1] showed that a Ser-Thr mutant (serine to threonine) of HCV RdRp was resistant to a 2'-C-methylated nucleoside analogue. Although this phenomenon is still widely discussed amongst virologists, one question remains: How does a single mutation in a viral RdRp cause resistance to a nucleoside analogue? The molecular escape mechanism behind this resistance was described by employing an all-atom, stochastic molecular software [3], a few nucleoside analogue inhibitors, and two *Flaviviridae* RdRps from HCV and TBEV. As the analogues approach the RdRp, subtle changes occur for a couple of water molecules and a metal ion cofactor. Within the active site, the all-atom simulations significantly showed that nucleoside analogues use water to camouflage itself within the active site of the wild type viral RdRp. The Ser-Thr mutant RdRp debunks this stealth mode since the hydrophobic methyl group of Thr (compared to Ser in the wild type) repels the water molecules from the analogue as it approaches the active site. Exposed, the analogue decreases its contacts with substrate-interacting residues that incorporate nucleosides during replication [4].

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Role of slipknot topology in Alkaline phosphatase-like superfamily

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Alkaline phosphatase-like superfamily joins five families of enzymes, which share five conserved active site residues and similar fold. These enzymes are found in various organisms from Bacteria to Animalia. A common feature of these proteins is non-trivial organization of a polypeptide chain which is known as slipknot topology. Slipknot is formed when one loop threads across another loop. The knots and slipknots in proteins are recognized based on probabilistic methods to close polypeptide chain and also on a branch of mathematics – knot theory [1]. Non-trivial knot and slipknot topology in proteins were shown to have an impact on protein stability and function [2]. Moreover, it was found that non-trivial topology is conserved among proteins from different kingdoms with the same function [3]. Therefore, understanding of the role of such "special" protein topology is an important task. Here we would like to shed light on the role of the slipknot topology in the superfamily of Alkaline phosphatase-like proteins. As an example member of this superfamily, we focused on Lipooligosaccharide phosphoethanolamine transferase A (LptA) [4]. This protein is found in multi-drug resistant gram-negative bacteria *Neisseria meningitidis*. LptA catalyzes modification of lipid A headgroup and therefore plays an important role in the resistance of gram-negative bacteria [4]. LptA enzyme as well as other members of Alkaline phosphatase-like superfamily possess trefoil slipknot topology. A detailed analysis of residues involved in forming slipknot revealed that they coincide with five residues forming active sites which are conserved across the whole superfamily.

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Effects of the cellular environment on PAPS-synthase stability

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The bifunctional enzyme PAPS-synthase provides the cell with PAPS (3'-phosphoadenosine-5'-phosphosulfate), a sulfate donor which is further used by sulfotransferases for modification of biomolecules (e.g. steroids) [1]. Mutations affecting PAPS-synthase activity result in disease states like bone and cartilage malformation as well as metabolic diseases. *In vitro* experiments showed that isoform PAPS-synthase 2 is naturally fragile but stabilized by binding of most of its ligands [2]. Compared to the homogeneous *in vitro* environment, the intracellular milieu is highly heterogeneous and crowded by large biopolymers leading to intermolecular interactions and excluded-volume effects affecting each biomolecule inside the cell [3].
Trying to understand how mutations affect the overall stability of this large enzyme (70 kDa), we use *Fast Relaxation Imaging* [4] to study the individual domains of PAPS-synthase directly inside the cell. Introducing disease relevant as well as catalysis inhibiting point mutations, we acquired new data providing thermodynamic insight into the enzymes stability inside the cellular environment, giving further insight into PAPS-synthase disease mechanisms and how they are affected by the cellular environment.

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Allostery: Monod's Second Secret

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Allostery is an emergent property of macromolecules in which the binding of one ligand facilitates or antagonizes the binding of another. Jacques Monod proposed a prescient model for the phenomenon 50 years ago, when we knew very little about macromolecular structures. The history of this model and its application in several molecular systems will be reviewed.

Allosteric mechanism of protein translocation though the bacterial SecYEG-SecA translocon

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Protein translocation through lipid bilayer is an essential process which mediates protein secretion and insertion into membranes. In bacteria this is achieved by the ubiquitous Sec machinery and the drive for translocation comes from ATP hydrolysis by the cytosolic molecular motor SecA and the proton motive force. Here, we combine molecular dynamics (MD) simulations with single molecule FRET and biochemical assays. We show that ATP binding by SecA causes opening of the SecY-channel at distance, while polypeptide substrate at the SecY-channel entrance feedback to regulate nucleotide exchange by SecA. This two-way communication is compatible with a new Brownian motor mechanism, whereby ATP binding and hydrolysis bias the direction of polypeptide diffusion. The model represents a solution to the problem of transporting inherently variable substrates such as polypeptides, and may underlie mechanisms of other motors that translocate heteropolymers such as proteins and nucleic acids. We have further investigated the dynamics of the SecYEG pore during initiation and translocation at single molecule level and time scales ranging from 0.1 ms to 100 seconds. This investigation revealed intrinsic dynamic on the millisecond time scale which is important for pore opening and initiation. Translocation of a typical substrate happens on a longer time scale with a rate of about 60 amino acids per second.

Reference: Allen et al (2016) Elife 5, e15598; https://doi.org/10.7554/eLife.15598.001

Structural Studies of Biomolecules in Deep Eutectic Solvents

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The investigation of the behavior of biomolecules in the absence of water has experienced a recent upsurge, with special emphasis on deep eutectic solvents (DES).[1] These solvents are generally formed from a eutectic mixture of organic compounds, where the melting point of the resulting mixture is considerably lower than those of the individual precursors. DES have been recently postulated as matrix where biomolecules may remain active, even in extreme conditions (e.g. cryogenic temperatures or total absence of water). Following on our previous work in surfactant self-assembly,[2,3] we have started a systematic study on phospholipid and protein behavior in pure and hydrated DES. Here we report the behavior of phospholipid monolayers at the air-liquid interface, and the conformation of proteins in DES.

We have employed Neutron and X-Ray reflectometry to study the behavior of DPPC, DMPC and DMPG at the air-liquid interface on a choline chloride:glycerol subphase. Circular dichroism and small-angle neutron scattering were used to examine the structure and conformation of bovine serum albumin and hen egg-white lysozyme in choline chloride-based DES.

Our investigations have shown the formation of stable, well-defined phospholipid monolayers of variable thickness and solvation depending on the surface coverage. The conformation of the proteins has been studied for pure and hydrated solvents. Our results have shown that proteins remain partially folded in pure DES, whereas in the presence of hydrated DES the protein effectively folds as in buffer, even at high DES concentrations.[4]

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Theoretical Investigations of Glutathione and Glutathione Analogues

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Glutathione (□-L-glutamyl-L-cysteinyl-glycine, GSH) is essential in many biochemical processes in living organisms (e.g. cell differentiation, proliferation, apoptosis, signal transduction) [1,2]. It is one of the most important antioxidants and accumulated in different cellular compartments [1,2]. There are many GSH analogues exist in different organisms which can work similarly as glutathione [3].

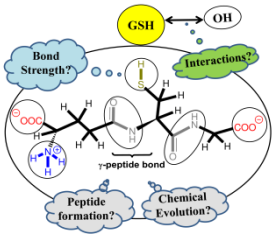

Figure 1. The studied properties of glutathione.

The research presented here is focused on different aspects of GSH and GSH analogues (**Figure 1**) which were studied by means of computational chemical tools [1-3].

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Formation Mechanism of the Simplest Carbohydrate in Interstellar Medium

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Glycolaldehyde or 2-hydroxyacetaldehyde ($HCOCH_2OH$), as known as a simplest sugar of the monosaccharide sugars, and its isomers have been detected in interstellar mediums, in dense clouds, in 2000 [1–2]. Molecular clouds are characterized by temperatures on the order of 15-20 K and the molecular density is $n=10^3$-10^6 cm^{-3} [3–4]. At these temperature, there is insufficient energy for collisions to overcome any activation barriers in reactions, and the only gas phase chemical reactions that can proceed at such low temperatures are radical-radical reactions and ion-molecule reactions, both of which are barrierless. Despite, the fact that the glycolaldehyde has significant astrobiological importance the actual formation mechanisms which is exists in interstellar medium remain unclear. The molecular composition reflects the balance between chemical evolution via reactions, destruction of molecules by light from stars or by cosmic rays, and condensation and subsequent reaction on dust grains. In this study, a new chemical model for the mechanism of the glycolaldehyde and its relevant isomers have been developed to represent all the possible formation reaction pathways.

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Theoretical Analysis of TRPV1 Ligands: Preliminary Results

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TRPV1 is a polymodal channel (**Figure 1**) that is involved in the signal transduction of noxious stimuli in the somatosensory nervous system, and apart from being activated by its ligands, it responds to noxious thermal stimuli, acidic pH, and depolarizing currents [1]. The channel has been shown to be tetrameric in structure [2], and each of the four subunits that make up the functional protein are comprised of six transmembrane helices. The ligand-binding pocket of the channel is located near the S3-S4 transmembrane region and the S4-S5 linker, where, upon binding of ligands, interactions between these two regions are disrupted, promoting the opening of the channel gate [3].

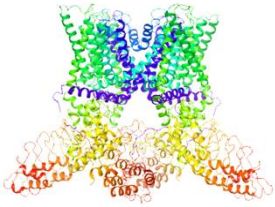

Figure 1*. Three-dimensional structure of the TRPV1 channel receptor*

Using computational methods, including molecular docking and structure calculation, we examined the interaction between TRPV1 and a number of its activators and inhibitors. In addition, we analyzed the antioxidant properties of individual ligands. The preliminary results of these studies will be presented, and therapeutic applications will be discussed.

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Reduction of Pt(IV) complexes by small biomolecules

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Pt(IV) complexes represent prospective anticancer pro-drugs. It is assumed that they must initially be reduced to obtain their anticancer activity and this fact can be used for tuning their properties via ligands in axial positions. In this contribution we focus on reduction of tetraplatin (tetrachlorodiaminocyclohexaneplatinum(IV)) in the presence of deoxyguanosine monophosphate (dGMP) [1-3]. The reaction starts with a substitution of one of chloro ligands by dGMP where both direct S_N2 and autocatalytic mechanisms are taken into account. In the next step, a nucleophilic attack of the 5' hydroxo or 5'phosphate group to the C8 position occurs. Subsequently the axial ligands are released simultaneously with reduction of the platinum complex. The reaction is completed by a hydrolysis of the chelate structure of oxidized dGMP leading to final 8-oxo-dGMP product. Nevertheless, reduction of satraplatin, one of the most investigated Pt(IV) pro-drug, cannot pass via the described mechanism. Therefore, we consider ascorbic acid as a reducing agent in this case. The studied mechanism for the process represents outer sphere or proton assisted electron transfer [4].

All the structures were optimized at the B3LYP/6-31G*/CPCM/Klamt computational level and single-point energy evaluations for optimized geometries at the B3LYP/6-311++G(2df,2pd)/IEFPCM/scaled-UAKS level [5]. Kinetic parameters of both the reactions are described using the evaluation of the side reaction model where dGMP and ascorbic acid are presented in different protonation forms in solution. In order to investigate changes in the electron density distribution the NPA, AIM and REF [6] analyses were also performed.

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Adsorption of oxalic acid on rutile surface

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Mineral-aqueous solution interfaces are of common occurrence and applying in natural and industrial environments. While experimental techniques cannot provide detailed view of ongoing processes on the molecular level, computer modeling of solid-liquid interface is able to provide atomistic details.

This work is continuation of our previous studies [1-2] based on the surface characteristics determination in the presence of ions. In our simulations we have used optimized classical molecular dynamics parameters of rutile (110) (TiO_2) surface and oxalic acid's deprotonated forms, namely, bioxalate ($HOOCCOO^-$) and oxalate ($COO-)_2$ [3]. In both cases we followed Electric Continuum Correction with Rescaling (ECCR) theory to include electronic polarization in the mean-field way by reducing the charges in our simulation setup [4].

The strong adsorption of oxalic acid on the rutile was observed for different surface charges densities, which correspond to pH values from macroscopic charging experiments of our colleagues. We identified the most favorable surface complexes, which were rationalized using a CD-MUSIC model combination. Finally, our results were compared with information inferred from published ATR-FTIR spectra.

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The Excision of OxoG Base with hOGG1 BER Enzyme; Towards Validation of the Catalytic Pathway

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One of the most abundant DNA lesions, the 8-oxo-2'-deoxyguanosine (OxoG), is repaired with human OxoG glycosylase 1 (hOGG1) base-excision repair enzyme. Catalytic mechanism employed by hOGG1 is unknown although several mechanisms were proposed. The catalytic scheme that employs pyramidal state of glycosidic nitrogen of OxoG allows substitution of N9-C1' bond with N9-H bond in a concerted synchronous manner [1-2]. The addition of proton to the glycosidic nitrogen operated by Lys 249 hOGG1 residue is OxoG/G specific as the nitrogen of OxoG is nucleophilic whereas that of normal G is electrophilic [3]. Measured 15N NMR shift of the glycosidic nitrogen owing to oxidative damage was accurately interpreted by means of theoretical calculations [4]. Recently, role of residues within hOGG1 catalytic core was studied employing NMR spectroscopy, which provided both necessary information on the mutant and coherent picture of the base excision strategy employed by hOGG1 [5].

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The mechanism of the glycosylase reaction with hOGG1: concerted effect of Lys249 and Asp268 during excision of oxoG

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In 2012 we proposed new reaction mechanism for cleavage of the N-glycosidic bond of 8-oxo-2′-deoxyguanosine (oxoG) catalyzed with the human 8-oxoguanine glycosylase 1 DNA repair protein (hOGG1) [1]. The reaction scheme suggested direct proton addition to the glycosidic nitrogen N9 of oxoG from the Nε-ammonium of Lys249 residue of hOGG1 that is enabled owing to the N9 pyramidal geometry. The synchronous effect of Lys249 and Asp268 residues on the excision of 8-oxoguanine (oxoG) by DNA glycosylase 1 (hOGG1) base-excision repair enzyme was studied by using the QM/MM (M06-2X/6-31G(d,p):OPLS2005) calculation method and nuclear magnetic resonance (NMR) spectroscopy. The excision of the oxoG base with $\Delta G^{\#}$ = 16.1 kcal/mol proceeded via substitution of the C1′-N9 N-glycosidic bond with an H-N9 bond where the negative charge on the oxoG base and the positive charge on the ribose were compensated in a concerted manner by NH_3^+(Lys249) and CO_2^-(Asp268), respectively [2]. The residue Asp268 was confirmed as the electrostatic stabilizer of ribose oxocarbenium through the initial base-excision step of DNA repair.

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Fluctuating Finite Element Analysis (FFEA): A Continuum Mechanics Software Tool for Mesoscale Simulation of Biomolecules

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Fluctuating Finite Element Analysis (FFEA) is an algorithm designed to perform continuum mechanics simulations of proteins and other globular macromolecules. These macromolecules are represented as continuous, tetrahedral 3D meshes, rather than fully atomistic structures. The meshes are simulated using conventional finite element methods, combined with stochastic thermal noise. The FFEA algorithm is appropriate for simulations of large proteins and protein complexes at the mesoscale (length-scales in the range of 10 nm to 1 μ m), where there is currently a paucity of modeling tools. It requires 3D volumetric information as input, which can be low resolution structural information such as cryo-electron tomography (cryo-ET) maps or much higher resolution atomistic co-ordinates, from which volumetric information can be extracted.

The details of the FFEA algorithm are due for release soon. We will also be releasing a software package under the GPLv3 free software license. This package will include include our own implementation of FFEA, written in C++, a set of tools to allow users to create and parameterise FFEA simulations from cryo-ET maps and atomistic data, a visualisation plugin for PyMOL, and a set of analysis tools. FFEA's release will be accompanied by a workshop at the University of Leeds in July.

Computational Modeling and Analysis of Backbone-Sidechain Interactions in Selected Amino Acid Diamide 5D Conformational Potential Energy Surfaces

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The conformational potential energy surface (PES) of a molecule provides insights into the relative stability of the possible conformers. However, the time and space complexity of electronic structure calculations, commonly used to generate PES, increases exponentially with an increasing number of atoms. The use of mathematical functions to model the topology of conformational PES is an alternative to these more computer-intensive quantum chemical calculations [1].

This study presents a mathematical representation of the conformational potential energy surfaces of seven amino acid diamides, using the model presented in [2], and analyze their properties, such as general topology, as well as the local minimum conformers and their relative stabilities.

This research lays an assessment for mathematical representation of amino acid PES, with less number of parameters and required computational resources. This may also be used to evaluate the conformational stability of longer peptides, with respect to its component amino acids.

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Quantum chemical study of hexopyranose configuration induced conformer distributions

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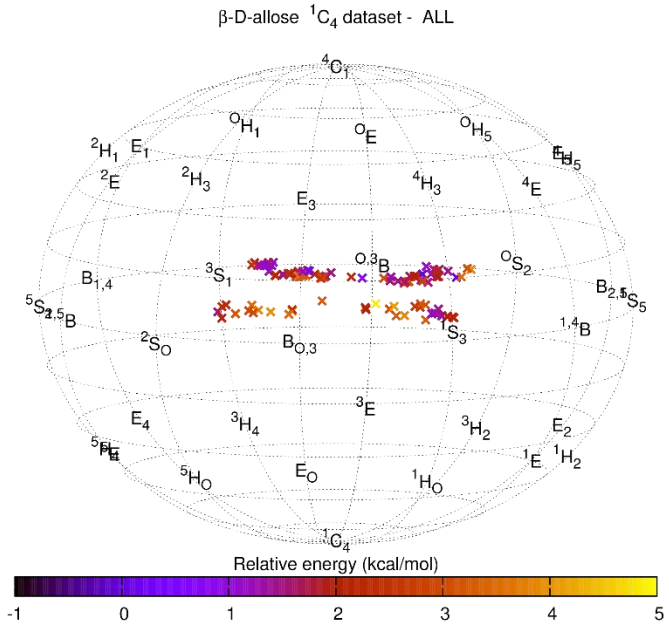
Stoddart published (Stereochemistry of carbohydrates, 1971) a method for comparing relative energies of conformations and configurations of hexopyranoses using empirically determined energy terms. These terms define for each destabilizing interaction the energy contribution (1,3-diaxial, gauche and anomeric effect) compared to the β-D-glucose. This approach is in a good agreement with experimental data for the α-β equilibria. Because the 1C-C1 equilibria are harder to determine (big energy difference), this data is harder to compare with empirical results. Our aim was to test the accuracy of the empirical data using pure quantum chemical (DFT) calculations using the Gaussian 09 software and determine (1) which functional works best and (2) what is the smallest basis set, (3) what can describe the systems accurately. The α-β and the $^1C_4$-$^4C_1$ equilibria were considered in the case of all 8 D-hexopyranoses. In each case the ΔG values for the α to β and $^1C_4$ to $^4C_1$ reactions were determined (32 forms). To check the consistency of the calculations the obtained ΔG values were plotted and the correlation was monitored. We have found that B3LYP functional worked better than the M062X. As the OH groups of the sugars in aqueous solutions usually oriented towards the water molecules, application of a solvent model was essential in combination with the filtering of the internal hydrogen bonds. The continuum solvent model, like IEFPCM improved the fitting accuracy, meanwhile the calculation demand didn't increased significantly. By using bigger bases, the energy values do converge to the experimental data, but there were still some differences. To eliminate this, we developed an approach which threats the observed sugar conformation as a Boltzmann ensemble and calculates the ΔG values as a weighted average of the selected conformers. Although starting from the usually very stable chair ($^1C_4$ to $^4C_1$) conformation, in many cases distortions and even inversion of the pyranose ring occurred, which can't be accounted by the empirical method. We have found that filtering out these "inappropriate" conformations from the averaging makes the fitting accuracy acceptable, but these conformations should exists in water solution.



Abstracts of poster presentations

The authors of the abstracts bear the full responsibility for the scientific and linguistic content.

Extraction of coffee ingredients from aqueous Ionic Liquid mixtures

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Several coffee ingredients including caffeine and various phenolic and flavonoid compounds exhibit interesting physiological activities. Extraction of those substances from spent coffee grounds with aqueous ionic liquid mixtures could provide an interesting alternative source while valorizing coffee production waste.

Molecular Dynamics Simulations of caffeine, gallic acid (representative for phenolics in coffee), quercetin (representative for flavonoids in coffee), protocatechuic acid and chlorogenic acid in various aqueous $C_2mim^+OAc^-$-mixtures were performed to analyze solvent – solute interactions and the solvation behavior. Distribution of the solvent as shown in Figure 1, average hydrogen bonding [1] during the simulation as depicted in Figure 2 and character of the first solvation shell were correlated to experimental extraction results [2].

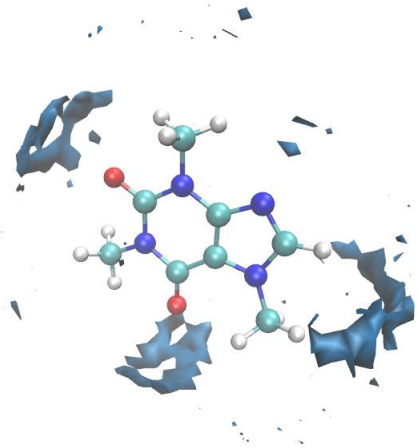

Fig.1: Preferred positions of water around the caffeine molecule in $C_2mim^+OAc^-$

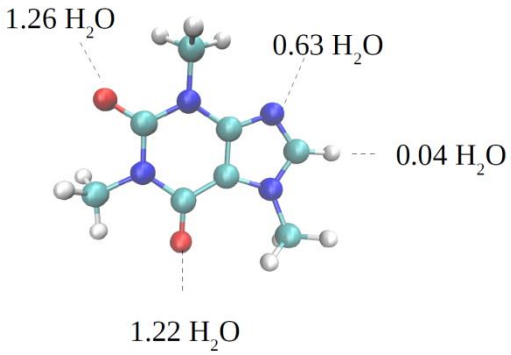

Fig.2: Average hydrogen bonding of the caffeine molecule with water in 1.1M $C_2mim^+OAc^-$ aqueous solution.

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Influence of disaccharides on water dynamics revealed by computational solvation dynamics

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The disaccharide trehalose is know to protect cells of some plants and microorganisms from freezing and desiccation injury via the protection of proteins, membranes and liposomes and is probably the most famous cryoprotectant among the carbohydrates. Proposed mechanisms range from water replacement or suppressed crystallization to changed water dynamics. Depending on the method of investigation (neutron diffraction, terahertz spectroscopy, NMR) different, sometimes even contradicting, conclusions about the range and nature of change in the solvent network induced by trehalose or other disaccharides such as maltose or sucrose have been drawn. New insight could be gained via computer simulation of time-dependent fluorescence spectroscopy of the disaccharide/water system using the molecular polarity probe N-methyl-6-oxyquinolinium betaine [1]. The simulation based on chromophore and solvent models from Ref. [2] yielded quantitative agreement to the experimental analog [3] for the trehalose/water system and showed differences between trehalose and maltose. The water retardation caused by the disaccharide could be decomposed into contributions from different locations, yielding insight into range of retardation, number of retarded solvent molecules and retardation factors which were found to depend heavily on the number of solvent molecules used for analysis. Thus, different findings in literature reporting various retardation factors can be traced back to the fact that the corresponding authors investigated different numbers of water molecules. Furthermore, the study revealed the limits of experimental solvation dynamics, as only half of the observed signal could be attributed to water molecules influenced by the presence of the disaccharide.

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Molecular dynamics study of anti-CD19 Chimeric Antigen Receptor (CAR).

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In a past few years CAR-T-based immunotherapy has achieved a significant progress in treatment of malignant hematological diseases and became one of the major frontier at the immuno-oncology field. CAR-T cells are genetically modified T-cells engineered to express a tumor-specific chimeric antigen receptor (CAR), which can activate them upon binding the antigen in MHC-independent manner. CAR is composed of an extracellular targeting domain, transmembrane domain and one or few costimulatory domains, that enhance the T-cell activation and cytokines production. Despite all the attention to such type of cell therapy from big pharma and academia, the lack of structural information about CAR organization and behavior slows down its engineering and development. Besides, the molecular mechanism of signal transduction caused by antigen binding still unknown.

The goal of the work was to create a reliable molecular dynamics model of the anti-CD19 CAR in the lipid bilayer membrane. The individual domains (anti-CD19 humanized scFv, CH2-CH3 spacer region and CD28 transmembrane region) were built with the MODELLER. Phospholipid bilayer was generated with CHARMM-GUI Lipid Builder using cholesterol, POPS, POPE, DOPC and DOPE lipids. Water layer was 17.5 Å high over and above the membrane. System charge was equilibrated by appropriate amount of Cl^- ions. For molecular dynamics simulation we used Amber16 software with Lipid14 and ff12SB force fields. After potential energy minimization a productive 100 ns MD was carried out.

As a result, we present a reasonable model of CAR in lipid bilayer environment. MD trajectories study revealed an increased internal dynamics of transmembrane helices that can lead to their homodimerization during binding of antigen. This model will be used in structural modification of existing CARs for providing an extended antibody-membrane linker flexibility and stability for signal transduction enhancing in order to make a new generation of more effective CAR-T cells.

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Structural peculiarities of cytochromes P450 in complex with antifungal azoles

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Cytochromes P450 (CYPs) – are the superfamily of heme-containing proteins that use different relatively small molecules as substrates in enzymatic reactions. In most cases they act as a terminal oxidase enzymes in electron-transfer chains [1]. CYPs play important roles in different organisms: animals, plants, fungi, protists, bacteria, archaea, and even in viruses. In human body cytochromes P450 are located either in the inner membrane of mitochondria or in the endoplasmic reticulum of cells and metabolize large amount of endogenous and exogenous chemicals such as drugs, xenobiotics, steroid hormones, prostaglandins and etc [2].

Azole-containing chemicals are widely used as antifungal agents and pesticides and work by inhibition of the fungal cytochrome P450 14α-demethylase. But at the same time they can tightly bind to other CYPs and lead to serious pathologies. At present study we used available crystal structures from Protein Data Bank of cytochromes P450 in complex with different azole-containing inhibitors to find out structural features of ligand-receptor interaction.

Analysis of data has shown that practically in all cases imidazole or triazole moiety of azole-containing chemical located perpendicular to the heme but plane containing heterocyclic five-membered ring can rotate around the axis passing along Fe-N bond. It was also found that distance between Fe atom of heme and nitrogen atom of azole is no more than 2.5 Å. Analysis of protein-ligand interaction interface showed that there are some residues conserved among CYP family that take part in ligand stabilization. The data obtained allowed us to create a new approach for analysis of binding different antifungal agents to cytochrome P450 7B1. It should be stressed that *in silico* data are in a good agreement with experimental results obtained by us earlier [4].

Given results cast light on structural basis of CYPs inhibition by azole-containing chemicals and can be used for *in silico* design of new antifungal drugs with low side effects frequency that allowed avoiding various pathologic states.

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A fluorescence spectroscopy, circular dichroism and molecular docking study of human alpha-1-microglobulin interactions with xenobiotics

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The proteins of lipocalin superfamily are characterized by a range of unique structural and functional properties and are involved in many physiological and pathological processes. Lipocalin alpha-1-microglobulin (A1M) is a plasma and tissue glycoprotein and a sensitive marker of renal diseases. It has been shown that A1M possesses immunoregulatory properties *in vitro* and has a reductase activity [1]. It has been established that A1M binds steroid and thyroid hormones [2]. Recently, the three-dimensional structure of recombinant human A1M has been determined [3]. A1M, like all lipocalins, share a highly conserved eight-stranded β-barrel with an internal hydrophobic cavity («lipocalin pocket») which has the ability to bind ligands. Over the past years, the interactions of small organic molecules with lipocalins have attracted much interest in the field of biochemistry and structural biology.

In the present study, we have estimated the ligand specificity of human A1M and demonstrated for the first time the ability of this lipocalin to bind xenobiotics including a broad range of insecticides of the neonicotinoid group. We used the well-known and most widely applied insecticide imidacloprid [4] and seven other xenobiotics [5]. This new compounds were structural analogues of imidacloprid containing 1,2,3- and 1,2,4-triazole cycles instead of 2-nitroiminoimidazolidine fragment and were characterized by different insecticide activities. The interactions of A1M with neonicotinoids were studied by fluorescence and circular dichroism (CD) spectroscopies and molecular docking. Fluorimetric titrations showed that increasing concentration of imidacloprid or five out of its seven analogues added to A1M (2 μM) quenched protein fluorescence excited at 280 or 297 nm, suggesting the binding of these substances to the protein. Two compounds did not cause any fluorescence quenching. The Stern–Volmer analysis at temperatures 293 and 303 K indicated that the fluorescence quenching of A1M by test compounds resulted from static mechanism. The binding constants (K_a) for the interaction of neonicotinoids with A1M were found to be 10^4-10^5 M^{-1}, indicating a moderate binding affinity. In comparison with the natural compounds, the K_a values of A1M – thyroid hormones complexes were 10^5-10^6 M^{-1}. It was found that one molecule of A1M combines with one molecule of imidacloprid or its analogue. The CD spectrum of A1M has a negative ellipticity at 213-214 nm, which are the typical characteristics of β-sheet structure of proteins. Ligands induced reduction of β-sheet content in A1M structure. The process of ligands binding to A1M was a spontaneous molecular interaction procedure ($\Delta G < 0$). The thermodynamic parameters such as enthalpy change (ΔH) and entropy change (ΔS) were calculated according to the van't Hoff equation and indicated that hydrophobic forces played a major role in stabilizing the A1M – neonicotinoid complexes. The data obtained by the molecular modeling study indicated that these ligands bound to a site located in the hydrophobic pocket of A1M.

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Combination of SAXS and molecular modeling in structure-function characterization of the plant DPP III orthologue

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Dipeptidyl peptidase III belongs to a family of zinc-exopeptidases, catalyzing the hydrolysis of dipeptides from N-terminus of small peptides with broad substrate specificity [1]. DPPs III are present in different organisms but their relevance still has not been fully understood. After the animal and bacterial orthologues, the first plant orthologue of DPP III, from the organism *P. patens*, *Pp*DPP III, has recently been discovered and characterized [2]. For the purpose of better understanding the DPP III's role(s), we are comparing structural and functional characteristics of DPP III orthologues from different domains of life.

Since the crystallization of *Pp*DPP III was not successful, a different approach has been attempted. This approach uses the combination of molecular modeling methods and SAXS (small angle X-ray scattering) measurements. SAXS measurements provide the overall 3D structural model with information about proper domain positioning, while the homology modelling and molecular dynamics (MD) simulations offer insights into the atomistic structure and dynamics of the plant orthologue.

We hope that the obtained results will enable us to correlate structure and dynamics of *Pp*DPP III with orthologues from other organisms and to better understand the function of DPP III in plants.

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Structure of proteins and phospholipid monolayers in deep eutectic solvents

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The investigation of proteins and enzymes in the absence of water has experienced a recent upsurge with the emergence of neoteric solvents, mainly deep eutectic solvents (DES).[1] DES are solvents obtained through the complexation of an organic salt and a hydrogen bond donor and the green character of the latter has attracted attention for possible applications in biochemistry and biocatalysis. We have therefore begun a systematic investigation on the behavior of biomolecules in these solvents.[2]

Here we will present our preliminary results on the formation of phospholipid monolayers at the air-DES interface and the conformation of proteins in DES. X-Ray and neutron reflectivity have been used to determine the characteristics of the phospholipid membranes (DPPC and DMPC), showing the formation of a stable monolayer with variable characteristics depending on the surface coverage. Circular dichroism and small-angle scattering have been used to determine the secondary structure and conformation of proteins (bovine serum albumin and lysozyme) in pure and hydrated DES, showing variable folding depending on water content.

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Adsorption of Methylamine at the Surface of Ice. A Grand Canonical Monte Carlo Simulation Study

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Gas/ice interactions are of fundamental importance to better understand the chemistry at the surface of ice-coated interstellar grain particles[1]. In particular, astrochemists focus their interest in the origin and formation of amino acids in the interstellar medium. Indeed, their presence may provide information on the delivery of prebiotic molecules to the early Earth, the origin of life on Earth, and the possibility of Earth-like life elsewhere in the Universe[2,3]. In this respect, the methylamine (CH_3NH_2) molecule has an important role in the chemical evolution of the simplest amino acid, glycine.

A series of 41 Monte Carlo simulations were performed in the grand canonical ensemble at 200 K to determine the adsorption isotherm and study in detail the adsorption of methylamine at the surface of I_h ice. The adsorption isotherm exhibits a plateau, corresponding to the saturated adsorption monolayer, in a broad range of chemical potentials and pressures. However, even this part of the adsorption isotherm deviates noticeably from the Langmuir shape. Shortly before condensation of methylamine occurs outer molecular layers also start building up. The remarkable stability of the adsorption monolayer is caused by the interplay of the hydrogen-bonding interaction between the adsorbed methylamine and surface water molecules and the dipolar interaction between neighboring adsorbed methylamines. As a consequence, the adsorbed methylamine molecules exhibit a rich orientational distribution relative to the ice surface, and the adsorption is accompanied by rather large energy change.

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The effect of substituents on the kinetics of the hydration reactions of trans-Platinum complexes

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Platinum anticancer drugs are administered in its inactive (chlorinated) form. The activation by hydrolysis takes place inside the cells. Affecting the speed of hydrolysis as the rate determining step of subsequent reactions is one of the ways to fine-tune the therapeutic effect.

We have studied the kinetics of the aquation reaction on the trans-$[Pt(NH_3)_2(pyr\text{-}X)Cl]^+$ and trans-$[Pt(NH_3)_2(NH_2\text{-}X)Cl]^+$ complexes with the substituent X being OH, Cl, F, Br, NO_2, NH_2, SH, CH_3, CCH, and dimethylamine (DMA). Mono- as well as some di- and tri-substituted complexes were studied. Reaction energy profiles, atomic charges, ligand binding energies and ETS-NOCV analyses were calculated. All the structures along the reaction pathway were optimized using B3LYP/MWB-60(f)/6-31+G* method. Single point energies and molecular properties were evaluated using B3LYP/MWB-60(2fg)/6-311++G (2df,2pd) both *in vacuo* and in implicit water environment (PCM). The effects of correcting dispersion using Grimme's empirical dispersion parameters (GD3BJ) [1] and M06-2X functional by Zhao & Truhlar [2] were compared.

The substituent ligand influences electron density on the pyridine ring and thus the electron donating ability of the heterocyclic nitrogen. Through the trans-effect the charge and binding energy of the Cl ligand are affected leading to the difference in the rate of the hydrolysis reaction of several orders of magnitude. On the opposite sides of the scale lie NO_2 and NH_2 ligands, as the least and most promoting substituents, respectively. Direct substitution shows similar behavior, with more pronounced sterical effects.

The results prove that the kinetic behavior of the platinum drug complexes can be easily engineered via the substituent trans-effect.

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Redshift of the wavelength in the Spinach aptamer complex, suggestions from QM:MM calculations

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Spinach aptamer was developed as an RNA analog of the Green Fluorescent Protein. The aptamer interacts with 3,5-difluoro-4-hydroxybenzylidene imidazolinone (DFHBI) molecule and modifies its electronic spectrum so that the chromophore emits bright light with wavelength of 501 nm. Song et al. have investigated modifications of the chromophore in their experimental study [1] and found that substitution of methyl group at position 2 by trifluoromethyl leads to emission wavelength of 523 nm in complex with the Spinach aptamer.

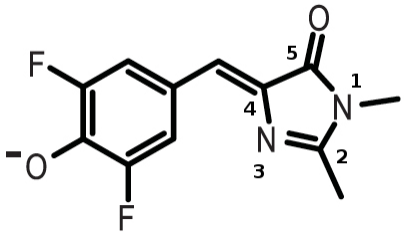

Figure 1: DFHBI molecule

The crystal structure of the Spinach aptamer in complex with its original ligand has been published in 2014 [2] and it enabled us to study the system computationally. In this contribution, we will report several new modifications of the chromophore that cause further redshift of the fluorescence wavelength of the complex [3]. Our results are based on combined quantum mechanical / molecular mechanical calculations in ONIOM with the choice of DFT as the quantum mechanics method. These were used for geometry optimization. The quantum mechanical (QM) region contained the chromophore and nearby nucleotides. Excitation energies were calculated by TDDFT method on the QM region, optimized in ONIOM, embedded in PCM continuum with solvent of medium dielectric constant.

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A molecular dynamics study on the effects of 1,4-dioxane on model membranes

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New industrial processes have led to the emission of numerous environmental pollutants, which decompose hardly or not at all decompose in nature. Understanding the effects of these pollutants are priorities of modern environmental protection. In this study, we examined the effects of 1,4-dioxane, a significant environmental pollutant, on biological membranes using molecular dynamics simulations.

For a better understanding of the effects of the highlighted molecule we used model membrane systems that were built up, either from DPPC or IPPC phospholipid molecules. Molecular dynamics simulations were performed for these systems in pollutant free and polluted (i.e. in presence of 100 1,4-dioxane molecules) environments in order to determine the influence of the lipid component or pollutant on the physical properties. The simulations were carried out in biologically relevant fluid-crystalline phase (T = 330 K, p = 1 atm, 50 water molecules/lipid) for 5×125 ns for each system using the GROMACS 5.1.2. program package with a CHARMM36 force field. Where possible the calculated values were compared with literature data which were generally in good agreement.

Overall it can be stated that the DPPC and IPPC model membranes have similar physical and structural parameters. The presence of 1,4-dioxane leads to systematic changes in both membrane systems, though generally the IPPC membranes retain their physical and structural properties more efficiently. In this work, the penetration mechanism of the dioxane molecule was also examined.

Fourier type potential energy functions for conformational change of selected organic functional groups

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The long-term aim for the present research is to find mathematical functions which describe the folding of peptide residues. We would like to eventually find the solution to the protein folding problem and to build up the conformational network for folding but it is reasonable to start with the description of small compounds, and aim for a bottom up solution.

In the 1970's, the Fourier-series were investigated by Radom and Pople to describe the energy changes of computed torsional functions [1]. In 1978, Cremer studied peroxide with a polarized large basis set [3] and it produced remarkedly accurate results yet Peterson and Csizmadia demonstrated that all critical points of the Potential Energy Hypersurface (PEHS) of three independent variables of n-butane could be reproduced at a modest level of theory [4]. In the late 1980's, Chung devised a method to compute torsional energies with n-term Fourier-series. For potential energy surfaces (2D) and hypersurfaces (3D) functions of two and three independent variables, respectively were necessary. Nevertheless, the practical question still remains; how many terms are needed to obtain reliable results for conformational PESs.

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Thermodynamics of Mixing of Formamide and Water in Computer Simulation

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The purpose of this work was to test several possible combinations of the existing formamide and water force fields in order to reproduce miscibility features of these systems in the entire composition range. The thermodynamic changes occurring upon mixing five models of formamide and three models of water were studied in computer simulations using an appropriately chosen thermodynamic cycle and the method of thermodynamic integration. From the performed simulations we were able to calculate the energy, entropy and the Helmholtz free energy of mixing. To validate our results, we used the fact that these compounds are fully miscible with each other [1], and the experimental values of their energy of mixing. Concerning both the miscibility and the energy of mixing of the studied model combinations, we could recommend a proper formamide-water model combination for the simulations of water-formamide mixtures which can be useful for further prebiotic studies as well as for understating industrial processes.

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Study on Reactivity of Methylene Diphenyl Diisocyanate (MDI) with Biomolecule Motifs

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Polyurethanes are the most versatile used plastics nowadays which can be formed in the polycondensation reaction of isocyanates and alcohols. Methylene diphenyl diisocyanate (MDI) is one of the most common raw material of polyurethane production. The most reactive isomer is the 4,4'-MDI which can easily react with nucleophiles. Such nucleophile motifs can be also presented in the respiratory system as part of proteins and glycolipids. Reactions of MDI with these biomolecules can probably be responsible for allergic respiratory diseases [1]. However, the relative reactivity and the reaction mechanism of the MDI with potential reactive sites of biomolecules are unknown yet.

Three types of peptide residue (serine-, threonine-, tyrosine-amide), a galactose-amine (N-acetylgalactoseamine) were proxies for the biomolecules in this theoretical study. The biomolecules were reacted via their hydroxyl group forming urethane bond with the isocyanate group. Air humidity can be serve as a sink of the MDI via the reaction between MDI water molecules, therefore this reaction was also examined. The reaction of hexandiol with MDI was used as a reference for each case.

The reaction pathways were explored including all transition states structures and intermediates using density functional B3LYP/6-31G(d) level of theory. The accurate thermodynamic functions were obtained using G3MP2B3 composite method. The G3MP2B3 activation energies of the different reactions were compared.

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Aniline metabolism in hepatocytes and erythrocytes

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Aniline is very important in the chemical industry, and is widely used by the pharmaceutical, polymer, and pesticide industry. Because of its wide spectrum of applications, the examination of its metabolism in the human body is justified.
During aniline metabolism, different types of products can form, depending on the organs and the organism in which the compound is found. Hydroxylation of the aromatic ring and the formation of isomeric phenol metabolites are major routes of aniline metabolism [1]. The hydroxylated products are rapidly conjugated to sulphates or glucoronides and excreted in the urine as water-soluble metabolites [1].

Aniline is rapidly oxidized in both the liver and in erythrocytes, through three competing transformation mechanisms: N-hydroxylation, ring hydroxylation, and N-acetylation followed by p-ring hydroxylation [2]. The products of these conversions, phenylhydroxylamine and p-aminophenol, as well as their oxidized forms, nitrosobenzene and p-iminoquinone, formed by reactions 1 and 2, are regarded as toxifying steps toward biologically active compounds, which N-acetylation is considered to be a detoxifying reaction [2]. The pharmacogenetic variation in the expression of N-Acetyl transferase explains the differential susceptibilities of human individuals to aniline toxicity.

The goal of our work was to analyze the mechanisms of aniline metabolism, using computational chemistry tools such as Gaussian 09 program package. The preliminary results of these calculations will be discussed, in an attempt to better understand the mechanisms of aniline toxicity, and to expand its applications in various industries.

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Development of carbon nanotube-coated core-shell structured nanocomposite catalysts

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In our work we synthesized catalyst support materials, which included nitrogen-doped carbon nanotubes (N-CNT) on their surfaces. In these core-shell structures, zeolite beads can be found as templates of the carbon nanotubes. In the case of N-CNT, the diffusion limitations can be overcome by the absence of microporosity [1]. On the micropores containing conventionally used catalyst supports, such activated carbon materials, the reactions were slow because the transport processes of the reactant molecules were limited, which is opposite to the case of carbon nanotubes [2]. The nitrogen content of N-CNT leads to stronger interactions between the catalytically active metal particles and the nanotubes. This phenomenon can be attributed the electron affinity of the incorporated nitrogen, which causes perturbations in the electron distribution of the carbon nanotube. The core-shell structured N-CNT-coated zeolite beads were synthetized from butylamine using the CCVD method, and these nanocomposites was impregnated with a palladium chloro complex. Following a reductive step, these nanocomposites were tested as catalysts in the hydrogenation of different olefins during gas phase and liquid phase reactions. The palladium-contained catalyst was characterized by thermogravimetry, scanning electron microscopy, and X-Ray diffractometry methods. The carbon nanotube content of the zeolit/N-CNT was 2.6 wt%, and the palladium content was 0.03 wt%. The average diameter of the palladium nanoparticles on the core-shell catalyst was 28 nm, and the palladium distribution showed homogenous distribution on the surface. The catalytic tests were followed by Fourier Transformed Infrared Spectroscopy, by examining the saturation of C=C double bonds in olefin molecules. During the hydrogenation tests, the catalyst showed significant activity. The maximum catalytic performance of the sample in the hydrogenation reaction of butane in gas phase was reached in less than 10 minutes. The hydrogenation of octadecene in liquid phase also occurred rapidly. The Pd-contained catalyst was active during hydrogenation tests, despite low palladium content (0.03 wt%); accordingly it is an economical catalyst.

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Computational study of the phosgenation reaction mechanisms of the MDA

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The polyurethane industry is mainly based on the reactions of isocyanates with different type of alcohols[1]. Besides toluene diisocyanate, methylene diphenyl diisocyanate (MDI) is the major isocyanate used to make polyurethanes, with this molecule having a 34% share of the global annual isocyanate production (4.4 million tonnes) [2]. Since MDI is mainly produced from methylene dianiline (MDA) by phosgenation [3], the side products of the phosgenation reaction can debase the MDI as well as the quality of the polyurethane produced. However, although the reaction mechanism for the phosgenation of MDA is relatively well-established, possible competing reaction channels have never been discussed in the literature. The aim of this work is to explore the energetics of these competing phosgenation reactions using computational chemical approaches. These reaction mechanisms were computed using the B3LPY/6-31G(d) level of theory as well as the more accurate and robust G3MP2B3 chemistry model. The obtained energy profile was compared with that of MDI production from MDA. All calculations were carried out using the Gaussian09 program package.

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Systematic theoretical investigation for high energy $C_2H_8O_4$ molecules

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Energy, the basic element that every nation needs, is now a debated subject pushing the scientific world to look for its sustainable forms, due to its declining resources combined with the increasing demand and fluctuating needs as well as its environmental impact [1,2]. Biofuels or biofuel additives can be attractive renewable energy alternatives [3,4] since are capable of storing large amount of energy in chemical bonds. In this work, we attempted to explore the energy content of all possible electronically singlet structures which can be described by the $C_2H_8O_4$ formula.

Initial structures of 131 constitutional isomers were generated from the general formula, $C_2H_8O_4$, using the Molgen 5.0 program, including not only unimolecular structures but also their molecular complexes. These structures were then optimized and their thermochemical properties such as heat of formation and heat of combustion were computed using the G3MP2B3 composite method of the Gaussian 09 quantum chemistry program package.

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Mechanistic Study of Formaldehyde Synthesis from Methanol

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Methanol oxidation is a multistep process which eventually leads to the formation of carbon dioxide and water. The reaction goes through valuable intermediates such as formaldehyde [1] which is important for the chemical industry in the production of more complex species. Formaldehyde production can be initiated with different catalysts (e.g. silver, iron oxide). In the case of the silver-catalyzed approach, formaldehyde is produced through two reaction channels [2]:

$$2CH_3OH + O_2 \rightarrow 2CH_2O + 2H_2O \qquad \text{eq1.}$$

$$CH_3OH \rightarrow CH_2O + H_2 \qquad \text{eq2.}$$

To get a more detailed view about these mechanisms, computational chemistry tools were used to study the reaction mixture and the interactions between the reactants and the products. Therefore, methanol, formaldehyde, and water were virtually mixed into the $C_3H_{14}O_5$ molecular formula and all the corresponding constitutional isomers were generated. Then, these structures (molecules and molecular complexes) were computed using G3MP2B3 composite methods, which are implemented in the Gaussian program package.

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Formation Mechanism of C_2H_5NO Isomers in Interstellar Medium

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Interstellar medium is one of the greatest discoveries in the mysterious space. There are over 150 different molecules that have been detected [1]. The dense molecular cloud has a temperature between 10K and 50K and a density between 10^4 and 10^6 cm^{-3} [2]. Acetamide (CH_3CONH_2) was detected in Sagittarius B2 in 2006, the molecule can be formed by the neutral-radical reaction for which is favoured at low temperature [3]. The reaction dynamics of interstellar molecules are still inconclusive and studying the formation of interstellar organic molecules will help to gain insight of astrochemistry and prebiotic chemistry. Standard enthalpy of formation, relative Gibbs free energy and entropy were calculated using G3MP2B3 as the level of theory in the Gaussian09 program package. Geometry optimizations and frequency calculations were carried out using B3LYP/6-31G(d) as the level of theory. This research will investigate the formation mechanisms of C_2H_5NO and its isomers in gaseous form under the extreme conditions of the dense molecular cloud by analyzing the potential energy surface, which allows us to study the relative stability of the possible isomers and develop all the possible barrierless formation pathways and the molecular structure of C_2H_5NO.

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Formation Mechanisms of Three-Carbon Sulfur-Containing Organic Compounds in ISM

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Some interesting sulfur-containing compounds were recently detected inside dense clouds by space radio spectrometry with the help of the development of telescopes and spectral instruments [1]. In this project, thioacetone (C_3H_6S), as one member of the sulfur-containing compounds family will be specifically discussed. Sulfur massively exists in organisms in the form of disulfide bonds in the construction of protein and iron-sulfur clusters in dissimilatory sulfur metabolism. Sulfur is essential to life because it makes up about 1% of the dry weight of organisms. Sulfur-containing compounds are also minor components of fats, body fluids, and skeletal minerals [2]. This research will help us find the origin of life in space, calculate the possibility of life form in other planets as well as understand new chemical models and mechanisms in different environment. However, not like on the Earth, the universe imposes extreme reaction conditions on those reactants; the average temperature of the universe is 2.73K and the average pressure is not uniformly distributed [3]. Thus, it is hard to do researches in labs. In this report, computational chemistry methods using the Gaussian09 program package will be used to help us understand the formation mechanism of the three-carbon sulfur-containing organic compound in space.

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Rational Design of Odorants – A Case Study of $C_2H_4O_2$

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Olfaction is a chemoreception composed of a sensory system that allows the organism to detect chemicals in its environment [1]. The odorant binds to the olfactory receptors (OR) which are part of the G-protein-coupled receptors (GPCR) superfamily [1]. There are two possible theories of olfaction, the structure odor relationship (SOR) and the vibrational theory of olfaction (VTO) [2]. The main explanation for smell was primarily based on the odorant molecule's surface but experiments have suggested that the ORs are activated by electron transfer through odorant vibrational excitation [3]. Molecules that have very different structures can still have similar odors and yet a minor change to the structure can alter the scent completely [2]. These studies suggest that the resulting process of olfaction is an amalgamation of both theories. To provide further insight using computational methods, a model structure of acetic acid was studied.

All the constitutional isomers of acetic acid with the $C_2H_4O_2$ molecular formula were generated using Molgen 5.0 program. The resulting structures were used as the initial structures for the local minima search on the multidimensional potential energy surface of the species. The quantum chemical calculations were carried out using G3MP2B3 composite methods which is implemented in the Gaussian program package.

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Computational Modelling and Analysis of 4D Conformational Potential Energy Surface of N-acetyl-valine-N-methylamide

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At the present time, antitumor drug development is an extremely active field of research and includes aspects of biochemistry, biology, and chemistry. Recent developments are discussed: D-valine acting as a significant factor in reducing water solubility, as well as the binding capacities of the tumor cell [1].

In this work, we focused on the conformational analysis of a valine diamide, N-acetyl-valine-N-methylamide, to obtain a stable valine structure for antitumor drug discovery, through narrowing and visually analyzing the global minimum energy of the 4D potential energy surface. The 4D potential energy surface diagram is built based on a comparison of relative energies (ΔE) using DFT electronic structure calculations under the B3LYP /6-31G(d) implementation in gas phase on the Gaussian09 software package [2]. A mathematical representation of the amino acid PES is then created through a linear combination of Fourier series and a mixture of Gaussian functions [3], to analyze the energy landscape more precisely. Specifically, a model is presented with respect to three independent variables corresponding to the backbone angles φ, ψ, and sidechain χ, i.e. E=f (φ, ψ, χ), to analyze the relative stabilities of the conformations of the molecule.

This study will gain insight into the valine residue in future research on tumor therapy though the conformational modelling an analysis of the valine diamide.

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Computational Modeling and Analysis of 5D Conformational Potential Energy Surface of N-Acetyl Glycylglycine N-Methylamide

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Proteins within a living cell are composed of amino acids bound together to form peptide chains that spontaneously fold into their active 3D state. Every protein has a specific energetic pathway along which it proceeds to correctly fold but sometimes a protein will follow the wrong path and become misfolded [1]. This may result in toxic proteins which tend to aggregate and form insoluble masses too large and complicated for the cell to eliminate [2,3], resulting in diseases like Alzheimer's disease [3]. The conformational potential energy surface (PES) of a protein can be used to determine its stable structures and its probable folding pathways. However, using traditional *ab initio* methods to calculate a protein's PES becomes increasingly complex with molecule size, and therefore computational time increases exponentially [1]. This study aimed to accurately determine the most stable structures of n-acetyl-glycylglycine n-methylamide and reduce the required computational time, through an analytical fitting of the PES following the model in [1]. The ultimate goal of this research is to use this method in future studies to efficiently analyze more complex proteins.

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Model Aided Biofuel Design: Thermodynamic Properties of the $C_5H_{10}O$ Constitutional Isomers

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Biofuel is the general term for energy sources that come from living matter. It has been a hot topic recently as it emits less greenhouse gases than other fuel sources, and it is a renewable energy source [1]. Several sources of biomass have been proven to be potential candidates for producing biofuel, including algae, jatropha tree, and fungi.

Biofuel production is a complex, multi-step process that combines both biological and chemical technologies. Phase change, sugar production [2], and sugar-ethanol conversion [3] are the main steps. Phase change makes the raw materials easy to transform through a chemical reaction to simple sugars. Afterwards, several types of alcohols can be produced by fermentation.

$C_5H_{10}O$ isomers are potential energy storage molecules, which can form during the biodegradation of several sources of biomass, and some of them might have high energy and high density structures. In this work, 75 initial structures of possible $C_5H_{10}O$ constitutional isomers were generated using the Molgen program, allowing hydrogen, carbon, and oxygen to have 1, 4, and 2 bonds respectively. Computational chemistry can then help to determine geometry of the molecule as well as its molecular properties. Thermodynamic properties of these isomers were determined by the G3MP2B3 model chemistry, and the enthalpy of formation, Gibbs free energy, and entropy can be calculated and analyzed according to the energy density of the molecular structure.

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Model Aided Biofuel Design: A Case Study of $C_6H_{12}O$

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Biofuels are renewable and sustainable sources of energy, which can be generated from inexpensive and abundant biomass feedstocks such as lignocellulose, and have a higher possibility of meeting the increasing demand of energy in current society [1,2]. In terms of environmental aspects, biofuels also have several advantages. For example, the combustion of biofuels generates less greenhouse gases compared with fossil fuels [1], and the subsequent feedstock growth consumes carbon dioxide [2], which contributes to the balance of the emission and absorption of carbon dioxide.

This research focus on identifying the specific structures with the chemical formula $C_6H_{12}O$ with higher thermodynamic energy and relative kinetical stablility by using computational chemistry. 211 constitutional isomers of mentioned molecular formula were generated by the Molgen 5.0 program. The G3MP2B3 level of theory was used to calculate the heat of formation, relative Gibbs free energy, and entropy of several promising isomers. Finally, the higher heating value and the lower heating value of standard reactions were calculated, which contributed to determining the ideal structure.

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Theoretical Investigation of Green Polyurethane in Industrial Applications

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Polyurethane (PUs) are polymers (**Figure 1**) that are used widely in various industrial and consumer applications due to their easily tunable rigidity and flexibility. They are applied as heat insulators in construction, seat cushion in automotives and elastomeric materials in medical industries. One of the main starting materials, isocyanates, have been found to be toxic and have raised severe health, environmental concerns [1]. In recent years, intensive research and development has been carried out to prepare non-isocyanate polyurethanes (NIPU) that can be sustainable for production.

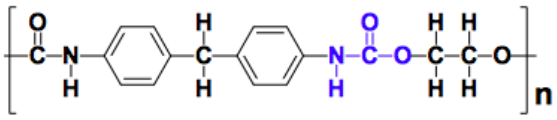

Figure 1 *Structure of conventional PU. The urethane linkage indicated in blue*

Numerous pathways can be taken to create NIPU. A successful example is the commercially available Green Polyurethane™, a hybrid material which combines the high chemical resistance properties of epoxy and advanced wear resistance properties of polyurethanes [2,3]. Another promising method is the crosslinking of hydroxyurethane modifier (HUM) based on renewable and vegetable raw materials [2,3]. The goal of the project is to study several existing structures of green polyurethane with chemical computational tools. These computed results can be used to discover new sustainable synthetic routes and physicochemical properties. Ultimately, this aids in the design of more environmental friendly polyurethanes for future industrial use.

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Designing voltage-sensitive probes for neuronal imaging

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Development of fluorescent molecular probes of cell membrane voltage promises to deliver the ability to observe the electrical activity of neuronal assemblies using an optical microscope. The aim of the present study is to give insight at a molecular level of the response to electric fields for existing probes and enhance their sensitivity. Nowadays, Among the most efficient fluorescent probes used for neuronal imaging is a combination of a GFP mutant and the voltage-sensitive domain (VSD) from the phosphatase protein.

We present here first the case of a Green Fluorescent Protein (GFP) and its mutant with increased number of negatively charged residues. Both constructs are anchored to the membrane by a lipid tail attached to the protein. Secondly, simulations were the activation of the VSD from the phosphatase in a presence of a transmembrane potential is present is shown. Finally, a new generation of probes is unveiled. Those are based on a transmembrane alpha-helix taken from a non voltage-sensitive protein. Mutations of non-charged amino-acids by arginines inside the membrane spanning region are conducted to enhance its sensitivity to the transmembrane voltage. Due to the complexity of the membrane environment and the many possible conformations of the proteins, enhanced sampling methods such as metadynamics were used along with virtual sites to reach micro-second time scale simulations. Constant homogeneous electric fields were applied during simulations to induce a difference in the transmembrane voltage.

Biochemical approaches to determine the role of long hydrophilic loop and C, N tail of *Saccharomyces cerevisiae* potassium translocating proteins

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Trk proteins are cation translocating proteins that allows *S. cerevisiae* (*S.c.*) to survive and grow under different environments from a few μM to hundreds of mM [K+] and maintaining internal [K+] relatively constant. In *S.c.* there are two specific K+ translocation proteins: Trk1 and Trk2. Trk1 is 1235 amino acids long and Trk2 is 889 amino acid long [1]. Fungal Trks are structurally related to prokaryotic Trk, Ktr and plant HKT proteins [2]. A unique feature of fungal Trks is that they contain a "Long Hydrophilic Loop" (LHL) which is not homologous between these proteins and differs largely in length i.e. 648 aa in Trk1 and 327 aa in Trk2 [1,3]. Trks also have C tail (66 a.a.) and N tail (49 a.a) which is not considered before in the structural model developed [5]. The objective of work is to study the role of LHL and to purify and crystallize C, N tails of Trk1.

To evaluate the role of LHL, GFP fusion constructs of Trk1 and 2 with and without LHL as well as constructs in which GFP was fused to LHL alone were prepared and used to transform *S.c.* BY4741[*Δtrk1,2, tok1*] cells [4]. Western blot analysis of plasma membrane fractions showed bands for GFP labelled Trk-proteins at their approximate expected sizes, indicating full-length expression of the constructs. Fluorescence microscopy was performed to compare the cellular localisation of the fusion proteins. Trk1/GFP and Trk2/GFP were observed evenly distributed at the cell periphery (plasma membrane), whereas Trk1[ΔLHL]/GFP showed a punctuate distribution pattern at the plasma membrane. GFP/LHL(Trk1) was found in the cytosol. C-tail and N-tail was purified as a fusion protein with GFP and attempts were made to crystallize the C-tail.

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Mechanism and Energetics of L-arginine Binding to Arginine Repressor Protein in *E. Coli*

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Arginine repressor protein provides feedback regulation of arginine metabolism upon activation by the negatively cooperative binding of L-arginine. Understanding this phenomenon requires the detailed analysis of each binding event and its effect on global motion of the complex.

Umbrella sampling technique was used to calculate binding energy (potential of mean force) of L-arginines to the ArgRC. Unbinding of L-Arg from ArgR was performed using steered dynamics. Potential of mean force (PMF) was calculated using weighted histogram analysis method in GROMACS. Differently ligated states were prepared either by deleting (from holo-ArgR crystal structure) or adding (to apo-ArgR crystal structure), using YASARA tool.

PMF for holo-5 state was ~12 kcal/mol, while in corresponding apo+1 state it was ~7 kcal/mol. PMF for holo-4 state and apo+2 state were ~4 kcal/mol and ~15 kcal/mol respectively.

The PMF of +1 and -5 states have similar values while that of -4 and +2 states are very different. The huge difference in the PMF between -4 and +2 states could be due to the differently occupied binding pockets in these two systems. Few more repetitions of +2 and -4 states are undergoing, once completed these will hopefully allow us to compare the binding affinity of differently liganded states of ArgRC and their effect on global motion of protein.

Computational Modeling of Phosphokinase Activity of Human 3'-Phosphoadenosine 5'- Phosphosulfate Synthase

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The sulfur nucleotide PAPS (3'-phosphoadenosine 5'-phosphosulfate) is the universal sulfuryl donor of the cell. In mammals 3'-phosphoadenosine 5'-phosphosulfate Synthase (PAPSS), using ATP, converts biochemically inert inorganicsulfate to metabolically active PAPS. PAPS synthase is a bi-functional enzyme and catalyzes the formation of PAPS in two sequential steps. As a bi functional dimer, the kinase domain of the PAPSS transfers a phosphate group from the γ phosphate of a second ATP to the sulfurylase domain product adenosine-5' phosphosulfate (APS) to form the PAPSS enzyme. A potential phosphorylation/allosteric motif is located close in space to the APS binding site. Concretely the respective motif is "TLDGD" (residues 85-89) for PAPSS1 and PAPSS2b (Venkatachalam unpublished/proposed), and docking studies show that these residues might play a key role in positioning the magnesium ion and the ribose prior to the first step of the reaction, the nucleophilic attack on ATP-γ phosphate by abstracting the proton from the 3'hydroxyl end of APS. MD simulations and G_MMPBSA calculations to were performed for WT and selected point mutations in this motif to determine the individual contributions to the interaction energy. Mutations in position 87 or 89 have an additional effect on the 62 Glycine residue of the P-loop that is responsible for the transfer of the Pi to APS. The potential role of this motif in phoshokinase activity is discussed

Computational Modeling of Sulfurylase Activity of Human 3'-Phosphoadenosine 5'- Phosphosulfate Synthase

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Human 3'-Phosphoadenosine 5'- Phosphosulfate Synthase (PAPSS) is a bifunctional protein consisting of N-terminal (1-260 aa) APS kinase domain and a C-terminal ATP sulfurylase domain (220-623 aa). In mammals and higher organisms, the enzyme is the sole means for bio- integration of inorganic sulfate through its transformation into an activated, organic form.[1] Although its overall mechanism and kinetics have been well studied in the past, more recent discoveries including the resolution of its crystal structure and research in its regulatory functions revealed previously unanticipated behaviors.[2] As the ubiquitous sulfate donor in most biological systems, the product of the enzyme, PAPS, plays an essential role in ECM formation, embryonic development and biomolecule secretion.[3] Moreover, PAPSS has also been shown to be involved with the pathophysiology of a number of diseases including HIV, hepatocellular carcinoma and non-small cell lung cancer.[4,5,6] A HXGH motif (amino acids 425-428) that is conserved in the ATP sulfurylase domain has been shown to be involved in enzyme activity. In H425A or H428A mutant enzyme activity is lost, where a N426K mutation resulted in an increased enzymatic activity. A G427A mutation decreased the enzyme activity by 30%. To reveal the functional importance of this motif, WT and mutant homology models using available PAPS1 crystal structures were built and used for atomistic molecular dynamics simulation to understand the dynamics of the system. Ligand binding affinity of the complex as determined by MMPBSA calculations correlates well with the experimentally observed enzyme activities, indicating that the initial binding of ATP might be the rate-limiting step in sulfurylation. In order to describe the sulfurylase reaction along the reaction coordinate ligand docking of reactants and products of the ATP sulfurylase domain has been performed to gain initial information about the positioning of the molecules in the respective binding pockets, followed then by a combination of molecular dynamics, hybrid QM/MM and quantum calculations. First results are discussed that give a realistic picture of sufurylase activity including molecular interactions, transition state structures and the reaction coordinate.

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Human 3'-Phosphoadenosine 5'- Phosphosulfate Synthase: Protein Expression, Purification, Crystallization and Activity

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The sulfur nucleotide PAPS (3'-phosphoadenosine 5'-phosphosulfate) is the universal sulfuryl donor of the cell. In mammals 3'- phosphoadenosine 5'-phosphosulfate Synthase (PAPSS), using ATP, converts biochemically inert inorganic sulfate to the metabolically active PAPS. It is a bi-functional enzyme and catalyzes the formation of PAPS in two sequential steps. In the first step, inorganic sulfate reacts with ATP to form APS and pyrophosphate. The resulting phosphoric-sulfuric anhydride bond has high energy that is the chemical basis of sulfate activation. The second step is catalyzed by the kinase domain of PAPSS and involves the reaction of APS with ATP to form PAPS and ADP. The proper function of PAPSS is essential for normal physiology in the human being. PAPSS deficiency in human results in osteochondrodysplasias or defective cartilage and bone metabolism as evidenced in the clinical condition of the recessively inherited, spondyloepimetaphyseal dysplasia (SEMD). To establish the enzyme kinetics and to demonstrate structural and functional consequences of point mutations in the enzyme, pure enzyme has to be prepared and purified. Briefly, this includes the design of primers to introduce the point mutations, PCR, recombinant protein expression in *E.coli* and protein purification using FPLC with affinity, ion exchange and gel filtration columns. The purity of the preparations is tested by SDS-PAGE and pure protein will be used for crystallization trials. Homogeneous preparations will be assayed for APS kinase, ATP sulfurylase, domain activities and PAPS synthase overall activity according to the published procedures of Venkatachalam (J. Biol Chem., 1998). The reaction products and the substrates will be separated on a reversed phase C-18 HPLC column. The products will be detected by UV absorbance and the corresponding peaks will be quantitated for products formation. First results are reported and possible strategies are discussed.

Ammonium Ligand Binding to dibenzo-18-crown-6 via Density Functional Theory

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Dibenzo-18-crown-6 (DB18C6) is a monofunctional, crown ether that acts as a host for a guest molecule. This system acts as a model for ligand binding to a protein and aids in higher understanding of binding processes. To support this study, density functional theory (DFT) calculations were utilized to determine the binding energy of DB18C6 with ammonium chloride in gas-phase as well as in implicit water solvent. Additionally, optimized structures of six conformers were found. Gas-phase calculations were done with the B3LYP, B3LYP-D, B3LYP-D3, m05-2X, and ωB97X-D DFT functionals and carried out with the open-source computational program package PSI4. Further DFT calculations with an implicit water solvent were done using the computational program package Gaussian 09. The gas-phase lowest energy conformer was found to lie approximately ~2 kcal mol^{-1} lower than the next lowest energy structure. This resulting lowest energy conformer, with C_2 symmetry, differs with the lowest energy structures found in multiple references. However, this structure is consistently the lowest energy conformer among all DFT functionals employed. A reordering of the relative energies of conformers resulted due to DB18C6 seeming to prefer a more open conformation when complexed and when in solvent. The study resulted in finding the lowest energy conformer of DB18C6 and the binding energy of the ammonium ligand to the crown ether as well as the respective geometry for each species. The calculated binding energy contributes to the overall study of DB18C6 and provides a deeper understanding of ligand binding.