Book of Abstracts

12th Visegrad Symposium on Biomolecular Interactions

Piešťany, Slovakia
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12^{th} Visegrad Symposium on Biomolecular Interactions

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Contents

Welcome to the Visegrad Symposium

12th Visegrad Symposium on Biomolecular Interactions carries on the long tradition of meetings of researchers and scientists held in Czechia, Hungary, Poland, and Slovakia.

The presented and discussed topics have changed and been updated since the first meetings to include not only use of computation of interactions in biological systems (e.g. quantum calculations and atomistic or coarse-grained simulations), but also experimental studies (e.g. spectroscopic techniques), machine-learning approaches, and big data science to describe and analyze the interactions in biomolecular materials and drug development and diagnostics.

This year edition of the symposium will offer presentations and posters from researchers from and outside the Central Europe region discussing the newest information on hot topics in the study of biomolecular interactions, both theoretically and experimentally, in the pleasant spa environment of Hotel MagnÃşlia in PieÅąÅěany.

Let us then welcome you to the Visegrad Symposium and hopefully you will enjoy and benefit from this scientific gathering.

Conference Program

Sunday, June 23rd

13:00–16:00	Registration	
16:00–16:10	Conference opening	
	Chairman: J. Urban	
16:10–16:50	**Anna Weinzinger**	Allosteric mechanisms of inward rectifier K^+ channel gating - *Invited speaker* - opening lecture
16:50–17:20	**Dénes Berta**	From model building to in silico ligand screening: targetting the SARS-CoV-2 helicase
17:20–17:50	**Abdenacer Idrissi**	Exploring the Interfacial Conformational Changes and Polymorphic Behavior of Curcumin - *Invited speaker*
17:50–18:20	**Béla Fiser**	Additives in Action - How to Make 'Greener' Polymers?
18:20–18:50	**László Forgách**	Development, In Vitro Characterization & In Vivo Testing of multimodal Prussian Blue nanoparticles in an animal model - *Invited speaker*
19:00	*Dinner Welcome party*	
20:30	*Poster session I.*	*even numbers*

Monday, June 24th

	Chairman: A. Idrissi	
09:00–09:30	**Marco Paolantoni**	Hydration properties of sugars: molecular structure and dynamics - Invited speaker
09:30–10:00	**Christian Schröder**	Computational spectroscopy of water
10:00–10:30	**Ari Paavo Seitsonen**	Vibrational spectroscopies in liquid water: On temperature and coordination effects in Raman and infra-red spectroscopies
10:30–10:50	*Coffee break*	
	Chairman: Christian Schröder	
10:50–11:20	**Esther Heid**	A systematic way of improving machine learning potentials through spatially resolved uncertainty - *Invited speaker*
11:20–11:45	**Marek Štekláč**	Docking power approximations: can molecular docking reproduce experiment?
11:45–12:10	**Berna Dogan**	Predicting Selectivity of Compounds Against HDAC Isoforms Quantitatively Using Deep Learning Approaches
12:10–12:30	**Ján Matúška**	Generalization improvement of the neural network constructed using SchNetPack 2.0
12:30–14:00	*Lunch*	

	Chairman: Jaroslav Burda	
14:00–14:30	**Zdenek Futera**	Conductance of Solvated Biomolecular Junctions
14:30–15:00	**David Řeha**	Mechanism of carbon dots synthesis from citric acid and ethylenediamine studied by QM calculations
15:00–15:30	**Filip Šebesta**	Electron Transfer via Artificial Tryptophan Pathways
15:30–16:00	**John P. Fetse**	Computational Scoring and Experimental Evaluation of Small Peptide Fragments Targeting PD-L1 for Cancer Immunotherapy - *Invited speaker*
16:00–19:00	Sightseeing tour in Piešťany	
19:00	*Dinner*	
20:00	Poster session II.	*odd numbers*

Tuesday, June 25th

	Chairlady: Jannette. Carey	
09:00–09:30	**Rózsa Zsófia Borbála**	Effect of Phospholipid Headgroups on the Permeation of Additives
09:30–10:00	**Zoltán Mucsi**	A GFP inspired fluorescent molecular sensor for the detection of Zn^{2+} by two-photon microscopy in biology
10:00–10:30	**Mohammad Uddin**	An Innovative Dual Action Antibiotic Adjuvant Strategy using Ionic Liquid towards Synergistic Restoration of Activity in MDRO and Enhancing Antimicrobial Activity against MRSA
10:30–10:50	*Coffee break*	
	Chairman: Béla Viskolcz	
10:50–11:15	**Dalma Dojcsák**	Diagnostic of Pediatric Appendicitis supported by altered serum N-glycome
11:15–11:40	**Marion Sappl**	Langevin behavior of the dielectric decrement of amino acids in electrolyte solutions
11:40–12:05	**Tímea Gerzsenyi**	Studies on adsorption and antibacterial effect of magnetic nanoparticles
12:05–12:30	**Péter Koska**	Bioremediation potential of heavy metal loaded waste water by C.vulgaris microalga
12:30–14:00	*Lunch*	

	Chairman: David Řeha	
14:00–14:30	**Babak Minofar**	Interaction of biomolecules in confined environments
14:30–15:00	**Tibor Kovács**	Development of NMR scaling factors and reaction mechanism computations
15:00–15:30	**Jaroslav Burda**	Modeling interaction of metals with biomolecules
15:30–15:50	*Coffee break*	
	Chairman: Babak Minofar	
15:50–16:15	**Milan Říha**	From QM to QM/MM: NHC gold(I) complexes as potential cancer therapeutics
16:15–16:40	**Ivan Klbik**	Impact of dimethylsulfoxide on ionic conductance in lipid bilayer-embedded ion channel: implications for cryopreservation
16:40–17:05	**Tamás Horváth**	Investigation of radioactive strontium decorporation by computational analysis of Calcium and strontium ion complexation
17:05–17:30	**Jannette Carey**	Allostery: Monod’s second secret of life
18:00–20:00	*Conference dinner*	

Wednesday, June 26th

10:00	Departure

Poster presentations

Sunday, June 23rd

2	Balsam Al-sabea	Carbohydrates-based Hydrogels in Drug Delivery System
4	Christian Fellinger	Binding Affinity Estimation using X-GRADE
6	Márta Gődény	Molecular dynamics simulations of the Influenza M2 proton channel: the role of the protonation state and the polarizability
8	Anikó Jordán	Investigation of polyester-model urethane biodegradation
10	Karina Kécskes	Investigation of the microorganism adsorption capacity of natural-based particles
12	Ádám Lévárdi	Docking Score Prediction of Molecules Using Machine Learning Methods
14	Milan Melicherčík	Chloroquine transport and mutations in PfCRT protein
16	Omid Moghaddam	Interactions of proteins with grafted poly(ethylene oxide) layer in two setups - A Molecular dynamics study
18	Hadeer Waleed Qasim	Experimental and Theoretical Study of Urethane Formation in the Presence of 2,2-dimorpholinodiethylether (DMDEE) and 1,4-dimethylpiperazine (DMP)
20	Jessica Trenkwalder	MD Simulations of SERT for the Analysis of Ligands
22	Adam Vavrečka	QM/MM study of the electron hopping processes of conjugated systems

Monday, June 24th

1	Nesreen Alkanakri	A Computational Study of Tropocollagen Hexamer and Heptamer
3	Marcell D. Csécsi	Computational and reaction kinetic study of carbodiimide formation
5	Fatemeh Heydari	Development and testing of polymer-encapsulated, amine-functionalized iron-based contrast materials in animal model
7	Ariyo P. Hidayanto	Investigation Of Cobalt bioacumulation by *Chlorella Vulgaris*
9	Dalal Karad	Theoretical study of the applicability of natural antioxidant additives
11	Natalia Kulik	Application of *in-silico* methods for enzyme engineering of the NtcA protein from *Synechocystis sp.*
13	Julie Mallouhi	Evaluation of *Sargassum*-Derived Activated Carbon and Biochar: Ecotoxicity, and Heavy Metal Adsorption Performance
15	Mike Owen	The Mediation of Amyloid-βDimer Conformations by Gangliosides
17	Princeton group	Random proteins bind random ligands
19	Ondřej Tichý	QM/MM study of the electron hopping processes of the Two Lowest Singlet Excited States of Cytosine, its Aza-Derivatives and Carotenoids
21	László Vanyorek	Development of antibacterial, core-shell structured transition metal ferrite nanoparticles

Abstracts of oral contributions

Allosteric mechanisms of inward rectifier K^+ channel gating

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G-protein-regulated inward rectifier K^+ channels (GIRKs) underlie the inhibitory effects of major $G_{i/o}$ coupled neurotransmitters in the heart, and brain and regulate heartbeat, and neuronal excitability. GIRKs are involved in drug and alcohol addiction, pain, depression, seizures, and cognitive impairment of Down syndrome. Phosphatidylinositol 4,5-bisphosphate (PIP_2), Gβγ and Na^+ ions are essential key regulators, required to open the pore. Despite the availability of x-ray structures revealing the atomistic interaction with all three key activators, gating related conformational changes are still poorly understood. Here we report on disease-linked mutations and an allosteric blocker that serve as tools for probing gating-related conformational changes. Molecular dynamics (MD) simulations on the G154S disease mutant provide insights into changes in the selectivity filter of the channel, which lead to loss of optimal K^+ coordination and subsequent water-mediated Na^+ flux. Further, aberrant filter dynamics are correlated with changes at the binding site of the Gβγ dimer, providing information on the constitutive activity of the mutant [1]. The antiepileptic drug ethosuximide (ETX) suppresses epileptiform activity in a mouse model of *GNB1* syndrome, through inhibition of GIRK channels [2]. Combining electrophysiology and µs-long MD simulations, we identified an allosteric binding site of the drug, close to the physiological activator PIP_2. Remarkably, Gβγ, the physiological activator of GIRKs, increases the potency of ETX block. Our research suggests that ETX is a potent allosteric GIRK blocker and can serve as a tool for probing gating-related conformational changes in GIRK.

Acknowledgments: Support from Austrian Science Fund grant nr. W1232 is acknowledged. Calculations were performed at the Vienna Scientific Cluster (VSC).

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2. Colombo S, Reddy HP, Petri S, Williams DJ, et al. *Front Cell Neurosci.* 2023 17,1175895.

From model building to *in silico* ligand screening: targeting the SARS-CoV-2 helicase

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Keywords: SARS-CoV-2, molecular dynamics, drug discovery

The SARS-CoV-2 RNA helicase, also known as non-structural protein 13 (NSP13), is part of the Orf1ab polyprotein, which is spliced to produce the necessary enzymes for viral replication. The helicase is involved in two essential functions: first, it performs the a step in the 5'-capping of the viral RNA by its triphosphatase function hydrolysing the 5'-triphosphate group to form diphosphate-RNA. Second, its main helicase function enables RNA translocation and unwinding in an ATP-dependent mechanism during viral replication. While the domains creating the ATPase site are widely conserved, the RNA recognition domains are specific to the coronaviruses, altogether making the helicase an ideal drug target [1].

I present accelerated effort the pandemic prompted from the scientific community on the example of the SARS-CoV-2 RNA helicase. A series of experimental structures are now available [2], at the start of the pandemic the structure of the holoenzyme was unknown. With a group of computational experts, we created a model with the ATP and single-stranded RNA bound, and studied the complicated dynamics of the complex [3]. We shed light on the difference of the RNA description by popular force fields and identified allosteric cryptic pockets, highlighting the importance of considering protein dynamics when looking for binding sites. The experimental identification of fragments binding to the helicase confirm some of these allosteric sites are indeed targetable [4].

The SARS-CoV-2 RNA helicase was selected the target of the second CACHE Challenge. Dozens of teams participated in the two-round competition with various computational screening methods. I will overview our strategy involving similarity search, docking, machine learning and binding energy calculation methods. The experimental evaluation of potential lead molecules, considering the lack of structural information of the helicase only four years ago, emphasises the efficiency of modern drug discovery strategies, in which computational methods play an valuable role.

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Investigating Conformational and Polymorphic Behavior of Curcumin at Vacuum and Supercritical CO_2 Interfaces Using Molecular Dynamics

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Industrial interest in polymorphism is rapidly growing because it impacts active pharmaceutical ingredient (API) form discovery, patenting (performance characteristics such as solubility and dissolution rate) and drug formulation. Several studied have shown the importance of the local structure at the interface of the API in determining the rate of nucleation and polymorph transformations.[1,2] As a consequence, the main objective of this work is to use molecular dynamics simulation to get insight at the molecular level of the conformation distribution of curcumin (CUR) at its interface with vacuum and supercritical CO_2 during the heating process that encompasses the melting temperature of CUR. We choose CUR because it has many different conformations that are driven by intra and inter molecular interactions. The tradeoff between hydrogen bonding and packing results in three polymorphs. To achieve this goal, three main steps are considered. The first one is related to the choice of the force field. CUR model has to reproduce the experimental melting temperature as close as possible and also to correctly describe the energy barriers of the key dihedral angles as obtained from quantum chemical results on the three conformations that are present in the three polymorphs of CUR. The second step is to perform melting simulations with vacuum and supercritical CO_2 interfaces. The last step is to identify truly interfacial molecules using the ITIM algorithm[3] and then quantify the conformation distributions in the bulk and at the vacuum and supercritical CO_2 interfaces.

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Additives in Action - How to Make 'Greener' Polymers

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Polymeric materials have been around longer than human civilizations. Initially, polymers were derived from natural substances and early biopolymers, with notable examples being cotton, starch, proteins, and wool. The "evolution" of polymers is closely linked to the use of additives, which are special chemicals that enhance the properties of final polymeric products. Additives can be incorporated into base polymer formulations to improve processability, extend shelf life, and enhance the physical and chemical properties of the final product. The most in-demand additives are plasticizers, flame retardants, biocides/antimicrobial agents, antistatic agents, antioxidants, UV stabilizers, colorants and catalysts. In our attempt to develop more environmentally friendly polymeric formulations, we focused on antioxidants and catalysts. We have employed computational and experimental tools to investigate the applicability of various compounds. We believe that the findings will promote the development of new, more environmentally friendly polymers.

Acknowledgements: This research was supported by the National Research, Development and Innovation Fund (Hungary) within the TKP2021-NVA-14 project. The GITDA (Governmental Information-Technology Development Agency, Hungary) is gratefully acknowledged for allocating computing resources used in this work. Further calculations have been carried out using resources provided by the Wroclaw Centre for Networking and Supercomputing. Furthermore, the research was funded in part by the National Science Centre, Poland under the MINIATURA 7 call within the project reg. No: 2023/07/X/ST4/01433.

Development, In Vitro Characterization & In Vivo Testing of multimodal Prussian Blue nanoparticles in an animal model

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The most commonly used Gd (III) materials lack the in vivo stability, causing toxic side-effects for the patiens; materials other than Gd (III) available lack the required in vivo contrast for the Magneti Resonance Imaging (MRI). A possible candidate for high performance MRI contrast materials could be the FDA authorized Prussian blue (PB), which is used to treat heavy metal poisoning since 2003. Its uniquie structure allows the control of size, shape and biocompatibility, nevertheless, non-functionalized PBNPs show less significant T1 and T2 signal changes in vitro; their measured longitudinal and transversal relaxation times did not suggest their in vivo use. Therefore, functionalizing PBNPs, to achieve multimodal contrast would highly increase their impact for preclinical applications, thus in the routine procedures MRI is oftentimes coupled with other modalities providing greater functional contrast. Native PBNPs were synthesized according to as described by Shokouhimehr [1], with modifications. For citrate coated PBNPs, the process as described by Shokouhimehr [1] was applied. A two-step PBNP preparation was made. The fluorescent labelling of PBNPs was conducted using Eosine Y, Rhodamine B and Methylene Blue (MB) that adsorbed onto the particle surface. For near infrared (NIR) fluorescent labelling, IR820 was used. For particle characterization, Dynamic Light Scattering (DLS), Atomic Force Microscopy (AFM) and Transmission Electron Microscopy (TEM) was used. The in vitro and in vivo imaging was performed using a nanoScan® PET/MR system with a 1T permanent magnetic field, and a Fluorescence-labeled Organism Bioimaging Instrument (FOBI) for the fluorescent measurements. Polyethylene glycol (PEG) stabilization was required for the fluorescent labelling of the PBNPs. Only MB conjugated PBNPs demonstrated promising in vitro stability and fluorescence. In vivo, the particles accumulated in the liver, spleen and gastrointestinal tract. For the NIR labelling, IR820 was successfully adsorbed onto the surface, facilitated by the porous nanoparticle surface. The particles showed similar characteristics in vivo, as compared to the

MB labelled PBNPs. The in vivo MRI measurements confirmed the presence of the particles in the thoracic region and the liver. This study demonstrates the synthesis and modification of biocompatible, stealth, fluorescent, and MRI-contrast-capable MB- and IR820-labeled PBNPs. The nano-system, including IR-820-conjugated PBNPs, exhibits enhanced in vitro and in vivo T1-weighted MR contrast. The study aims for clinical translation, utilizing QbD during synthesis and validating with nude mice, offering a finely tuned platform for clinical applications with potential for further development and implementation.

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Simulation of IR and Raman signals in liquid water from molecular dynamics: Effect of the hydrogen bond network

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Ari Paavo Seitsonen[a,d,*]

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Water is a ubiquitous liquid that has several exotic and anomalous properties. Despite its apparent simple chemical formula, its capability of forming a dynamic network of hydrogen bonds leads to a rich variety of physics. Here we study the vibrations of water using molecular dynamics simulations via the Raman and infra-red spectroscopic signatures, and compare our simulated results with the measured ones [1]. Recently we investigated [2] the consequences of the temperature on the vibrational frequencies, and we entered the details of the hydrogen bonding coordination in order to gain quantitative insight on the dependence of the frequencies on the hydrogen bonding network. We have also explored the collective dynamics of water [3].

Here we extend the previous study by using machine learning interaction potentials (MLIP) together with dipole moments and electronic polarisability fitted with machine learning to describe the liquid water [4]. This allows us to expand the simulation time in the trajectories, thus yielding better statistics of the effects due to the varying hydrogen bond network on the infra-red and Raman spectra.

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Computational spectroscopy of water

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We present a comparative study on the dielectric and far-infrared (terahertz) spectra of widely used water models through extensive classical molecular dynamics simulations [1]. Our analysis encompasses both polarizable and nonpolarizable models, as well as the multipole AMOEBA water. By applying a two-relaxation model and the Cole-Cole functional form, we accurately characterize the main dielectric relaxation and the fast relaxation processes, assessing deviations from the Debye behavior.

In the terahertz region, we compute the infrared absorbance which can be decomposed into contributions from permanent and induced dipoles and their cross-correlations. Furthermore, we attributed the various absorbances of the force field models to molecular librational motions and identified qualitatively distinct spectral features among the models. Notably, we decompose the librational band into contributions from water molecules with varying hydrogen-bonded neighbors, revealing significant spectral complexity.

This study aims to serve as a comprehensive reference for future simulation studies on the dielectric properties of aqueous solutions and mixtures, enhancing the understanding of how different water models influence spectroscopic features. The insights gained could inform the selection and development of water models for various computational applications, particularly those involving complex aqueous environments. Our ultimate goal is the development of a polarizable water model, which allows for proton transfers.

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Hydration properties of sugars: molecular structure and dynamics

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The hydration of sugars has been extensively investigated due to the relevance of this class of compounds in various fields encompassing, life and medical sciences, biology, pharmaceutics, and food technology.

Despite being a classical topic in solvation science, understanding how and to what extent sugars affect the H-bonding network of water remains elusive. This is evidenced by the presence of contradictory views reported in the recent literature. Different spectroscopic approaches often lead to different interpretations concerning both the structure and dynamics of the water hydrogen bonding, making a thorough molecular-level understanding of the sugar hydration is still incomplete.

In the last decade, the Extended frequency range Depolarized Light Scattering (EDLS) technique has been developed in Perugia (Italy) to study the fast dynamics of water in aqueous solutions of biorelevant systems, including peptides, proteins and sugars [1-3]. EDLS has proven to be suitable for distinguishing the motion of the solute from that of the solvent and, in turn, separating the contribution of hydration water from the bulk [1-3], leading to quantitative information on the structural dynamics around different biorelevant compounds.

Recently, novel structural information has been gathered on glucose and trehalose aqueous solutions by using various vibrational techniques such as Raman, Mid-IR and Near-IR spectroscopies. In addition to serving as classic hydrophilic model systems, glucose and trehalose, are especially relevant in biopreservation theories, which frequently involve water-mediated effects [4-5]. Solute-correlated (SC) [6] spectra have been derived from both Raman and IR data to emphasize the spectral contribution of the hydration water and its structural characteristic.

The results of this comparative analysis will be discussed, and a coherent view will be proposed regarding the impact of glucose and trehalose on both hydrogen bonding structure and dynamics of water, addressing relevant inconsistencies found in the literature.

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A systematic way of improving machine learning potentials through spatially resolved uncertainty

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Machine learning potentials can nowadays easily be used as a drop-in replacement for quantum-mechanical calculations, once they are trained on a sufficient and representative amount of data. Systematically improving a potential, on the contrary, is still an open question, where especially the identification of erroneous predictions that should be added to the training set is difficult. We present a new method to obtain model uncertainties that directly correlate with the absolute error in the prediction per data point, as well as spatially resolved for each atom within a data point.[1] We demonstrate the applicability of the method on a wide range of problems, ranging from gas phase reactions, to bulk liquid and solid surfaces. By reliably identifying uncertain sub-regions in a simulation, the potential can be improved in an active learning loop by adding only little amounts of additional training data, outperforming other uncertainty metrics for data selection.

Acknowledgments: This research was funded in part by the Austrian Science Fund (FWF) 10.55776/COE5 and 10.55776/F81.

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Generalization improvement of the neural network constructed by SchNetPack 2.0

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In general, properties of chemical compounds are not distributed evenly in an arbitrary data set. Even worse, the most desired property values are populated the least. This heavily complicates construction and reliability of a training data sets for machine learning[1]. One way of mitigating the problem is by maximazing the generalization capacity of the prediction model. Here, Generalization of the neural network designed using the SchNetPack 2.0[2] package with respect to top docking scores prediction is investigated. Hyperparameter tuning is performed to improve prediction of the top scoring compounds, which have characteristically a low incidence in randomized data sets composed of chemical compounds. The prediction robustness is evaluated using the mean square error (MSE) and the entropy of the average loss landscape. The most impactful hyperparameter is the cutoff (5 Å is reported as the optimal choice). Other optimized hyperparameters (e.g., number of radial basis functions, number of interaction layers of the neural network, feature vector size or its batch size) do not improve prediction accuracy for the top scoring compounds in any comparable magnitude relative to the cutoff. Admittedly, there is the penalty in form of worsening the overall prediction error. As an alternative to hyperparameter optimization, modification of the training data set in form of oversampling and undersampling is investigated, but results indicate that the cutoff modification performs better than training data set resampling.

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Predicting Selectivity of Compounds Against HDAC Isoforms Quantitatively Using Deep Learning Approaches

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Histone deacetylases (HDACs) are important enzymes for normal body functions and are considered in targeted drug therapy, especially for cancer diseases. Promising inhibitors have already been developed for these enzymes, and there are approved drugs that target this class of enzymes. However, these therapeutics act on multiple isoforms of the enzyme, potentially leading to dose-dependent side effects in patients due to their nonselective inhibition. Hence, the design and discovery of selective inhibitors especially for HDAC6 isoform is desired and well explored though without any approved selective drug as of yet. In this study, we aimed to apply molecular modeling tools for novel HDAC6 inhibitor discovery. We developed various machine learning approaches to predict the selectivity of compounds for HDAC6 over other common isoforms like HDAC1 and HDAC4. Especially, deep learning-based deep belief network (DBN) models were novel algorithms we utilized for selectivity modeling here and used to screen a virtual molecule library. Other tools such as molecular docking and molecular dynamics simulations were also employed to determine the interactions of selected molecules from the molecule library with HDAC6 enzyme. Thus, in this study, we proposed three compounds that DBN models predicted to be selective. Several compounds exhibited favorable interactions with HDAC6 comparable to the interactions of those known inhibitors. Hence, our approach predicted novel potentially effective and selective HDAC6 inhibitors.

Docking power approximations - can molecular docking reproduce experiment?

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The years following the outbreak of SARS-CoV-2 saw an unprecedented response from the scientific community, with the number of researchers studying coronaviruses increasing by nearly 500% annually. This surge in research led to a significant rise in publications, primarily driven by in silico studies. By the end of 2020 alone, approximately 16,500 scientific articles focused on molecular docking approaches to combat SARS-CoV-2 [1]. This raises questions about the extent to which molecular docking can accurately describe studied systems. A benchmarking set was created using 671 3-dimensional crystal structures of SARS-CoV-2 main protease Mpro have been downloaded from RCSB Liganded structures were then cleaned of disordered atoms, crystallization artifacts, and water molecules, and split into monomeric structures. The ligands were manually inspected and corrected for incorrect bond orders, isomerism, and missing fragments. The successfully corrected 424 structures were categorized based on their interaction type with the target protein (covalent vs. noncovalent) and the ligand's position relative to the Mpro catalytic dyad (cavity vs. surface bound). Performance of molecular docking protocols implemented in AutoDock [2], AutoDock Vina [3], and PLANTS [4], was evaluated, focusing on their docking and screening power, with newly defined parameters such as geometrically and/or energetically approximated docking power.

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Conductance of Solvated Biomolecular Junctions

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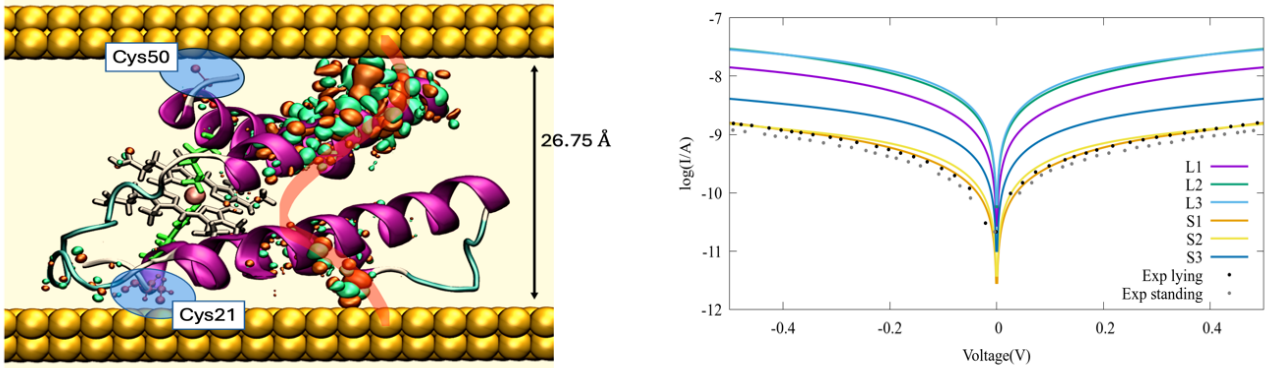
Electronic conductance of redox proteins in their native environment is typically facilitated by the organometallic cofactors, like hemes in cytochromes, which can be reversibly reduced / oxidized. The electrons thus flow through such proteins by sequential hopping that can be well described by the Marcus theory. However, when these proteins are immobilized on metal surfaces, and their conductance is probed by a scanning tunneling microscope (STM) or its electrochemical variant (EC-STM), magnitudes, shapes, and temperature dependencies of the measured current-voltage curves suggest coherent tunneling as the undergoing transport mechanism rather than hopping. To elucidate these data and investigate the factors affecting charge transport in biomolecular junctions, we developed a computational procedure based on multiscale modeling involving classical molecular dynamics (MD), electronic-state calculations within density functional theory (DFT), and electronic coupling calculations. Here, we demonstrate its feasibility in the study of single-heme cytochrome b_{562}, for which the EC-STM data were previously reported in the literature.



(a) (b)

Figure 1: Cytochrome b_{562} junction between gold contacts: (a) optimized solvated structure with indicated chemisorbed cysteines and conduction channel, (b) calculated current-voltage curves on several lying (L) and standing (S) junction models and their comparison with experiment.

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Application of in-silico methods for enzyme engineering of the NtcA protein from Synechocystis sp.

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Carbon dots (CDs) have found applications across various fields, including light emitting devices, bioimaging, and environmental research. However, the large structural variability of CDs poses significant challenges for their precise characterization. Despite substantial efforts, synthetic routes for fabrication of CDs still lack the desired control, and precise structural regulation toward property-tailored CDs remains elusive. We explored the early evolution of CDs from citric acid and ethylenediamine precursors. Density functional theory calculations of explicitly micro-hydrated reaction systems were performed to estimate reaction barriers and thermodynamics during the initial reaction steps. We have identified several intermediates towards to synthesis of IPCA molecular fluorophore and estimated the Gibbs energy profile of the reaction. Furthermore we have demonstrated the importance of explicit micro-solvation for the computational investigation of this type of reactions.

The theoretical calculations aligned well with the experimental data attained by NMR and MALDI-TOF mass spectrometry measurements, which identified six- and five-membered-ring heterocyclic intermediates, IPCA molecular fluorophore as well as larger products.

The presented results provide a comprehensive insight into the initial steps of CD formation, revealing new reaction intermediates and pathways, and rationally predicting the formation of specific structural arrangements attributable to the structural composition of amorphous CDs.

Hole Hopping via Artificial Tryptophan Pathways in Azurin Mutants. A QM/MM/MD study

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Aromatic amino acid pathways typically mediate an electron transfer (ET) in proteins from its inner region toward its surface. Such an ET is based on the Trp/Tyr reduction potential gradient toward the surface. In azurin protein mutants, the artificial pathways containing Trp have been built on the surface where a rhenium-based organometallic photoxidant photosensitizer is appended. Despite significant ET acceleration by Trp residues, yields are quite low compared to ET in photolyases or cryptochromes.

This talk is focused on searching for dynamical factors promoting or limiting ET in two azurin mutants Re124W122 and Re126W124W122 containing one and two Trp residues in the ET pathway, respectively. Based on experimentally determined kinetics [1], hole transfers between the electronically excited photosensitizer ($\mathrm{(Re(His)(CO)_3(4,7\text{-dimethyl-}1,10\text{-phenanthroline})^+)}$) and adjacent Trp and subsequently, in the case of Re126W124W122, between neighboring W124 and W122 in hole hopping pathways toward the Cu(I) center were investigated. [2, 3] The ground- and optically excited states, and two charge separated states with the hole localized on the first, and, for Re126W124W122, also on the second Trp residues were initially modeled using classical molecular dynamics (MD) with optimized charge distributions for the cofactors. Subsequent 3 ps long QM/MM/MDs simulated temporal evolution of the lowest triplet state describing the active cofactors at the PBE0-GD3/6-31+G(d) level using unrestricted Kohn-Sham equations or time-dependent DFT exhibiting switches of the state character along several trajectories. Accompanying changes in protein and solvent arrangements, and electrostatic potentials generated by surroundings reveal the key role of solvent fluctuations and flexibility of the cofactors for ET feasibility. Large magnitudes of calculated electronic couplings indicate rather adiabatic course of the hole hopping steps.

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Development of NMR scaling factors and reaction mechanism computations

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A new flexible germacranolide (**22**, lobatolide H) was isolated from the aerial parts of *Neurolaena lobata.* The structure elucidation was performed by classical NMR experiments and DFT NMR calculations. Altogether, 80 theoretical level combinations with existing ^{13}C NMR scaling factors were tested, and the best performing ones were applied on **22**. ^{1}H and ^{13}C NMR scaling factors were also developed for two combinations utilizing known exomethylene containing derivatives, and the results were complemented by homonuclear coupling constant (J_{HH}) and TDDFT-ECD calculations to elucidate the stereochemistry of **22**.

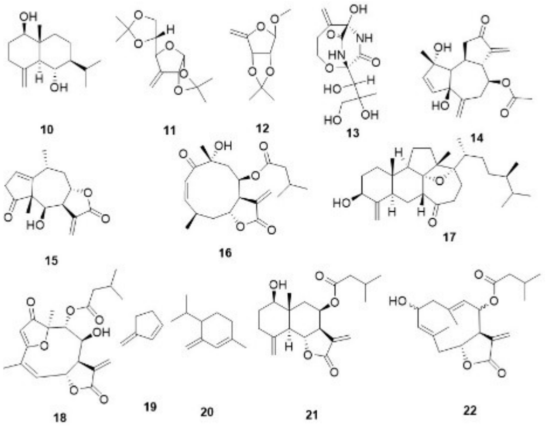

Figure 2: Structures of the compound(s) of the scaling factor test (**10**), of the reference set of the novel NMR shift methods, of the validation of the new scaling factors (**21**) and of lobatolide H (**22**)

Domino Knoevenagel-cyclization reactions of two chroman derivatives was carried out. The cyclization step occurred with different mechanisms: a stepwise polar [2+2] cycloaddition, or a multi-step nitro hetero Diels-Alder-ring-opening-Cadogan-type cyclization sequence. The latter reaction provided a new route to hydroxyindoles by an inverse Cadogan-type cyclization, in which the nitro group is deoxygenated by a nitro IMHDA-ring-opening sequence. The cyclization mechanisms and their stereoselectivity were studied by DFT calculations, based on which we proposed a mechanism for the multi-step cyclization to hydroxyindoles and explained the observed diastereoselectivity.

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Effect of NSO-HET Molecules on Membrane Models with Different Phospholipid Headgroups

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Aromatic hydrocarbons are present in several industrial processes. The family of these molecules is diverse and includes species such as polyaromatic hydrocarbons (PAHs), chlorinated aromatic hydrocarbons, furans and also the less focused nitrogen, sulfur or oxygen containing heterocyclic hydrocarbons (NSO-HETs). The presence of heteroatoms makes these compounds more water soluble and mobile compared to their homocyclic analogues, leading to a lasting and easily spreading contamination in industrial sites and to an enhanced risk of contaminated drinking water resources. Due the lipophilic nature of NSO-HETs the primary site of their toxicity is the cell membrane. Accumulation of compounds in the membrane may lead to the alteration of the membrane structure and function, which can lead to the penetration of additional molecules. 1,4-dioxane, morpholine, oxane and phenol are industrially applied NSO-HET molecules which are able to passively permeate inside biomembranes, which has previously been investigated in the case of DPPC composed model membranes by using molecular dynamics simulations [1].

At the same time lipid composition is involved in regulating membrane mechanical properties and cellular functioning. Different lipid components affect membrane properties, contributing to their biological diversity. Variation of the lipids can have wide-reaching effects over the entire membrane structure due to the extensive intermolecular interactions [2]. The objective of this study is to examine the impact of NSO-HET molecules on four distinct membrane models, varying solely in their headgroup composition, DPPA - containing a phosphatidic acid headgroup, DPPE - containing a phosphatidylethanolamine headgroup, DPPC - containing a phosphatidylcholine headgroup, and DPPG - containing a phosphatidylglycerol headgroup.

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A GFP inspired fluorescent molecular sensor for the detection of Zn^{2+} by two-photon microscopy in biology

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Zn ions posses important roles in living organisms, they are involved in cell division, neurotransmission, immunity, etc. They also play roles in diseases as Alzheimer's or Parkinson's disease and in viral infections such as SARS-CoV-2. Detection of metal ions in biological systems usually relies on fluorescent microscopy aided by fluorescent chemosensors, which illuminate the analytes. The number of fluorescent probes for Zn used in Two-Photon (2P) microscopy is limited and many of the known probes are compromised by issues such as weak signal to noise ratio, selectivity or solubility. This work presents the synthesis of a library of fluorescent Zn sensors for 2P microscopy based on the fluorescent chromophore of the green fluorescent protein (GFP). A detailed spectroscopical characterization is provided by spectrophotometric and fluorimetric studies. Fluorescence-enhancement up to 75-fold are reported by excitation at $\lambda = 450$ nm, while emission at $\lambda = 510$ nm was detected selectively in presence of Zn at the physiologically relevant pH range. The Zn complexes display high stability (K = 0.15-15 nM) and water-solubility of the probes was excellent (> 1 g/L), strong fluorescence under two-photon excitation was detected as well. Finally, the practical applicability of the probes has been demonstrated by live cell imaging of Zn in fluorescent microscopy.

An Innovative Dual Action Antibiotic Adjuvant Strategy using Ionic Liquid towards Synergistic Restoration of Activity in MDRO and Enhancing Antimicrobial Activity against MRSA

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PURPOSE Antimicrobial resistance has emerged as a global critical issue that needs to be addressed. It develops when microorganisms evolve resistance and tend to cease response to treatments in humans. [1] Multi Drug Resistant Organisms (MDRO) such as Methicillin Resistant Staphylococcus Aureus (MRSA) and gram-negative bacteria are nonresponsive to various classes of antimicrobial agents thereby limiting the diverse treatment options for patients. These organisms are also responsible for around 23,000 deaths annually in US alone along with 70,837 inpatient deaths caused by MDRO sepsis. [2] Ionic liquids (IL) have recently surfaced as the salts possessing antimicrobial properties with varying degrees of solubility and they are used as adjuvants in this study to enhance the antibiotic activity. [3] Cetylpyridinium chloride is a quaternary ammonium compound possessing antimicrobial activity due to it's hydrophobicity, [4] and the effective concentration against microbes is between 0.05% and 0.1%. [5] In this research Cetylpyridinium chloride is used as IL adjuvant. This study elucidates how novel ionic liquids, either alone or in combination with current antibiotics restore their effect against MDRO, thereby enhancing antimicrobial activity.

METHODS To synthesize the Ionic Liquid- Antibiotic complex Cetylpyridinium chloride was initially dissolved in methanol and passed through an ion exchange column to form a Cetylpyridinium hydroxide solution which was then added to the Antibiotic- Colistin, similar process was performed for Amoxicillin. Bacterial strains such as S. aureus, MRSA, and both Colistin-resistant and -sensitive MCR-1 encoded E. coli were utilized to investigate bacterial growth. Colonies from the strains were collected and suspended using PBS as diluent to read the absorbance at 0.1@ 600 nm. the minimum inhibitory concentration of the antimicrobial agent. Electron microscope was used to study the bacterial surface. To monitor bacterial growth curves against Colistin sensitive & resistant E.Coli, S.Aureus and MRSA the groups established include a control group, an antibiotic group, antibiotic-Ionic liquid group and an Ionic liquid-only group. All groups were monitored from 4 hours up to overnight for both strains of bacteria.

RESULTS The antibiotic adjuvant effects on E. coli membrane have shown physical changes in bacteria when treated with different antibiotics. While untreated cells showed normal shape of bacteria, E. coli treated with Colistin and IL adjuvant were clumped and showed damaged cell integrity under the electron microscope. There were minimal changes such as elongation when treated with colistin alone and IL adjuvant alone. Based on the growth curve results for both Colistin resistant and sensitive bacteria, it is observed that the IL when combined with antibiotic Colistin a strong synergistic effect was observed after 24hours indicating a notably lower bacterial growth i.e $p < 0.0001$. However, the results also show that the IL alone has moderate effect when compared with combination of IL and antibiotic in inhibiting the growth of both sensitive and resistant bacteria. Similar effects were observed when Amoxicillin was added to antibiotic adjuvant with peak effects at 4hours i.e $p < 0.0001$. The data obtained suggests that the novel antibiotic adjuvant IL possesses the bactericidal activity and restores colistin action against MDRO.

CONCLUSION Ionic liquids possessing antimicrobial properties are now emerging as effective adjuvants. The in-vitro assessment of the Novel antibiotic IL demonstrated its dual-purpose functionality as an adjuvant for possessing bactericidal activity and exerting synergistic activity against E. Coli strains when combined with Colistin. Quantitatively, the most significant effect was observed at 24hrs for IL and Colistin complex and at 4hrs for IL and Amoxicillin complex, mitigating the antibiotic resistance. These results successfully demonstrated that the IL and antibiotic complex combat the antimicrobial resistance effectively.

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Studies on adsorption and antibacterial effect of magnetic nanoparticles

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The use of magnetic nanoparticles (MNPs) in molecular biology and microbiology has contributed to the advancement of medicine, pharmaceutical and environmental science in the recent decades with a large amount of research results. While MNPs have numerous potential uses (e.g. MRI contrast agent, drug delivery, sewage treatment), our research has demonstrated that they can be effectively used as a solid phase for DNA isolation as well. For the extraction of pDNA from bacterial cells, 5 types of ferrite-based magnetic nanoparticles were tested with three different buffer compositions for adsorption and desorption, then the most optimal protocol for each nanoparticle was determined. Concentration and purity of the extracted pDNA was measured by UV-Vis spectrophotometry, while the integrity of the samples was examined with agarose gel electrophoresis and restriction digestion reactions using fast and traditional endonucleases. MNPs can not only provide an adsorbent surface for DNA isolation but can also be used for the removal of microorganisms from water. Research publications have already shown that some magnetic nanoparticles also have antibacterial activity. Our aim is to determine the bacterial adsorption efficiency of MNPs on both Gram-negative and Gram-positive microorganisms and to investigate their antibacterial effect. Ultimately, we aim to gain a comprehensive understanding of how their antibacterial effect works in cells.

Langevin behavior of the dielectric permittivity of amino acids in electrolyte solutions

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It is a well-known effect that electrolyte mixtures of salts and water show a dielectric decrement depending on the salt concentration. The dielectric permittivity shows a dependency to the salt concentration according to the function [1]

$$\varepsilon(c) = \varepsilon_{water} - \beta L\left(\frac{3\alpha c}{\beta}\right) \tag{1}$$

where L is the Langevin function

$$L\left(\frac{3\alpha c}{\beta}\right) = coth\left(\frac{3\alpha c}{\beta}\right) - \frac{1}{\frac{3\alpha c}{\beta}} \tag{2}$$

with ε being the static dielectric permittivity, c being the concentration of the salt, β being the difference of the static dielectric permittivity between water and the molten salt and α being the excess polarization [1]. Our group showed that this behavior is not only true for classic salts but also for mixtures of ionic liquids and water [2]. Recently, we investigated dielectric spectra of ternary solutions in collaboration with researchers from the Max Planck Institute for Polymer Research in Mainz, consisting of solvated, zwitterionic amino acids, water, and different salts to study ion specific effects. To compare results our collaborators determined the dielectric spectra experimentally and computer simulations were executed by our group. Even though ternary solutions exhibit complex behavior, the dielectric decrement of the solutions still followed behavior according to eq. (1) in the experiment and the simulations. Moreso, the individual contributions of water and the amino acids to the dielectric permittivity showed this behavior as well. It was found that the ion type played a significant role and that the value of parameters α and β show ion specific effects. Five salts with different concentrations were used in this study. KCl, KBr and KI were examined to study effects of anions. $LiCl$, $NaCl$ and KCl were examined to study effects of the cations. The amino acids under investigation were arginine, lysine, and serine. We found that cations had a bigger influence on the dielectric permittivity than anions considering the contribution of the amino acid. Furthermore, we found that the cation type had a huge influence, especially on the contributions of the amino acids, with lithium having the biggest effect followed by sodium and potassium. The major influence of the cation type is probably due to strong electrostatic interactions between the carboxylate group of the amino acids and the cations. In the case of the anions no significant influence of the type of ion could be established as the influence was comparably small.

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Potential of Chlorella vulgaris in bioremediation of heavy metal contaminated industrial waste waters

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Keywords: Collagen, Gromacs, tropocollagen, Hydroxyproline, Proline

Toxic heavy metal contamination of freshwaters is a serious threat to ecological systems and human health. Since heavy metals cannot be metabolized, they are accumulating in living organs of food chain. The most critical source of heavy metals are the industrial waste waters of electronic production plants, accumulator industry, metallurgical industry.

To hindrance the emission of heavy metals from these plants, necessitates to develop an efficient wastewater cleaning process, which capable of removing heavy metal ions, organic compounds, phosphate, nitrate at the same time.

Microalgae have wide range metabolic spectrum which can grow both on versatile type of organic carbon sources and autotrophically on carbon-dioxide and can be characterized by high resilience to heavy metals like cadmium, cobalt nickel, and manganese. Although great heavy metal removing capability of microalgae are widely studied, the mechanism behind the phenomenon remains elusive.

In our laboratory an air lift photobioreactor system was developed which is a suitable tool for optimization the main parameters of microalgal heavy metal cleaning process, e.g. retention time, phosphate/nitrogen ratio, pH etc.

With the application of this microalgal bioreactor system, we can achieve high density cell culture of microalga ($5 \cdot 10^8 - 10^9$ cell/ml) in 5 days. In this way we can gain reproducible data for further optimization scale up parameters, for higher volume microalgal processes.

Diagnostic of Pediatric Appendicitis supported by Altered Serum N-glycome via Magnetic Nanoparticle based Automated Sample Preparation process

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Magnetic nanoparticles (MNPs) have become increasingly popular in medical applications such as MRI and biosensors, as well as in the chemical industry. Due to their nanoscale size and the large specific surface area making their surfaces highly functionalizable and effective for binding DNA, proteins, or glycans. This advantageous properties have made MNPs a valuable alternative in bioanalytical glycosylation research for glycan sample preparation. For comprehensive glycan profiling, hydrophilic interaction liquid chromatography (HILIC) is the most suitable due to its ability to separate oligosaccharides based on their hydrophilic properties. Many analytical studies focus on glycosylation changes, specifically examining serum-derived glycan molecules in inflammatory diseases. The altered peak ratios on chromatograms post-separation can indicate disease presence, making glycosylation changes potential biomarkers for inflammatory conditions. Appendicitis, an inflammation of the appendix at the junction of the large and small intestines, is one of the most common causes of acute abdominal pain in both adults and children. Additionally, similar symptoms from other conditions (e.g., viral gastroenteritis) and comorbidities (e.g., soft tissue infection) further complicate the diagnosis.

The aims of this research was to develop a new, cost-effective, and automatable protocol for glycosylation study sample preparation. Four different amine-functionalized MNPs were tested for their automatable potential, comparing their initial media, dispersibility, glycan binding efficiency, and fluorescent dye binding efficiency. The most efficient MNP for sample preparation was further examined for glycan elution efficiency, testing various elution volumes and buffer compositions. The goal was to use this optimized protocol to compare glycosylation patterns in serum samples from children diagnosed with appendicitis with those from healthy individuals using HILIC separation and MS detection.

The tests were performed by a PNGase F deglycosylation, fluorescent labelling and MNP based sample purifications. The purified glycans were analysed by ultra-performance liquid chromatography (HILIC method) instrument equipped with a fluorescence detector and mass spectrometer. The well-separated glycan structures were identified by their m/z values with Xevo-G_2-XS QTOF mass spectrometer. The chromatograms were integrated and the generated area distribution data were used for multi-dimensional statistical tests (LDA, Kruskal-Wallis test, ROC curve).

Overall the relative area percentages of significantly different glycan levels were determined to classify control and appendicitis patient groups. The control group was further divided into normal and abdominal pain subgroups, revealing that the monosialylated glycans' level was significantly lower and the trisialylated glycans' level was higher in appendicitis patients compared to both control groups. These results align with previous studies showing that higher sialylation is associated with the inflammation.

Effect of crowding agents and ionic liquids on the structure of bio-macromolecules

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Aqueous solutions of different additives such as salts, buffers, sugar molecules organic solvents, and ionic liquids can alter the permittivity of aqueous solutions therefore such changes in solution properties can influence the activity and structural stability of biomolecules. Enzymatic reactions that occur in such environments can be significantly influenced by the presence of low concentrations of different biomolecules salts, sugar, and solvents. In biological systems salts, buffers, and sugar molecules occupy approximately 40% of cell volume. These molecules can stabilize or destabilize macromolecules, influencing their folding, solubility, and activity in the solutions. For example, ionic liquids can enhance enzyme stability and activity, facilitate protein crystallization, and stabilize DNA. Understanding these interactions provides insights into changing the environment for biotechnological and pharmaceutical applications where the potential biomolecular activity and stabilization can be elevated. [1,2,3]

In this contribution, the effect of salts, sugars, organic solvents, ionic liquids, and deep eutectic solvents on the solvation structure and dynamics of different biomolecules such as DNA and proteins investigated by molecular dynamics simulations to help to understand the complex phenomena from experimental findings.

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The Description of the Reactions in Solutions with Constant pH; Ab initio/DFT approach

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Keywords: thioredoxin reductase, gold(I) complexes, DFT, grand-canonical ensemble

Interactions of metallodrugs in cellular environment have to regarded as reactions in solutions with constant pH. In this way the correct description goes within grand-canonical ensemble, which represents from the thermodynamic point necessity of additional Legendre transformation of Gibbs free energy to the new thermodynamic state function - Gibbs-Alberty potential. The evaluation passes via determination of corresponding equilibrium constant K'.[1-4] Within this description, chemical reactions do not run into the equilibrium concentrations as suppose according to the Guldberg-Waage dynamic principle but to the chosen value of proton chemical potential. In this work we applied the above-mentioned method on coordination of gold(I) NHC complex family with Cys and Sec amino acids. Here it is demonstrated how the preference for metal interaction with seleno-site of Sec changes with pH in favor to sulphur site of Cys, which is the Sec neighbour in TrxR enzyme.

In the other part properties of dinuclear Pt(II) complexes with tetrazole heterocycle where some kinetic and thermodynamic aspects are explored. Also, in this case the pH-dependent approach is applied on reaction energy profile.

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Impact of dimethylsulfoxide on ionic conductance in lipid bilayer-embedded ion channel: implications for cryopreservation

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Cryopreservation extends the shelf-life of biological material, including cells, tissues, and organs, by storing them at low subzero temperatures. While cell cryopreservation is well-established, preserving tissues and organs presents significant challenges. The primary issues arise during freezing and thawing, which can severely compromise cell viability due to ice and eutectics formation, as well as osmotic changes from freeze-concentration of salts and other solutes. Cryoprotective agents (CPAs) are critical for maintaining post-thaw cell viability. These agents include low-molecular-weight substances and macromolecular additives, with dimethylsulfoxide (DMSO) being a gold standard CPA for many cell types. Despite their widespread use, the mechanisms of cryoinjury and cryoprotection are not fully understood. CPAs reduce ice and eutectics formation, minimize salt freeze concentration, and stabilize cell membranes. This study focuses on a specific cryoinjury mechanism by examining DMSO's effect on ion conductance via the potassium KcsA membrane channel. Previous theoretical work suggested that hypertonic activation of membrane ion channels involved in cell volume regulation contributes to the observed salt loading of cryopreserved cells exposed to freeze-concentrated solutions [1]. This salt loading can affect osmotic cell volume, potentially leading to hypotonic-like cell lysis upon thawing when the cell medium osmolality returns to normal. The hypothesis posits that CPAs modulating ionic conductance across cell membranes could significantly influence salt loading and possibly prevent osmotic lysis. Using the GROMACS module CompEL, classical molecular dynamics simulations were employed to study the DMSO's impact on the potassium conductance of the KcsA channel embedded in a DMPC membrane under sustained transmembrane voltage. The simulations employed AMBER force fields: OPC water model, Li&Merz ions (12-6 IOD), LIPID21, and ff19SB to represent molecular interactions. The results indicated that the average single-channel potassium conductance was 19.84 pS without DMSO. However, adding 10 vol.% DMSO significantly abolished potassium conductance (1.8 pS). This inhibitory effect appears to result from DMSO partitioning into the channel cavity, blocking it without significantly altering the structural conformation of the channel protein. These findings suggest that DMSO's ability to inhibit potassium conductance may be a fundamental mechanism of its protective action against osmotic aspects of cryoinjury, expanding the current knowledge of fundamental principles of cryobiology.

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From quantum mechanics to QM/MM: NHC gold(I) complexes as potential cancer therapeutics

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Keywords: thioredoxin reductase, gold(I) complexes, DFT, QM/MM, WHAM

One possible treatment for cancer is inducing programmed cell death (PCD) through increased oxidative stress. Apoptosis, a form of PCD, is governed by thioredoxin systems. Specifically, thioredoxin reductase, a crucial component of this system, can be inhibited by gold(I) complexes, leading to therapeutic oxidative stress.[1,2]

Our study focused on how a specific gold(I) complex interacts with the enzyme's active site. We used the density functional method to analyze the structures of reactants, transition states, and products in various redox states and (de)protonation levels of the active site. Alongside calculating the Gibbs activation energies and standard reaction energies for the reaction profiles, we also performed additional analyses such as Natural Population Analysis (NPA) and Bond Critical Point (BCP) analysis.

Recently, we extended our research by applying QM/MM calculations with the Umbrella sampling technique to more complex systems. In these systems, the quantum core included a tetrapeptide sequence at the C-terminal of the enzyme along with the gold(I) complex. We expanded our model to include the entire protein and explicit solvent, which were optimized using classical molecular dynamics force fields, while the tetrapeptide (quantum core) continued to be optimized via QM. We utilized the Weighted Histogram Analysis Method (WHAM), Umbrella Integration (UI) techniques, and Free Energy Perturbation (FEP) corrections for comprehensive analysis. Additionally, beyond the original scope, we also focused on the influence of explicit water by studying hydration reactions to further understand their role in the enzymatic activity and stability.

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Investigation of radioactive Strontium decorporation through computational analysis of alkaline earth metal complexation

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Industrial activities release various metal ions into the ecosystem, which are known to have a significant polluting effect on the environment and are particularly toxic to humans. Radioactive strontium is one of the most dangerous isotopes in terms of its physiological effects considering its ability to replace calcium in the skeleton. Searching for a chelating agent that can be used for the removal of this ion is crucial for nuclear safety and sustainable development of nuclear energy. In this study we investigate the chelate effect of the compounds (7,16-bis-malonate)-1,4,10,13-tetraoxa-7,16-diazacyclooctadecane-bis-malonic acid (XI) and 5-[bis(carboxymethyl)amino]-3-(carboxymethyl)-4-cyanothiophene-2-carboxylic acid (ranelic acid, RAN) on alkaline earth metal cations, namely calcium (Ca^{2+}) and strontium (Sr^{2+}). The structural and binding characteristics of the chelating agents with the ions were investigated by hybrid density functional (DFT) calculations at B3LYP/def2-TZVP level of theory in an aqueous SMD polarizable continuum model. The validation of the model and the level of theory was carried out using calcium and strontium complexed by ethylene diamine tetraacetic acid (EDTA). Our calculations reveal that the exchange reaction from Ca^{2+} to Sr^{2+} with the chelating agent XI has an exergonic (-8.7 kJ/mol) Gibbs free energy profile. Further exploration of the chelates has also started using molecular dynamics (MD) simulations. A comparison of DFT and MD results was performed with the aim to obtain accurate cation-complex structures in molecular dynamics simulations. Based on our analysis, we have found that the selected force fields, when compared with the DFT results, show a good agreement, and were used to determine the free energy profile of chelation using well-tempered metadynamics simulations. Our simulations revealed the mechanism of chelation and indicate that stable chelates are formed, meaning the molecules can bind the examined ions.

Allostery
Monod's second secret of life

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An historical view of Monod's famous model will be used to introduce its basic principles; to connect them with modern understanding of protein dynamics and thermodynamics; and to frame contemporary examples.

Abstracts of poster contributions

QM/MM study of the electron hopping processes of the Two Lowest Singlet Excited States of Cytosine, its Aza-Derivatives and Carotenoids

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The electron hopping of selected heterocyclic compounds and selected carotenoids was studied using QM/MM simulations of excited states dynamics. Tully electron hopping and semiempirical methods OMx in combination with MRCISD method was used in the simulations [1]. Calculations were performed using Newton-X, MNDO99, MNDO2020 and Gromacs. The lifetimes of the excited states were estimated based on the simulations. The results showed that used methods correctly describes the time evolution of excited states of heterocyclic compounds. Deexcitation from S2 state was ultrafast with femtosecond calculated lifetimes. Following deexcitation to ground state were longer, with 0.83 ps lifetime for Cytosine and with ~ 1 ps and tens of picosecond lifetimes for Aza-Derivatives. This is great progress compared with the previous study[2] as the lifetimes were too long studying the dynamics in gas phase. The results also strongly suggest that electronic structures are very sensitive to the substitution on the triazine ring and that the photophysical properties of nucleic acid analogues depend highly on their molecular structures. In case of carotenoids only deexcitation from first excited state was described correctly. The results also suggest that the length of the pi conjugated system correlates with lifetime (the longer the chain the shorter the lifetime).

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QM/MM study of the electron hopping processes of conjugated systems

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Mean lifetimes of the two lowest singlet excited states of linear conjugated polyenes from ethene to docosaundecaene were explored. The semiempirical OM2/MNDO method using MRCISD computational level was combined with Gromacs description of environment to per- form QM/MM dynamics[1] of these molecules in n-hexane. In each step the time-dependent Schrödinger equation was solved and the transitions between states were carried out by the Tully's fewest switches algorithm. Mean lifetimes were determined by fitting the time dependent occupancies of the excited states according to the exponential decay law. The lifetimes of the S2 state are very short: from hexatriene (7 fs) to octatetraene (51 fs). As for the S1 state lifetime, ethene has the shortest one, 89 fs, in contrast to octatetraene, whose S1 state lifetime is 1275 fs. The lifetimes are significantly shorter in comparison with the previous gas-phase simulations[2].

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Binding Affinity Estimation using X-GRADE

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This work introduces a novel descriptor and machine learning pipeline for rapid prediction of protein-ligand binding affinity, focusing on pKi, pKd and ÎŤG. The key innovation lies in the "eXtended GRAil[1]-based DEscriptor (X-GRADE)", which captures critical interactions through *extended pharmacophore-based (Ligandscout)*[2] GRAIL *scores, van der Waals interactions,* electrostatic energies, and a receptor-independent ligand fingerprint. This fixed-length X-GRADE enables efficient training of various machine learning models. Evaluated on the PDBbind database[3], the proposed approach demonstrates comparable accuracy to existing methods while achieving significantly faster prediction times. Additionally, compatibility with GRAIL Maps and GPU acceleration further enhance its efficiency, facilitating rapid scanning of large databases in the future. By providing a fast and accurate structure-based approach, X-GRADE has the potential to streamline early-stage drug discovery workflows.

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MD Simulations of SERT for the Analysis of Ligands

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This project originated from *in vitro* testing of amphetamine derivatives on the serotonin transporter (SERT), revealing notable variations in their activity, particularly among respective enantiomers. Motivated by these findings, our project aimed to elucidate the underlying mechanisms of those protein-ligand interactions at an atomistic level using molecular dynamics (MD) simulations combined with force distribution analysis. Sixteen potential serotonin-releasing amphetamine analogues were docked using AutoDock Vina 1.1 into the outward-open and occluded conformation of SERT and simulated in an all-atom representation for 100ns using the AMBER ff99SB-ILDN force field. The poster will present our insights into the dynamic behavior of SERT-ligand complexes, highlighting critical residues within the binding site that influence occlusion of SERT. This was achieved through a comprehensive analysis of individual atomic pairwise forces that allows a much more sensitive view of the protein-ligand complex. Such insights offer significant implications for understanding the SERT-substrate interactions, thereby providing valuable guidance for future drug design strategies.

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A Computational Study of the Tropocollagen Hexamer and Heptamer

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Keywords: Collagen, Gromacs, tropocollagen, Hydroxyproline, Proline

Collagen is the main protein found in the connective tissues of mammals [1]. Over the past 20 years, it has become increasingly evident that collagen matrices exhibit significant heterogeneity in their hierarchical structures, which play a crucial role in their structure and function[2]. In this study, we used the GROMACS software to investigate the impact of hydroxyproline and proline on the structure of tropocollagen hexamers and heptamers. According to the results, proline and hydroxyproline have an important role in stabilizing the 3-helix of collagen. Hydroxyproline is more effective than proline in this regard. While collagen is not entirely hydrophobic, some regions of its structure exhibit this property. We delved into the impact of proline and hydroxyproline on Lennard-Jones and Coulomb interactions. These interactions are heavily influenced by the distinctive molecular properties of these amino acids, which in turn can affect the stability and conformation of collagen molecules.

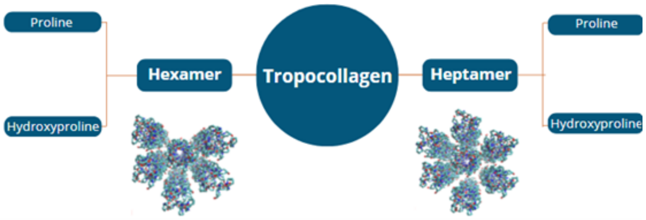

Figure 1. The systems were studied using GROMACS software.

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Computational and reaction kinetic study of carbodiimide formation

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The base ingredients in polyurethane industry include polyols and diisocyanates (TDI, MDI). From the latter, MDI can be modified in the production process to reach favorable properties to its popular and widely used products: the polyurethane foams [1]. Carbodiimides are compounds with N=C=N cumulated double bonds. In this research, the carbodiimide bond formation progress is explained through a model reaction using 2 eq. *mono*isocyanate (phenyl isocyanate), not a *di*isocyanate. The reaction is a homogenous catalytic reaction, where carbon dioxide gas evolves. Ortho-dichlorobenzene (ODCB) is a common solvent used in the isocyanate industry, therefore it was applied in our experiments too. The reaction can be catalyzed by several type of molecules (organometallics, phospholanes, phospine oxides etc.), however *phospholene oxides* (in our case, 3-methyl-1-phenyl-2-phospholene-1-oxide, MPPO) show higher conversion value and less byproducts than others. [2]

In the first part of this study, detailed computational chemistry calculations were carried out using density functional theory (DFT) to determine the mechanism and thermochemistry of the reaction. The level of theory and basis set were chosen to B3LYP/6-31G(d), which is a well-known, hybrid density functional. The aim was to calculate the potential energy surface (PES) and reaction enthalpy. Both gas and ODCB solvent phase calculations were performed. To conclude it, 6 transition states and 8 intermediates can be differentiated, and activation zero-point energies for gas and ODCB phase calculations are 54.5 and 58.7 kJ/mol, respectively, which can be further calculated to enthalpies. To validate the computational results, an experimental apparatus was developed, where the CO_2 gas evolving is measured with a conventional gas burette, at 5 different temperatures (40-80 °C). As more gas is produced by the time and by increasing temperature, the read volumes are used to calculate reaction kinetics, rate constants and Arrhenius equation is for activation energy. Applying initial rates method, reaction follows second order kinetics and the activation energy is 55.8 kJ/mol.

Comparing our results, it was revealed that the computationally and experimentally got results show pretty good match within 5% relative difference, which verifies our both developed methods and its applicability in carbodiimide chemistry. The experiences and lessons of this study encourage us for further research.

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Investigation of the microorganism adsorption capacity of natural-based particles

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Preventing water scarcity and protecting natural water sources are significant challenges we face today. To solve this issue, some industrial sectors are increasingly focusing on waste management and wastewater treatment. The reuse of treated industrial wastewater (e.g. as process water or irrigation water) is under strict regulations, which require a number of chemical, physical and biological parameters to be taken into account. Among these parameters is the determination of the microbiological composition of the treated water and the limitation of the concentration of the microorganisms. For example, the removal of microorganisms that degrade the quality of natural and artificial waters can be achieved by using appropriate adsorbents. The properties of the adsorbents can be tailored to collect the specific bacteria from the effluent on their surface and remove them from the system by sedimentation with the adsorbent. A montmorillonite clay, modified by the intercalation of zirconium ions between silicate layers, was investigated for the removal of Gram-negative (*Escherichia coli*) and Gram-positive (*Micrococcus luteus*) cells from aqueous saline solution. The removal efficiency of the Na-Zr 2.5 clay particles was approximately 60% for *E. coli* and 55% for *Micrococcus* cells. The bacterial cells adsorbed onto the clay surface by electrostatic attraction. The results show that the modified clays are effective in reducing microorganism levels in aqueous solutions and can be used as low-cost, environmentally friendly adsorbent in wastewater treatment. The possibility of regenerating these particles after adsorption was also investigated. Among the regeneration methods, thermal regeneration was chosen, as this method not only regenerates the particles but also sterilizes them. No loss of adsorption capacity was observed during reuse, indicating that the particle can be reused for the adsorption of microorganism.

Evaluation of *Sargassum*-Derived Activated Carbon and Biochar: Ecotoxicity, and Heavy Metal Adsorption Performance

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Keywords: Activated carbon, biochar, toxicity test, Escherichia coli, Adsorption test, pH dependence

Activated carbon AC and biochar BC are porous substances that can be derived from any carbonous material. In this study, AC and BC were produced from *Sargassum spp.* by chemical activation and pyrolysis process. In order to ensure the activated carbon and biochar produced are safe, all samples were tested for possible toxic substances, where a bacteria-based toxicity test was applied by using *Escherichia coli.* The results indicated that all samples showed no ecotoxicity towards *Escherichia Coli.* In addition to that, adsorption tests were carried out to determine the adsorption capacity of activated carbon and biochar for the removal of toxic metals from water (Ni, Pb, Co). The effect of initial metal concentration (20 mmol/l), and pH, have been studied at room temperature. As the pH increases, the deprotonation of the metal binding sites increases the negative charge on the surfaces of activated carbon and biochar, which enhances adsorption. However, precipitation could occur above a certain pH in each experiment in the case of Ni, Pb, and Co ions and hydroxy precipitates can form during the process. Therefore, it was shown that the adsorption capacity was highly dependent on pH. However, the results showed that all samples could absorb the metal ions. By comparing all samples AC and BC showed better adsorption capacity of Pb ions than Ni and Co ions where at optimal pH the adsorbed amount of Pb ions was 87.1 mg/g and 38.9 mg/g which is larger than nickel and cobalt adsorption which was 15.4 mg/g, 11.8 mg/g for AC and 26.5, 9.1 for BC.

Experimental and theoretical study of urethane formation in the presence of 2,2-dimorpholinodiethylether (DMDEE) and 1,4-dimethylpiperazine (DMP)

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Polyurethane (PU) foams are widely used in automotive, housing, and other industries. PU is found in several forms such as rigid, or flexible foam. Polyurethanes are segmented polymers, containing a soft segment and a hard segment. The soft one provides flexibility, while the hard one provides strength. Typically, catalysts have an essential role in industrial chemical transformations, especially in polyurethane synthesis being required to maintain a balance between the reaction of the isocyanate and polyol. Thus, catalysis plays a vitally important role in the synthesis of polyurethanes, urethane-isocyanurate, urethane-urea, and other isocyanate-based polymers in the form of foams, coatings, adhesives, and the catalysts affect the reaction rates. In this work the reaction of phenyl isocyanate (PhNCO) and butan-1-ol (BuOH) in acetonitrile without and in the presence of two amine catalysts, 2,2-dimorpholinodiethylether (DMDEE) and 1,4-dimethylpiperazine (DMP), has been studied, using computational and experimental tools. Kinetic investigation of the alcoholysis of phenyl isocyanate (PhNCO) using stoichiometric butan-1-ol (BuOH) in acetonitrile was examined as well. The same reaction was investigated by using density functional theory and composite methods (BHandHLYP/6-31G(d) and G3MP2BHandHLYP) in combination with the SMD implicit solvent model. The calculated values for the proton affinities (PAs) of the catalytic nitrogen showed that the PA affected the relative energy of the corresponding reaction steps. The results showed that the barrier height of the reaction significantly decreased (> 120 kJ/mol) in the presence of catalysts. The computed and measured thermodynamic properties were in good agreement with each other. This proves the important role of amine catalysts in urethane synthesis. The achieved result further proves the validity of the proposed mechanism and the method selection as well. Based on these results it can be seen that computational tools are applicable to describe similar systems in upcoming studies.

Theoretical study of the applicability of natural antioxidant additives

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Oxidative stress is a physiological condition that occurs when there is an imbalance between the production of reactive oxygen species (ROS), or free radicals, and the body's ability to detoxify them or repair the resulting damage. Antioxidant additives are molecules that can neutralize ROS and prevent or repair cellular damage. In this study, the antioxidant potential of commonly used natural antioxidant additives, including curcumin [1] and ascorbic acid (Asc) [2], and α-tocopherol and trolox has been studied and compared using computational tools. The geometries of molecules and their corresponding radicals, radical cations, and anions were optimized by employing the M06-2X functional in combination with the 6-311+G(d,p) and 6-311++G(2d,2p) basis sets in the gas phase. Three antioxidant mechanisms have been considered: hydrogen atom transfer (HAT), single electron transfer-proton transfer (SET-PT), and sequential proton loss electron transfer (SPLET). The bond dissociation enthalpy (BDE), ionisation potential (IP), proton dissociation enthalpy (PDE), proton affinity (PA), and electron transfer enthalpy (ETE) have all been considered for every possible hydrogen donor site. Overall, the BDE values indicate that the O-H bonds have a greater ability to contribute H-atoms to free radicals compared to the C-H bonds. Moreover, both structures contain C-H sites that have a comparable antioxidant capability to the O-H sites, depending on their location within the structures. As the bond dissociation enthalpy (BDE) decreases, the antioxidant capacity increases. When comparing with regularly utilized polymers, each species that was analyzed has at least one X-H bond with a lower BDE value than those found in commonly used polymers such as PE and PP.

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Investigation Of Cobalt bioacumulation by *Chlorella Vulgaris*

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Toxic metal pollution of soil and water is a serious environmental challenge which threatens human health. Exposure of a living organ to high concentration of heavy metal exerts toxic effects by causing oxidative stress, impairing protein structure and function and causes DNA damage. One of the main sources of heavy metal contamination is industrial wastewater and sludge. Therefore, it is crucial to develop efficient processes for removal of heavy metals from industrial wastewater [1]. In recent work we have examined the ability of microalga Chlorella vulgaris to remove Co^{2+} from the model medium. The model medium was inoculated by *C.vulgaris* culture, OD680 value which indicates the cell density, was adjusted to 0.3-0.5. *C.vulgaris* reached plateau phase of growing at the 5th. day of culturing by photoautotrophic growth. OD680 values were determined by spectrophotometer. Cell free supernatants were obtained by centrifugation of cultures. The cobalt concentration of the supernatants was analyzed by inductive plasma coupled spectrophotometer. Examination revealed that Co^{2+} concentration significantly decreased within 24 hours. The result shows that *C.vulgaris* can be employed to cobalt decontamination crucially found in the most water bodies [2].

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Carbohydrate-based Hydrogels in Drug Delivery Systems

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Hydrogels are three-dimensional crosslinked materials with extracellular matrix-like physicochemical characteristics. Changes in hydrogel material, crosslinking, molecular weight, chemical surface, and functionalization can imitate native tissue mechanical properties. For medication delivery and cancer treatment, hydrogels made of natural polysaccharides (chitosan, alginate, hyaluronic acid, cellulose, and carrageenan) are popular. Carbohydrate-responsive drug carriers were investigated for selective delivery of anticancer drug molecules to the tumor site, which increases local drug dosages and kills cancer cells while minimizing chemotherapy's negative effects on other tissues, improving patient quality of life.[1-3]

Molecular dynamics modeling was applied to predict the molecule conformations of cellulose, chitosan and hyaluronic acid polymers and their interactions with the surrounding environment. The aforementioned polymers were simulated as monomers, homodimers, and heterodimers in water with 150 mM NaCl. The initial configurations of the systems were created using the CHARMM-GUI online server. To perform MD simulations, GROMACS 2023.2 was used with the Charmm27 force field. As a final step, a production simulation of 200 ns was run for all studied systems. Analyses were conducted on H-bonding, gyration radius, root mean square deviation, root mean square fluctuation, and solvent accessible surface area. The analysis revealed that the cellulose monomer exhibited the best level of stability. The mobility and flexibility of molecule terminals exceeded that of cores. The hyaluronic acid monomer exhibited the highest amount of hydrogen bonding and hydrophilicity among the simulated systems of monomers.

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Investigation of polyester-model urethane biodegradation

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Polyurethane (PU) and other polymers are part of our everyday lives, but their accumulation in the environment, particularly in water and soil, poses a serious problem. Although numerous waste management techniques exist, biological degradation is still not widely used. This method has several advantages, such as the possibility of complete mineralization of plastics and low energy requirements. The primary agents of biological waste management are microorganisms, which participate in the degradation or depolymerization of various natural and synthetic polymers through different enzymatic reactions. To date, the best degradation results have been observed with biologically renewable plastics (PLA, PHA, PHB), PET, and PUR.

To test the plastic-degrading abilities of bacteria, model substrates (chemically similar molecule to the original plastic) are often used. Impranil DLN substrate is commonly used for the preliminarily testing of PET and PUR biological degradation. In our research, we examined *Bacillus* strains, several of which showed the ability to degrade Impranil DLN SD substrate. Among them, *Bacillus velenensis* GZCB6 proved to be the most promising strain. We investigated the degradation of Impranil DLN SD by this strain in both solid medium (by measuring the size of the clear zone) and in liquid fermentation (by following the OD changes of the initially opaque Impranil containing medium spectrophotometrically at 600 nm). We compared the sizes of the clear zones and the changes of the liquid OD with the concentration of the cells applied for the degradation tests. Liquid fermentation was also monitored using the ATR-FTIR method, with samples taken at 24, 48, and 72-hour intervals to identify the degradation products and confirm the process of degradation. Nonetheless, further analysis is required to fully exploit the potential of microorganisms in polymer and polyurethane waste management, which could be the new solution for sustainable economy.

Molecular dynamics simulations of the Influenza M2 proton channel: the role of the protonation state and the polarizability

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We developed a Python tool called Protex to enable the simulation of proton transfers to investigate how proton transfer contributes to the conductivity of protic ionic liquids. Now we are extending Protex to work with more complex systems, with a main focus on ion channels. Proton transfer has a key role in the function of proton conducting ion channels. On the one hand, protons can be transferred through the channel by the Grotthuss mechanism. Here protons hop mainly between water molecules, but the channel's acidic side chains can also be involved. On the other hand, whether the channel itself is open is decided by the protonation state of a few key residues.

We investigated the M2 channel of the Influenza A virus. It consists of four identical helices, and it has already been shown that the protonation state of one histidine side chain per helix determines to what degree the channel is open. To better understand the system, we carried out a series of simulations of the ion channel embedded in a membrane and surrounded by water with various protonation states of these residues. We also investigated the effect of polarizability by conducting both polarizable and non-polarizable simulations, as well as ones where only parts of the system were polarizable. We analyzed the size of the channel and the RMSD of the four helices to see how they are pushed apart from each other upon protonation of the histidines.

At the same time, we were also working on updating Protex. In order to simulate proton transfer in water, we developed our own polarizable water model with two possible protonation sites (corresponding to the lone pairs of a real water molecule). Protex is now able to handle multiple protonation sites per molecule, as well as more than two possible protonation states (such as OH^-, H_2O and H_3O^+ in the case of water). We conducted our first simulations of proton transfer in an aqueous solution. At the moment, we are working on refining Protex further to be able to handle proteins correctly, by incorporating some special, biomolecule-specific forces we have not needed up until now (NBFIX and NBTHOLE in CHARMM terms).

Chloroquine transport and mutations in PfCRT protein

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Previous results showed possibility of spontaneous transport of chloroquine molecule into cavity of PfCRT protein [1]. Yet the transport to cytoplasmatic side wasn't successful, the chloroqine molecule remained in central cavity for several microseconds and interacted with several residues. Several results was observed in different authors e.g. Berger [2]. Within several repetitions was most frequent contact area around modified residue A220S (with chloride atom from chloroquine molecule). This mutation is present in nearly all chloroquine-resistent mutant species. Yet this mutation does not significantly manifest in structural changes in apo protein. To clarify effect of several mutations, we ran several repeated simulations of microsecond scale, each with single mutation which differs resistant Dd2 and 3D7 Plasmodium falciparum strains from wild type chloroquine-sensitive 3D7 strain - E75N, T76N, A220S, S326D, S326N, S356I. Several of them blocked transport to central cavity (E75N, T76N, S356I), while other allowed the entrance to cavity, but caused different interactions than with surroundings of S220 (S326D, S326N).

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Docking Score Prediction of Molecules Using Machine Learning Methods

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The study aims to predict the docking score (DS) of inhibitors of the SARS-CoV-2 main protease, potential drug candidates for COVID-19 disease, using a machine learning (ML) method: Kernel Ridge Regression (KRR) in combination with the Smooth Overlap of Atomic Positions (SOAP) descriptor. The performance of various combinations of models and test setups was evaluated, paying particular attention to the performance on the lower extreme DS ranges of potentially the most potent compounds. Results of Multi-Instance Learning (MIL) with resampling already indicate improvement over popular ML workflows in this field[1]. The methodology was also tested on different conformers generated by Molecular Dynamics.

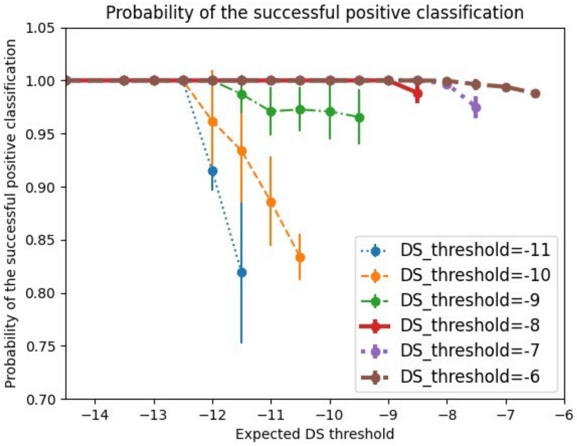

Figure 1: The probability of successful positive classification is the ratio of compounds that meet two criteria: their predicted DS falls below a given DS threshold and their expected DS remains below the expected DS threshold, to the total number of compounds that have an expected DS below the threshold.

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Development of antibacterial, core-shell structured transition metal ferrite nanoparticles

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Ensuring sufficient and high-quality drinking water is a significant challenge today. In addition to chemical contaminants caused by human activities, the proliferation of pathogenic microorganisms in water also poses serious health risk. This work describes the development of antibacterial core-shell structured nanoparticles that contain silver particles inside and have a surface layer composed of amine functionalized transition metal ferrite ($CoFe_2O_4$ and $NiFe_2O_4$) particles (Figure 1). The magnetic nanoparticles were synthesized by solvothermal method in an ethylene glycol medium using monoethanolamine at 200°C.

Thanks to the presence of silver core, these particles exhibit outstanding antibacterial activity against pathogens, and due to the ferrite particles crystallized on their surface, they possess magnetic properties, making them easily and quickly separable from purified water samples using magnetic field. An additional advantage of magnetic nanoparticles is that their surface is rich in amine functional groups, which protonate when pH decreases, resulting in a positive electrokinetic potential. Due to the electrostatic interaction between the particles and the negatively charged membrane of bacteria, the dead cells can also be physically removed through magnetic separation.

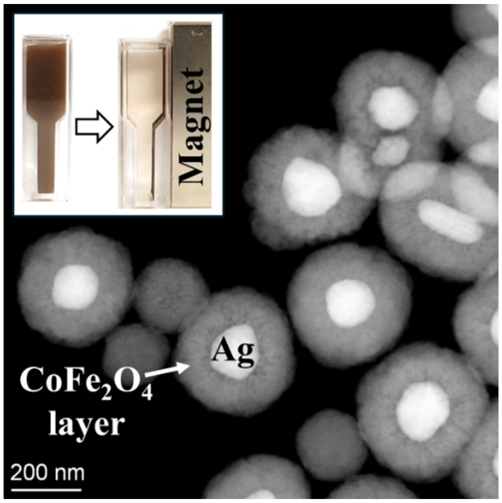

Figure 1: HAADF-TEM picture and magnetic separation of the Ag/$NiFe_2O_4$ core-shell structured nanoparticles.

Antibacterial tests were performed with *Escherichia coli* Gram negative bacteria and the extent of the antibacterial effect was determined by measuring the size of the inhibition zone.

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Development and testing of polymer-encapsulated, amine-functionalized iron-based contrast materials in animal model

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Magnetic nanoparticles (NPs), particularly superparamagnetic variants such as magnetite, maghemite, and various ferrite NPs, emerge as promising alternatives to traditional Magnetic Resonance Imaging (MRI) contrast agents (CAs). Their heightened specificity and biocompatibility make them attractive candidates. The escalating demand for stable and precisely tuned magnetic NPs in biomedical applications highlights their significance. However, the preparation of these NPs remains a persistent challenge.

Two different solvothermal methods (12 h reflux and a 4 min microwave, MW) were used to synthesize amine-functionalized ferrite, superparamagnetic NPs, doped with Zn^{2+} and Cu^{2+} ions. To overcome stability problems in the colloidal phase, the ferrite NPs were embedded in polyvinylpyrrolidone and could be easily redispersed in water. The morphological characterization of the NPs was executed by Atomic Force Microscopy (AFM) and Dynamic Light Scattering (DLS). For detecting the supramolecular interactions and crystalline structure, Fourier Transform Infrared Spectroscopy and X-ray Powder Diffraction was utilized. The in vitro and in vivo MRI measurements were performed with a PET/MR system.

In case of the Zn-doped NPs, the conventional synthesis ($ZnFe_2O_4$-NH_2 Refl.) resulted in a more stable system as compared to the microwave-assisted synthesis ($ZnFe_2O_4$-NH_2 MW). These results were supported by DLS and AFM measurements, as well as in vitro MRI measurements, where inhomogeneities in the signal were detected. The $CuFe_2O_4$-NH_2 samples however showed increased colloidal stability as well as homogenous MRI signal in vitro and in vivo. After injection, both samples exhibit a concentrated presence in the hepatic region of the animals; comparable biodistribution and pharmacokinetics with other SPION NPs is suspected.

Our investigation shows that the ferrite NPs are a feasible candidate for a new generational, multimodal MRI CA. Their chemical properties, owning an -NH2 group holds great options for surface modifications with chelators for isotopes or fluorescent pigments for multimodal molecular imaging purposes. It must be highlighted that the preparation method as well as the nature of the applied precursors play a crucial role in the synthesis of a stable system.

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1. Shokouhimehr M. Prussian blue nanoparticles and its analogues as new-generation T1-weighted MRI contrast agents for cellular imaging (Master's thesis, Kent State University).

The effect of GM1, GM2, and GM3 gangliosides on the stability of Amlyloid-β42 dimers

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Neuronal membranes has been shown to enhance or prevent the formation of oligomers of the amyloid-β42 (Aβ) peptide, the neurotoxic species implicated in Alzheimer's disease (AD). The relation between the membrane composition and its effect on Aβ oligomerization remains unclear, though it is possible that key Aβ residues may affect this transition. Gangliosides are glycolipids that contain sialic acid, and have an important role in brain development, regeneration, and the progression of AD, but its role and AD pathology must also be clarified. The aim of this work is to use MD simulations to unravel the first steps in the interaction between clusters of GM1, GM2, and GM3 gangliosides and Aβ dimers.

Clusters of GM2 reduced the attractive interaction energy between Aβ dimers more than those of GM1 or GM3 did. This was particularly evident in the central polar region of the peptides within the Aβ dimer. These results suggest that the GM2 clusters have the strongest propensity to destabilize the Aβ dimer. While the effect on the secondary structure of the dimer was minimal, the effect of the dimer-ganglioside interactions on the key residues that affect Aβ oligomerization provides new insight on how these gangliosides affect Aβ dimer stability and possibly AD progression.

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1. Shokouhimehr M. Prussian blue nanoparticles and its analogues as new-generation T1-weighted MRI contrast agents for cellular imaging (Master's thesis, Kent State University).

Random proteins bind random ligands

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Is small-molecule binding by proteins the result of exhaustive evolutionary fine-tuning specific to each protein-ligand pair? Or are proteins inherently able to bind a wide range of ligands, even if their crystal structures present no specific binding sites? Indirect evidence suggests that protein surfaces are teeming with potential binding sites for small molecules of all kinds, even when binding crevices are not detected in crystal structures. To test this hypothesis, we used docking and molecular dynamics simulations (MD) to explore whether arbitrary ligands appear to bind to arbitrary proteins. We selected 14 random E. coli protein crystal structures from the Protein Data Bank (PDB) that were water soluble, under 40 kDa, solved at resolution 1.5 to 2.0 ÃĚ, and with few unresolved residues. Each protein was docked with each of the 20 natural amino acids individually; none of the proteins has an amino acid as a natural ligand. AutoDock Tools version 1.5.7 predicted amino acid poses. By visual examination promising poses were chosen for diverse and extensive bonding interactions, and depth of recess from the surface. Each pose was evaluated with all-atom molecular dynamics simulations of each complex for up to one microsecond using GROMACS. Nine proteins bound one or more amino acids for 100ns or longer based on a stable RMSD level that could be replicated in an independent MD simulation. Binding energies were calculated in vacuum using an empirical scoring function in AutoDock Vina. Amino acid binding energies ranged from -2.70 to -5.44 kcal/mol. Several proteins bound the amino acid methionine (among others). Those proteins were then tested with S-adenosyl methionine (SAM). With several proteins SAM demonstrated a notable increase in residue interactions and calculated binding energies compared to methionine alone, staying bound for most of one microsecond and with calculated binding energies of -3.31 to -5.50 kcal/mol. Proteins that bound SAM were also tested with adenosine monophosphate (AMP). Several proteins exhibited favorable binding energies (-5.31 to -8.80 kcal/mol) in one-microsecond simulations, comparable to the energy found in a onemicrosecond MD simulation with the known AMP-binding protein cystathione b-synthase (PDB:3DDJ; -6.23 kcal/mol). The docking and simulations indicate that randomly selected E. coli proteins can bind random small ligands like amino acids, SAM, and AMP persistently and with binding energies comparable to those calculated for a natural AMP-binding protein. Unexpectedly, all ligands remain mobile within their binding locations, with changing bonding partners but similar binding energies. The energies calculated until now are a weakness of the results because they neglect contributions from solvent and cannot be commensurate with experimental affinities. We are presently evaluating better tools for energy calculations; calculating binding energy for a native SAM-binding protein; docking and simulating a new group of random proteins; and deciding on methods to examine the binding of SAM and AMP to the proteins experimentally. These results indicate that protein surfaces indeed present potential binding sites for random small molecules. This conclusion suggests that many, perhaps all, soluble proteins may be viable targets for design of small molecules that can modulate protein function. Even when binding crevices do not pre-exist in crystal structures, ligands may exploit protein dynamics to find binding sites that can be further optimized to alter function directly or indirectly (allosterically).

Application of *in-silico* methods for enzyme engineering of the NtcA protein from *Synechocystis sp.*

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Nitrogen control A (NtcA) is a cyanobacterial transcription factor belonging to the CRP (cAMP receptor protein) family. It is a global regulator of nitrogen assimilation and metabolism. Activation of NtcA is induced under N starvation, accompanied by accumulation of 2-oxoglutarate (2-OG) and release of the PipX protein. Binding of 2-OG and PipX protein activates the NtcA transcription factor. The apo-conformation of NtcA in the formed complex changes to holo with higher affinity to a number of promoters that induce transcriptional responses (such as carbon metabolism and photosynthesis) [1].

This defines the main direction of computational enzyme engineering: improving the binding to DNA and changing the conformation of NtcA to active by point mutation(s).

Although the structure of the active (tetrametic) NtcA-PipX complex of the homolog has been solved [2], the structure of the NtcA-PipX complex with DNA is not available. Preliminary homology models of NtcA and PipX from *Synechocystis sp.* PCC 6803 (79.2% and 56.8% sequence identity of the corresponding structures to the *S. elongatus* homolog - pdb code 2xko[2]) were used to construct the NtcA-PipX complex bound to DNA. The position of the DNA was defined on the basis of the DNA bound to NtcA from the Anabaena transcriptional activation complex (8h3v). Preliminary mutant screening of NtcA on the contact surface with DNA using FoldX [3] revealed 3 potential mutants with improved interaction energies.

Another strategy focuses on mutating the 2-OG binding site and shifting the NtcA equilibrium towards holo-like structure. Proposed mutations in the 2-OG binding site were generated using Rosetta Design [4]. Suggested mutations were visually analysed using YASARA and potential mutants were selected. AlphaFold3 was used to generate mutants. Molecular dynamics simulation with GROMACS was used to validate the ability of NtcA mutants to maintain active conformation based on RMSD calculation and PCA analysis.

Our preliminary scan revealed several potential mutants that will be used for primer construction and *in vivo* testing and validation of our strategy.

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Interactions of proteins with grafted poly(ethylene oxide) layer in two setups - A Molecular dynamics study

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The protection of surfaces against undesired protein adsorption has received a great deal of scientific attention mainly due to the application in biomedicine, biomedical devices, biomaterials, and biotechnology. Covering surfaces with a biocompatible polymer layer appears to be the most effective, versatile, and tunable way of surface modification. Poly(ethylene oxide) (PEO) belongs to the most frequently used anti-fouling polymers. Despite numerous studies, either experimental or theoretical, that have been devoted to PEO-modified surfaces, no unambiguous explanation of observed behavior has yet been proposed. Conversely, contradictory results are often published [1]. The uniqueness of PEO protective layers comes from the perfect solubility of PEO in water its biocompatibility, and nontoxicity [2]. The interactions of proteins with PEO-modified surfaces are rather complex, as they depend on a large number of phenomena, some of which act in the opposite way. Protein interactions with the PEO-covered substrates are an interplay of steric repulsion as well as hydrodynamic and van der Waals attractions between the protein and the PEO chains. In addition, the water affinity, size, shape, and secondary structure of a protein add more complexity. The properties of PEO layer can be tailored by the coverage density, molecular mass, and distal chemistry of the grafted PEO chains. Atomistic molecular dynamics (MD) simulations represent a suitable tool for studying the mechanism of protein adsorption to or protein repulsion from PEO-modified surfaces at the atomistic level and to provide an explanation of the observed behavior. The MD approach also allows comparison of interactions between peptides and PEO-grafted surfaces under experimental conditions with conditions in living bodies. The specific and non-specific effect of water in these interactions can also be understood. Proteins in two setups, free form and anchored to graphene surface, interacting with planar surfaces irreversible covered by the PEO chains at grafting densities that include pancake and brush regimes were studied using atomistic MD simulations. The differences of two setups were elucidated.

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