VISEGRAD SYMPOSIUM ON BIOMOLECULAR INTERACTIONS

PROGRAM & ABSTRACTS

19th – 22nd June 2022

Nové Hrady

Czech Republic

Organization

Laboratory of Structural Biology and Bioinofmatics
Institute of Microbiology
Czech Academy of Sciences

Academy and University Center of Nove Hrady

Scientific Orginizing Committee:

Babak Minofar, Academy of Sciences, Nove Hrady, Czech Republic
David Řeha, Academy of Sciences, Nove Hrady, Czech Republic
Ján Urban, Comenius University in Bratislava, Slovakia
Béla Viskolcz, University of Szeged, Hungary
Joanna Sułkowska, University of Warsaw, Warsaw, Poland

Local Organizing Team

David Řeha
Babak Minofar
Dagmar Kaftanová

Inivted Speakers

Barbara Rossi, Elettra Synchrotron Trieste, Italy
Christian Schröder, University of Vienna, Austria
Eva Pluhařová, J.H. Institute of Physicsl Chemistry of CAS, Prague, Czech Republic
Vojtech Spiwok, University of Chemistry and Technology, Prague, Czech Republic
Jan Vacek, Palacky University Olomouc, Czech Republic
Per-Olof Syrén, KTH, Stockholm, Sweden
Hashem Alssab, Taif University, Saudi Arabia
Mario Vazdar, University of Chemistry and Technology, Prague, Czech Republic
Mehdi D. Davari, Leibniz Institute of Plant Biochemistry, Germany
Miklós Nagy, University of Miskolc, Hungary
Mohammad Nasir Uddin, Mercer University, Atlanta, GA, USA
Mike Ries, University of Leeds, UK

Symposium program

Sunday, June 19

15.00-18.00	**Registration**
18.00-18.10	**Conference opening**

Chairperson: Béla Viskolcz

18.10-18.40	**Christian Schröder (Wien)**: Polarizable proton transfer molecular dynamics simulations of 1-methylimidazolium acetate
18:40-19:10	**Vojtěch Spiwok (Prague)**: Metadynamics Driven by AlphaFold Collective Variables
19.10-	**Poster session, welcome party**

Monday, June 20

Chairperson: Christian Schröder

09:00-09:30	**Barbara Rossi (Trieste)**: Synchrotron-based UV Resonance Raman spectroscopy is a powerful tool to probe biomolecular interactions
09:30-10.00	**Jan Vacek (Olomouc)**: Serum Albumin as a Multifunctional and Model Protein
10:00-10:30	**Hashem Alssab (Taif)**: Imaging tools for cancer research and drug discovery
10:30-11:00	Coffee break

Chairperson: Zdeněk Futera

11:00-11:30	**Jaroslav Burda (Prague)**: Electronic excited states of conjugated molecules and their lifetimes
11:30-12:00	**Mario Vazdar (Prague)**: Arginine-rich peptides at biological membranes – what did we learn after 10 years of investigation?
12:00-12:30	**Eva Pluhařová (Prague)**: Molecular simulations of the influence of crowders on enzyme structure and kinetics
12:30-14:00	**Lunch**

Chairperson: Per-Olof Syrén

13:40-14:10	**Mehdi D. Davari (Halle)**: Transforming protein engineering through the power of *in silico* methods: Enzyme engineering for biocatalysis in organic solvents
14:10-14:40	**Michal Owen (Mickolc)**: The glycan headgroup affects the nanoscopic segregation of gangliosides
14:40-20:00	**Free time, individual trips and excursions**
20:00-21:00	**Dinner**
21:00-	**Free time, beer**

Tuesday, June 21

Chairperson: Barbara Rossi

09:00-09:30 **Per-Olof Syrén (Stockholm)**: TBA

09:30-10:00 **Mohammad N. Uddin (Atlanta)**: Evaluating bacterial quorum sensing precursor molecule (S)-DPD and its analogues as potential vaccine adjuvants

10:00-10:30 **Fatima Matroodi (Trieste)**: Toxic Effect of Phthalic Acids Esters: Structural Changes of ds-DNA

10:30-11:00 Cofee break

Chairperson: Jaroslav Burda

11:00-11:30 **Zdeněk Futera (Č.Budějovice)**: Electron Transport on Bio/Metallic Interfaces

11:30-12:00 **Zsófia Borbála Rózsa (Miskolc)**: Molecular-level understanding of membrane permeation of some chemicals

12:00-12.30 **Milan Melicherčík (Bratislava)**: Malarial resistance to chloroquine and protein mutations

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

Chairperson: Francesca Goudou

14:00-14:30 **Béla Fiser (Miskolc)**: Growth Mechanisms of Persistent Organic Pollutants – A Case Study of Benzo(a)pyrene

14:30-15:00 **Dalma Dojcsák (Miskolc)**: NH2-Functionalized Magnetic Nanoparticles For The N-glycomic Analysis of Multiple Sclerosis Using Hydrophilic-Interaction Liquid-Chromatography

15:00-15:30 **Tímea Gerzsenyi (Miskolc)**: Development of magnetic nanoparticles aided nucleic acid isolation techniques

15:30-16:00 Coffee break

Chairperson: Jan Vacek

16:00-16:30 **Miklós Nagy (Miskolc)**: Aromatic isocyanides as novel potential anti-fungal and anti-cancer agents

16:30-17:00 **Štěpán Timr (Prague)**: Multi-Scale Simulations Provide Insights into Protein Conformation under Crowding

17:00-17:30 **Francesca Goudou (Pointe-à-Pitre)**: Chlordecone and B-Hexachlorocyclohexane Interaction Functionalized Activated Carbon by Molecular Modelling and Molecular Dynamics Simulation

17:30-18:00 **Michael Ries (Leeds)**: Dissolving Cellulose with Ionic Liquids (on-line)

18:00-18:30 **Closing remarks**

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

Wendsday, June 22: **Departure**

List of posters

1. **Milán Szőri (Miskolc):** Adsorption of some organic molecules on icy surface
2. **Dalal K. Thbayh (Miskolc)**: Theoretical investigation of antioxidant potential of BHA, TBHQ, BHT, and curcumin
3. **Hadeer Q. Waleed (Miskolc)**: Experimental and Theoretical Study of Tertiary Amine Catalysed Urethane Formation
4. **Julie Mallouhi (Miskolc)**: Polyurethane Degradation – A Computational Study
5. **Dimah Zakaraia (Miskolc)**: Collagen as a Bio-Material: An All-Atom MD Approach to Understanding Biopolymers
6. **Anastasiia Shaposhnikova (Č.Budějovice)**: The interaction of crowding agents and salts on structure and dynamics of biomolecules
7. **Volkan Cirik (Č.Budějovice**): Effect of phthalic acid esters on DNA and removal of them with cyclodextrins
8. **Riana Uddin (Atlanta)**: Evaluation of ionic liquid (1,4-Diazabicyclo octane (DABCO)) as permeability enhancer in orally dissolving films (ODFs) for buccal administration of antihistamine Diphenhydramine HCl drug for children

Abstracts of oral presentations

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

Polarizable proton transfer molecular dynamics simulations of 1-methylimidazolium acetate

Florian Joerg, Laurens van Dam, Konstantinos Kanellopoulos, Richard Jacobi, Christian Schröder

University of Vienna, Department of Computational Biological Chemistry, Währingerstr. 17, 1090 Vienna

Ionic liquids play a promising role in future battery and fuel cell generations and can contribute positively to the current challenges of alternative and sustainable energy usage. Of particular interest are protic ionic liquids , a subclass of ionic liquids composed of a Brønsted acid and Brønsted base. These liquids can transfer a proton and form hydrogen-bonded networks resulting in high conductivities.

In principle, ab initio molecular dynamics (MD) simulations are a good tool for examining proton transfer reactions, as bond breaking and formation occur naturally. However, this kind of simulation is limited in system size and simulation period and hence not suitable to compute the conductivity of these systems. Although constant pH simulations in classical MD simulations have been used for several years, they are designed for single protonation sites of a protein. In ionic liquids, however, several hundred cations and anions are subject to protonation and deprotonation processes.

We combine polarizable MD simulations with proton transfer events in our new approach. The equilibrium between 1-methylimidazolium acetate and the neutral components 1-methylimidazole and acetic acid can be determined by a series of polarizable MD simulations of various compositions. In addition, several proton reactions of charged and neutral species are possible, resulting in a complex reaction network. Probabilities and equilibrium concentrations of the involved species are modeled by the combination of reducible Markov chains and quantum-mechanical calculations. In a final step, these results are combined to run a polarizable proton transfer MD simulation.

Metadynamics Driven by AlphaFold Collective Variables

Vojtěch Spiwok[1], Martin Kurečka[2], Aleš Křenek[2]

[1] *Department of Biochemistry and Microbiology, University of Chemistry and Technology, Prague, Technická 3, Prague 6, 166 28, Czech Republic*

[2] *Institute of Computer Science, Masaryk University, Šumavská 416/15, 602 00 Brno, Czech Republic*

spiwokv@vscht.cz

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

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

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

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Synchrotron-based UV Resonance Raman spectroscopy is a powerful tool to probe biomolecular interactions

Barbara Rossi

Elettra-Sincrotrone Trieste, S.S. 114 km 163.5, Basovizza, 34149, Trieste, Italy

Raman spectroscopy is a non-destructive experimental technology very useful to provide molecular information on several kind of systems, such as liquids, gels, polymers and bio-macromolecules trough the investigation of their vibrational dynamics. Thanks to the resonance effect, UV Resonance Raman (UVRR) spectroscopy offers several advantages with respect to spontaneous Raman one, such as the significant increment of the detection limit and the selectivity needed to incisively monitor specific chromospheres within the sample. This determines the usefulness of UVRR spectroscopy, especially to probe biomolecular interactions in complex systems such as macromolecules in aqueous solutions. In the past few years, there was a growing in the use of UVRR spectroscopy thanks to the advancements in laser technology and the development of high efficiency array detectors for the entire UV-visible region. However, the conventional laser sources suffer from the limitation of providing fixed wavelength energies, while tunable excitation radiation in the UV range allows to "map" the whole resonance landscape of the samples for matching with the best experimental conditions.

In this contribution, I will present the potentialities of the unique in the world UVRR setup exploiting the wide and fine tunability of the synchrotron radiation source that is available at Elettra Sincrotrone Trieste (www.elettra.eu). The synchrotron-based UVRR setup results in an innovative spectroscopy facility for approaching open issues in physics, chemistry, materials and life sciences [1,2]. Selected case studies will be discussed in order to show possible applications of UVRR method with particular attention to biophysical and biochemical systems.

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Serum Albumin as a Multifunctional and Model Protein

Jan Vacek

Department of Medical Chemistry and Biochemistry, Faculty of Medicine and Dentistry, Palacky University, Hnevotinska 3, 775 15 Olomouc, Czech Republic

Serum albumin is a multifunctional protein. The main functions of this protein include maintaining oncotic pressure, the transport and distribution of fatty acids and hormones, bilirubin and ions, and last but not least, it binds a range of drugs. Albumin is used in a number of biochemical and analytical laboratories as a model (reference) protein, blocker of nonspecific binding sites, delivery system, protein component of culture media, molecular weight marker, and applications in clinical practice have been proposed. For this reason, we have noticed serum albumin to be a "first choice" protein in a number of studies, without having sufficiently assessed its suitability for a given application or being sufficiently acquainted with its physicochemical properties under the given experimental conditions. This can lead to a number of artifacts and misinterpretations. The aim of this paper is to describe and critically assess the applications of HSA and BSA as model proteins and to discuss the following facts: a) Under native conditions, serum albumin occurs not only in the form of monomers, but also as oligomeric associates. b) Because the protein is isolated, its isolates also differ quite significantly in terms of quality and quantity. c) Serum albumin can be oxidized to a greater or lesser degree natively, but also during isolation. d) The crystal structure differs from the structure in aqueous solution. e) Also, if serum albumin interacts with surfaces, its structure is subject to local deformations. f) The protein is highly soluble in water, but it also contains hydrophobic domains. From the modest list of points a-f above, it follows that despite the simple isolation and purification of albumin, the standardization and interpretation of the results obtained with it can be quite difficult [1-6]. The intention of the author of this contribution is to remind us of the dangers that arise from specific experimental mistakes that we could make when working with albumin. The multifunctionality of albumin under physiological conditions suggests that there will be many modalities and variables of this protein in our experiments.

***Acknowledgements**: This work was supported by the Czech Science Foundation, grants 19-21237Y and 19-09212S.*

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Imaging Tools for Cancer Research and Drug Discovery

Hashem O. Alsaab

Department of Pharmaceutics & Pharmaceutical Technology, Taif University, Saudi Arabia

The goal of this session will focus on how newer imaging methodologies support capturing drug response in real-time with imaging techniques. Also, I will have a sub-section on models that represent the tumor microenvironment and highlight its applications, advantages, and translational challenges. In addition, each modality's salient benefits, considerations, and best applications will be discussed and demonstrated with examples from our research group. For instance, theranostics and photodynamic therapy (PDT) is a promising new modality that combines a photosensitizing chemical and visible light to manage various solid malignancies. PDT has some advantages over conventional tumor therapy in terms of its excellent safety and lower toxicity in treating malignant lesions. Results revealed that PDT could be considered a reasonable option in treating malignant and pre-malignant conditions. It is also valuable for the treatment of some other cancers. I will also highlight how different imaging techniques could support drug discovery, especially in cancer, utilizing nanomedicine and various pharmaceutical biotechnology for drug targeting.

Electronic excited states of conjugated molecules and their lifetimes

Ondřej Tichý, Kateřina Fatková, Jaroslav V. Burda

Department of Chemical Physics and Optics Faculty of Mathematics and Physics, Charles University, Ke Karlovu 3, 121 16 Prague, Czech Republic

Vertical absorption spectra of selected molecules (pyrimidine nucleobases and 5-azacytosin, 2-amino-1,3,5-triazine, 2,4-diamino-1,3,5-triazine, 2,4,6-triamino-1,3,5-triazine, and s-triazine) were performed using TD-DFT, post-HF, and semiempirical OM2/MNDO methods. In the second part of the project also electronic excited states in the series of the conjugated polyenes from C2 to C22 were explored. From comparing a relatively large set of functionals from the different rungs of Jacob's DFT ladder, the important role of the exact exchange interaction is demonstrated. TD-DFT results with the ωB2GP-PLYP functional (and a few others) are comparable with the post-HF methods. An important finding is also a good accuracy of the semiempirical OM2 approximation, which is used for estimations of lifetimes of the excited states for the same set of molecules. The lifetimes' determination is based on a stochastical treatment of a swarm of MD trajectories. The time-dependent Schrödinger equation is solved for the electronic degrees of freedom while the dynamics of nuclei is treated classically in each trajectory step applying Tully's fewest switch algorithm. The probability of individual states in time is determined and compared with both experimental and computational studies. Our results are in fair accord with available experiments. The nucleobases relatively quickly deactivate – all the relaxation times are below 0.5 ps (in very good accord with measured values). Much longer lifetimes (a few hundred ps) were obtained for other molecules: 5-azacytosin, 2,4-diamino-1,3,5-triazine, and 2,4,6-triamino-1,3,5-triazine. Also, in agreement with experimental data, 2-amino-1,3,5-triazine returns to the ground state on a nanosecond scale. As to polyenes, the longest lifetime of the first excited state (S1) was found for decapentaene (about 5 ps). Further elongation of the conjugated chain caused a mild decrease of this value to ca 1.5 ps for docosaundecaene.

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Arginine-rich peptides at biological membranes – what did we learn after 10 years of investigation?

Mario Vazdar

Department of Mathematics, Faculty of Chemical Engineering,
University of Chemistry and Technology, Prague, Czechia

Adsorption of arginine-rich positively charged peptides onto neutral zwitterionic phosphocholine (PC) bilayers is a key step in the translocation of those potent cell penetrating peptides into the cell interior. In the past, we have shown both theoretically and experimentally that polyarginines adsorb to the neutral PC-supported lipid bilayers in contrast to polylysines. However, the comparison of our results with previous studies showed that the results often do not match even at the qualitative level.[1, 2] The adsorption of arginine-rich peptides onto POPC may qualitatively depend on the actual experimental conditions where binding experiments have been performed.

In this work, we systematically studied the adsorption of R_9 and K_9 peptides onto the POPC bilayer, aided by molecular dynamics (MD) simulations and fluorescence cross-correlation spectroscopy (FCCS) experiments.[3] Using MD simulations, we tested a series of increasing peptide concentrations, in parallel with increasing Na^+ and Ca^{2+} salt concentrations, showing that the apparent strength of adsorption of R_9 decreases upon the increase of peptide and/or salt concentration in the system. The key result from the simulations is that the salt concentrations used experimentally can alter the picture of peptide adsorption qualitatively. Using FCCS experiments with fluorescently labeled R_9 and K_9, we first demonstrated that the binding of R_9 to POPC is tighter by almost two orders of magnitude compared to that of K_9. Finally, upon the addition of excess of either Na^+ or Ca^{2+} ions with R_9, the total fluorescence correlation signal is lost which implies the unbinding of R_9 from the PC bilayer, in agreement with our predictions from MD simulations.

Acknowledgements*: We gratefully acknowledge funding by the Czech Science Foundation (EXPRO Grant 19-26854X) and IOCB for Sabbatical Program for MV.*

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Molecular simulations of the influence of crowders on enzyme structure and kinetics

Štěpán Timr[1], Adam Nori[1], Babak Minofar[2], Eva Pluhařová[1]

[1]*J. Heyrovský Institute of Physical Chemistry of the Czech Academy of Sciences Dolejškova 2155/3, 182 23 Prague 8, Czech Republic*

[2]*Laboratory of Sructural Biology and Bioinformatics, Institute of Microbiology of the Czech Academy of Sciences, Zámek 136, 37333 Nové Hrady*

The cellular environment is crowded because macromolecules occupy up to 40 % of the cell volume [1]. Proteins are frequently studied in aqueous solutions without the presence of other macromolecules both *in vitro* and *in silico*. However, including these crowding agents is required for a realistic description because they influence the overall stability of proteins, conformational landscapes, enzyme kinetics, etc [2].

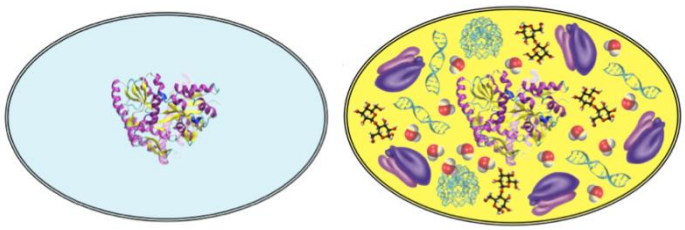

Figure: *Illustration of a protein present in the conventional aqueous buffer (left) and in the cell (right).*

We focus on two enzymes, glutamate dehydrogenase and citrate synthase, whose activities are influenced by crowding. By means of molecular simulations, we study these enzymes in aqueous solutions of glucose and dextran. Our simulations show that crowding slightly alters protein structure and fluctuations in comparison with aqueous solution. We observe that the crowder molecules preferentially interact with polar amino acid residues at the protein surface as well as with the substrate molecules present in the active site. The dynamics of the protein–crowder interaction quantified by a residence time is pronouncedly heterogeneous. The results shall provide deeper understating of enzymes' functioning in the crowded cellar interior.

Acknowledgements*: We gratefully acknowledge funding by the Czech Science Foundation (GAČR) in the context of Project No. 21-15936.*

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Transforming protein engineering through the power of *in silico* methods: Enzyme engineering for biocatalysis in organic solvents

Mehdi D. Davari

Department of Bioorganic Chemistry, Leibniz Institute of Plant Biochemistry, Weinberg 3, 06120 Halle, Germany

Email: mehdi.davari@ipb-halle.de

.Biocatalysis is increasingly gaining more attention in the sustainable ("green") production of biofuel, building blocks for fine chemicals and pharmaceutical compounds, due to its remarkable advantages of excellent chemoselectivity, enantioselectivity, and mild (aqueous) reaction conditions. Hydrolases are industrial biocatalysts within the chemical industry because of their high stability, catalytic efficiency, broad substrate specificity, and commercial availability in a wide application in synthetic transformations. Hydrolases are well-known for their remarkable ability to carry out a wide variety of chemo-, regio-, and enantio-selective transformations, occurring in aqueous mediums and as non-aqueous mediums. However, the use of aqueous systems limits the applications of enzymes within the chemical industry. Water is barely an ideal solvent in particular for hydrophobic substrates, limiting productivity and space-time yield of the possible processes. Moreover, water may interfere with other reaction steps and/or downstream processing. Therefore, biocatalysis using non-aqueous reaction media, such as organic solvents, provides a potential solution. However, enzyme activity is limited because of inefficient substrate binding, lack of solubility, and inactivation by organic solvents.

Enzyme engineering (directed evolution and rational design) is used routinely in academia and industry to develop biocatalysts for biotechnological, biomedicine, and life sciences applications[1]. However, the reliable prediction of beneficial amino acid substitutions, their combination, and their effect on functional properties still pose significant challenges in protein engineering[2]. To accelerate enzyme evolution and efficiently explore its potential, rational design approaches are developed to redesign enzymes through focused libraries by predicting sequence-function relationships. In this presentation, I describe the challenges and opportunities reported in reengineering enzymes for catalysis in organic solvents. I discuss our discovered principles from combined *in silico*[3] and directed evolution study on the molecular level that enable redesigned enzymes to operate efficiently in organic solvents[4]. The latter should provide an overview of the structure-function relationships, first insights on general design principles, and successful protein engineering strategies for stabilizing enzymes in organic media for sustainable catalysis.

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The glycan headgroup affects the nanoscopic segregation of gangliosides

Michael C. Owen[1,2,3], Robert Vacha[3]

[1]*Institute of Chemistry, Antal Kerpely Doctoral School of Materials Science & Technology, University of Miskolc, Hungary*

[2]*Higher Education and Industrial Cooperation Centre, University of Miskolc, Hungary*

[3]*CEITEC – Central European Institute of Technology, Masaryk University, Kamenice 753/5, Brno 625 00, Czech Republic.*

Gangliosides form an important class of receptor lipids containing a large oligosaccharide headgroup whose ability to self-organize within lipid membranes results in the formation of nanoscopic platforms. Despite of their biological importance, the molecular basis for the nanoscopic segregation of gangliosides is not clear. In this work, we investigated the role of the ganglioside headgroup on the nanoscale organisation of gangliosides. We studied the effect of the reduction in the number of sugar units of the ganglioside oligosaccharide chain on the ability of gangliosides GM_1, GM_2 and GM_3 to spontaneously self-organize into lipid nanodomains. To reach nanoscopic resolution and to identify molecular forces that drive ganglioside segregation, we combined an experimental technique MC-FRET offering high lateral and trans-bilayer resolution with molecular dynamics (MD) simulations. We show the ganglioside headgroup plays a key role in ganglioside self-assembly despite the negative charge of the sialic acid group. The nanodomains range from 7 to 120 nm in radius and are majorly composed of the surrounding bulk lipids, with gangliosides being a minor component of the nanodomains. The interactions between gangliosides are dominated by the hydrogen bonding network between the headgroups that stabilizes the nanodomains. The N-acetylgalactosamine sugar moiety of GM_2, however, impairs the stability of these nanodomains by disrupting hydrogen bonding of neighbouring sugars, which is reflected in a broad size distribution of GM_2 nanodomains. The formation of nanodomains is accompanied by several conformational changes in the gangliosides, which, however, have little impact on the solvent exposure of these receptor groups. Overall, this work identifies the key physicochemical factors that drive nanoscopic segregation of gangliosides.

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Evaluating bacterial quorum sensing precursor molecule (S)-DPD and its analogues as potential vaccine adjuvants

Mohammad Nasir Uddin

College of Pharmacy, Mercer University, Atlanta, GA 30341, USA

Adjuvants potentiate the immune response against co-inoculated antigens in the vaccine formulation. Based on the mechanism of action, the adjuvants are classified as immunostimulatory adjuvants and vaccine delivery systems. (S)-4,5-Dihydroxy-2,3-pentanedione (DPD) is the precursor of bacterial quorum sensing molecule, autoinducer (AI)-2. Autoinducer-2 (AI-2) is an important class of QS signals that are produced by many bacteria and are purported to be interspecies signals. In this research project, we tested the immunogenicity and adjuvant potential of microparticulate formulation of (S)-DPD and sevral of its analogues in an in vitro study. The (S)-DPD and its analogues ent-DPD, n-butyl-DPD, isobutyl-DPD, n-hexyl-DPD and phenyl-DPD were formulated in microparticle. The microparticles were tested for their immunogenicity and cytotoxicity. The immunogenicity was tested by quantify the immunostimulatory markers MHCI and MHCII and CD40 and CD 80. The cytotoxicity was tested by using MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) assay. We further tested their adjuvant effect by combining it with particulate vaccines for measles and gonorrhea and compared the adjuvant effect observed with the microparticulate formulations of the FDA-approved adjuvants alum, MPL A®, and MF59®. The adjuvant efficacy was evaluated by nitrite test. Microparticulate (S)-DPD and all the analogues were found to be non-cytotoxic towards the antigen-presenting cells. The results also showed (S)-DPD and its analogues ent-DPD has the higher adjuvant effect. Further studies with additional bacterial vaccines and the in vivo evaluation will confirm the potential of microparticulate (S)-DPD and its analogues as a potential vaccine adjuvant candidate.

Toxic Effect of Phthalic Acids Esters: Structural Changes of ds-DNA

Fatima Matroodi[1], Simone Mearini[2], David Řeha[3],Volkan Cirik[3,4], Isabella Swartz[5], Babak Minofar[3], Jan Vacek[6], Barbara Rossi[2]

[1]*Department of Physics, Faculty of Science, Shahid Chamran University of Ahvaz, Ahvaz 6135783151, Iran*

[2]*Elettra-Sincrotrone Trieste, S.S. 114 km 163.5, Basovizza, 34149 Trieste, Italy.*

[3]*Center for Nanobiology and Structural Biology, Institute of Microbiology of the Czech Academy of Sciences, Zámek 136, Nové Hrady, Czech Republic*

[4]*Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic*

[5]*Princeton University, Princeton, NJ 08544, USA*

[6]*Department of Medical Chemistry and Biochemistry, Faculty of Medicine and Dentistry, Palacky University, Hnevotinska 3, 775 15 Olomouc, Czech Republic*

Phthalic acid (PAEs) esters are a group of plasticizers which widely used in food packaging, beauty products, paints, adhesives as well as toys [1-3]. There are many reports on the toxicity of phthalates in rats, fish and also, and humans. In the present report, the effect of phthalic acids on the disruption of DNA double helix structure is monitored in an aqueous solution by probing nucleobases stacking/unstacking interactions and H-bonding through the DNA unfolding thermal pathway. In our study, we have considered the effect of two types of phthalic acids, dimethylphthalate (DMP) and diethylphthalate (DEP) effects on two representative models of natural DNA, i.e. large DNA molecules (salmon-DNA) and a 30-base pair double-stranded DNA structure. The joint combination of Circular Dichroism (CD), UV-absorption techniques and synchrotron-based UV Resonance Raman spectroscopy allowed us to obtain information on both cooperative and local changes occurring in the DNA structure as a function of temperature (291 to 373 K). Molecular dynamics (MD) simulations showed that PAE molecules interact with nucleobases in the minor and major grooves of double-stranded by π- π stacking interactions. The results showed alteration in DNA melting and pre-melting temperatures and the effect is more dominant in the case of DEP. Raman spectroscopy results show a red shift for dAI ,dGI and dT markers due to the perturbation of the hydrogen shell around the DNA Raman-active groups [5].

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Electron Transport on Bio/Metallic Interfaces

Zdeněk Futera[1], Outi Vilhelmiina Kontkanen[1], Denys Biriukov[1,2]

[1]*Faculty of Science, University of South Bohemia, Branišovská 1760, 370 05 České Budějovice, Czech Republic*

[2]*Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Flemingovo náměstí 542, 160 00 Prague 6, Czech Republic*

Many life-driving processes such as respiration, photosynthesis, and various enzymic catalytic reactions are based on electron transfer reactions facilitated by redox proteins. Especially metalloproteins are known for their efficiency to transfer electrons over relatively long ranges in biological systems. Thanks to these properties, redox-active proteins have been recently utilized in nanobioelectronic devices where they are placed in contact with metal surfaces forming the protein junctions. Interestingly, new physical phenomena emerged in such devices [1]. While the electrons incoherently hop among the available redox sites when the proteins are in their native environment, coherent electron transport was detected on the bio/metallic interfaces and junctions [2].

We study these processes using computer simulations based on combinations of classical molecular dynamics (MD), hybrid quantum-mechanical/molecular-mechanical (QM/MM) approaches, and density functional theory (DFT) [3]. The thermally activated incoherent hopping mechanism is described by Marcus theory, where redox potentials, reorganization free energies, and electronic couplings are the key parameters for predicting transfer rates and electron fluxes. On the other hand, coherent current calculations are based on Landauer formalism requiring evaluation of transmission functions. We demonstrate the performance of these computational techniques by investigating the charge transport properties of small tetraheme cytochrome (STC) and blue-copper protein azurin in contact with the gold electrodes.

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Molecular-level understanding of membrane permeation of some chemicals

Zsófia Borbála Rózsa, Milán Szőri

Institute of Chemistry, University of Miskolc, Miskolc-Egyetemváros A/2, H-3515 Miskolc, Hungary

Due to this day, several xenobiotics are emitted into the environment without proper handling and understanding the dangers they can pose to biological entities. The motivation our work [1], [2] was to better understand the pollution mechanism of potential and proven environmental pollutants on biological membranes. In this presentation the membrane altering effects of 10 chemicals are shown and compared, obtained by classical Molecular Dynamics (MD) simulations and Well-Tempered Metadynamics. All investigated species, both from the classical chemical industrial era (1,4-dioxane, Oxane, Phenol and Morpholine) and the byproducts from a potential synthesis pathway (telomerisation of 1,3-butadiene and carbon-dioxide) were found to pose potential dangers to cellular membranes. During our simulations the concentration dependence of the membrane permeation was investigated in the case of 5 compounds, and it can be clearly observed, that the free energy of membrane permeation has concentration dependence. Based on our studies at a given concentration the narcotic potential and the permeation order of the compounds were set.

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Malarial resistance to chloroquine and protein mutations

Michal Damek, Milan Melicherčík

Department of Nuclear Physics and Biophysics,
Faculty of Mathematics, Physics and Informatics, Comenius University in Bratislava, Slovakia

Malaria is 6th most common reasons for death in low income countries (by WHO statistics) [1]. It is caused by single cell organism from *Plasmodium* species. Even people fight this disease, which today causes cca. 600 000 death every year, from paleolithic era [2], we still don't have drugs for successful treatment of every patient because of many resistant strains.

The digestion of hemoglobin is done in digestive vacuole. Chloroquine (CQ) is assumed to block biocrystalization of free heme into inert hemozoin and causes that parasite cell poisons itself. *Plasmodium falciparum chloroquine resistance transporter* protein (PfCRT) probably causes resistance of CQ and other drugs. It transports peptides and other molecules from digestive vacuole to cytosol and render drugs ineffective. There are strains with different mutations (usually less than 10) with different sensitivity for drugs.

New possibility brought discovery of PfCRT structure from resistant to CQ 7G8 strain [3]. To study this protein from different strains, 3D7 (CQ sensitive) and Dd2 (CQ resistant) strain-specific proteins were also created. MD simulations were carried out in Gromacs using Amber FF in DOPC/DPPC membrane for 8 μs. Simulations however didn't revealed any major structural changes in transmembrane part of protein. However the changes the size and shape of internal cavity of protein, where ligands bounds.

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Growth Mechanisms of Persistent Organic Pollutants - A Case Study of Benzo(a)pyrene

Edina Reizer[1,2], Béla Viskolcz[1,2], Béla Fiser[1,2,3]

[1]*Institute of Chemistry, University of Miskolc, H-3515 Miskolc, Miskolc-Egyetemváros, Hungary*

[2]*Higher Education and Industrial Cooperation Centre, University of Miskolc, H-3515 Miskolc-Egyetemváros, Hungary*

[3]*Ferenc Rákóczi II. Transcarpathian Hungarian College of Higher Education, UA-90200 Beregszász, Transcarpathia, Ukraine*

Persistent organic pollutants (POPs) represent a major class which include various contaminants that have been produced and emitted into the environment. A subclass of POPs is the toxic polycyclic aromatic hydrocarbons (PAHs) which are formed under different incomplete combustion processes. The structure of PAHs is mostly based on benzene ring units but can include penta-rings as well. The larger the structure, the more toxic is the PAH. Thus, it is important to understand and describe the growth mechanisms of PAHs. In suitable conditions, these species can grow and from smaller PAHs larger and larger molecular structures can be formed, while at the end of the process soot particles are reached. In this work, a computational study was carried out to understand the growth of PAHs. To determine potential reaction initiation points of a carefully selected set of structures, all unique C-H BDE (bond dissociation enthalpy) values are calculated and compared [1]. Furthermore, the formation of benzo(a)pyrene (BaP), which is one of the most carcinogenic polycyclic aromatic hydrocarbons, has been described starting from smaller species. Chrysene (Chr) and benzo(a)anthracene (BaA) are considered as starting structures, and the reaction routes are built up based on the HACA (hydrogen abstraction acetylene addition) [2], MAC (methyl addition cyclisation) [3], HAERA (hydrogen abstraction ethynyl radical addition) [2], and Diels-Alder mechanisms [2]. The results contribute to the deeper understanding of PAH formation and growth.

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NH2-Functionalized Magnetic Nanoparticles For The N-glycomic Analysis of Multiple Sclerosis Using Hydrophilic-Interaction Liquid-Chromatography

Dalma Dojcsák[1], Ágnes Ilosvai[2], László Vanyorek[2], Ibolya Gilányi[3], Csaba Oláh[3] and Csaba Váradi[1]

[1]*Advanced Materials and Intelligent Technologies Higher Education and Industrial Cooperation Centre, University of Miskolc, 3515 Miskolc, Hungary*

[2]*Institute of Chemistry, Faculty of Materials Science and Engineering, University of Miskolc, 3515 Miskolc, Hungary*

[3]*Borsod-Abaúj-Zemplén County Center Hospital and University Teaching Hospital, 3526 Miskolc, Hungary*

Multiple sclerosis (MS) is a progressive autoimmune disorder, affecting the central nervous system and spinal cord, leading to damage of myelin and axons without identified cause (1). One of the main problems with MS is that disease identification requires multiple tests due to the lack of specific biomarkers (2). Glycosylation is a critical quality attribute of proteins and reportedly altered in several inflammatory and malignant disorders including MS as well. High-throughput quantitative measurement of protein glycosylation is challenging as glycans lack fluorophore groups thus require fluorescent labeling. The attachment of fluorescent tag to each glycan moiety necessitates the use of sample clean-up for reliable quantitation. The use of magnetic particles in glycan sample preparation is reportedly a cost-effective and automatable solution to accomplish large-scale biomarker discovery studies. Our goal was to develop a novel, cost-effective and automatable glycan sample preparation protocol using in-house synthetized magnetic nanoparticles. This platform will be applied to analyze clinical samples to identify potential glycosylation-based alterations.

Our future plan is to annotate the glycan structures which are significantly different between MS patients and healthy controls and identify the origin of these alterations. Using affinity chromatography, we are aiming to analyze IgG and IgM antibodies from serum samples of MS patients to reveal molecular alterations which can be linked to this disease.

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Development of magnetic nanoparticles aided nucleic acid isolation techniques

Tímea Gerzsenyi[1], Ágnes M. Ilosvai[2], László Vanyorek[2], Béla Viskolcz[1,2], Emma Szőri-Dorogházi[1]

[1]*Higher Education and Industrial Cooperation Centre, University of Miskolc*

[2]*Institue of Chemistry, University of Miskolc, 3515 Miskolc-Egyetemváros, Hungary*

One of the basic needs of molecular biological processes is to be able to isolate DNA molecules from various sources as quickly, simply and free of contaminants as possible. The aim of our work is to test newly synthesized magnetic nanoparticles (MNPs), which can be used in such DNA extraction methods due to their ability to bind nucleic acids. We would like to determine the efficiency of the nucleic acid binding capacity of the mentioned MNPs and compare them with commercially available ones. A strain of Escherichia coli DH5α Gram-negative, non-pathogenic bacterium containing pBAD24 or pBluescript KS(+) plasmids were used as model organisms.

From the obtained MNPs we tested 7 different ones, differing in their composition (NiFe2O4-NH2, CoFe2O4, CoFe2O4-NH2, MnFe2O4, MnFe2O4-NH2, CuFe2O4) and their production time (MgFe2O4-NH2 (12-hour synthesis time), MgFe2O4-NH2 (4-minute synthesis time)). All of the examined nanoparticles were able to reversibly bind DNA. For succesful DNA isolation, MNPs have to form uniformly distributed dispersions, therefore the stability of the dispersions under isolation conditions was investigated. Among the tested magnetic nanoparticles, the amino-functionalized MgFe2O4 (12-hour synthesis) had adequate colloidal stability, so further tests were performed with this MNP.

We verified the presence of DNA in the elution fractions of the isolations through agarose gel electrophoresis and certified that only the extracted, fluorescent-dyed DNA molecules can be seen under the 254 nm UV light. In parallel, we developed a background measurement method for DNA concentration determination of the elution fractions with a conventional photometric method (at λ260 nm). For the DNA isolation with MgFe2O4-NH2 (12-hour synthesis time) MNP we determined the volume of the elution fraction in term to reach the maximum concentration of isolated DNA. We compared the DNA extraction efficiency of MgFe2O4-NH2 MNPs with the commercially available ones. The downstream usability of the DNA isolated with the tested MNPs was also inspected for enzymatic and amplification (PCR) reactions as well.

Aromatic isocyanides as novel potential anti-fungal and anti-cancer agents

Miklós Nagy

Institute of Chemistry, University of Miskolc, Miskolc-Egyetemváros, 3515-Hungary

Isocyanoaminoarenes (ICAAr-s) are a novel and versatile group of solvatochromic flourophores. Despite their versatile applicability, such as antifungals, cancer drugs and analytical probes, they still represent a mostly unchartered territory among Intramolecular Charge-Transfer (ICT) dyes. Recently, we have synthesized a series of new solvatochromic fluorophores based on the preliminary concept of ICT in which the donor amine and the acceptor isocyano groups are connected *via* the naphthalene moiety in its 1,5-positions to yield 1,5-isocyanoamino- (1,5-ICAN) derivatives [1,2]. The 1,5-ICANs exhibit large solvatochromic and Stokes shifts and turned out to be one of the most versatile "smart" fluorophore dye family. They enable the selective detection of Hg^{2+} and at the same time is able to indicate the presence of Ag^{+}, which is unprecedented among fluorescent sensors [3]. The simultaneous presence of the amino, isocyano and naphthalene groups yielded a most effective antifungal drug, the efficacy of which was demonstrated in vivo in mice against *Candida* strains [4]. Moreover, the small modification of acridine orange (the aromatic core is very similar to the anthracene in this study) resulted a very efficient physiological pH-probe and the new isocyano-aminoacridines opened up a new pathway in cancer treatment based on phototoxicity [5].

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Multi-Scale Simulations Provide Insights into Protein Conformation under Crowding

Štěpán Timr[1,2,3], Fabio Sterpone[2,3]

[1] *J. Heyrovský Institute of Physical Chemistry, Czech Academy of Sciences, Dolejškova 3, Prague 8 18223, Czech Republic*

[2] *Laboratoire de Biochimie Théorique (UPR 9080), CNRS, Université de Paris, 13 rue Pierre et Marie Curie, 75005 Paris, France*

[3] *Institut de Biologie Physico-Chimique, Fondation Edmond de Rothschild, PSL Research University, 13 rue Pierre et Marie Curie, 75005 Paris, France*

The function of proteins is tightly linked with the stability of their native conformations, which can be modulated by the dense and heterogeneous environment inside living cells [1,2]. Using multi-scale molecular simulations, we inquire into the effects of macromolecular crowding on the conformations of model proteins such as chymotrypsin inhibitor 2 and superoxide dismutase 1. We consider environments of growing complexity, including crowded protein solutions mimicking the composition of a bacterial cytoplasm. Our simulations reveal a delicate balance between two factors—the entropic excluded volume and weak transient interactions—resulting in a temperature-dependent effect of crowding on protein stability. Moreover, we find that interactions with the surrounding crowded environment can enhance the population of partially unfolded states, which might have an impact on the processes of toxic aggregation and oligomerization. Our computational approach opens the way for a detailed description of how the cellular environment shapes protein conformations and conformational transitions.

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Chlordecone and β-Hexachlorocyclohexane Interaction Functionalized Activated Carbon by Molecular Modelling and Molecular Dynamics Simulation

F. Goudou[2], A. Duro[2], K. Melchor-Rodríguez[1], C. Carmenate-Rodríguez[1], A. Ferino-Pérez[1], J. J. Gamboa-Carballo[1], B. Minofar[4], S. Gaspard[2], U. J. Jáuregui-Haza[3]

[1]*INSTEC, University of Havana, Avenida Salvador Allende, 1110, Quinta de los Molinos, Plaza de la Revolución, A.P. 6163, La Habana, Cuba.*

[2]*Laboratoire COVACHIM M2E, EA 3592, Université des Antilles, 97157 Pointe à Pitre Cedex, Guadeloup*
.

[3]*Instituto Tecnológico de Santo Domingo, Ave. de los Próceres, Santo Domingo, República Dominicana*

4 Institute of Microbiology of the Czech Academy of Science, Zámek 136, Nové Hrady, Czech Republic

e-mail:francesca.goudou@univ-antilles.fr

A molecular modeling study of the influence of acidic [1-2] and basic [3] surface groups (SG) of activated carbon (AC) model on chlordecone (CLD) and β-hexachlorocyclohexane (β-HCH) adsorption is presented, in order to help understanding the adsorption process considering pH and hydration effect. A coronene molecule, with the functional groups under study in the edge, were used as a simplified model of AC. Multiple Minima Hypersurface methodology was employed to study the interactions of CLD and β-HCH with SGs on AC using PM7 semiempirical Hamiltonian. A further re-optimization of obtained structures was done for pesticide-AC complexes by means of Density Functional Theory. The Quantum Theory of Atoms in Molecules was used to characterize the interaction types using the Nakanishi criteria. As results, the interactions are governed by dispersive interactions of chlorine atoms of the pollutants with the graphitic surface and by electrostatic interactions with COO^- and O^- acidic groups and water molecules. For oxygenated basic SGs, like pyrone, chromene and ketone, no interactions have been shown at acidic pH for both pollutants whereas dispersive interactions have been found at neutral and basic pH. For nitrogenous basic SGs, the results showed a greater association of both pesticides with the primary amine in comparison with the pyridine, secondary and tertiary amine in the absence and presence of water molecules, and this behavior increase at acidic pH conditions where the amines and pyridine are protonated. As conclusion, significant associations of acidic SGs with CLD suggest a chemical sorption at slightly acidic and neutral pH conditions. On the other hand, the interactions of both pollutants with basic SGs on AC are similar with the physisorption process. Finally, an increase in carboxylic SGs content is suggested to enhance CLD and β-HCH adsorption onto AC. Molecular dynamics simulation is as well employed to understand experimental findings.

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Dissolving Cellulose with Ionic Liquids

Mike Ries

School of Physics and Astronomy, University of Leeds, Leeds, LS2 9JT, United Kingdom

The dissolution of cellulose is of much interest both academically and industrially. Cellulose is widely used to produce films, fibres, various derivatives, sponges, adhesives, food thickeners and membranes. Cellulose does not melt, so to process this biopolymer requires the use of a solvent, with materials being formed through the method of wet-casting. In 2002 several imidazolium-based ionic liquids were found to be non-derivatising solvents for cellulose, and therefore these offer potential routes to "green" methods of processing cellulose. [1] Ionic liquids are salts that, below 100 °C, are in the liquid state. In this work we have studied the dissolution of glucose, cellobiose, cellulose and flax yarn by the ionic liquid 1-ethyl-3-methylimidazolium acetate [C2mim][OAc]. Nuclear magnetic resonance was used to determine the effects of the presence of carbohydrates on the dynamics (translational and rotational) of the ions. We demonstrate that the key factor in determining the motion of the ions, and indeed their chemical environment, is the density of OH groups coming from the dissolved carbohydrate. [2] For the dissolution of flax yarn, through quantifying how much material dissolved as a function of time and temperature, we found an activation energy for dissolution of 100±10 kJ/mol. [3]

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Abstracts of poster presentations

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

Adsorption of some organic molecules on icy surfaces

Milán Szőri[1] and Pál Jedlovszky[2]

[1]*Institute of Chemistry, University of Miskolc, Miskolc-Egyetemváros A/2, H-3515 Miskolc, Hungary*

[2]*Department of Chemistry, Eszterházy Károly University, Leányka u. 6, H-3300 Eger, Hungary*

The enrichment and self-aggregation of molecules are critical issues in the molecular understanding of the chemical evolution. Adsorption of biologically relevant species on icy surface can be one of the plausible mechanisms for that purpose. Since 2014, the adsorption properties of several molecules had been investigated by grand canonical Monte Carlo technique such as HCN [1], methylamine [2,3], formamide [4], cyanamide [5], propylene oxide [6], benzonitrile [7] and acetamide [8,9] which may allow us to review and restructured the knowledge gained from these simulations to derive a better theory to predict the physicochemical properties of the adlayer(s).

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Theoretical investigation of antioxidant potential of BHA, TBHQ, BHT, and curcumin

Dalal K. Thbayh[1,2,3], Béla Viskolcz[1,3], Béla Fiser[1,3,4]

[1]*Institute of Chemistry, University of Miskolc, 3515 Miskolc-Egyetemváros, Hungary*

[2]*Polymer Research Center, University of Basrah, Basrah, Iraq*

[3]*Higher Education and Industrial Cooperation Centre, University of Miskolc, 3515 MiskolcEgyetemváros, Hungary*

[4]*Ferenc Rákóczi II. Transcarpathian Hungarian College of Higher Education, 90200 Beregszász, Transcarpathia, Ukraine*

Pure polymeric materials are frequently found to have inferior properties, which would lead to their commercial failure. In the absence of additives, polypropylene (PP) and other polyolefins would not be able to keep their properties and decay [1]. Therefore, additives play a significant role in processing and improving the properties of polymers used in many applications. Antioxidant additives are substances that are able to prevent oxidative stress induced deterioration of the materials. There are two main types of antioxidants, synthetic and natural. The antioxidant potential of commonly used synthetic and natural antioxidant additives, including butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tert-butylhydroquinone (TBHQ), and natural additive, curcumin have been studied and compared by calculating the bond dissociation enthalpy (BDE), ionization potential (IP), proton dissociation enthalpy (PDE), proton affinity (PA), and electron transfer enthalpy (ETE) values for each potential hydrogen donor site. Three different density functional theory (DFT) methods have been used, the B3LYP functional in combination with the 6-31G(d) basis set, and the M06 and M06-2X in combination with the 6-311+G(d,p) basis set to study all the species in gas phase. The results indicate that, in each additive an O-H group has the highest antioxidant potential (lowest BDE value). The studied molecules can be ranked based on their antioxidant potential as follows: BHT > BHA ≈ TBHQ > curcumin A > curcumin D. By comparing with commonly used polymers, in each studied species, there is at least one X-H bond which has a lower BDE value than in the corresponding polymeric material. Thus, all studied additives are potentially applicable to protect polymeric materials. BHT is the best radical scavenger additive in case of the hydrogen atom transfer (HAT), and the sequential proton loss electron transfer (SPLET) mechanisms to donate a H atom, but in single electron transfer proton transfer (SETPT) curcumin could also be suitable.

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Experimental and Theoretical Study of Tertiary Amine Catalysed Urethane Formation

Hadeer Q. Waleed[1,2], Zsolt Fejes[1], Béla Viskolcz[1,2], Béla Fiser[1,2,3]

[1]*Institute of Bioorganic Chemistry of NAS of Belarus, Minsk, Belarus*

[2]*Vitebsk state medical university, Vitebsk, Belarus*

[3]*Belarusian Research Center for Pediatric Oncology, Hematology and Immunology, Minsk, Belarus*

Polyurethanes (PUs) are one of the most versatile and unique polymer types being a part of our daily life and over two million tons of PUs synthesized every year in the European Union[1]. Despite of the relatively long history of polyurethanes since its discovery by Otto Bayer and his research group in the 1930s, there are still numerous ongoing research aiming to design better types with superior physical and mechanical properties, reduced carbon footprint and less environmental issues[2], and thus, various synthetic procedures including one or more catalysts are applied to prepare them. For PUs foams, the most important catalysts are nitrogen-containing compounds. Synthesizing PUs from isocyanates and alcohol under industrial conditions requires a combination of catalysts which will expedite the chemical reactions[3]. In this work a kinetic and mechanistic investigation of the alcoholysis of phenyl isocyanate (PhNCO) using stoichiometric butan-1-ol (BuOH) in acetonitrile in the presence of different tertiary amine catalysts was undertaken. The reaction mechanisms without and in thepresence of the experimentally applied catalysts were described by using the G3MP2BHandHLYP composite method. The apparent activation energies obtained from the calculations were in good agreement with the experimental data ($\Delta\Delta E0 < 2$ kJ/mol). Thus, a reliable mechanistic description of the catalytic process was achieved. Both experimental and theoretical results prove the important effect of tertiary amine catalysts on urethane formation. Furthermore, based on the results, the design of new catalysts will be possible in the near future.

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Polyurethane Degradation – A Computational Study

Julie Mallouhi[1], Emma Szőri-Dorogházi[2], Béla Viskolcz[1,2], Béla Fiser[1,2,3]

[1] *Institute of Chemistry, University of Miskolc, 3515 Miskolc-Egyetemváros, Hungary*

[2] *Higher Education and Industrial Cooperation Centre, University of Miskolc, 3515 Miskolc-Egyetemváros, Hungary*

[3] *Ferenc Rákóczi II. Transcarpathian Hungarian College of Higher Education, 90200 Beregszász, Transcarpathia, Ukraine*

The applications of polyurethane (PU) continues to grow at a rapid pace throughout the world, and because of its excellent mechanical, physical, biological, and chemical properties, it used as furniture, coatings, adhesives, and constructional materials.[1] PU contains several urethane bonds (–NH–COO–), which connect the building blocks of the polymer. Thus, the chemical recycling and the degradation of the polymer depend on the strength of the urethane bond.[1] Polyurethanes are exposed to outdoor conditions like sunlight, oxygen, water, chemicals, bacteria, fungi, *etc.* and all these factors can induce the degradation of the material. Biodegradation happens when the main structure undergoes changes in its bonds and the microbe excretes extracellular enzymes, attaches the enzyme to the plastic's surface, and hydrolyzes short polymer intermediates, which are subsequently assimilated as carbon source by microbial cells, producing CO_2.[2]

To better understand polyurethane degradation at the molecular level, three model systems were created, carbamic acid, methyl *N*-methylcarbamate, and phenyl *N*-phenylcarbamate, and studied by using computational chemical tools. Each of the model compounds contains one urethane bond. Thus, the effect of various functional groups around this motif can be studied by comparing the corresponding bond dissociation enthalpies (BDE) within these compounds.[3] The BDEs were computed by using the B3LYP density functional theory (DFT) method in combination with the 6-31G(d) basis set in the gas phase. It was found that in the case of phenyl *N*-phenylcarbamate the degradation is more probable, because the corresponding BDEs are lower by >XXX kJ/mol than in the other two compounds.

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Collagen as a Bio-Material: An All-Atom MD Approach to Understanding Biopolymers

Dimah Zakaraia[1], Michael C. Owen[1,2]

[1]*Institute of Chemistry, Antal Kerpely Doctoral School of Materials Science & Technology, University of Miskolc, Hungary*

[2]*Higher Education and Industrial Cooperation Centre, University of Miskolc, Hungary*

Collagen is a structural protein found in abundance in all mammals [1]. It is a significant fibrous material that is accountable for the structural integrity of many body parts like skin, bone and teeth [2]. In this work we will study the structure of collagen to learn more about its behavior at different temperatures. Molecular dynamics (MD) simulation is a rapidly growing branch of science that has proven to be a reliable method for understanding the dynamic behavior of biomolecules [3]. MD simulation was used to investigate a collagen-like model peptide $(Pro\text{-}Pro\text{-}Gly)_9$ without hydroxyproline residues by the GROMACS 2020 program to know the properties and behavior of this protein also the interactions that happen in this protein. The system was equilibrated and simulated at 300 K, 310 K, 320 K, and 330 K for 100 ns. The results indicates that collagen is a hydrophobic polymer. Cluster analysis revealed that the largest cluster was at 300 K, which suggests that the structure of the protein was the most stable at this temperature. Moreover, the RMSD was lowest at 300 K, which in another indicator of stability. Using MD simulations to investigate the behavior of collagen at different temperatures gives information on the behavior, structure, and stability of collagen and gain insight into the stability of polymers in general.

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The interaction of crowding agents and salts on structure and dynamics of biomolecules

Anastasiia Shaposhnikova[1,2], Fatemeh Fadaei[1,2], Babak Minofar[2]

[1]*Faculty of Science, University of South Bohemia in Ceske Budejovice, Czech Republic*

[2]*Laboratory of Structural Biology and Bioinformatics,*
Institute of Microbiology of the Czech Academy of Sciences, Czech Republic

The influence of specific salts and macromolecular crowding can have a significant impact on the structural stability and dynamic properties of biomolecules. We studied the stability of human serum albumin (HSA), human rhinovirus-14 protease 3C (HRV), and glutathione (GSH) with the effects of salts and macromolecular crowding. Molecular dynamics (MD) simulation of aqueous solutions of biomolecules was performed for comparison with the experimental methods.

The GROMACS package was used to carry out the molecular dynamics simulations of systems. After the simulation in our studies, the following methods have been used: the root mean square deviation (RMSD) to show structural fluctuations which are associated with biological function; the root mean square fluctuation (RMSF) analysis to compute the fluctuations of each subset of the structure (atom or residue) relative to the average structure of the simulation; the number of hydrogen bonds (HB) in time and radial distribution function (RDF) to investigate the distribution of one molecule or an atom around one specific molecule or atom.

Our results show that salts and crowding agents are able to influence the dynamics, folding and structural stability of biomolecules. It was confirmed by experimental and computational methods.

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Effect of phthalic acid esters on DNA and removal of them with cyclodextrins

Volkan Cirik[1], Isabella Swartz[2] David Reha[1,3], Babak Minofar[1,3]

[1] *Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic*

[2] *Princeton University, Princeton, NJ 08544, USA*

[3]*Laboratory of Structural Biology and Bioinformatics, Institute of Microbiology of the Czech Academy of Sciences, Zámek 136, Nové Hrady, Czech Republic*

Phthalic acid esters (PAE) are a group of plasticizers that are widely used in the manufacture of products such as personal care products, packaging materials, and plastics to soften them [1]. There are many studies that report the toxicity of PAEs in humans [2] and their damage to DNA [3]. In the first part of this study, the effect of PAEs on a 30-base pair of double-stranded DNA was investigated using molecular dynamics simulations. The aromatic ring of PAEs and the aromatic ring of nucleotide bases show π- π stacking interaction. To measure the interaction between DNA and PAEs, a distance and radial distribution function analysis was used. The effect of this interaction on the structure of DNA was analyzed by calculating the root mean square deviation.

In the second part of the study, the removal of PAEs with cyclodextrins was investigated. The unique hydrophobic cavity of cyclodextrins is capable of hosting molecules inside. It makes them one of the most efficient ways to remove them. Molecular dynamics simulations were used to investigate the interaction between cyclodextrins and PAEs.

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Evaluation of ionic liquid (1,4-Diazabicyclo octane (DABCO)) as permeability enhancer in orally dissolving films (ODFs) for buccal administration of antihistamine Diphenhydramine HCl drug for children

Riana Uddin[1], Sarthak Shah[2], Paula Lince[2], Mohammad Nasir Uddin[2]

[1] *Northview High School, Atlanta, GA 30005, USA*

[2] *College of Pharmacy, Mercer University, Atlanta, GA, USA*

Ionic Liquids (IL) are salts composed of a cation and anion. IL has a wide range of applications from engineering to pharmaceutical sciences. In pharmaceutical sciences, IL can be used as transdermal enhancers, permeability and stability enhancers. Increasingly, novel methods of delivery are emerging every day and moving away from the traditional injection delivery method. Specially for child patient who has needle fear, injection is a big issue. Injecting needle is a common fear among almost all the children. Buccal administration can be a good alternative to injection that can overcome this fear issue. Buccal administration is an exciting route of administration. This is great site of delivering a drug or vaccine which can avoid first pass effect and can reach the blood circulation quickly. In this project, we formulated an orally dissolving film that can be administered in the buccal area to deliver an antihistamine drug. For the buccal administration, easy drug permeation through the membrane to blood circulation is the most important determinant. In this research project, we explored ionic liquid (1,4-Diazabicyclo octane (DABCO)) as permeability enhancer in ODF films to delivery an antihistamine drug, diphenhydramine hydrochloride. We investigated this IL to determine its safety and permeability enhancement ability in an ODF formulations.In the second part of the study, the removal of PAEs with cyclodextrins was investigated. The unique hydrophobic cavity of cyclodextrins is capable of hosting molecules inside. It makes them one of the most efficient ways to remove them. Molecular dynamics simulations were used to investigate the interaction between cyclodextrins and PAEs.

An orally dissolvable film dosage forms was prepared by the solvent casting method in petri dishes. Kollidon F90 and Kollidon VA64 was used as hydrophilic polymeric carriers, PEG 2000 was added as plasticizer for the oral films, HPMC grade K4M was added to the films to enhance flexibility of the films, diphenhydramine hydrochloride was used as the drug. In the formulations, different concentrations of (1,4-Diazabicyclo octane (DABCO)) was added. The films were characterized in terms of fold endurance, thickness, morphology, weight variation, disintegration time, tensile strength, % elongation, in vitro percent drug release, and ex vivo drug permeability capability using porcine buccal mucosa. Cytotoxicity assay was done using the 2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. The results showed that formulation with IL exhibited a significantly higher permeability versus the control.