



VISEGRAD SYMPOSIUM ON BIOMOLECULAR INTERACTIONS

PROGRAM & ABSTRACTS

19th – 22nd June 2022

Nové Hradý

Czech Republic

Organization



*Laboratory of Structural Biology and Bioinformatics
Institute of Microbiology
Czech Academy of Sciences*



Academy and University Center of Nove Hradý

Scientific Organizing Committee:

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Local Organizing Team

David Řeha

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Invited Speakers

Barbara Rossi, Elettra Synchrotron Trieste, Italy

Christian Schröder, University of Vienna, Austria

Eva Pluhařová, J.H. Institute of Physics and Chemistry of CAS, Prague, Czech Republic

Vojtech Spiwok, University of Chemistry and Technology, Prague, Czech Republic

Jan Vacek, Palacký 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 (Mickole):** 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)**: Plastic degradation using enzymes
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 Coffee 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

- 14:00-14:15 **Béla Fiser (Miskolc)**: Growth Mechanisms of Persistent Organic Pollutants – A Case Study of Benzo(a)pyrene
14:15-14:30 **Rachid Hadjadj (Miskolc)**: Monoethanolamine assisted CO₂ hydrogenation to methanol - A mechanistic study
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. **Ágnes Ilosvai (Miskolc):** Sonochemical combined synthesis of nickel-ferrite and cobalt-ferrite magnetic nanoparticles and their application in glycan analysis
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

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Christian Schröder

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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 Spiwok¹, Martin Kurečka², Aleš Křenek²

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

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

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

[1] A. Laio, M. Parrinello, *Proc. Natl. Acad. Sci. USA*, **2002**, 99, 12562–12566.

[2] P. Raiteri, A. Laio, F.L. Gervasio, C. Micheletti, M. Parrinello, *J. Phys. Chem. B*, **2005**, 110, 3533–3539.

[3] Z. Šučur, V. Spiwok, *J. Chem. Theory Comput.*, **2016**, 12, 4644–4650.

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

Barbara Rossi

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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 through the investigation of their vibrational dynamics. Thanks to the resonance effect, UV Resonance Raman (UVR) 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 chromophores within the sample. This determines the usefulness of UVR 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 UVR 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 UVR 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 UVR 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 UVR method with particular attention to biophysical and biochemical systems.

[1] IUVS beamline@Elettra, <https://www.elettra.trieste.it/it/lightsources/elettra/elettra-beamlines/iuvs/iuvs.html>

[2] B. Rossi, C. Bottari, S. Catalini, A. Gessini, F. D’Amico, C. Masciovecchio, Synchrotron based UV Resonant Raman scattering for material science, Molecular and Laser Spectroscopy, Volume 2 (eds V. P. Gupta, Y. Ozaki), Elsevier, **2020**, Chapter 13, pages 447-478

Serum Albumin as a Multifunctional and Model Protein

Jan Vacek

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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.*

- [1] Hernychova L., et al.: *Int. J. Biol. Macromol.* 203 (2022) 116–129.
- [2] Zatloukalova M., et al.: *Redox Biol.*, 46 (2021), 102097.
- [3] Vacek J. and Hrbac J.: *Bioanalysis*, 12 (2020) 1337–1345.
- [4] Vacek J., et al.: *Curr. Opin. Electrochem.* 12 (2018) 73–80.
- [5] Vacek J., et al.: *Anal. Biochem.* 550 (2018), 137–143.
- [6] Havlikova M., et al.: *Anal. Chem.* 87 (2015) 1757–1763.

Imaging Tools for Cancer Research and Drug Discovery

Hashem O. Alsaab

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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

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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 ω B2GP-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 stochastic 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

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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 R₉ and K₉ 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 Ca²⁺ salt concentrations, showing that the apparent strength of adsorption of R₉ 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 R₉ and K₉, we first demonstrated that the binding of R₉ to POPC is tighter by almost two orders of magnitude compared to that of K₉. Finally, upon the addition of excess of either Na⁺ or Ca²⁺ ions with R₉, the total fluorescence correlation signal is lost which implies the unbinding of R₉ 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.*

[1] M. Vazdar et al, *Journal of Physical Chemistry B*, **2013**, 117, 11530-11540.

[2] A. D. Robison et al, *Journal of Physical Chemistry B*, **2016**, 120, 9287-9296.

[3] M. N. T. Hong et al, *submitted*.

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

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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].

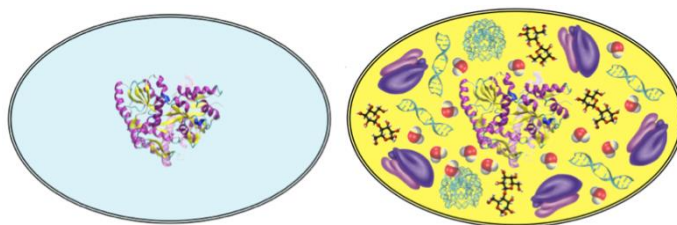


Figure: Illustration of a protein present in the conventional aqueous buffer (left) and in the cell (right).

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 cellular interior.

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

[1] S. B. Zimmerman, A. P. Minton, *Annu. Rev. Biophys. Biomol. Struct.* **1993**, 22, 27–65.

[2] R. J. Ellis, *Trends Biochem. Sci.* **2001**, 26, 597–604.

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

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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.

References

- [1] S. Pramanik, F. Contreras, M. D. Davari, U. Schwaneberg, *Protein Engineering: Tools and Applications* **2021**, 153-176.
- [2] U. Markel, L. Zhu, V. J. Frauenkron-Machedjou, J. Zhao, M. Bocola, M. D. Davari, K.-E. Jaeger, U. Schwaneberg, *Catalysts* **2017**, 7, 142.
- [3] H. Cui, M. Vedder, U. Schwaneberg, M. D. Davari, in *Enzyme Engineering*, Springer, **2022**, pp. 179-202.
- [4] aH. Cui, H. Cao, H. Cai, K. E. Jaeger, M. D. Davari, U. Schwaneberg, *Chemistry* **2020**, 26, 643; bH. Cui, T. H. Stadtmüller, Q. Jiang, K. E. Jaeger, U. Schwaneberg, M. D. Davari, *ChemCatChem* **2020**, 12, 4073-4083; cH. Cui, L. Zhang, L. Eltoukhy, Q. Jiang, S. K. b. Korkunç, K.-E. Jaeger, U. Schwaneberg, M. D. Davari, *ACS Catalysis* **2020**, 10, 14847-14856; dH. Cui, L. Eltoukhy, L. Zhang, U. Markel, K. E. Jaeger, M. D. Davari, U. Schwaneberg, *Angewandte Chemie International Edition* **2021**, 60, 11448-11456; eH. Cui, K. E. Jaeger, M. D. Davari, U. Schwaneberg, *Chemistry—A European Journal* **2021**, 27, 2789-2797; fH. Cui, M. Vedder, L. Zhang, K. Jaeger, U. Schwaneberg, *ChemSusChem* **2022**.

The glycan headgroup affects the nanoscopic segregation of gangliosides

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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 GM₁, GM₂ and GM₃ 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 GM₂, however, impairs the stability of these nanodomains by disrupting hydrogen bonding of neighbouring sugars, which is reflected in a broad size distribution of GM₂ 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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Plastic degradation using enzymes

Per-Olof Syrén

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In this contribution I will present recent results from my group, and from ongoing collaborations at KTH (Prof. István Fűr  and Prof. Minna Hakkarainen), on biocatalytic modifications of building blocks and enzymatic plastic recycling adhering to the 12 principles of green chemistry.

Biocatalytic synthesis of monomers and upgrading of plastics into added-value compounds holds great promise in tackling the “plastic problem” by mild green chemistries. Still, designer enzymes for material applications remain scarcely explored. Hence, enzymatic degradation of plastics only works for a few polymer types[1] at rates typically prohibitively slow for industrial applications. Strong secondary structures displayed by bulky polymers is a major challenge obstructing enzymatic degradation of man-made materials. Also, the backbone of polymers adopts different conformations. The extent of conformational change of enzyme and substrate required for efficient biocatalysis remains poorly understood. Herein we demonstrate[2] how enzyme design can be combined with chemical engineering to generate matching productive enzyme and polymer conformations, resulting in orders of magnitude increased enzymatic depolymerization rate, using polyesters as model system.

The forefront in sustainability science lays in the development of biocatalytic processes based on engineered enzymes, to enable efficient generation of biomaterials and fine chemical synthons from renewable resources under mild reaction conditions. Still, the bulk (ca 98%) of all man-made materials are fossil-based and their generation is associated with resource inefficiency, accumulation of non-recyclable plastic waste and severe environmental impacts. Hitherto more than 5 billion tons of polymer waste has accumulated in land- and aquatic ecosystems with severe environmental consequences. Our concept of focusing on generating matching enzyme and substrate conformations could facilitate monomer synthesis[3,4] and enable rapid biocatalytic depolymerization of plastics into synthons apt for further enzymatic valorization.

References:

- [1] J nsson, C.; Wei, R.; Biundo, A.; Landberg, J.; Schwarz Bour, L.; Pezzotti, F.; Toca, A.; Jacques, L. M.; Bornscheuer, U.T.; Syr n, P.-O. *ChemSusChem* **2021**, *14*, 4028–4040.
- [2] Guo, B.; Vanga, S.; Saenz-Mendez, P.; Lopez-Lorenzo, X.; R nnblad Ericsson, S.; Fang, Y.; Ye, X.; Schrieffer, K.; B ckstr m, K.; Biundo, A.; Zubarev, R. A.; Fűr , I.; Hakkarainen, M.; Syr n, P.-O. *ACS Catal.* **2022**, *12*, 3397–3409.
- [3] Stamm, A.;  hlin, J.; Mosbech, C.; Ols n, P.; Guo, B.; S derberg, E.; Biundo, A.; Fogelstr m, L.; Bhattacharyya, S.; Bornscheuer, U. T.; Malmstr m, E.; Syr n, P.-O. *JACS Au* **2021**, *11*, 1949–1960.
- [4] Schrieffer, K.; Saenz-Mendez, P.; Srilakshmi R.; Hendrikse, N. M.; Hudson, E. P.; Biundo, A.; Schnell, R.; Syr n, P.-O. *J. Am. Chem. Soc.* **2021**, *143*, 3794–3807

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

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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 several 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

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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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References

- [1] A. Paloma, E. Tudurí, L. Marroquí, I. Quesada, R. M. Sargis, and A. Nadal, **2019**, 764-775.
- [2] R. W. Benson, "Hazards of Food Contact Material: Phthalates." Encyclopedia of food safety 2, **2014**, 438-443.
- [3] S. Saeidnia, "Phthalates." Wexler, P., Encyclopedia of Toxicology, 3rd ed., Academic Press, London, **2014**, 928-933.
- [4] C. Bottari, S. Catalini, P. Foggi, I. Mancini, A. Mele, D.R. Perinelli, A. Paciaroni, A. Gessini, C. Masciovecchio, B. Rossi, J. Mol. Liq., **2021**, 330, p.115433.

Electron Transport on Bio/Metallic Interfaces

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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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[1] K. Garg, et al., *Chem. Sci.* **2018**, 9, 7304-7310.

[2] Z. Futera, et al., *J. Phys. Chem. Lett.* **2020**, 11, 9766-9774.

[3] O. V. Kontkanen, D. Biriukov, Z. Futera, *J. Chem. Phys.* **2022**, 156, 175101.

[4] Z. Futera, X. Wu, J. Blumberger, *Small* **2022**, submitted.

Molecular-level understanding of membrane permeation of some chemicals

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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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- [1] Z. B. Rózsa, E. Szőri-Dorogházi, B. Viskolcz, and M. Szőri, “Transmembrane penetration mechanism of cyclic pollutants inspected by molecular dynamics and metadynamics: the case of morpholine, phenol, 1,4-dioxane and oxane,” *Phys. Chem. Chem. Phys.*, vol. 23, pp. 15338–15351, 2021.
- [2] Z. B. Rózsa, L. J. Németh, B. Jójárt, K. Nehéz, B. Viskolcz, and M. Szőri, “Molecular Dynamics and Metadynamics Insights of 1,4-Dioxane-Induced Structural Changes of Biomembrane Models,” *J. Phys. Chem. B*, vol. 123, no. 37, pp. 7869–7884, 2019.

Malarial resistance to chloroquine and protein mutations

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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 μ s. 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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[1] WHO: The top 10 causes of death, **2020**, <https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death>.

[2] Harper K, Armelagos G, Int. J. Environ. Res. and Pub. Health, **2011**, 7 (2): 675–97.

[3] Kim, J., Tan, Y. Z., Wicht, K. J. et al. Nature, **2019**, 576, 315–320.

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

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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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[1] E. Reizer and B. Fiser, *Arab. J. Chem.*, **2022**, 15, 6, 103839.

[2] E. Reizer, I. G. Csizmadia, K. Nehéz, B. Viskolcz, and B. Fiser, *Chem. Phys. Lett.*, **2021**, 772, 138564.

[3] E. Reizer, I. G. Csizmadia, Á. B. Palotás, B. Viskolcz, and B. Fiser, *Molecules*, **2019**, 24 (6), 1040.

Monoethanolamine assisted CO₂ hydrogenation to methanol - A mechanistic study

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The use of hydrogen produced from the electrolysis of water in carbon dioxide reduction can be an interesting solution for both to reduce carbon emission into the atmosphere and to solve the problem related to the storage of renewable energy [1]. The synthesis of methanol from CO₂ is a complicated process which is usually performed catalytically in gas phase. Nowadays, many scientists expressed interest in realizing the reaction also in aqueous phase [2]. In this direction, monoethanolamine (MEA) can be used as a solvent to capture carbon dioxide in an aqueous phase [3]. In the current work, the possibility of hydrogenation of one of the species (HOC₂H₄NH-COOH) forming during the capture process has been investigated. A non-catalysed and a catalysed-like hydrogenation mechanism of the selected intermediate molecule leading to methanol (and MEA+water) has been studied by using computational chemistry tools in aqueous phase. The calculations predicted that the highest relative Gibbs free energy barrier in the catalysed-like process is equal to 114.67 kJ/mol, which is 227.71 kJ/mol lower than the corresponding value of the non-catalysed process. Furthermore, the energy storage efficiency of the catalysed-like pathway has been calculated and it was found to be equal to 96.68%, while the non-catalysed mechanism was only 12.75% efficient.

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[1] A. I. Osman, N. Mehta, A. M. Elgarahy, M. Hefny, A. Al-Hinai, A. H. Al-Muhtaseb, D. W. Rooney, *Environmental chemistry letters*, **2022**, 20, 153–188.

[2] G. Rigbi, R. Magri, A. Selloni, *J. Phys. Chem.*, **2019**, 123, 9875.

[3] B. Lv, B. Guo, Z. Zhou, G. Jing, *Environ. Sci. Technol.*, **2015**, 49, 17.

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

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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 synthesized 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.

Acknowledgements: *This study was supported by the 2020-1.1.6-Jövő-2021-00009 project.*

[1] Koch, L.C.; Rumrill, P.D.; Chan, F. Multiple sclerosis in the COVID-19 era: Vocational rehabilitation strategies to meet the needs of a growing and immunevulnerable clientele. *Journal of Vocational Rehabilitation*.

[2] Murray, T.J. The history of multiple sclerosis: From the age of description to the age of therapy. *Primer on multiple sclerosis* 2016, 3-10.

Development of magnetic nanoparticles aided nucleic acid isolation techniques

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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* DH5 α 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 successful 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 λ 260 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

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Isocyanaminoarenes (ICAAr-s) are a novel and versatile group of solvatochromic fluorophores. Despite their versatile applicability, such as antifungals, cancer drugs and analytical probes, they still represent a mostly uncharted 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-isocyanamino- (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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[1] Rácz, D.; Nagy, M.; Mándi, A.; Zsuga, M.; Kéki, S., *Journal of Photochemistry and Photobiology A: Chemistry* **2013**, 270, 19-27.

[2] Nagy, M.; Rácz, D.; Nagy, Z.L.; Fehér, P.P.; Kalmár, J.; Fábián, I.; Kiss, A.; Zsuga, M.; Kéki, S., *Sens. Actuators B Chem.* **2018**, 255, 2555–2567.

[3] Nagy, M.; Kovács, S. L.; Nagy, T.; Rácz, D.; Zsuga, M.; Kéki, S., *Talanta* **2019**, 201, 165-173.

[4] Nagy, M.; Szemán-Nagy, G.; Kiss, A.; Nagy, Z. L.; Tálas, L.; Rácz, D.; Majoros, L.; Tóth, Z.; Szigeti, Z. M.; Pócsi, I.; Kéki, S., *Molecules* **2020**, 25, (4), 903.

[5] Bankó, C.; Nagy, Z. L.; Nagy, M.; Szemán-Nagy, G. G.; Rebenku, I.; Imre, L.; Tiba, A.; Hajdu, A.; Szöllősi, J.; Kéki, S.; Bacso, Z., *Cancers* **2021**, 13, (22), 5652.

Multi-Scale Simulations Provide Insights into Protein Conformation under Crowding

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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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[1] J. Danielsson, X. Mu, L. Lang, H. Wang, A. Binolfi, F.-X. Theillet, B. Bekei, D. T. Logan, P. Selenko, H. Wennerström, M. Oliveberg, *Proc. Natl. Acad. Sci. U.S.A.*, **2015**, 112, 12402–12407.

[2] D. Gnutt, S. Timr, J. Ahlers, B. König, E. Manderfeld, M. Heyden, F. Sterpone, S. Ebbinghaus, *J. Am. Chem. Soc.*, **2019**, 141, 4660–4669.

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

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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.

References

- [1] J.J. Gamboa-Carballo *et al.*, Theoretical Study of Chlordecone and Surface Groups Interaction in an Activated Carbon Model under Acidic and Neutral Conditions. *J. Mol. Graph. Mod.* 65 (2016) 83-93.
- [2] K. Melchor-Rodríguez *et al.*, Chlordecone adsorption on functionalized activated carbons: Computational chemistry as a tool for understanding the adsorption process. *Quim. Nova.* 44(2) (2021) 172-179.
- [3] K. Melchor-Rodríguez *et al.*, Role of Basic Surface Groups of Activated Carbon in Chlordecone and β -Hexachlorocyclohexane Adsorption: A Molecular Modelling Study. *Molecules.* 26 (2021) 6969.

Dissolving Cellulose with Ionic Liquids

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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]

[1] Journal of the American Chemical Society, 2002, 124 (18), 4974–4975.

[2] Journal of Physical Chemistry B, 2018, 122, 8763-8771.

[3] Carbohydrate Polymer Technologies and Applications, 2021, 2, 100021, 1-8.

Abstracts of poster presentations

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

Sonochemical combined synthesis of nickel-ferrite and cobalt-ferrite magnetic nanoparticles and their application in glycan analysis

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The combination of sonochemical activation of $\text{Ni}(\text{NO}_3)_2$ and $\text{Co}(\text{NO}_3)_2$ in the presence of $\text{Fe}(\text{NO}_3)_3$ and poly(ethylene-glycol) and consecutive heat treatment of the formed metal hydroxides offer a cheap and efficient method for the preparation of nickel-ferrite and cobalt-ferrite magnetic nanoparticles, which can be successfully applied in the selective capture of fluorescently derivatized N-glycans from human serum. XRD measurement revealed that besides the ferrite phase, nickel and cobalt oxides also form during heat treatment. The amount of simple metal oxides can be well controlled by the temperature of the heat treatment, since increasing temperature yielded higher spinel content. For both nickel and cobalt, the best heat treatment temperature was found to be 673 K, where the samples contained 84.1 % nickel ferrite, and in the case of cobalt almost pure (99.6 %) cobalt ferrite could be prepared. FT-IR and zeta potential measurements indicated the presence of surface OH groups, which aid the dispersion of the particles in water and in addition can promote the adsorption of polar compounds. The practical applicability of the magnetic nanopowders was demonstrated in the purification of fluorescently derivatized N-glycans (from human serum). Cobalt-ferrite was found to be the most effective. Owing to the easy preparation and the simplicity of magnetic separation the pure cobalt-ferrite magnetic nanoparticles could be efficient tools for the selective enrichment of serum N-glycans in HPLC measurements.

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

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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 \approx 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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[1] H. Bockhorn, A. Hornung, U. Hornung, D. Schawaller, Journal of Analytical and Applied Pyrolysis, 1999, 48, 93-109.

Experimental and Theoretical Study of Tertiary Amine Catalysed Urethane Formation

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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 Union¹. 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 issues², 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 reactions³. 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 the presence 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 ($\Delta\Delta E_0 < 2$ 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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[1] Y. Yanping, Mater. Sci. Mater. Rev., 2018, 1, 1–8.

[2] M. F. Sonnenschein. John Wiley & Sons, Inc., First Edit., 2015.

[3] B. Eling, Ž. Tomović and V. Schädler, Macromol. Chem. Phys., 2020, 221, 1–11

Polyurethane Degradation – A Computational Study

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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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[1] A. Reghunadhan, S. Thomas, *Elsevier Inc.*, **2017**, 1-16.

[2] K. Skleničková, S. Abbrent, M. Halecký, V. Kočí, H. Beneš, *Crit. Rev. Environ. Sci. Technol.* **2020**, 1–46.

[3] J.B. Huang, G.S. Zeng, X.S. Li, X.C. Cheng, H. Tong, *IOP Conf. Ser. Earth Environ. Sci.*, **2018**, 167, 1-7.

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

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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-Pro-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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- [1] M. D. Shoulders and R. T. Raines, “Collagen structure and stability,” *Annu. Rev. Biochem.*, vol. 78, pp. 929–958, 2009.
- [2] C. Hongo, K. Noguchi, K. Okuyama, Y. Tanaka, and N. Nishino, “Repetitive interactions observed in the crystal structure of a collagen-model peptide, [(Pro-Pro-Gly)₉]₃,” *J. Biochem.*, vol. 138, no. 2, pp. 135–144, Aug. 2005.
- [3] van Gunsteren, “Molecular dynamics simulations.”

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

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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.

[1] Z.E. Eremenko, E.M. Ganapolskii. Method of microwave measurement of dielectric permittivity in small volume of high loss liquid using hemispherical cavity resonator. *Measurement Science and Technology*, **2003**, 14, 2096 - 2103.

[2] E. Dusekova, B. Minofar, E. Sedlak et al. Specific anion effect on properties of HRV 3C protease. *Biophys Chem.*, **2022**, 287:106825.

[3] S. Catalini, B. Rossi, F. Bruni et al. Aqueous solvation of glutathione probed by UV resonance Raman spectroscopy. *Molecular Liquids*, **2019**, 283, 537-547.

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

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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.

References

- [1] Gao DW; Wen ZD, Phthalate esters in the environment: A critical review of their occurrence, biodegradation, and removal during wastewater treatment processes, *Sci Total Environ.* 2016;541:986–1001.
- [2] Øie L, Hersoug LG, Madsen JØ, Residential exposure to plasticizers and its possible role in the pathogenesis of asthma, *Environ Health Perspect*, 1997;105(9):972–8.
- [3] Li L, Zhang T, Qin XS, Ge W, Ma HG, Sun LL, et al, Exposure to diethylhexyl phthalate (DEHP) results in a heritable modification of imprint genes DNA methylation in mouse oocytes, *Mol Biol Rep*, 2014;41(3):1227–35.

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

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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.