universität innsbruck

ISQBP2022

President's meeting 2022

Innsbruck, July 11 – 14, 2022

International Society of Quantum Biology and Pharmacology

Schrödinger JCTC JCIM

Welcome to the ISQBP President's meeting 2022 in Innsbruck.

We are happy to welcome you to the 2022 Meeting of the International Society of Quantum Biology and Pharmacology (ISQBP).

For the past 50 years, the ISQBP has organized dynamic biennial meetings providing a forum to discuss and extend the impact of computational methodologies in the fields of biology, chemistry, chemical biology, and pharmacology.

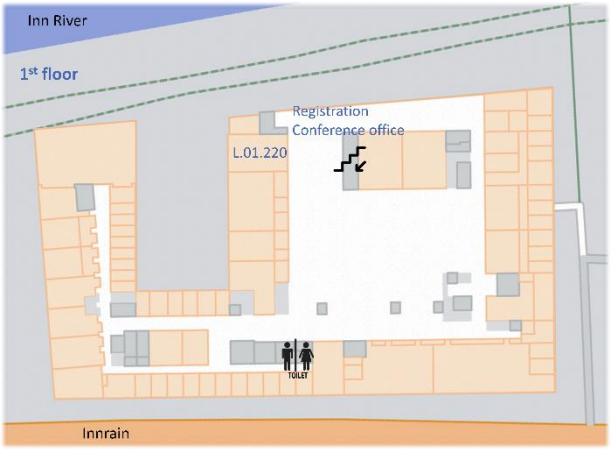
We are happy to host the 2022 meeting in Innsbruck, Austria from July 11-14th.

Klaus Liedl

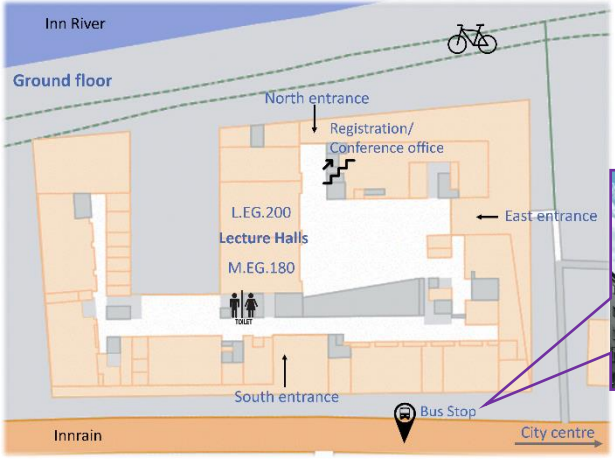
President of the ISQBP

Map of the Venue

1st Floor



Ground Floor



Schrödinger JCTC JCIM

Schrödinger

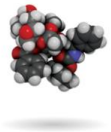
Schrödinger's industry-leading computational platform facilitates the research efforts of biopharmaceutical and industrial companies, academic institutions, and government laboratories worldwide. Schrödinger also has wholly-owned and collaborative drug discovery programs in a broad range of therapeutic areas.

Schrödinger is deeply committed to investing in the science and talent that drive its computational platform. Schrödinger was founded in 1990, has over 500 employees and is engaged with customers and collaborators in more than 70 countries. To learn more, visit https://www.schrodinger.com/. Follow us on LinkedIn and Twitter.

Find the solutions you're looking for:

DRUG DISCOVERY

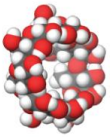
Comprehensive solutions to accelerate lead discovery and optimization, validated by our collaborators' success across a wide array of targets.



Learn more

MATERIALS SCIENCE

Molecular simulations of chemical systems to design materials essential to modern life.



Learn more

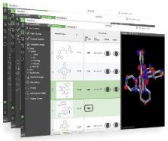
SOLUTION SPOTLIGHT:
LIVEDESIGN

LiveDesign, a linchpin of our platform, enables interactive and collaborative molecule design, aggregation and sharing of data, and end-to-end discovery project coordination between chemists, modelers, and biologists. Learn more about our user-friendly enterprise informatics solution.

Learn more

REQUEST INFO

Our platform is used by industry leaders globally. See how our physics-based methods can help you open new worlds of possibility.



Learn More

ACADEMIC LICENSING

We support the next generation of scientists through special licensing for academic institutions.

Learn More

Time	Monday 11.7.2022	Tuesday 12.7.2022	Wednesday 13.7.2022	Thursday 14.7.2022
Session Chair	*Klaus Liedl*	*Monte Pettitt*	*Zoe Cournia*	*Thomas Cheatham*
09:00-09:20	**Gerhard Hummer** *(Computational Biology Award)*	**Nigel Richards**	**Tom Kurtzman**	**Michele Parrinello**
09:20-09:40				
09:40-10:00	**Rodrigo Ochoa**	**Nadja Katharina Singer**	**Franz Waibl**	**Christine Peter**
10:00-10:20	**Anastasia Croitoru**	**Callum Matthew Ives**	**Cedric Vallee**	
10:20-10:50	*Coffee Break*			
Session Chair	*Lennart Nilsson*	*Tom Kurtzman*	*Edina Rosta*	*Nigel Richards*
10:50-11:10	**Krystel El Hage**	**Lennart Nilsson**	**Sílvia Osuna**	**Jana Shen**
11:10-11:30	**Martin Spichty**			
11:30-11:50	**Stephanie Linker**	**Roland Stote**	**Vladimir Sladek**	**Monica Fernández-Quintero**
11:50-12:10	**Chris Oostenbrink**	**Patrick K. Quoika**	**Giulia D'Arrigo**	*Best Poster Prize*
12:10-12:30	*Conference Photo*	*Lunch Break*		
13:00-14:00	*Lunch Break*			
Session Chair	*Jana Shen*	*Alexander Mackerell*	*Chris Oostenbrink*	
14:00-14:20	**Alexander Mackerell**	**William L. Jorgensen**	**Sereina Riniker**	
14:20-14:40				
14:40-15:00	**Carmen Domene**	**Flash Talks (P17 – P32)**	**Edina Rosta**	
15:00-15:20	**Flash Talks (P1 – P16)**			
15:20-16:00		*Coffee Break & Poster Session*	*Coffee Break*	
Session Chair	*Jana Shen*		*Klaus Liedl*	
16:00-16:20	*Coffee Break & Poster Session*		**Zoe Cournia**	
16:20-16:40				
16:40-17:00			**Monte Pettitt**	
17:00-17:20			**Stephan Ehrlich**	
17:20-17:40		**Charles L. Brooks**	**Thomas Cheatham** *(Loew Lectureship)*	
17:40-18:00				
18:00-18:20	**Yun Lyna Luo**			
18:20-18:40				
18:40			*Gala Dinner*	

Contents

Conference Program 1
Posters 5
Oral contributions' abstracts 8
Posters' abstracts 30
Participants list 62
Lunch Suggestions 65

Conference Program

Sunday 10.7.2022

Welcome Session		
16:00-19:00		Registration & Welcome Reception

Monday 11.7.2022

Session 1 (Chair: Klaus Liedl)		
09:00-09:40	**Gerhard Hummer** *(Computational Biology Award)*	*Molecular simulations in the era of AI and exascale computing: are we ready?*
09:40-10:00	**Rodrigo Ochoa**	*Protocol for iterative optimization of modified peptides bound to protein targets*
10:00-10:20	**Anastasia Croitoru**	*Parametrization of nonstandard amino acids for the CHARMM force field*
10:20-10:50		Coffee Break
Session 2 (Chair: Lennart Nilsson)		
10:50-11:10	**Krystel El Hage**	*Targeting RNA:Protein Interactions using an Integrative Approach: Identification of Potent YB-1 Inhibitors*
11:10-11:30	**Martin Spichty**	*The fate of oxidized methionine: insights from computer simulations and spectroscopic experiments*
11:30-11:50	**Stephanie Linker**	*Lessons for Oral Bioavailability: How Conformational Flexible Cyclic Peptides Enter and Cross Lipid Membranes*
11:50-12:10	**Chris Oostenbrink**	*Hot, Hotter, BuRNN: A new scheme for polarizable QM/MM simulations with machine-learning*
12:10-12:30		Conference Photo
12:30-14:00		Lunch Break

Session 3 (Chair: Jana Shen)		
14:00-14:40	**Alexander Mackerell**	*Site Identification by Ligand Competitive Saturation (SILCS): From ligand design to modeling of macromolecular interactions*
14:40-15:00	**Carmen Domene**	*How can cells detect and respond to oxygen levels? Computer simulations studies of oxygen diffusion into prolyl hydroxylases*
15:00-16:00	**Flash Talks (P1 – P16)**	
Session 4 (Chair: Jana Shen)		
16:00-18:00		Coffee Break & Poster Session
18:00-18:40	**Yun Lyna Luo**	*Binding free energies of Piezo1 channel agonists at protein-membrane interface*

Tuesday 12.7.2022

Session 5 (Chair: Monte Pettitt)		
09:00-09:40	**Nigel Richards**	*Expanding the Genetic Alphabet*
09:40-10:00	**Nadja Katharina Singer**	*The Turn-On Fluorescence Mechanism of an Allosteric Modulator Screening Tool for GABAa Receptors*
10:00-10:20	**Callum Matthew Ives**	*A Co-Operative Knock-On Mechanism Underpins Ca2+-Selective Cation Permeation in TRPV Channels*
10:20-10:50		Coffee Break
Session 6 (Chair: Tom Kurtzman)		
10:50-11:30	**Lennart Nilsson**	*Nucleotide stacking revisited*
11:30-11:50	**Roland Stote**	*Interference of the peroxisome proliferator-activated receptor alpha 1 from Atlantic cod (Gadus morhua) by per- and polyfluoroalkyl substances*
11:50-12:10	**Patrick K. Quoika**	*Physicochemical properties of thermosensitive polymers in molecular simulations are determined by water model*
12:10-14:00		Lunch Break

Session 7 (Chair: Alexander Mackerell)		
14:00-14:40	**William L. Jorgensen**	*Evolution of Free Energy Calculations from Butane to Drug Discovery*
14:40-15:20	**Flash Talks (P17 – P32)**	
Session 8 (Chair: Alexander Mackerell)		
15:20-17:20		Coffee Break & Poster Session
17:20-18:00	**Charles L. Brooks**	*New methods for high-throughput ligand discovery and refinement*

Wednesday 13.7.2022

Session 9 (Chair: Zoe Cournia)		
09:00-09:40	**Tom Kurtzman**	*How can water structure and thermodynamics inform lead drug discovery and design?*
09:40-10:00	**Franz Waibl**	*Advances in Grid Inhomogeneous Solvation Theory for Versatile and Interpretable Calculation of Solvation Free Energies*
10:00-10:20	**Cedric Vallee**	*Characterisation of Single Ion Permeation in ASIC1*
10:20-10:50		Coffee Break
Session 10 (Chair: Edina Rosta)		
10:50-11:30	**Sílvia Osuna**	*Conformationally-driven computational enzyme design*
11:30-11:50	**Vladimir Sladek**	*Protein Residue Networks – Recent Advancements and Use in Molecular Biology*
11:50-12:10	**Giulia D'Arrigo**	*Estimation of Protein-Protein Dissociation Rates from τRAMD Simulations*
12:10-14:00		Lunch Break
Session 11 (Chair: Chris Oostenbrink)		
14:00-14:40	**Sereina Riniker**	*Efficient free-energy calculation with replica-exchange enveloping distribution sampling*

14:40-15:20	**Edina Rosta**	*Using Machine Learning for Transition State Analysis: Applications for Ligand Unbinding Kinetics*
15:20-16:00		Coffee Break
Session 12 (Chair: Klaus Liedl)		
16:00-16:40	**Zoe Cournia**	*Predicting protein-membrane interfaces using ensemble machine learning and coarse-grained metadynamics simulations*
16:40-17:00	**Monte Pettitt**	*Components of the Electrostatic Potential of Proteins in Solution: Experiment vs Theory*
17:00-17:20	**Stephan Ehrlich**	*Exploring Large Chemical Spaces for Lead Optimization*
17:20-18:00	**Thomas Cheatham** *(Loew Lectureship)*	*Successes (and failures) in simulations of nuclei acids (and proteins)*
18:40		Gala Dinner

Thursday 14.7.2022

Session 13 (Chair: Thomas Cheatham)		
09:00-09:40	**Michele Parrinello**	*to be announced*
09:40-10:20	**Christine Peter**	*Combining molecular dynamics simulations with machine learning driven analysis to study biomolecular interactions*
10:20-10:50		Coffee Break
Session 14 (Chair: Nigel Richards)		
10:50-11:30	**Jana Shen**	*Molecular dynamics and machine learning tools for targeted covalent drug design*
11:30-11:50	**Monica Fernández-Quintero**	*The dynamic nature of antibodies - paratope states and interface movements in antibody design*
11:50		Best Poster Prize

Posters

P1. Theoretical study on the mechanism of action of Re(I) antibacterial complexes
Daniel Alvarez Lorenzo, Pablo Campomanes Ramos, Stefano Vanni

P2. Utilizing machine learning algorithms and fragmentation techniques to probe disordered proteins' phase space of NMR chemical shifts
Michael Bakker

P3. Modelling ionic and DNA transport through nanopores using a coarse-grained force field
Nathalie Basdevant, Cagla Okyay, Delphine Dessaux, Jérôme Mathé, Rosa Ramirez

P4. Improving Accuracy, Accessibility, and Throughput of MSλD via Neural Network Potentials and Charge Renormalization
Luis F. Cervantes, Jonah Z. Vilseck, Charles L. Brooks III

P5. Association and binding pathways of neomycin with the RNA aptamer – two-step binding mechanism
Piotr Chyży, Marta Kulik, Ai Shinobu, Suyong Re, Yuji Sugita, Joanna Trylska

P6. Increase of radiative forcing through mid-IR absorption by stable CO2 dimers?
Dennis F Dinu, Pit Bartl, Patrick K Quoika, Maren Podewitz, Klaus R Liedl, Hinrich Grothe, Thomas Loerting

P7. The influence of the antibody humanization on shark variable domain (VNAR) binding site ensembles
Monica Lisa Fernández-Quintero, Anna-Lena M. Fischer, Janik Kokot, Franz Waibl, Clarissa Amanda Seidler, Klaus R. Liedl

P8. Multiscale NMR calculations of spin-spin couplings and the phophprylation induced chemical shifts changes in disordered proteins
Amina Gaffour, Vojtěch Zapletal, krishnendu BERA, JANA PAVLÍKOVÁ PŘECECHTĚLOVÁ

P9. Potential dual-inhibitors of human neutrophil elastase and proteinase 3
Parveen Gartan, Fahimeh Khorsand, Pushpak Mizar, Luis F. Cervantes, Ruth Brenk, Bengt Erik Haug, Charles L. Brooks III, Nathalie Reuter

P10. Towards molecular design of peptide-based therapeutics against striated muscle disorders: unraveling the biophysics of the inotropic peptide S100A1ct by molecular modeling and simulation
Manuel Glaser, Michael Egger, Lukas Jarosch, Rafael Salazar, Sara Đaković, Patrick Most, Rebecca C. Wade

P11. Impact of Gaussian accelerated molecular dynamics on dynamic allosterism
Oriol Gracia I Carmona, Franca Fraternali, Chris Oostenbrink

P12. A MM/3D-RISM Approach to Characterize Antibody CH3-CH3 Interface Stability
Lukas J. Grunewald, Valentin J. Hörschinger, Franz Waibl, Monica L. Fernández-Quintero, Klaus R. Liedl

P13. Computational Characterization of Antibody CH3-CH3 Interfaces

Valentin J. Hörschinger, Franz Waibl, Nicolas Melcher, Monica L. Fernández-Quintero, Klaus R. Liedl

P14. Insights into the protic ionic liquid 1-methylimidazolium (trifluoro-)acetate

Florian Jörg, Christian Schröder

P15. Transformato: an MD engine independent tool for calculating relative binding free energies

Johannes Karwounopoulos, Marcus Wieder, Stefan Boresch

P16. Simulations of the Nucleosomal DNA: Mapping the Radical Cation Guanine

Maxime Kermarrec, Natacha Gillet

P17. Characterizing conformational diversity of antibody paratopes as a response to viral threats

Janik Kokot, Robert F. Wild, Monica L. Fernández Quintero, Klaus R. Liedl

P18. Antibody Interdomain Movements Co-determining Antigen Recognition

Monica L. Fernández-Quintero, Katharina B. Kroell, Johannes R. Loeffler, Patrick K. Quoika, Franz Waibl, Nancy D. Pomarici, Martin C. Heiss, Florian Hofer, Jakob R. Riccabona, Alexander Bujotzek, Ekkenhard Moessner, Guy Georges, Hubert Kettenberger, Klaus R. Liedl

P19. BuRNN: Buffer Region Neural Network Approach for Polarizable QM/MM Simulations

Bettina Lier, Peter Poliak, Philipp Marquetand, Julia Westermayr, Chris Oostenbrink

P20. Building the most accurate zirconia force field for surface simulations of a nanovaccine platform

Tamás Milán Nagy, Livia Naszályi Nagy, Aranit Harizaj, Zsuzsanna Veres, Judith Mihály, Zsuzsanna Czégény, Emma Jakab, Zoltán Varga, Katalin Jemnitz, José C. Martins, Kevin Braeckmans, Krisztina Fehér, Hendrik Heinz

P21. Coarse-grained modelling of DNA translocation through a protein nanopore

Cagla Okyay, Jérôme Mathé, Nathalie Basdevant

P22. Bispecific Antibodies - Effects of Point Mutations on CH3-CH3 Interface Stability

Nancy Pomarici, Monica L. Fernández-Quintero, Klaus R. Liedl

P23. Novel P-glycoprotein inhibitors with anti-cancer properties

Ashish Radadiya

P24. Alchemical Free Energy Calculations to predict the Effect of Point Mutations in Antibody CH3-CH3 Interfaces

Jakob R. Riccabona, Philipp Graf, Franz Waibl, Valentin J. Hörschinger, Monica L. Fernández-Quintero, Klaus R. Liedl

P25. Ab Initio calculations of anharmonic vibrational spectra of carbonic acid and carbonic acid methyl ester

Jonas Schlagin, Dennis F. Dinu, Thomas Loerting, Klaus R. Liedl

P26. Computing free energy differences between levels of theory by optimized non-equilibrium work methods

Andreas Schöller

P27. Structural Characterization of Nanobodies in Different Stages of Affinity Maturation

Clarissa A. Seidler, Monica L. Fernández-Quintero, Klaus R. Liedl

P28. Exploring common dynamic determinants of quorum quenching enzymes activity and their rational engineering to efficiently combat antibiotic resistant bacteria

Bartlomiej Surpeta, Michal Grulich, Andrea Palyzová, Helena Marešová, Jan Brezovsky

P29. Prediction of macrocycle passive cell membrane permeability with machine learning

Xuechen Tang, Monica L. Fernández-Quintero, Franz Waibl, Anna S. Kamenik, Patrick K. Quoika, Dennis Dinu, Klaus R. Liedl

P30. Biophysical Characterization of Antibody Constant Domains

Florian S. Wedl, Monica L. Fernández-Quintero, Klaus R. Liedl

P31. Benchmarking New and Emerging Nucleic Acid Force Fields Using DNA Mini-Dumbbells

Lauren Grace Winkler, Rodrigo Galindo-Murillo, Thomas E. Cheatham III

P32. Guanine-Cytosine dynamics during DNA strand separation

Max Winokan, Louie Slocombe, Jim Al-Khalili, Marco Sacchi

Oral contributions' abstracts

Protocol for iterative optimization of modified peptides bound to protein targets

Rodrigo Ochoa[1], Pilar Cossio[2], Thomas Fox[1]

[1]Medicinal Chemistry, Boehringer Ingelheim Pharma GmbH & Co KG, 88397 Biberach/Riss, Germany; [2]Center for Computational Mathematics, Flatiron Institute, New York 10010, United States of America

The inclusion of chemical modifications on peptides is a strategy to overcome common issues such as degradation and instability. Converting natural to non-natural amino acids is one valid approach to avoid these inconveniences, and potentially improve the affinity towards a protein of relevance. Here we present a computational open source pipeline to optimize peptides based on adding non-natural amino acids while improving their binding affinity. The workflow makes single point mutations on the peptide sequence using modules from the Rosetta framework. The modifications can be guided based on the monomer properties and previous knowledge of the biological system. After each mutation, the affinity to the protein is estimated by sampling the complex and applying a consensus metric using various open protein-ligand scoring functions. The mutations are accepted based on the score differences, allowing the iterative optimization of the initial molecule. The sampling/scoring schema was benchmarked with a set of protein-peptide complexes where experimental affinity values have been reported. In addition, a basic application using a known protein-peptide complex is also provided. The structure- and dynamic-based approach allows users to optimize bound peptides, with the option to personalize the code for further applications.

Parametrization of nonstandard amino acids for the CHARMM force field

Anastasia Croitoru[1], Sang-Jun Park[2], Anmol Kumar[3], Jumin Lee[2], Wonpil Im[2], Alexander D. MacKerell Jr.[3], Alexey Aleksandrov[1]

[1]Laboratoire d'Optique et Biosciences (CNRS UMR7645, INSERM U1182), Ecole Polytechnique, Institut Polytechnique de Paris, F-91128 Palaiseau, France; [2]Departments of Biological Sciences, Chemistry, Bioengineering, and Computer Science and Engineering, Lehigh University, Bethlehem, Pennsylvania 18015, United States; [3]Department of Pharmaceutical Sciences, School of Pharmacy, University of Maryland, 20 Penn Street, Baltimore, Maryland 21201, United States

Nonstandard amino acids are both abundant in nature, where they play a key role in various cellular processes, and can be synthesized in laboratories, for example, for the manufacture of a range of pharmaceutical agents. In this talk, I will present our recent work to extend the additive all-atom CHARMM36 and CHARMM General force field (CGenFF) to a large set of 333 nonstandard amino acids. I will discuss both amino acids with nonstandard side chains, such as post-translationally modified and artificial amino acids, as well as amino acids with modified backbone groups, such as chromophores composed of several amino acids. I will also present how model compounds representative of the nonstandard amino acids were parametrized for protonation states that are likely at the physiological pH of 7 and, for some more common residues, in both D- and L-stereoisomers. Considering all protonation, tautomeric, and stereoisomeric forms, a total of 406 nonstandard amino acids were parametrized. Emphasis was placed on the quality of both intra- and intermolecular parameters. Partial charges were derived using quantum mechanical (QM) data on model compound dipole moments, electrostatic potentials, and interactions with water. Optimization of all intramolecular parameters, including torsion angle parameters, was performed against information from QM adiabatic potential energy surface (PES) scans. Special emphasis was put on the quality of terms corresponding to PES around rotatable dihedral angles. Validation of the force field was based on molecular dynamics simulations of 20 protein complexes containing different nonstandard amino acids. Overall, the presented parameters will allow for computational studies of a wide range of proteins containing nonstandard amino acids, including natural and artificial residues.

Targeting RNA:Protein Interactions using an Integrative Approach: Identification of Potent YB-1 Inhibitors

Krystel El Hage, David Pastré
INSERM U1204, Université Paris-Saclay, Evry, France

While targeting Protein:Protein Interactions have provided a basis for the development of small molecules of therapeutic interest, targeting RNA:Protein Interactions critically involved in pathological mechanisms is a promising strategy to find novel classes of drug candidates that remains largely unexploited. Nevertheless, challenges arise from the drug discovery process such as finding a druggable pocket in RNA-binding interfaces, the quality of the computational models, the strategies used in the *in silico* screening, and the lack of methods and feedback between computational and experimental techniques essential to orient the drug design procedure toward the most relevant molecules. Here, we tackle these challenges by developing a drug screening approach that integrates chemical, structural and cellular data from both advanced computational and experimental techniques while overcoming limitations in a concerted manner. And we demonstrate its robustness by targeting Y-box Binding Protein 1 (YB-1), a mRNA-binding protein involved in cancer progression and resistance to chemotherapy. Based on our discovery of a druggable pocket located at the RNA-protein interface by molecular dynamics simulations, we implemented a large-scale computational approach that balances accuracy and computational cost to screen potent compounds from small molecule libraries. Using absolute binding free energy simulations, we were able to identify 22 potential hits, of which 15 are active *in vitro* (validated by NMR spectroscopy) and 11 are active in cancer cells at 10 micromolar; and one of our leads is Niraparib, an FDA-approved PARP-1 inhibitor, with a Kd ~ 6 µM *in vitro* and an IC_{50} ~ 10 µM in cells. Future efforts will focus on rationally optimizing the identified leads in order to increase their affinity and selectivity for YB-1. Together, these results show the efficiency of the proposed drug screening approach and paves the way for the development of small molecules that target RNA-Protein Interactions.

The fate of oxidized methionine: insights from computer simulations and spectroscopic experiments

Martin Spichty[1], Dmytro Neshchadin[2], Chantal Houée-Levin[3], Georg Gescheidt[2]

[1]Laboratoire d'Innovation Moléculaire et Applications, Mulhouse, France; [2]Technische Universität Graz, Austria; [3]Laboratoire de Chimie Physique, Orsay, France

L-Metionine is an important natural antioxidant and a component in many proteins. The antioxidant effect of methionine is based on its primary (one-electron) oxidation and the subsequent formation of methionine sulfoxide, which, then is converted back to parent methionine by sulfoxide reductases.[1] The one-electron oxidation leads to a short-lived radical cation intermediate.[2] Here we investigate the fate of this intermediated by combining QM/MM-based computer simulations and EPR/CIDNP-experiments.[3] The results point to a multitude of subsequent reaction pathways including decarboxylation and deprotonations at carbon sites. The C-H acidity of this elusive intermediate is quantified with the aid of a thermodynamic cycle and free-energy calculations.

References:

[1] R.L. Levine, J. Moskovitz, E.R. Stadtman, *IUBMB Life*, **2000**, *50*, 301.

[2] P. Archirel, C. Houee-Levin, J.L. Marignier, *J. Phys. Chem. B*, **2019**, *123*, 9087.

[3] D. Neshchadin, A.-M. Kelterer, C. Houée-Levin, E. Stadler, M. Spichty, G. Gescheidt, *Appl. Magn. Reson.*, **2022**, *in press*.

Lessons for Oral Bioavailability: How Conformational Flexible Cyclic Peptides Enter and Cross Lipid Membranes

Stephanie Maria Linker[1], Christian Schellhaas[1], Hans-Jörg Roth[2], Marianne Fouché[2], Sereina Riniker[1]

[1]Laboratory of Physical Chemistry, ETH Zürich, Vladimir-Prelog-Weg 2, 8093 Zürich, Switzerland; [2]Novartis Institutes for BioMedical Research, Novartis Pharma AG, Novartis Campus, 4056 Basel, Switzerland

Cyclic peptides have the potential to vastly extend the scope of druggable proteins and to lead to new therapeutics for currently untreatable diseases. However, the design of cyclic peptides with oral bioavailability is challenging and often only achieved via a tedious trial-and-error process. Experimental methods lack the resolution to track the pathway of macrocycles through lipid membranes. Thus, little is known about the permeability process of cyclic peptides which poses a major obstacle for their rational design.

We use molecular dynamics (MD) simulations as a computational microscope to uncover how cyclic decapeptides enter and cross a POPC membrane. In a first step, we performed unbiased MD simulations to obtain the permeation pathway(s). Subsequently, this knowledge of possible pathways was utilized to seed biased simulations to further enrich for permeation events.

Based on our simulations, we show how specific side-chain residues can act as "molecular anchors", which establish the first contact between a cyclic peptide and the membrane before entrance. After anchoring, cyclic peptides can insert into the membrane by taking advantage of transient gaps between the lipids. The cyclic peptides then position themselves at the interface between the polar headgroup and the apolar tail region, and show a preference for one of two distinct orientations. Interestingly, this unique environment created by the polar/apolar interface alters the conformational dynamics of cyclic peptides in comparison to pure solvent systems. In particular, the interface can catalyze the interconversion from open conformations to the permeable "closed" conformation, and thus facilitate membrane permeation. Only this permeable conformation is able to cross from the outer to the inner membrane leaflet, which again requires a unique anchoring mechanism. Our findings allow us to propose a membrane permeability model for flexible cyclic peptides, and reveal unique design considerations for each of the process steps.

Hot, Hotter, BuRNN: A new scheme for polarizable QM/MM simulations with machine-learning

Bettina Lier[1], Peter Poliak[1], Philipp Marquetand[2], Julia Westermayr[3], Chris Oostenbrink[1]

[1]University of Natural Resources and Life Sciences, Vienna (BOKU), Austria; [2]University of Vienna, Austria; [3]University of Warwick, UK

In hybrid quantum mechanics / molecular mechanics (QM/MM) approaches, the molecular system is partitioned into regions that are treated at different levels of theory. At the interfaces between these regions, artifacts may occur. Examples are an overpolarization of the QM region due to near partial charges in the MM region, the lack of polarization in the MM region or unbalanced interactions between particles in the different regions, leading to an intrusion of MM particles into the QM region, or an accumulation or depletion of QM particles if particles are allowed to change character.

We have recently introduced a buffered embedding scheme, in which a buffer region between the inner (QM) and outer (MM) region is defined for which the interactions are computed both at the QM and MM level. This comes at the cost of introducing a second QM-calculation at every timestep of the simulation. The use of neural networks to describe molecular potential energies, allows for an elegant solution to this problem. We train a neural network directly on the difference between the two QM calculations, ensuring that the network reproduces the QM-interactions of the inner region, with itself and with the buffer region as well as the polarization of the buffer region due to the inner region. Any remaining artifacts largely cancel in the trained differences and are far removed from the inner region of interest. The use of the Buffer Region Neural Network (BuRNN) approach, furthermore, allows us to apply alchemical free-energy calculations at the QM-level of theory. In this presentation, I will demonstrate our most recent advances with BuRNN.

Lier, B., Poliak, P., Marquetand, P., Westermayr, J., Oostenbrink, C. (2022) BuRNN: Buffer Region Neural Network Approach for Polarizable-Embedding Neural Network/Molecular Mechanics Simulations. *J Phys Chem Lett* **13**, 3812-3818. doi: 10.1021/acs.jpclett.2c00654

How can cells detect and respond to oxygen levels? Computer simulations studies of oxygen diffusion into prolyl hydroxylases

Carmen Domene
Department of Chemistry, University of Bath, United Kingdom

The human body is able to sense changes in atmospheric oxygen levels and adjust its metabolic activities to suit the local environment. This is why we are able to live at a variety of altitudes ranging from below sea level to up on mountains high. How can cells detect and respond to oxygen levels?

Prolyl Hydroxylase Domain-2 (PHD2) is the most important of the human PHDs that enzymes involved in oxygen sensing. PHDs are a member of the 2OG-dependent dioxygenase family of enzymes that use dioxygen to catalyze a post-translational hydroxylation reaction in the human oxygen sensing cycle. The mechanism of catalysis involves a slow diffusive entry of dioxygen into the active site of PHD2. Work using equilibrium classical molecular dynamics simulations, coupled with biased sampling methods, non-equilibrium steered MD (SMD) and adaptive biasing force as well as Markov state models have been used to study the mechanism and kinetics of oxygen diffusion from the bulk solvent to the metal active center. The results provide a quantitative mechanism to help understand the oxygen-sensing properties of PHD/2OG-dependent dioxygenases.

Acknowledgments

CD acknowledges HECBiosim, RES (https://www.bsc.es/) and finally, PRACE for awarding access to computational resources in the 17th and 20th Project Access Calls.

The Turn-On Fluorescence Mechanism of an Allosteric Modulator Screening Tool for GABAa Receptors

Nadja Katharina Singer[1,2], Pedro A. Sánchez-Murcia[3], Margot Ernst[4], Leticia González[1,5]

[1]Institute of Theoretical Chemistry, University of Vienna, Vienna, Austria; [2]Vienna Doctoral School in Chemistry (DoSChem), University of Vienna, Vienna, Austria; [3]Division of Physiological Chemistry, Otto-Loewi Research Center, Medical University of Graz, Graz, Austria; [4]Department of Molecular Neurosciences (Center for Brain Research), Medical University of Vienna, Vienna, Austria; [5]Vienna Research Platform on Accelerating Photoreaction Discovery, University of Vienna, Vienna, Austria

$GABA_A$ (γ-aminobutyric acid type A) receptors are ligand-gated ion channels mediating fast inhibitory transmission in the mammalian brain. However, investigating the multiple binding sites of this large and complex family of drug targets is challenging.[1] Here we report the molecular and electronic mechanism that governs the turn-on emission of a fluorescein-based imaging probe able to target the human $GABA_A$ receptor.[2] We identify the binding mode of the probe using classical molecular dynamics and find that the imaging probe goes through a drastic conformational change from folded in solution to rod-like upon binding to the receptor. Further, quantum mechanics/molecular mechanics (QM/MM) simulations evidence that the unfolding of the probe removes intramolecular ππ-stacking interactions responsible for quenching fluorescence in solution. Our studies provide fundamental insight into the photophysical properties of fluorescence probes, therefore assisting the design of new photoactivatable screening tools for $GABA_A$ receptors.[3]

References:

[1] M. Ernst, F. Steudle, K. Bampali, eLS, 1–12 (**2018**)

[2] S. Sakamoto, K. Yamaura, T. Numata, F. Harada, K. Amaike, R. Inoue, S. Kiyonaka, I. Hamachi, ACS Cent. Sci. **5**, 1541–1553 (**2019**)

[3] N. K. Singer, P. A. Sánchez-Murcia, M. Ernst, L. González, Angew. Chem. Int. Ed., e202205198, (**2022**)

A Co-Operative Knock-On Mechanism Underpins Ca2+-Selective Cation Permeation in TRPV Channels

Callum Ives, Neil Thomson, Ulrich Zachariae
University of Dundee, United Kingdom

The selective exchange of ions across cellular membranes is a vital biological process. Due to the significance of Ca^{2+} in a broad array of cellular processes, strict concentration gradients are maintained across the plasma and organelle membranes. Therefore, Ca^{2+} signalling relies on permeation through selective ion channels that control the flux of Ca^{2+} ions. A key family of Ca^{2+}-permeable membrane channels are the polymodal signal-detecting Transient Receptor Potential (TRP) ion channels. Whilst most members of this family permeate a broad range of cations non-selectively, TRPV5 and TRPV6 are unique due to their strong Ca^{2+}-selectivity. Here, we address the question of how some members of the TRPV subfamily show a high degree of Ca^{2+}-selectivity whilst others conduct a wider spectrum of cations. We present results from all-atom molecular dynamics simulations of continuous ion permeation through two Ca^{2+}-selective and two non-selective TRPV channels. Using a new method to quantify permeation co-operativity based on mutual information, we show that Ca^{2+}-selective TRPV channel permeation occurs by a three binding site knock-on mechanism, whereas a two binding site knock-on mechanism is observed in non-selective TRPV channels. Each of the ion binding sites involved displays greater affinity for Ca^{2+} over Na^{+}. As such, our results suggest that coupling to an extra binding site in the Ca^{2+}-selective TRPV channels underpins their increased selectivity for Ca^{2+} over Na^{+} ions.

Nucleotide stacking revisited

Lennart Nilsson

Karolinska Institutet, Sweden

In the mid 1990'ies we performed a series of studies of the stacking behavior of all 32 RNA and DNA dinucleotide monophosphates using umbrella sampling to obtain the potential-of-mean-force (PMF) along a simple geometric reaction coordinate, the distance between the N1/N9 atoms of the two bases.

The CHARMM force field is quite stable, but in the quarter century that has passed there have been some updates. Here we present the results of PMF calculations using the subsequent FF generations, up to CHARMM36, also including the recently developed polarizable Drude force field.

Interference of the peroxisome proliferator-activated receptor alpha 1 from Atlantic cod (Gadus morhua) by per- and polyfluoroalkyl substances

Ana Milinski[1], Annick Dejaegere[1], Sofie Söderström[2], Odd André Karlsen[2], Roland H. Stote[1]

[1]Department of Integrated Structural Biology, IGBMC-CNRS, France; [2]Department of Biological Sciences, University of Bergen, Bergen, Norway

Per- and polyfluoroalkyl substances (PFAS) are synthetic chemicals that have been used in industrial and consumer products since the late 1940s. Often referred to as endocrine-disrupting chemicals (EDCs), they show structural similarities to fatty acids and they can interfere with hormone systems. Their toxicity has been linked to their ability to activate proteins of the nuclear hormone receptor family and, in particular, peroxisome proliferator-activated receptors (PPARs), which are major regulators of metabolism. It is believed that upregulation of PPARs could perturb metabolic pathways at critical stages of the life cycle and cause adverse effects.

Atlantic cod (*Gadus morhua)* is a major financial resource for some nations and studies have linked exposure to persistent chemicals, such as PFAS, to declining fish populations. It has recently been demonstrated that some PFAS can activate the PPARα1 isoform of *Gadus morhua* PPARα, but not the PPARα2 isoform. Binary mixtures of activating and non-activating PFAS can result a greater activation compared to the activating compound alone (1).

We investigated the interactions between PFASs and homology models of *Gadus morhua* PPARα1 and PPARα2 through docking calculations and molecular dynamics simulations; the results suggest that activating PFAS stabilize the PPARα1 ligand binding domain, but not that of PPARα2. We also identified a putative allosteric binding site that could play a role in the potentiating effects of the non-activating ligand. Together with the experimental data, this study provides novel mechanistic insight into how PFASs may modulate the PPAR signaling pathway by either binding the canonical ligand-binding pocket or by interacting with an allosteric binding site.

Improving our understanding of the mechanistic characteristics of endocrine disruptors will produce novel scientific data that can support the risk assessment of future PFAS chemicals.

1. Søderstrøm S, Lille-Langøy R, et al doi: 10.1016/j.envint.2022.107203.

Physicochemical properties of thermosensitive polymers in molecular simulations are determined by water model

Patrick K. Quoika[1,2]

[1]Technical University Munich, Germany; [2]University of Innsbruck, Austria

Polymers that show a phase transition with lower critical solution temperature (LCST) are commonly called thermosensitive polymers. Accordingly, these polymers exhibit good solvent quality below the LCST, whereas they exhibit bad solvent quality above. Their macroscopic phase behavior is commonly associated with a conformational transition of the polymer chains, i.e., the Coil-Globule transition. Accordingly, these polymers show a conformational collapse above the LCST. We investigated the Coil-Globule transition of a well-known thermosensitive polymer (N-Isopropylacrylamide) in atomistic simulations with eight different water models. We found that the properties of the polymers dramatically depend on the choice of water model. Indeed, the thermosensitive Coil-Globule transition temperature spreads over a range of 100 K between different water models. The Coil-Globule transition is particularly sensitive to the choice of in-silico water models, since the solvation thermodynamics are crucial for the process. Decisive quantities—such as the polymer-water interaction strength, but also the bulk entropy of the solvent—vary between water models. We found that the quadrupole moment of the water model correlates well with the Coil-Globule transition temperature: Water models with a high quadrupole moment lead to high Coil-Globule transition temperatures. Our results suggest that the Coil-Globule transition thermodynamics are linked to the solvent phase diagram.

New methods for high-throughput ligand discovery and refinement

Charles L. Brooks III

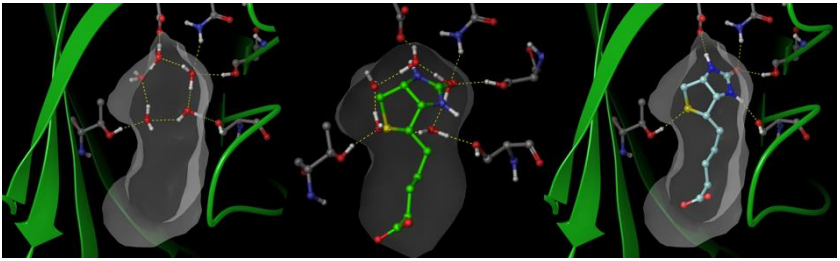
Department of Chemistry and Biophysics, University of Michigan, United States

In this talk I will provide an overview and review of methods developed in our research group directed toward the integration of ligand discovery and refinement. Advances in Flexible Receptor docking through CDOCKER and multi-site lambda dynamics will be presented with select applications of these approaches to problems in drug discovery and refinement.

How can water structure and thermodynamics inform lead drug discovery and design?

Tom Kurtzman
Lehman College CUNY, United States

Water plays an instrumental role in the recognition between small molecule drugs and their biomolecular targets. When a drug is unbound, the structure and thermodynamics of water in the binding site reveal information that can be used to inform the rational discovery of lead drug compounds and their subsequent optimization. We will discuss how computer simulations and statistical mechanical liquid state theory can be used to map out the properties of water on the surface of proteins and how these 'maps' are used to improve modern drug discovery and optimization efforts.



Advances in Grid Inhomogeneous Solvation Theory for Versatile and Interpretable Calculation of Solvation Free Energies

Franz Waibl, Valentin J Hoerschinger, Johannes Kraml, Monica L Fernández-Quintero, Klaus R Liedl
University of Innsbruck, Austria

The free energy of solvation defines the propensity of a molecule to be transferred from the gas phase to a solution. It affects all processes that involve solute-solvent interactions, such as accumulation in membranes, ligand binding to proteins, and chemical reactions in solution.

Grid inhomogeneous solvation theory (GIST) has proven useful to calculate solvation properties of systems ranging from small molecules to antibodies and serine proteases. It provides spatial resolution as well as separate contributions of enthalpy and entropy. Thereby, it facilitates interpretation of solvation properties in large molecules and identification of hydrophobic regions on protein surfaces.

Many processes, such as accumulation in membranes, depend on the transition between different solvent phases. However, GIST implementations were previously limited to water as a solvent. Here, we present generalizations towards arbitrary rigid solvent molecules as well as salt-solvent mixtures. Furthermore, we improved the convergence of the entropy algorithm, which is especially relevant for larger solvents with a lower number density.

We demonstrate that GIST can be used to compute partition coefficients between water and other solvents, which are important for the prediction of permeation and accumulation in biological membranes in drug design.

Furthermore, we show how to incorporate co-solutes such as ions in GIST calculations. This allows for a realistic treatment of electrostatics around charged systems. Additionally, it enables us to investigate the effect of salt on the hydration properties. Using an extension to GIST that computes parts of the second order entropy, we show that water-salt correlations contribute significantly to the salting-out effect.

We conclude that our extensions to GIST strongly increase the field of applications, and expect that they will help to elucidate solvation phenomena in a wide range of biochemical applications.

Characterisation of Single Ion Permeation in ASIC1

Cedric Vallee, Brendan J Howlin, Rebecca Lewis
University of Surrey, United Kingdom

Acid Sensing Ion Channels (ASICs) are proton-gated ion channels mainly permeable to sodium (Na^+) and calcium (Ca^{2+}) with a higher selectivity toward Na^+ [1]. They are involved in several important physiological roles; hence they are one of the most studied channels of the Epithelial Sodium Channel/Degenerin (ENaC/DEG) superfamily [2-3]. The ASIC1 subunit can function as a homotrimeric channel and its structure is currently the most established of the whole ENaC/DEG family. By computing single ion free energy profile on different ASIC1 structures, we recently showed that the channel is cation-selective and that the histidine of the conserved 'HG' motif from the re-entrant loop plays an important role for binding Na^+ [4]. Based on these results, we investigated ion selectivity by computing single ion free energy for K^+, as well as the impact of external electric fields on the potential of mean force. Finally, because Na^+ is partially hydrated in the newfound ion binding site, we investigated the classical effect of heavy water on the single ion free energy profile. Our results suggest that ASIC1 is more selective to Na^+ than K^+ and that negative external electric fields act as catalyst and lower the barrier that the cation need to overcome which should facilitate the permeation. Furthermore, switching from normal water to heavy water considerably lowers the affinity of Na^+ in the ion binding site. These results highlight the significant role of the water shell surrounding the cation for ion permeability and selectivity.

References:

[1] R. Waldmann, G. Champigny, F. Bassilana, C. Heurteaux and M. Lazdunski, Nature, 1997, 386, 173—177

[2] J. A. Wemmie, J. Chen, C. C. Askwith, A. M. Hruska-Hageman, M. P. Price, B. C. Nolan, P. G. Yoder, E. Lamani, T. Hoshi and J. H. Freeman Jr, et al., Neuron, 2002, 34, 463—477

[3] E. Deval, X. Gasull, J. Noël, M. Salinas, A. Baron, S. Diochot and E. Lingueglia, Pharmacol. Ther., 2010, 128, 549—558

[4] C. Vallée, B. J. Howlin and R. Lewis, Physical Chemistry Chemical Physics, 2022, 24, 13824-13830, doi: 10.1039/D2CP01563C

Protein Residue Networks – Recent Advancements and Use in Molecular Biology

Vladimir Sladek
Institute of Chemistry, Slovak Academy of Sciences, Slovak Republic

Several tasks in molecular biology do, in one way or another, benefit from knowing the role of particular residues, e.g. amino acids, in large biomolecules or their complexes. A good example would be the assessment of residue importance in the formation of protein complexes. Convoluted experimental assays such as alanine scans with surface plasmon resonance measurements can be used for this purpose. On the other hand, having a theoretical model of the proteins that would allow to carry out such assessment in a reproducible, qualitative and quantitative way *a priori* of experimental work would certainly prove beneficial.

Protein Residue Networks (PRN) are network models of proteins wherein each residue is a node in the network and their interactions, i.e. connections, are edges. Recent advances in this field allow us to build such PRNs by combining modern (quantum) chemical calculations and network analysis. The capabilities of specialised computational techniques such as Fragment Molecular Orbital (FMO) methods are well suited to obtain all residue-residue pair interaction energies (PIE). These are used to assign weights to the network edges. Subsequently, a wide range of network analytical approaches can be used on the model. The results are used to rank the importance of nodes in the networks topology and draw conclusions on the structural role of the residues in e.g. secondary, tertiary and quaternary protein structure.

In this contribution we focus on our own Network Differential Analysis (NDA) method as well as on variants of singular value decomposition techniques suited to accomplish these tasks. Both were recently implemented into the free, open source PyMOL plugin pyProGA https://gitlab.com/Vlado_S/pyproga. Our model for the case study will be the protein complex TR-2 – UL141 and we will show how the network predictions correlate with experimental data.

Funding from projects APVV 20-0230 and 19-0376, VEGA 2/0031/19, SAS-MOST/JRP/2019/882/GM-INHIB.

Estimation of Protein-Protein Dissociation Rates from τRAMD Simulations

Giulia D'Arrigo[1], Daria B. Kokh[1], Ariane Nunes-Alves[1,2], Rebecca Wade[1,3]

[1]Molecular and Cellular Modeling Group, Heidelberg Institute for Theoretical Studies, Schloss-Wolfsbrunnenweg 35, 69118 Heidelberg, Germany; [2]Technical University of Berlin, Strasse des 17. Juni 135, 10623, Berlin, Germany; [3]Center for Molecular Biology (ZMBH), DKFZ-ZMBH Alliance, and Interdisciplinary Center for Scientific Computing (IWR), Heidelberg University, Im Neuenheimer Feld 282, 69120 Heidelberg, Germany

Due to their pivotal role in cell functioning and multiple diseases, protein-protein interactions (PPIs) have become a highly pursued target for therapeutic strategies [1]. The dissociation rate, or its inverse, the residence time ($\tau=1/k_{off}$), is a measure of the duration of a PPI and, as such, is key for determining biological function. Computing protein-protein dissociation rates is therefore crucial for a deeper knowledge of PPIs and their role in complex signaling pathways, as well as for drug design purposes. Here we apply the τRAMD (τ-Random Acceleration Molecular Dynamics) methodology [2], which was previously validated on protein-small molecules complexes [3], to estimate the relative residence time of protein-protein complexes. τRAMD enables the dissociation of molecular partners on the nanosecond timescale and is here validated on three well characterized protein-protein complexes and related mutants [4]. Our results show the ability of the τRAMD procedure to estimate the relative residence times of protein-protein complexes, in accordance with experimental data. In addition, when coupled to MD-IFP interaction fingerprint analysis, it enables the identification of transient states along the dissociation path, thus being a valuable tool for the in-depth exploration of unbinding mechanisms and their determinants. These findings provide a basis for modulating and selectively targeting PPIs, as well as for drug and protein design

Components of the Electrostatic Potential of Proteins in Solution: Experiment vs Theory

Monte Pettitt

Ubniversity of Texas Medical Branch, United States of America

Components of the protein electrostatic potentials in solution are analyzed with NMR paramagnetic relaxation enhancement experiments and compared with continuum solution theory, and multiscale simulations. To determine the contributions of the solution components we analyze different ionic strengths from 0 to 745 mM. A theoretical approximation allows the determination of the electrostatic potential at a given proton without reference to the protein structure given the ratio of PRE rates between a cationic and an anionic probe. The results derived from simulations show good agreement with experiment and simple continuum solvent theory for many of the residues. A discrepancy including a switch of sign of the electrostatic potential was observed for particular residues. By considering the components of the potential we found the discrepancy is mainly caused by angular correlations of the probe molecules with these residues.

Exploring Large Chemical Spaces for Lead Optimization

Pieter H. Bos et al.[1], Stephan Ehrlich[2]

[1]Schrödinger, Inc., 1540 Broadway, New York, New York 10036, United States;
[2]Schrödinger GmbH, Glücksteinallee 25, 69163 Mannheim, Germany

The lead optimization stage of a drug discovery program generally involves the design, synthesis, and assaying of hundreds to thousands of compounds. The design phase is usually carried out via traditional medicinal chemistry approaches and structure-based drug design (SBDD) when suitable structural information is available. Two of the major limitations of this approach are (1) difficulty in rapidly designing potent molecules that adhere to myriad project criteria (the multiparameter optimization problem) and (2) the relatively small number of molecules explored compared to the vast size of chemical space. To address these limitations, we have developed AutoDesigner, a *de novo* design algorithm. AutoDesigner employs a cloud-native, multistage search algorithm to carry out successive rounds of chemical space exploration and filtering. Millions to billions of virtual molecules are explored and optimized while adhering to a customizable set of project criteria such as physicochemical properties and potency. Once the search space is sufficiently narrowed down, the workflow ranks virtual molecules via efficient and accurate active learning free energy perturbation (AL-FEP) calculations, narrowing down the selection to a final set on the order of tens of compounds for visual inspection and synthesis prioritization.

To assess the effectiveness of AutoDesigner, we applied it to the design of novel inhibitors of D-amino acid oxidase (DAO), a target for the treatment of schizophrenia. The compounds generated by AutoDesigner that were synthesized and assayed subsequently not only meet the desired physicochemical criteria, clearance, and central nervous system (CNS) penetration (Kp,uu) cutoffs. They also meet potency thresholds and fully utilize structural data to discover and explore novel interactions and a previously unexplored subpocket in the DAO active site. The reported data demonstrate that AutoDesigner can play a key role in accelerating the discovery of novel, potent chemical matter within the constraints of a given drug discovery lead optimization campaign.

Bos, P. H. *et al.*, JCIM **2022**, *62* (8), 1905-1915.

Molecular dynamics and machine learning tools for targeted covalent drug design

Jana Shen
University of Maryland School of Pharmacy, Baltimore, USA

Targeted covalent inhibitors have gained increasing popularity owing to the potential of overcoming several significant challenges of traditional reversible inhibitors and expanding the druggable space. In this talk, I will discuss our recent efforts in the development of a computational tool set based on molecular dynamics and machine learning for discovering covalently druggable sites in the entire proteome.

Mapping Guanine Oxidation in Nucleosomal DNA using Multiscale Simulations

Natacha Gillet, Maxime Kermarrec, Laleh Allahkaram, Tao Jiang, Elise Dumont
Laboratoire de Chimie UMR 5182 CNRS ENS de Lyon, France

Light, oxidative stress or exogenous molecules modify the well-designed structure of DNA by inducing nucleobases lesions. Because of the complexity of the DNA molecule in its biological context, the elucidation of the damages formations mechanisms becomes rapidly combinatorial, involving sequence, structural and dynamical effects. Indeed, the histone core [1] mechanically constrains the nucleosomal DNA conformation and creates an heterogeneous electrostatic field. Then, the physicochemical properties of the nucleobases, especially redox ones, depend on their position around the histone core (see for example [2]).

The recent efforts in computational power have permitted the first all-atom classical simulations of a nucleosome at a microsecond timescale, including damages. [3–5] A first part of our work consists in a large conformational sampling of the histone tails, which are flexible and rich in positively charged amino acids. We also present here our guanine oxidation study using an efficient FO-DFTB/MM approach [6] over 20 µs all-atom simulations. We focus on the importance of different parameters: sequence, nucleobase position, tail or ion proximity... All our data will be used to feed machine learning algorithms to provide a better understanding of these environmental factors and to facilitate the analysis of our complex simulations.

References

(1) R. K. McGinty, S. Tan, *Chem. Rev.* 2015, 115, 2255

(2) J. Hu, S. Adar, C. P. Selby, J. D. Lieb, A. Sancar, *Genes Dev.*2015, 29, 948.

(3) E. Bignon, V. E. P. Claerbout, T. Jiang, C. Morell, N. Gillet, E. Dumont, *Sci. Rep.*2020, 10, 17314.

(4) E. Bignon, N. Gillet, T. Jiang, C. Morell, E. Dumont, *J. Phys. Chem. Lett.* 2021, 12, 6014

(5) E. Matoušková, E. Bignon, V. E. P. Claerbout, T. Dršata, N. Gillet, A. Monari, E. Dumont, F. Lankaš, *J. Chem. Theory Comput.* 2020, 16, 5972

(6) T. Kubar, M. Elstner, *J. R. Soc. Interface* 2013, 10, 20130415

Posters' abstracts

P1. Theoretical study on the mechanism of action of Re(I) antibacterial complexes

Daniel Alvarez Lorenzo[1,2], Pablo Campomanes Ramos[1], Stefano Vanni[1]

[1]University of Fribourg, Switzerland; [2]University of Oviedo, Spain

The lack of research for new antibacterial drugs contributes to making antimicrobial resistance one of the greatest threats to public health. In this regard, metal complexes are promising candidates and recently, Re(I) tricarbonyl complexes have been demonstrated as potential therapeutics for antibacterial drugs. In particular, some candidates display impressive activity against MRSA and low toxicity towards the host cells. Given the cationic nature of the most active complexes, their selectivity may be due to the anionic lipids present in higher concentrations at the bacterial membranes. However, their mechanisms of action and the properties related to their activity and selectivity are not known.

Molecular Dynamics (MD) simulations are a powerful tool to investigate this issue. They provide insights into the energy barriers that have to be overcome to enter the bacteria and the host cells, as well as into the interaction of the drugs with the lipids present in their membranes, their disposition inside those bilayers, and the changes that they may induce in those membranes. This is critical for understanding the selectivity and permeability of the antibacterial compounds and their mechanisms of action, which could be related to the disruption of the membrane.

Our goal is to unravel the outstanding activity and selectivity of the antibacterial Re(I) tricarbonyl complexes by studying the interaction of active and non-active complexes with models of bacterial and eukaryotic membranes. For this purpose, we will use all atom MD simulations with newly developed parameters for the Re(I) complexes, which will be parameterized based on QM calculations. To compute the free energy of insertion into the membrane, we will employ umbrella sampling. This research is critical for the use of these compounds in clinical trials. On top of that, it can help to design new complexes with even better activity.

P2. Utilizing machine learning algorithms and fragmentation techniques to probe disordered proteins' phase space of NMR chemical shifts

Michael Bakker
Charles University, Czech Republic

In the previous decades, significant interest has grown in understanding structural properties of intrinsically disordered proteins (IDPs). Many of these proteins play key roles in degenerative diseases such as Alzheimer's and Parkinson's. While several investigations into the in-vivo post-translational processes are underway, there is a significant advantage in atomic level in-silica structural computations. Such techniques have previously implemented some form of NMR calculations of chemical shifts (CS), although many complications persist as barriers to such investigations, e.g. computational costs and the structural disorder within the protein.

Several techniques have emerged to circumvent these issues. To simulate the chaotic nature of the molecule, a molecular dynamics (MD) trajectory was implemented and several structures were sampled to create a more complete phase space. Additionally, the sheer size of many of these molecules disallows the use of direct NMR computations, so fragmentation techniques such as ADMA was introduced so only the local influences are considered in a DFT calculation for expediency.

In this investigation we formulated a viable and practical approach to computing 31P, 15N, 13C, and H NMR chemical shifts in IDPs through a combined MD/ADMA/DFT technique. We also expanded this technique using a machine learning algorithm known as cluster analysis. Through the k+1 nearest neighbor approach, the complete set of conformations were compared using RMSD, and a significantly (10x) smaller subset of frames were computed and compared to those obtained at a regular interval. In comparisons between the two subsets, the accuracy of the chemical shifts was not reduced while the computational cost was severely reduced, producing great promise for future implementation among other structures with great disorder.

P3. Modelling ionic and DNA transport through nanopores using a coarse-grained force field

Nathalie Basdevant[1], Cagla Okyay[1], Delphine Dessaux[1,2], Jérôme Mathé[1], Rosa Ramirez[1]

[1]LAMBE, Université d'Evry, Université Paris-Saclay, CNRS, Evry, France; [2]present address: Toulouse Biotechnology Institute, Université de Toulouse, CNRS, INRAE, INSA, Toulouse, France

Molecular dynamics simulations are highly valuable for understanding the physical processes implied in Nanopore Force Spectroscopy experiments. These experiments consist in applying an electric potential difference which guides a charged biopolymer through an artificial or biological nanopore, inserted in a solid or lipid membrane, surrounded by an ionic solution. When a macromolecule passes through the pore, it partially blocks it and induces a decrease in the ionic current, according to the nature of the molecule and the pore properties. The alpha-hemolysin toxin channel is a membrane protein widely used for NFS experiments, due to its commercial availability. It enables the passage of a single-stranded DNA and has been the subject of several DNA translocation and unzipping experiments in our lab.

To understand these experiments in microscopic details, coarse-grained models are a good alternative to classical all-atom models because they enable longer and faster simulations for large systems, closer to the experimental characteristic times. In collaboration with experimentalists of our lab, we performed coarse-grained molecular dynamics simulations to study the ionic transport through alpha-hemolysin, inserted into a lipid bilayer surrounded by solvent and ions, using the MARTINI and PW water. Our system is reduced to 90,000 coarse grains instead of around 400,000 atoms. We simulated 12 different systems, for which several charged residues were neutralized, each of them in the presence of 9 different electric fields to mimic the electric potential difference, for 1.5 microscond. We were able to observe several specific features of this pore, current asymmetry and anion selectivity, in agreement with previous studies and experiments, and also identified the charged amino-acids responsible for these current behaviours using ionic density maps. Moreover, to elucidate the mechanisms involved in DNA translocation experiments, we are now performing steered molecular dynamics of transport of single-stranded DNA molecules of different lengths through alpha-hemolysin.

P4. Improving Accuracy, Accessibility, and Throughput of MSλD via Neural Network Potentials and Charge Renormalization

Luis F. Cervantes[1], Jonah Z. Vilseck[2,3], Charles L. Brooks III[1,2]

[1]Department of Medicinal Chemistry, College of Pharmacy, University of Michigan, Ann Arbor, Michigan 48109, United States; [2]Department of Chemistry, University of Michigan, Ann Arbor, Michigan 48109, United States; [3]Department of Biochemistry and Molecular Biology, Center for Computational Biology and Bioinformatics, Indiana University School of Medicine, Indianapolis, Indiana 46202, United States

Multisite λ-Dynamics (MSλD) allows for free energy prediction via a combinatorial exploration of chemical space. Among other applications, this method is particularly useful for small molecule lead development since multiple variations at different sites of a lead scaffold can be modeled and tested within a single simulation. While MSλD has been proven to be as accurate and even more efficient in predicting binding free energies compared to other methods like TI or FEP, it is not yet widely used because of the complexity of setting up the simulation models associated with the hybrid topology of the perturbing ligand. Herein we present a novel, fully automated, open-source, and user-friendly tool called *msld_py_prep* as both a standalone Python implementation and a PyMOL plugin for the efficient setup of MSλD simulations using a charge renormalization algorithm. Through use of this tool, MSλD simulations are conveniently set up and run for a large variety of receptor/ligand experimental datasets. This has led to identification of major sources of error within the force fields used for these simulations, particularly dihedral parameters. To this end, a recently developed neural network potential (NNP) – ANI2x – torsional scanning engine, additional features, and a potential energy surface fitting protocol have been implemented and benchmarked in the TorsionDrive algorithm via pyCHARMM, an integrated implementation of CHARMM as a python callable library. We believe the use of both *msld_py_prep* and custom parameterization via the ANI2x implementation in TorsionDrive will enhance accuracy, throughput and user accessibility for MSλD simulations.

P5. Association and binding pathways of neomycin with the RNA aptamer – two-step binding mechanism

Piotr Chyży[1], Marta Kulik[1], Ai Shinobu[2], Suyong Re[2,3], Yuji Sugita[2,4], Joanna Trylska[1]

[1]University of Warsaw, Warsaw, Poland; [2]RIKEN Center for Biosystems Dynamics Research, Kobe, Japan; [3]National Institutes of Biomedical Innovation, Health and Nutrition, Osaka, Japan; [4]RIKEN Cluster for Pioneering Research, Wako, Japan

The RNA aptamers are of vast interest in biology due to their ability to bind specific molecules with high affinity. We investigate an aminoglycoside-binding aptamer that constitutes a synthetic 27-nucleotide long N1 riboswitch regulating translation. Experiments showed that mutations, particularly the A17 to G substitution, hinder the riboswitch activity [1]. But why do the A17G mutants reduce the translation regulation efficiency almost 6-fold?

We have recently explained how the differences in the dynamics of the N1 riboswitch and its mutants [2] coincide with the aminoglycoside dissociation constants [1,3]. Here, we present the ligand association pathways obtained from two-dimensional replica-exchange molecular dynamics (2D-REMD) with varying solute temperature (between 310 and 380 K) and RNA-ligand distance (from 9 to 30 Å). We describe with atomistic details the two-step binding mechanism of neomycin, dominated by conformational selection [4].

2D-REMD simulations show that neomycin dissociates from the binding site via one pathway although the shape of the RNA indicates two potential directions. The free-energy surface suggests that RNA forms two stable low-energy conformations during neomycin binding. The global minimum describes bound state [2]. The local minimum probably corresponds to an intermediate state detected experimentally [4]. In the N1 riboswitch, C6:A17 stacking appears in both minima. In the A17G mutant, the corresponding C6:G17 stacking is present in the global minimum, but in the local one the G17 base stacks with U7. Neomycin conformations in and close to the binding site differ between the A17G mutant and N1 riboswitch. These differences likely reduce the binding affinity of neomycin to the A17G mutant.

We acknowledge support from National Science Centre Poland (DEC-2017/26/M/NZ1/00827).

[1] Weigand, J.E., et al. (2014), *Chembiochem*,15:1627-1637

[2] Chyży, P., et al. (2021), *Front. Mol. Biosci*., 8:633130

[3] Kulik, M., et al. (2018), *Nucl. Acids Res.*,46:9960-9970

[4] Gustmann, H., et al. (2019), *Nucl. Acids. Res.*,47:15-28

P6. Increase of radiative forcing through mid-IR absorption by stable CO2 dimers?

Dennis F Dinu[1,2,3], Pit Bartl[2], Patrick K Quoika[1,4], Maren Podewitz[3], Klaus R Liedl[1], Hinrich Grothe[3], Thomas Loerting[2]

[1]Department of General, Inorganic and Theoretichal Chemistry, University of Innsbruck, Austria; [2]Department of Physical Chemistry, University of Innsbruck, Austria; [3]Department of Materials Chemistry, TU Vienna, Germany; [4]Department of Theoretical Biophysics (T38) - Moleculardynamics, TU Munich, Germany

We have demonstrated that the combination of matrix isolation infrared (MI-IR) spectroscopy and vibration configuration interaction (VCI) calculations [1-3] is a feasible approach [4] to accurately assign vibrational transitions of single molecules, such as water [5], fluoroethane [6], carbon dioxide and methane [7].

Relying on our integral experimental-computational methodology, we recently investigated the carbon dioxide dimerization [8] including MI-R spectroscopy of carbon dioxide monomers CO_2 and dimers $(CO_2)_2$ trapped in neon and in air. Based on our VCI calculations accounting for mode-coupling and anharmonicity, we identify additional infrared-active bands in the MI-IR spectra due to the $(CO_2)_2$ dimer.

In a systematic carbon dioxide mixing ratio study using neon matrices, we observe a significant fraction of the dimer at mixing ratios above 300 ppm, with a steep increase up to 1000 ppm. In neon matrix, the dimer increases the IR absorbance by about 15% at 400 ppm compared to the monomer absorbance alone. This suggests a high fraction of the $(CO_2)_2$ dimer in our matrix experiments. In atmospheric conditions, such increased absorbance would significantly amplify radiative forcings and, thus, the greenhouse warming.

In the context of planetary atmospheres, our results improve understanding of the greenhouse effect for planets of rather thick CO_2 atmospheres such as Venus, where a significant fraction of the $(CO_2)_2$ dimer can be expected. There, the necessity of including the mid-IR absorption by stable $(CO_2)_2$ dimers in databases used for modelling radiative forcing, such as HITRAN, arises.

References

[1] G. Rauhut, JCP, **121**, 19 (2004)

[2] M. Neff *et al*, JCP, **131**, 12 (2009)

[3] H. J. Werner *et al*, JCP, **152**, 14, (2020)

[4] D. F. Dinu *et al*, TCA, **139**, 12, (2020)

[5] D. F. Dinu *et al*, JPCA, **123**, 38 (2019)

[6] D. F. Dinu *et al*, JMS, **367**, (2019)

[7] D. F. Dinu *et al*, PCCP, **22**, 32 (2020)

[8] D. F. Dinu *et al*, JPCA, **126**, 19, (2022)

P7. The influence of the antibody humanization on shark variable domain (VNAR) binding site ensembles

Monica Lisa Fernández-Quintero, Anna-Lena M. Fischer, Janik Kokot, Franz Waibl, Clarissa Amanda Seidler, Klaus R. Liedl
University of Innsbruck, Austria

Sharks and other cartilaginous fish produce new antigen receptor (IgNAR) antibodies, as key part of their humoral immune response and are the phylogenetically oldest living organisms that possess an immunoglobulin (Ig)-based adaptive immune system. IgNAR antibodies are naturally occurring heavy-chain-only antibodies, that recognize antigens with their single domain variable regions (VNARs). In this study, we structurally and biophysically elucidate the effect of antibody humanization of a previously published spiny dogfish VNAR (parent E06), which binds with high affinity to the human serum albumin (HSA). We analyze different humanization variants together with the parental E06 VNAR and the human Vk1 light chain germline DPK9 antibody to characterize the influence of point mutations in the framework and the antigen binding site on the specificity of VNARs as reported by Kovalenko et al. We find substantially higher flexibility in the humanized variants, reflected in a broader conformational space and a higher conformational entropy, as well as population shifts of the dominant binding site ensembles in solution. A further variant, in which some mutations are reverted, largely restores the conformational stability and the dominant binding minimum of the parent E06. We also identify differences in surface hydrophobicity between the human Vk1 light chain germline DPK9 antibody, the parent VNAR E06 and the humanized variants. Additional simulations of VNAR-HSA complexes of the parent E06 VNAR and a humanized variant reveal that the parent VNAR features a substantially stronger network of stabilizing interactions. Thus, we conclude that a structural and dynamic understanding of the VNAR binding site upon humanization is a key aspect in antibody humanization.

P8. Multiscale NMR calculations of spin-spin couplings and the phophprylation induced chemical shifts changes in disordered proteins

Amina Gaffour[1], Vojtěch Zapletal[2], krishnendu BERA[2], JANA PAVLÍKOVÁ PŘECECHTĚLOVÁ[1]

[1]Department of Biophysics and Physical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic; [2]CEITEC Masaryk university, Kamenice 753/5, Brno, 625 00, Czech Republic

Intrinsically disordered proteins (IDPs) are post-translationally modified polypeptide chains that fail to form a stable single well-defined three-dimensional (3D) structure. These proteins are crucial for understanding the progression of neurodegenerative diseases such as Alzheimer and Parkinson. Despite their unstable structure, they serve an important function in a variety of processes, including molecular recognition and the regulation of transcription. Both NMR experiment interpretations and computational approaches are used to characterize the structural properties of IDPs.

The aim of our work is to design a reliable multiscale protocol for the calculation of NMR spectroscopy parameters in IDPs. We use (1) the Map2c protein fragment consisting of residues (159-245) to compute 1H, 13C and 15N NMR chemical shifts and the (2) Tau(210-240) protein frgament to calculate 3JHN-Ha spin-spin couplings for this purpose. To obtain the NMR parameters, we designed a computational protocol that combines classical molecular dynamics and density functional caluclations.. We use the MD trajectory to construct structural ensembles that represent the IDP conformational space. Prior to the NMR calculations, all ensemble structures are subject to protein fragmentation in order to constract molecular clusters that are computationally feasible. In particular, we apply the fragmentation by the Adjustable Density Matrix Assembler that splits a protein into individual aminoacids and further into their backbone and side chain parts. The resulting fragments are embedded in the protein surroundings including the ions and explicit solvent.

In the poster contribution, we will demonstrate the performance of MD/DFT calculations for NMR chemical shifts in both phosphorylated and non-phosphorylated Map2c. Additionally, we will compare the calculated spin-spin couplings with the experimental data and with empirically derived predictions based on Karplus equations for Tau proteins.

P9. Potential dual-inhibitors of human neutrophil elastase and proteinase 3

Parveen Gartan[1], Fahimeh Khorsand[2], Pushpak Mizar[3], Luis F. Cervantes[4], Ruth Brenk[2], Bengt Erik Haug[3], Charles L. Brooks III[5], Nathalie Reuter[1]

[1]Department of Chemistry, University of Bergen, Bergen, Norway, Computational Biology Unit, University of Bergen, Bergen, Norway; [2]Department of Biomedicine, University of Bergen, Bergen, Norway; [3]Department of Chemistry, University of Bergen, Bergen, Norway; [4]Department of Medicinal Chemistry, College of Pharmacy, University of Michigan, Ann, MI, USA; [5]Department of Chemistry, University of Michigan, Ann Arbor, MI, USA, Biophysics Program, University of Michigan, Ann Arbor, MI, USA

Chronic obstructive pulmonary disease (COPD) is a progressive lung disease that comprises emphysema, asthma, and bronchiectasis. Two of the neutrophil serine proteases namely human neutrophil elastase (HNE) and proteinase 3 (PR3) have emerged as potential drug targets. Recent studies have revealed the excess amount of PR3 released by neutrophils compared to HNE and its role in damaging tissue structure in the lungs. Different generations of elastase inhibitors have been developed by both academia and industry but, similar efforts have not been put forth for PR3. Noticeably, the new generations of elastase inhibitors, such as those developed by Bayer HealthCare AG (Nussbaum et al., 2016) have not been tested against PR3. While HNE and PR3 share 57% sequence identity, there are noticeable differences in their binding sites, and it is unclear if these compounds inhibit PR3. We used molecular docking, equilibrium molecular dynamics simulations, and free energy calculations to evaluate the binding of these compounds on PR3. Multisite lambda dynamics (MSLD) was used to predict the relative binding free energies with CHARMM and the CHARMM General Force Field (CGenFF). Organic synthesis of one of the compounds and in vitro assay provided affinity data used to obtain absolute binding free energy. A comparison of the absolute binding free energy for individual compounds between the enzymes revealed that some of the compounds have similar or higher binding affinities to PR3 than to HNE. The analysis of enzyme-inhibitors hydrogen bonds and hydrophobic contacts from equilibrium MD simulations further revealed the effect of the differences in the binding site of both enzymes on their different affinity for the compounds. The main difference arising from the Leu/Lys99 mutation in the binding pocket of HNE to PR3. We are awaiting synthesis and in vitro assays of additional compounds to evaluate our computational predictions.

P10. Towards molecular design of peptide-based therapeutics against striated muscle disorders: unraveling the biophysics of the inotropic peptide S100A1ct by molecular modeling and simulation

Manuel Glaser[1], Michael Egger[2,3], Lukas Jarosch[1], Rafael Salazar[1], Sara Đaković[1], Patrick Most[2,3], Rebecca C. Wade[1,4]

[1]Molecular and Cellular Modeling (MCM) Group, Heidelberg Institute for Theoretical Studies (HITS), 69120 Heidelberg, Germany; [2]Division of Molecular and Translational Cardiology, Department of Medicine III, Heidelberg University Hospital, 69120 Heidelberg, Germany; [3]German Center for Cardiovascular Research (DZHK), Partner site Heidelberg, 69120 Heidelberg, Germany; [4]Center for Molecular Biology of Heidelberg University (ZMBH), DKFZ-ZMBH Alliance and Interdisciplinary Center for Scientific Computing (IWR), 69120 Heidelberg, Germany

Background and motivation: S100A1, an EF-hand calcium sensor protein, has been shown to influence cardiac performance. The S100A1ct peptide derived from its C-terminal helix is able to convey similar positive effects in cardiomyocytes (e.g. improved contractility) by affecting, e.g. the sarcoplasmic/endoplasmic reticulum calcium ATPase 2a (SERCA2a). Thus, S100A1ct has potential for the treatment of acute heart failure. We investigated S100A1ct's conformational preferences and its potential interaction with SERCA2a by computational approaches to provide insights for its therapeutic exploitation and development of peptidomimetics.

Methods and results: Enhanced sampling with Gaussian accelerated molecular dynamics (GaMD) of S100A1ct in aqueous environment indicated that an α-helix is its energetically most stable conformation but that another free energy basin was occupied by conformations kinked close to its cysteine residue, with the shallow energy landscape indicating that S100A1ct may undergo conformational switching.(1) We then customized a docking pipeline for generating putative structures of the S100A1ct-SERCA2a complex, applying global rigid-body docking (ClusPro) followed by semi-flexible refinement in a membrane environment (Rosetta MPDock). We identified two prominent possible binding sites for S100A1ct on SERCA2a: a groove lined by the transmembrane helices which is the site at which SERCA peptide regulators (e.g., phospholamban) bind in experimentally determined structures, and a groove on the opposite side of SERCA2a, where a disulfide bridge formed with SERCA2a would be possible.

Conclusions: We hypothesize that S100A1ct may bind similarly to known SERCA peptide regulators although it has different functional effects. We suggest that the conformational switching behaviour of S100A1ct observed may be important for its function.

(1) M. Glaser, N. J. Bruce, S. B. Han, and R. C. Wade, J. Phys. Chem. B. 125, 18, 4654-4666 (2021)

P11. Impact of Gaussian accelerated molecular dynamics on dynamic allosterism

Oriol Gracia I Carmona[1,2], Franca Fraternali[2], Chris Oostenbrink[1]

[1]University of natural resources and life sciences, Vienna, Austria; [2]King's College London, London, UK

Allosterism is the process by which a modification, namely an organic molecule binding or a mutation, that happens outside the binding site of the protein generates a change in the global behaviour of the protein. These modifications in the behaviour can be generated by big conformational shifts, or by changes in the internal motions of the proteins without any drastic change in the overall protein conformation, sometimes referred to as "dynamic allosterism".

Dynamic allosterism has remained more elusive due to the complexity of correctly assessing these subtle internal changes experimentally, making molecular dynamics simulations (MD) an ideal solution to tackle these kinds of systems. Several improvements regarding the study of allosteric pathways have been published, especially focusing on describing how the allosteric signal traverses the protein. However, despite all the improvements, these methods require long time scales to converge, hampering its applicability to drug design pipelines.

To tackle this challenge one can use enhanced sampling techniques such as Gaussian Accelerated Molecular Dynamics (GAMD). GAMD is an enhanced sampling technique that works by adding an harmonical boosting potential to lift up the energy wells, allowing to produce microseconds worth of sampling in nanoseconds time scales. GAMD has been successfully applied to different systems, however there is no knowledge of how the adding of this bias could affect these subtle internal motions nor how short the GAMD simulations can be to still capture the allosteric signal properly. In this work we have performed an in-depth analysis of the effects of GAMD on an allosteric model system, the Pyruvate kinase M2 (PKM2), and provided a comprehensive list of advantages and caveats of different possible settings.

P12. A MM/3D-RISM Approach to Characterize Antibody CH3-CH3 Interface Stability

Lukas J. Grunewald, Valentin J. Hörschinger, Franz Waibl, Monica L. Fernández-Quintero, Klaus R. Liedl
University of Innsbruck, Austria

Antibodies and other novel antibody-derived formats, such as bispecific antibodies, have emerged as one of the most important classes of biopharmaceuticals. Bispecific antibodies can target two separate epitopes utilizing two different antigen-binding sites, while conventional IgG1 antibodies are monospecific with two identical antigen-binding sites.

The development of these bispecific antibodies is challenging, because it requires the correct pairing of two different heavy chains and two different light chains. The pairing of all available chains can theoretically result in a variety of up to ten different molecules with only one being the desired bispecific antibody. Therefore, the presence of non-functional pairings results in a decrease of bispecific antibody yield. Over the past decades, several strategies have been developed to induce heterodimerization due to the effect of different point-mutations in the C_H3-C_H3 region. However, the resulting interactions in these different pairings at an atomistic scale are still not well understood.

Using molecular dynamics simulations in combination with 3D-Reference Interaction Site Model (3D-RISM), we estimate the binding free energy in the C_H3-C_H3 interface. We then compare our calculated results with the experimental data to identify the effect of different mutations, which are responsible for heterodimerization.

We find that the estimated electrostatic (ES) and Van der Waals (VdW) binding energies correlate inversely with our calculated hydration binding free energy. Furthermore, we consistently find that our approach can distinguish low-yielding homodimers from high-yielding heterodimers. By localization of the hydration free energy and ES/VdW energy we provide insights into the interactions that are critical for the C_H3-C_H3 interface formation.

P13. Computational Characterization of Antibody CH3-CH3 Interfaces

Valentin J. Hörschinger, Franz Waibl, Nicolas Melcher, Monica L. Fernández-Quintero, Klaus R. Liedl

University of Innsbruck, Austria

Antibodies are a fast-growing class of biotherapeutic proteins, opening novel treatment avenues to tackle various diseases. They adopt a Y-shaped structure consisting of various interacting domains. The protein-protein interactions driving the associations of these domains in solution are not yet fully understood, as they are experimentally not easily accessible. New insights into the underlying processes could improve our understanding of the antibody fold and aid in the development of novel bispecific antibodies or other antibody formats.

In this study we aim to characterize the interactions of the isolated C_H3-C_H3 dimer interface in solution in silico. Towards this, we employ a physics-based procedure utilizing structural information extracted from MD simulations. When applied to a set of human IgG1 bispecific candidates, this procedure can reliably differentiate beneficial from unbeneficial mutations.

In our approach, hydrophobic interactions are taken into account as hydration free energies derived from grid inhomogeneous solvation theory (GIST). Electrostatic and Van der Waals interactions are calculated directly from molecular mechanics energies.

By combining these two sources of information we provide a clear picture of the IgG1 C_H3-C_H3 interface and identify major structural interaction hotspots.

P14. Insights into the protic ionic liquid 1-methylimidazolium (trifluoro-)acetate

Florian Jörg, Christian Schröder
University of Vienna, Austria

The protic ionic liquid 1-methylimidazolium is in equilibrium with the corresponding neutral species 1-methylimidazole and acetic acid. The composition correpsonding to the equilibrium of this system was investigated. While several experiments suggest that the neutral species dominate the equilibrium, a significant conductivity is found. The newly developed polarizable force field was used to get insight into several mixtures of the neutral and charged species. Not only single value properties, like density, diffusion coefficients or conductivity were calculated and compared to experimental values, but also the complete frequency-dependent dielectric spectrum was used to determine the equilibrium composition [1].

The effects of varying the content of neutral species in the system was also investigated and quantified by means of the mentioned properties. Furthermore, the effect of exchanging acetic acid/acetate molecules with trifluoroacetate, which is more acidic, was investigated.

A new routine is under developement to allow for instantaneous proton transfer events during an MD simulation [2]. This allows the equilibrium to form, while the simulation is running. Additionally, deeper insight into proton transfer mechanisms can be gained.

[1] F. Joerg and C. Schröder, PCCP (2022), accepted

[2] R. Jacobi, F. Joerg and C. Schröder, PCCP (2022), 9277-9285

P15. Transformato: an MD engine independent tool for calculating relative binding free energies

Johannes Karwounopoulos[1], Marcus Wieder[2], Stefan Boresch[1]

[1]University Vienna, Faculty of Chemistry, Institute of Computational Biological Chemistry, Austria; [2]University Vienna, Faculty of Life Science, Department of Pharmaceutical Sciences, Austria

Predicting relative protein-ligand binding affinities is one of the major tasks in computer-aided drug design projects. In principle, alchemical free energy (AFE) calculations can fulfill this task, but in practice, a lot of hurdles prevent them from being applied routinely. AFE calculations need significant computing resources and can be comparatively slow. Their setup is not straightforward, even for expert users.

Several frontends to biomolecular simulation packages help to set up AFE simulations, e.g. [1,2,3]. We present a related Python based tool called Transformato that automates the setup and calculation of relative binding free energy calculations (RBFE). Our approach is based on the common core / serial-atom-insertion (CC/SAI) method [4], which avoids the need for special purpose code related to alchemical transformations. Thus, in principle, Transformato makes it possible to carry out RBFE calculations with any molecular dynamics (MD) engines.

We demonstrate Transformato's utility as a setup tool for RBFE calculations and validate its performance on a set of 76 pairwise mutations from well established datasets. We find that our results compare reasonably well with a root mean square error (RMSE) of 1.17 kcal/mol and a mean absolute error (MAE) of 0.85 kcal/mol to experiment. Thus, Transformato provides an open source and user-friendly implementation of the CC/SAI approach to rapidly setup up and calculate relative binding free energies, without being tied to a particular simulation program.

[1] D. Seeliger, et. al., Biophys. J., 2010, 98(10), 2309-2316

[2] V. Gapsys, et. al., Chem. Sci., 2020; 11(4), 1140-1152

[3] L. Wang, et. al., JACS, 2015; 137(7), 2695-2703

[4] M. Wieder, et. al., 2022, J. Comput. Chem., 43(17), 1151-1160

P16. Simulations of the Nucleosomal DNA: Mapping the Radical Cation Guanine

Maxime Kermarrec, Natacha Gillet

Université de Lyon, ENS de Lyon, CNRS UMR 5182, Université Claude Bernard Lyon 1, Laboratoire de Chimie, F69342, Lyon, France

An overexposure of DNA to an oxidative stress may results to various diseases related to mutations, especially cancers. [1] Therefore, understanding the behavior of the DNA oxidative damages is of medical and pharmaceutical interest. Guanine has the lowest ionization potential thus it is the most likely to be oxidized among the 4 nucleobases. As a result of this, the formation of an 8-oxo-Guanine or even a protein-DNA cross link can occur. Here we investigate the interactions between histones and guanines and what it generates on the ionization energy. Consequently, we want to understand what factors and effects can modulate the ionization potential of guanine in the nucleosome. We have done classical MD simulations to explore the conformational landscape. And then we used a QM/MM method : FO-DFTB/MM[2,3], in order to calculate the ionization potential and eventually do charge transfer simulations.

We have undertaken to study the influence of the near environment on the ionization potential of guanines within the nucleosome. From these simulations, we pinpoint that the histones tails have a considerable impact on this potential. Some others possible effects are under consideration such as: the sequence, the position or even the closeness of positive charges.

References

[1] Greenberg, M. M. In Vitro and in Vivo Effects of Oxidative Damage to Deoxyguanosine. Biochem.

Soc. Trans. 2004, 32 (1), 46–50. https://doi.org/10.1042/bst0320046.

[2] Kubar, T., Woiczikowski, P. B., Cuniberti, G., & Elstner, M. (2008). Efficient calculation of charge transfer matrix elements for hole transfer in DNA. *The Journal of Physical Chemistry B*, *112*(26), 7937-7947. https://doi.org/10.1021/jp801486d

[3] Kubar, T., & Elstner, M. (2008). What governs the charge transfer in DNA? The role of DNA conformation and environment. *The Journal of Physical Chemistry B*, *112*(29), 8788-8798. https://doi.org/10.1021/jp803661f

P17. Characterizing conformational diversity of antibody paratopes as a response to viral threats

Janik Kokot, Robert F. Wild, Monica L. Fernández Quintero, Klaus R. Liedl
Universität Innsbruck, Austria

If the past two years have taught us anything, then, that viral threats should not be underestimated. The impact of SARS-CoV 2 upon the world's finances, physical and mental health seems to be incomprehensible. Further, other rapidly mutating viruses e.g. the influenza A virus pose the constant threat of also mutating into a pandemic-threat variant. That's why continuous research to better understand both the threat itself and options to combat it is essential.

As one of nature's most versatile answers to pathogens, antibodies have become a centrepiece for human drug development. Nowadays numerous strategies are employed on antibodies and antibody related structures to increase efficacy and human compatibility. Here we showcase how computational methods can be used to generate invaluable insights for antibody research and drug development.

To this end, bias exchange metadynamics simulations followed by conventional molecular dynamics simulations were performed to efficiently traverse and sample the energy landscape. Two systems of SARS-CoV2 neutralizing antibodies with similar heavy chain sequences but distinct light chain germlines were simulated to investigate the effect of different light chains on the properties of the antigen binding site – the paratope. The experimentally observed loss in affinity is reflected in a completely different set of interchain contacts. Furthermore, the impact of a point mutation on the affinity and specificity of broadly neutralizing antibodies against influenza A was investigated. We were able to observe differences in flexibility and conformational diversity and identify possible mechanisms behind the experimentally observed behaviour.

Both examples show the importance of characterizing antibody paratope ensembles in solution to elucidate the respective dynamics involved in antigen recognition at atomic resolution.

P18. Antibody Interdomain Movements Co-determining Antigen Recognition

Monica L. Fernández-Quintero[1], Katharina B. Kroell[1], Johannes R. Loeffler[1], Patrick K. Quoika[1], Franz Waibl[1], Nancy D. Pomarici[1], Martin C. Heiss[1], Florian Hofer[1], Jakob R. Riccabona[1], Alexander Bujotzek[2], Ekkenhard Moessner[3], Guy Georges[2], Hubert Kettenberger[2], Klaus R. Liedl[1]

[1]Institute of General, Inorganic and Theoretical Chemistry, University of Innsbruck, Austria; [2]Roche Pharma Research and Early Development, Large Molecule Research, Roche Innovation Center Munich, Penzberg, Germany; [3]Roche Pharma Research and Early Development, Large Molecular Research, Roche Innovation Center Zurich, Schlieren, Switzerland

Antibodies have emerged as one of the most important classes of biotherapeutic proteins. They have revolutionized the treatment of various diseases like influenza, covid, autoimmune diseases and cancer. Therefore, understanding of the biophysical properties of an antibody to the respective antigen binding process is crucial to design new formats, which are required to address novel health challenges. Structurally, antibodies consist of two heavy and two light chains and can be subdivided into two antigen binding fragments and one crystallizable fragment. It has already been shown that for antibodies a single-static structure is not sufficient to capture its high flexibility and, hence, must be understood as a conformational ensemble. An extremely variable and diverse part of the antigen binding fragment is the complementarity determining region, which consists of six hypervariable loops forming the antigen-binding site, the paratope. However, not only these loops characterize the shape of the paratope but also the V_H-V_L and C_H1-C_L interfaces as well as the elbow angle are involved in antigen binding.

Here, we investigate the influence of different paratope states, framework mutations, different heavy- and light-chain pairings, affinity maturation, humanization, affinity maturation and different light chain types framework mutations on the three angles of the antigen binding fragment (V_H-V_L, C_H1-C_L, elbow angle). Examining the impact of paratope states on the relative V_H-V_L orientation reveals that due to the rearrangement of the CDR loops different paratope states favour distinct angles. As framework mutations and different heavy- and light-chain pairings affect the shape of the paratope too, alterations of the V_H-V_L interface angle and its distribution also occur as a result of these two effects. Additional to the V_H-V_L interface angle the elbow angle is rigidified as a consequence of affinity maturation and shifted by cause of humanization. The analysis of the dynamics of 5 antigen binding fragments with different light chain types and highly diverse elbow angles in the crystal structures reveals that both V_H-V_L and C_H1-C_L interfaces as well as the elbow angle interconvert between each other.

Thus, this study has broad implications in the field of antibody engineering, as it clearly shows the importance of considering the flexibility of antibody interdomain movements to revolutionize the understanding of the antigen binding process.

P19. BuRNN: Buffer Region Neural Network Approach for Polarizable QM/MM Simulations

Bettina Lier[1], Peter Poliak[1,2], Philipp Marquetand[3], Julia Westermayr[4], Chris Oostenbrink[1]

[1]University of Natural Resources and Life Sciences, Vienna, Austria; Institute of Molecular Modeling and Simulation; [2]Slovak University of Technology in Bratislava, Bratislava, Slovakia; Institute of Physical Chemistry and Chemical Physics; [3]University of Vienna, Vienna, Austria; Institute of Theoretical Chemistry; [4]University of Warwick, Coventry, U.K.; Department of Chemistry

Hybrid quantum mechanics/molecular mechanics (QM/MM) methodologies are powerful tools and advanced the field of computational chemistry. QM/MM simulations provide both a dynamic view on large systems at relevant time scales as well as precise energetics for a small quantum region. However, they are computationally expensive and suffer from artifacts at the interface between the regions that are treated at different levels of theory.

We have developed the buffer region neural network (BuRNN) approach as an alternative to existing QM/MM schemes. BuRNN introduces an additional buffer region between the QM and MM region, that experiences full electronic polarization by the QM region. The buffer is treated at both levels of theory to smooth the transition between the regions. Artifacts at the QM interface are minimized with BuRNN; essentially, they are expected to cancel at the edges of the buffer region in the difference of two QM calculations. To circumvent costly QM calculations at every time step, deep neural networks (NN) are employed. The energy difference of the two QM calculations can directly be predicted by a single evaluation of a trained NN. Besides, training on interaction energies and forces achieves outstanding accuracies. A second NN derives charges for including the mutual polarization also at longer ranges. Thus, the BuRNN approach allows for highly efficient and accurate hybrid NN/MM simulations.

We have demonstrated BuRNN by performing NN/MM simulations of the hexa-aqua iron complex. It is a relatively small and simple system but includes a transition metal and coordinative bonds, which are difficult to describe by classical force fields. We show that BuRNN can be applied for metal-ligand interactions instead of parameterization of additional force field terms. Furthermore, the good agreement of structural features and the high stability of long time-scale hybrid MD simulations makes BuRNN a promising method for the future.

P20. Building the most accurate zirconia force field for surface simulations of a nanovaccine platform

Tamás Milán Nagy[1], Livia Naszályi Nagy[2], Aranit Harizaj[3], Zsuzsanna Veres[4], Judith Mihály[5], Zsuzsanna Czégény[5], Emma Jakab[5], Zoltán Varga[5], Katalin Jemnitz[4], José C. Martins[2], Kevin Braeckmans[3], Krisztina Fehér[1,2], Hendrik Heinz[6]

[1]MTA-DE Molecular Recognition and Interaction Research Group, University of Debrecen, H-4032 Debrecen, Hungary; [2]NMR and Structure Analysis research group, Department of Organic and Macromolecular Chemistry, Ghent University, B-9000 Ghent, Krijgslaan 281 S4, Belgium; [3]Ghent Research Group on Nanomedicines, Laboratory of General Biochemistry and Physical Pharmacy, Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, Ghent University, Ottergemsesteenweg 460, B-9000 Ghent, Belgium; [4]Functional Pharmacology Research Group, Institute of Organic Chemistry, Research Centre for Natural Sciences (IOC RCNS), H-1117 Budapest, Magyar tudósok körútja 2., Hungary; [5]Institute of Materials and Environmental Chemistry, Research Centre for Natural Sciences (IMEC RCNS), H-1117 Budapest, Magyar tudósok körútja 2., Hungary; [6]Department of Chemical and Biological Engineering, University of Colorado Boulder, Boulder, Colorado 80303-0596, United States

Zirconia is a biocompatible and biodegradable ceramic metal-oxide often used as orthopedic and dental implants [1] and also studied as a promising drug carrier platform [2,3]. In our recent paper, we carried out the production of silica@zirconia (core@shell) nanoparticles [4] in the size range of 20-200 nm. These inorganic cargo molecules have attractive properties to immobilize, transport and release drug molecules such as immunoadjuvant oligodeoxynucleotides (ODN), which are promising candidates for targeting cancerous diseases.

Together with the investigation of biological responses we support the screening and selection of potential ODNs with all-atom molecular dynamics simulations. For studying the non-bonded and covalent interactions between the carrier and the adjuvant a unique molecular mechanics force field is required that can handle both the zirconia as well as the nucleotide atom types. This study follows the parametrization workflow of the INTERFACE simulation platform (IFF) [5], a well-performing extension of common harmonic force fields. Here, an order-of-magnitude higher accuracy is achieved since the parameters for inorganic compounds are carefully interpreted on a physical-chemical basis.

To begin with, the latest silica force field set was selected for the iterative adjustment of atomic charges and Lennard-Jones parameters to reproduce experimental (X-ray) lattice parameters with high accuracy (<1% error). Bonded terms were also introduced to keep the density close to the literature value. Representative cleavage planes were selected and the surface chemistry was also considered by hydroxylation. Currently, specific bulk (elastic modulus, cleavage energy) and surface (water contact angle, surface and hydration energy) properties are being checked in the final validation stage.

[1] *Dental Materials* 2018, 34:171-182. [2] *Trends Biomater. Artif. Organs* 2006, 20, 24–30. [3] *J. Mater.Sci. Mater. Med.* 2007, 19, 531–540.[4] *Nanomaterials* 2021, 11, 2166.

[5] *Langmuir* 2013, 29, 1754.

Nanomaterials **2021**, *11*, 2166.

P21. Coarse-grained modelling of DNA translocation through a protein nanopore

Cagla Okyay, Jérôme Mathé, Nathalie Basdevant
Université Paris-Saclay, Univ Evry, CNRS, LAMBE UMR8587, Évry-Courcouronnes 91025, France

Molecular-scale nanopores are discovered to be strong candidates for detection at single molecule level, due to their intrinsic sensing properties. Recent studies have demonstrated their potential for nanoscale detection in various fields of biology, biophysics, biotechnology and nanotechnology Boskovic and Keyser (2021); Yusko et al. (2016). Nanopore technology is therefore regarded as a powerful and precise method for single-molecule sensing, among which DNA sequencing has received a great research interest and is proving to be successful Jain et al. (2018). However, the dynamics of DNA sequencing through protein nanopores remains insufficiently explored. Our aim is to understand the molecular details of DNA translocation through α-hemolysin (αHL) nanopore, a broadly-used nanopore Di Muccio et al. (2019), using coarse-grained (CG) molecular dynamics (MD) simulations. The MARTINI force-field Monticelli et al. (2008); Uusitalo et al. (2015) was used for all CG-MD simulations and our system is composed of an αHL nanopore, a lipid membrane, a single stranded DNA (ssDNA) molecule in 1M ionic solution, (500,000 atoms,140,000 CG beads). In order to study in detail the translocation process as a function of DNA length and direction at the nanopore entry, several systems with different ssDNA lengths and orientations were prepared and simulated for 500 ns using steered MD to drive the DNA through the nanopore. Our preliminary results shed light on the dynamics of driven translocation of DNA through αHL nanopore with the aim of developing efficient detection devices.

P22. Bispecific Antibodies - Effects of Point Mutations on CH3-CH3 Interface Stability

Nancy Pomarici, Monica L. Fernández-Quintero, Klaus R. Liedl
Universität Innsbruck, Austria

A new emerging format of therapeutic proteins are bispecific antibodies, in which two different heavy chains heterodimerize to obtain two different binding sites. Therefore, it is crucial to understand and optimize the third constant domain (C_H3-C_H3) interface to favor the heterodimerization process over homodimerization, and consequently the production of bispecific antibodies. Here we use molecular dynamics simulations to investigate the dissociation process of 19 C_H3-C_H3 crystal structures that differ from each other in only few point mutations. We describe the dissociation of the dimeric interface as a two-steps mechanism. Apart from the bound and the dissociated state, we observe an additional intermediate state, which corresponds to an encounter complex. The analysis of the interdomain contacts shows that the electrostatic interactions dominate the C_H3-C_H3 interface, and it reveals key residues that stabilize the interface. A Markov state model confirms the presence of three states, providing additional information about kinetics and thermodynamics of the dissociation process. We expect that our results will improve the understanding of the C_H3-C_H3 interface interactions and thus advance the developability and design of new antibodies formats.

P23. Novel P-glycoprotein inhibitors with anti-cancer properties

Ashish Radadiya

Cardiff University, United Kingdom

Multidrug resistance (MDR) remains a deadly obstacle in cancer chemotherapy despite recent advances in our understanding of the molecular mechanisms underlying this phenomenon. P-glycoprotein (Pgp), an ATP binding cassette (ABC) transporter, is a key contributing ATP dependent efflux pump responsible for cancer multi-drug resistance. As part of efforts to identify human Pgp (hPgp) inhibitors, we chemically synthesized a series of novel triazole-conjugated dihydropyrimidinones (DHPM) and tested them against colorectal adenocarcinoma Caco-2 cell lines and assayed for their ability to modulate hPgp based on calcein-AM assay. The most potent DHPM derivative exhibits a five-fold higher activity against Caco-2 cells than carboplatin, gemcitabine and daunorubicin. Moreover, this compound has a 2-fold and 3-fold increase in its inhibitory potency against hPgp when compared with the well characterized hPgp modulators Verapamil and Cyclosporin A, respectively. Molecular docking and molecular dynamics (MD) studies employing a homology model of hPgp suggest that these DHPM derivatives prefer the M- rather than the R- or H-drug binding sites (DBSs). Our combined experimental and computational studies demonstrate that DHPM derivatives have dual function as hPgp inhibitors and anti-cancer agents.

P24. Alchemical Free Energy Calculations to predict the Effect of Point Mutations in Antibody CH3-CH3 Interfaces

Jakob R. Riccabona, Philipp Graf, Franz Waibl, Valentin J. Hörschinger, Monica L. Fernández-Quintero, Klaus R. Liedl
University of Innsbruck, Austria

In recent years, monoclonal antibodies have become one of the most successful biopharmaceutical proteins and are designed to tackle different severe diseases. Nowadays it is critical to design new antibody formats, like bispecific antibodies based on human IgG1, to achieve significant advances in the field of antibody engineering. Recent design strategies focused on engineering antibodies that can bind either two distinct antigens or two different epitopes on the same antigen. One of the major challenges in designing bispecific antibodies is to optimize the binding preferences between two distinct heavy and light chains to obtain heterodimers rather than homodimers.

We performed alchemical free energy calculations to understand the effect of point mutations on the C_H3-C_H3 interface stability. We started off by introducing one alanine point mutation to see which residues determine the steric or charge complementarity in the interface and whether calculated results agree with experimental data.

Furthermore, we compared the performance of the AMBER force fields ff14SB and ff19SB together with their respective parameterized water models TIP3P and OPC. The results for the different force fields were very consistent and encourage to apply this very robust protocol to more mutations to prospectively predict the interface stability.

P25. Ab Initio calculations of anharmonic vibrational spectra of carbonic acid and carbonic acid methyl ester

Jonas Schlagin[1], Dennis F. Dinu[1], Thomas Loerting[2], Klaus R. Liedl[1]

[1]Department of General, Inorganic and Theoretical Chemistry, University of Innsbruck, Austria; [2]Department of Physical Chemistry, University of Innsbruck, Austria

The formation and reactivity of carbonic acid has been subject of interest for over a century. In the late 20th century Hage, Hallbrucker and Mayer [1] postulated a polymorphism during their formation studies of H_2CO_3. This claim remained unchallenged for almost 20 years, however the recent works of Bernard [2], and Köck [3] suggested that instead of a polymorphism, a methylation takes place. Their works are partially based on the comparison of matrix isolation IR data to calculated vibrational spectra, but only within the harmonic approximation. These calculations are reliant on scaling factors to match the experimental data and only show good agreement for wavenumbers below 2000 cm^{-1}. Therefore, the aim of this work is to provide the anharmonic vibrational spectra in order to corroborate their argument.

The spectra are calculated with a Vibrational Configuration Interaction (VCI) approach [4] based on a multi-mode potential energy surface (PES) with up to 4-mode couplings using CCSD(T) – F12 / VTZ – F12 level of theory for 1- and 2-mode PES terms and CCSD(T) – F12 / VDZ – F12 for 3- and 4-mode PES terms. The accuracy of the VCI approach allows for a correct assignment of the bands due to smaller deviation from the experiment than the harmonic approximation and has therefore no need for empirical scaling factors [5].

[1] W. Hage, A. Hallbrucker, E. Mayer, J. AM. Chem. Soc. 1993, 115, 8427-8431

[2] J. Bernard, Ph.D. thesis, University of Innsbruck, available online at http://www.loerting.at/publications/bernard14-dissertation.pdf (Innsbruck, Austria), 2014

[3] E. Köck, Chemistry A European Journal 2019, 26, 285-305

[4] G. Rauhut, J. Chem. Phys. 2004, 121, 9313

[5] D. Dinu, Theor. Chem. Acc. 139, Article Number: 174

P26. Computing free energy differences between levels of theory by optimized non-equilibrium work methods

Andreas Schöller
University of Vienna, Austria

Force field (MM) based free energy simulations are becoming a routine method in drug development. Often, it would be desirable to carry out free energy simulations (FES) with a mixed (semi-empirical) quantum mechanical / MM Hamiltonian ((S)QM/MM). However, the computational cost quickly becomes excessive (even using SQM methods), and several "tricks" possible with pure force field based approaches cannot be used. The use of **indirect cycles** avoids some of these complications; the central step here is the calculation of free energy differences $\Delta A^{MM \rightarrow SQM}$ between the MM and the SQM representation of the system. Here we present strategies and methods to optimize such computations using **non-equilibrium work methods (NEW)**, focusing on the **optimal use of computational resources**, searching for **optimized switching protocols**, and the use of **Mulliken charge intermediates**.

In initial work we focused on the **optimal use of computational resources** in gas-phase simulations [1]. These results are summarized; in addition, we present first data how to optimize protocols for simulations in explicit solvent. The short lengths (2ps) of our non-equilibrium switching simulations may be too short to allow for complete solvent reorientation and relaxation. To compensate, we (i) explored various non-linear switching schemes to **optimize switching protocols**. Further, (ii), we employed **Mulliken charge intermediates** i.e., we split the calculation of $\Delta A_{X\ solv}^{MM \rightarrow SQM}$ into two steps, $\Delta A_{X\ solv}^{MM \leftrightarrow MULL}$ and $\Delta A_{X\ solv}^{MULL \rightarrow SQM}$. Inserting this intermediate stage avoids poor convergence due to the the lack of phase-space overlap between the SQM/MM and MM levels of theory. The use of Mulliken charges makes the (intermediate) low level of theory much more high-level like at affordable computational cost.

[1] Schöller, A., Kearns, F., Woodcock, H. L. & Boresch, S. *J. Phys. Chem. B*, **2022**, *126*, 2798-2811.

P27. Structural Characterization of Nanobodies in Different Stages of Affinity Maturation

Clarissa A. Seidler, Monica L. Fernández-Quintero, Klaus R. Liedl
University of Innsbruck, Austria

Antibody derived therapeutics belong to the fastest growing class of drugs and are therewith in the focus of the pharmaceutical industry. Since with the handling of such proteins a large number of problems is encountered, novel formats are under development. One promising example are nanobodies – the smallest-known functional antibody fragments with high therapeutic potential. Compared to conventional antibodies, nanobodies such as camelid V_HH domains are more stable, more soluble, they can work inside cells and can recognize cryptic epitopes.

In this study, we investigate camelid heavy chain antibody variable domain (V_HH) binding to hen egg-white lysozyme (HEL). We structurally and dynamically characterize the conformational diversity of four V_HH variants to elucidate the antigen binding process. For two of these antibodies not only dissociation constants are known, but also experimentally determined crystal structures of the V_HH in complex with HEL are available. We performed well-tempered metadynamics simulations in combination with molecular dynamics simulations to capture a broad conformational space and to reconstruct thermodynamics and kinetics of conformational transitions in the antigen binding site, the paratope. By kinetically characterizing the loop movements of the paratope, we find that with an increase in affinity the state populations shift towards the binding competent conformation. Additionally, these investigated nanobodies clearly follow the conformational selection paradigm, as the binding competent conformation pre-exists within the structural ensembles without the presence of the antigen.

P28. Exploring common dynamic determinants of quorum quenching enzymes activity and their rational engineering to efficiently combat antibiotic resistant bacteria

Bartlomiej Surpeta[1,2], Michal Grulich[3], Andrea Palyzová[4], Helena Marešová[4], Jan Brezovsky[1,2]

[1]International Institute of Molecular and Cell Biology in Warsaw; [2]Department of Gene Expression, Faculty of Biology, Adam Mickiewicz University in Poznan; [3]Laboratory of Modulation of Gene Expression, Institute of Microbiology, v.v.i., Academy of Sciences of the Czech Republic; [4]Laboratory of Molecular Structure Characterization, Institute of Microbiology,v.v.i., Academy of Sciences of the Czech Republic

Constantly developing bacterial antibiotic resistance is prioritized by WHO as one of the most alarming global health issues. Antibiotics exert high selective pressure that promotes resistance development due to their lethal effect on the microbiota. Clearly, effective alternatives to substitute or at least support antibiotics are needed. In this work, we target the bacterial communication process – quorum sensing. Degradation of microbial signaling molecules, commonly known as quorum quenching (QQ), has been shown to hamper the expression of genes controlling virulence factors and limit biofilm formation. Moreover, its less-selective mode of action is believed to limit the risks of conventional resistance mechanisms to appear. Through comprehensive modeling including molecular docking, molecular dynamics simulations of apo-enzymes and their complexes with signaling molecules, followed by hybrid quantum mechanics/molecular mechanics molecular dynamics simulations of the initial reaction steps we proved QQ activity of two biotechnologically well-established enzymes – *E. coli* and *Achromobacter spp.* penicillin G acylases (PGAs).[1] Importantly, proposed computational investigation was further enriched by experimental verification, that confirmed the prediction. Benefiting from such methodology that accounted for protein dynamics as a crucial factor of catalytic function and by comparing PGAs with prototypical acyl-homoserine lactone acylase possessing native activity towards bacterial signaling molecules, we determined common determinants responsible for QQ activity of N-terminal serine hydrolases and highlighted reasons for relatively low activity of PGAs. Building on top of these findings, we performed in-silico site-saturation mutagenesis and proposed rationally-engineered variants of *E. coli* PGAs with improved activities towards bacterial signaling molecules bringing us closer to antimicrobial agents supporting antibiotics in medicine, and potentially eliminating their usage in agriculture, industry or aquaculture applications.

This work was supported by the National Science Centre, Poland (2017/25/B/NZ1/01307 and 2021/41/N/NZ2/01365). The computations were performed at the Poznan Supercomputing and Networking Center.

1. Surpeta B., et al. ACS Catal., 6359–6374(2022)

P29. Prediction of macrocycle passive cell membrane permeability with machine learning

Xuechen Tang[1], Monica L. Fernández-Quintero[1], Franz Waibl[1], Anna S. Kamenik[2], Patrick K. Quoika[3], Dennis Dinu[1], Klaus R. Liedl[1]

[1]University of Innsbruck, Austria; [2]ETH Zürich, Switzerland; [3]Technical University Munich, Germany

Macrocycles are attractive for difficult targets with flat binding sites. Another interesting aspect is that they can reach hot spots within cells. This membrane permeability is aided by characteristic chameleonic behaviors, which describe conformational changes when crossing lipid membranes. During lead optimization, where polar groups are restricted by desired binding mode, permeability cliffs induced by small structural changes can zoom into these dynamic adaptions. Here we combine machine learning with enhanced sampling to develop a workflow to predict permeability of two different macrocycle sets which are based on different lead optimization strategies. The first dataset comprises 47 macrocycles and the second consists of 6 macrocycles, both with experimentally recorded passive cell permeability measured by parallel artificial membrane permeability assays (PAMPA). We perform accelerated molecular dynamics simulations in chloroform and in water, thereby mimicking the plasma membrane environment, to capture macrocycle chameleonicity. To characterize the effect of different optimization strategies, such as linker growth, N-, C- methylations and sidechain modifications, we use 72 permeability descriptors, including variations of polarized surface area (PSA) and intramolecular hydrogen bonds (IMHB). Structural and statistical key determinants of permeability are then extracted with machine learning to derive efficient prediction models for permeability. The obtained machine learning model for the first macrocycle datasets yields a Pearson correlation of 0.97 for the test set and highlights the increased sensitivity to chameleonicity at higher PSA. Additionally, we show transferability of our method to the second dataset, which has initially been studied with simulations and grid inhomogeneous solvation theory.

To sum up, we provide a widely applicable workflow to build reliable macrocycle permeability prediction models based on conformational ensembles, to characterize structural determinants on the stem structure which reifies structural rules previously only accessible with more expensive syntheses of exclusive macrocyclic series. This study has broad implications in accelerating permeability refinements during lead optimization, allowing easier characterization of structural determinants and guide synthesize of promising compounds with favorable permeability profile for difficult targets.

Reference:

Le Roux, A. et al. (2020), Journal of Medicinal Chemistry, 63(13), pp. 6774–6783.

Kamenik, A.S. et al. (2020), Journal of Chemical Information and Modeling, 60(7), pp. 3508–3517.

Tyagi, M. et al. (2018), Organic Letters, 20(18), pp. 5737–5742.

Eibe Frank, Mark A. Hall, and Ian H. Witten (2016), Morgan Kaufmann, Fourth Edition, 2016.

Rossi Sebastiano, M. et al. (2018), Journal of Medicinal Chemistry, 61(9), pp. 4189–4202.

P30. Biophysical Characterization of Antibody Constant Domains

Florian S. Wedl, Monica L. Fernández-Quintero, Klaus R. Liedl

Department of General, Inorganic and Theoretical Chemistry, Center for Molecular Biosciences Innsbruck (CMBI), University of Innsbruck, Innsbruck, Austria

Contributing Autors: Monica L. Fernández-Quintero, Patrick K. Quoika, Florian S. Wedl, Clarissa A. Seidler, Katharina B. Kröll, Johannes R. Löffler, Nancy D. Pomarici, Valentin J. Hörschinger, Franz Waibl, Alexander Bujotzek, Guy Georges, Hubert Kettenberger and Klaus R. Liedl

Antibodies are Y-shaped proteins and play an important role in our immune system. Due to their long half-life and their specific and unique binding properties, they represent an essential tool in the modern pharmaceutical industry. In order to optimize the development of antibody therapeutics and other new formats, the understanding and the prediction of biophysical properties in a computational cost efficient and accurate way is critical.

Especially, when designing new formats, a targeted modulation of pairing preferences, which is governed by biophysical properties, is mandatory. In general, the most used antibody format in case of pharmaceutical treatments is the immunoglobulin G. The classical format depends on homodimerization of two identical heavy chains in the crystallizable fragment region. In modern approaches, the interface of the third constant domain (C_H3-C_H3) is modified by mutations on each domain to form rather heterodimers than homodimers. Additionally, the C_H1-C_L interfaces of the constant domains of the antigen binding fragments share the same fold and a similar structure with the C_H3-C_H3 dimer and can further be modified by mutations.

Thus, the focus of this thesis is the comparison of these two antibody constant domains to identify differences that allow to crosslink the knowledge between these two interface types. We apply state-of-the-art biophysical analysis techniques in combination with machine learning approaches on single static antibody structures and their respective ensembles in solution to facilitate and advance rational design of antibodies.

P31. Benchmarking New and Emerging Nucleic Acid Force Fields Using DNA Mini-Dumbbells

Lauren Grace Winkler, Rodrigo Galindo-Murillo, Thomas E. Cheatham III
University of Utah, United States of America

Molecular dynamics (MD) simulations of duplex DNA repeatedly show convergence and agreement with experimental data. However, many of the most interesting and functional nucleic acid (NA) structures, such as noncanonical DNA motifs or RNA, are not pure helical duplexes and are highly sensitive to the balance of forces between water, ions, and NA atoms. Despite significant advances in simulation methods and available force fields (FF), *none* of the currently available FFs are able to consistently and accurately model non-duplex NAs. This greatly limits MD simulation in its application to drug development and biological discovery. Thus, in an effort to understand current FF limitations and focus FF development, this project has benchmarked eight of the newest FFs (BSC0, BSC1, OL15, OL21, Tumuc1, HBFIX/CuFIX, Charmm36, and Drude Polarizable) and three FF modifications using the DNA mini-dumbbell model. The DNA mini-dumbbells form two type II loops, where the first and fourth residues form a loop-closing base pair, while the second and third residues fold into the minor groove and either base stack or form a mismatch base pair. Hence, these mini-dumbbells were chosen for their variety of noncanonical interactions and ability to capture anomalous structures, while being computationally cost-effective at only 8 bases. FFs were evaluated using the AMBER MD machinery utilizing adaptive ensemble methods and analysis methods. Benchmarking of two DNA mini-dumbbell sequences at the microsecond timescale has shown surprisingly good results with some of the FFs. These include a high percentage of trajectory frames with less than 1 Å agreement to NMR re-refined structures and torsional angles modeled close to X-ray crystallographic measurements. This project, and understanding the current state of NA FFs, has implications in focusing FF development so noncanonical NAs and RNA may be one day be modeled with the accuracy that has now been achieved with duplex DNA.

P32. Guanine-Cytosine dynamics during DNA strand separation

Max Winokan[1], Louie Slocombe[2], Jim Al-Khalili[3], Marco Sacchi[2]

[1]Leverhulme Quantum Biology Doctoral Training Centre, University of Surrey; [2]Department of Physics, University of Surrey, Guildford; [3]Department of Chemistry, University of Surrey, Guildford

Proton transfer between the DNA bases can lead to non-standard, potentially mutagenic tautomeric forms [1, 2]. If the tautomers successfully pass through the replication machinery, they are thought to adopt a Watson-Crick-like shape and mismatch with the wrong base, thus evading proof-reading and potentially leading to replication error [3]. There is heated debate over the true biological impact of the tautomeric forms. Previously it was proposed that if the tautomeric lifetime is much shorter than the helicase cleavage time, no tautomeric population would successfully pass the enzyme [4]. Density functional theory (DFT) results suggest that the proton transfer energy landscape drastically changes during the first two Angstrom cleavage of the base. Molecular dynamics simulations indicate that cleavage time is much quicker than previously thought, with our models describing aqueous DNA. Our results indicate that a static picture of the proton transfer oversimplifies the biological event.

[1] L. Slocombe, J. S. Al-Khalili, M. Sacchi, Phys. Chem. Chem. Phys., (2021), 23(7), pp.4141-4150.

[2] O. Brovarets', D. Hovorun, J. Biomol. Struct. Dyn., (2018), 37(7), pp.1880-1907.

[3] P. Löwdin, Rev. Mod. Phys., (1963), 35(3), pp.724.

[4] O. Brovarets', D. Hovorun, J. Biomol. Struct. Dyn., (2014), 32(9), pp.1474-1499.

Participants list

Name	Affiliation	Country	
Alexey Aleksandrov	CNRS	France	*(Online Participation)*
Daniel Alvarez Lorenzo	Université de Fribourg	Switzerland	
Maciej Baginski	Gdansk University of Technology	Poland	
Michael Bakker	Charles University	Czech Republic	
Prabin Baral	University of Maryland	United States	*(Online Participation)*
Nathalie Basdevant	Université d'Evry Val d'Essonne	France	
Charles L. Brooks	University of Michigan	United States	*(Invited Speaker)*
Marco Cecchini	University of Strasbourg	France	
Luis Fernando Cervantes	University of Michigan	United States	
Payal Chatterjee	University of Maryland Baltimore	United States	*(Online Participation)*
Thomas Cheatham	University of Utah	United States	*(Invited Speaker)*
Piotr Chyży	University of Warsaw	Poland	
Zoe Cournia	Biomedical Research Foundation Academy of Athens	Greece	*(Invited Speaker)*
Anastasia Croitoru	Ecole Polytechnique	France	
Giulia D'Arrigo	HITS gGmbH	Germany	
Dennis Dinu	University of Innsbruck	Austria	
Carmen Domene	University of Bath	United Kingdom	
Stephan Ehrlich	Schrödinger GmbH	Germany	
Krystel El Hage	University of Paris-Saclay - University of Evry Val d'Essonne	France	
Monica L. Fernández-Quintero	University of Innsbruck	Austria	*(Invited Speaker)*
Anna-Lena Fischer	University of Innsbruck	Austria	
Leon Franke	Universität Konstanz	Germany	
Amina Gaffour	Faculty of Pharmacy Charles University, Hradec Králové	Czech Republic	
Parveen Gartan	University of Bergen	Norway	
Guy Georges	Roche	Germany	
Natacha Gillet	CNRS ENS de Lyon	France	
Manuel Glaser	Heidelberg Institute for Theoretical Studies (HITS)	Germany	
Oriol Gracia I Carmona	University of natural resources and life sciences, Vienna	Austria	
Jana Grünbacher	University of Innsbruck	Austria	
Lukas Grunewald	University of Innsbruck	Austria	
Florian Hofer	University of Innsbruck	Austria	

Johanna Hörberg	University of Gothenburg	Sweden	*(Online Participation)*
Valentin Jakob Hörschinger	University of Innsbruck	Austria	
Gerhard Hummer	Max Planck Institute of Biophysics	Germany	*(Invited Speaker)*
Callum Matthew Ives	University of Dundee	United Kingdom	
Florian Jörg	University of Vienna	Austria	
William L. Jorgensen	Yale University	United States	*(Invited Speaker)*
Alessia Karasani	University of Innsbruck	Austria	
Johannes Karwounopoulos	University Vienna	Austria	
Maxime Kermarrec	Ecole Normale Superieure de Lyon	France	
Johannes Kraml	BioNTech SE	Germany	
Katharina B. Kroell	University of Innsbruck	Austria	
Anmol Kumar	University of Maryland Baltimore	United States	*(Online Participation)*
Tom Kurtzman	Lehman College CUNY	United States	*(Invited Speaker)*
Nathan Levinzon	University of Utah	United States	
Klaus Liedl	University of Innsbruck	Austria	*(President)*
Bettina Lier	University of Natural Resources and Life Sciences, Vienna	Austria	
Stephanie Linker	ETH Zurich	Switzerland	
Yun Lyna Luo	Western University of Health Sciences	United States	*(Invited Speaker)*
Gillian Lynch	University of Texas Medical Branch at Galveston	United States	*(Online Participation)*
Alexander MacKerell	University of Maryland	United States	*(Invited Speaker)*
Kenneth M. Merz, Jr.	Michigan State University	United States	
Ana Milinski	IGBMC	France	*(Online Participation)*
Tamás Milán Nagy	University of Debrecen	Hungary	
Lennart Nilsson	Karolinska Institutet	Sweden	*(Invited Speaker)*
Taiji Oashi	Kyowa Kirin Co., Ltd.	Japan	*(Online Participation)*
Rodrigo Ochoa	Boehringer Ingelheim	Germany	
Cagla Okyay	Universite Paris-Saclay-Universite d'Evry	France	
Chris Oostenbrink	University of Natural Resources and Life Sciences, Vienna (BOKU)	Austria	
Asuka A Orr	University of Maryland, Baltimore	United States	*(Online Participation)*
Sílvia Osuna	University of Girona	Spain	*(Invited Speaker)*
Michele Parrinello	ETH Zürich	Switzerland	*(Invited Speaker)*
Negar Parvizi	University of Maryland	United States	*(Online Participation)*
Christine Peter	University of Konstanz	Germany	*(Invited Speaker)*

Monte Pettitt	Ubniversity of Texas Medical Branch	United States	
Nancy Pomarici	University of Innsbruck	Austria	
Patrick K. Quoika	Technical University Munich	Germany	
Ashish Radadiya	Cardiff University	United Kingdom	
Jakob R. Riccabona	University of Innsbruck	Austria	
Nigel Richards	Cardiff University	United Kingdom	*(Invited Speaker)*
Sereina Riniker	ETH Zürich	Switzerland	*(Invited Speaker)*
Edina Rosta	University College London	United Kingdom	*(Invited Speaker)*
Manabendra Sarma	Indian Institute of Technology Guwahati	India	*(Online Participation)*
Jonas Schlagin	University of Innsbruck	Austria	
Andreas Schöller	University of Vienna	Austria	
Clarissa Amanda Seidler	University of Innsbruck	Austria	
Jana Shen	University of Maryland School of Pharmacy	United States	*(Invited Speaker)*
Mingzhe Shen	University of Maryland, Baltimore	United States	*(Online Participation)*
Nadja Katharina Singer	University of Vienna	Austria	
Vladimir Sladek	Institute of Chemistry, Slovak Academy of Sciences	Slovakia	
Martin Spichty	CNRS	France	
Roland Stote	IGBMC-CNRS	France	
Bartlomiej Surpeta	International Institute of Molecular and Cell Biology	Poland	
András Szabadi	University of Vienna	Austria	
Xuechen Tang	University of Innsbruck	Austria	
Cedric Vallee	University of Surrey	United Kingdom	
Franz Waibl	University of Innsbruck	Austria	
Yin Wang	University of Innsbruck	Austria	
Florian Stefan Wedl	University of Innsbruck	Austria	
Robert Franz Wild	University of Innsbruck	Austria	
Lauren Grace Winkler	University of Utah	United States	
Max Winokan	University of Surrey	United Kingdom	
Razie Yousefi	Univeersity of Texas Medical Branch	United States	*(Online Participation)*
Wenbo Yu	University of Maryland Baltimore	United States	*(Online Participation)*
Mingtian Zhao	University of Maryland, Baltimore	United States	*(Online Participation)*

Lunch Suggestions

Uni-Café Bistro, Innrain 55, $$
Saisonal Food, Burger, Sandwich

Taj Indian Restaurant Muskete, Franz-Fischer-Straße 54, $
Indian Food, food delivery possible

Posidonas, Innrain 38, $$
Greek Food, Food delivery possible

D-Werk, Innrain 30a, $
Special kebab and bowls, also vegan dishes

Machete – Burrito Kartell, Anichstraße 29, $
Mexican Burritos

Thai-Li-Ba, Adolf-Pichler-Platz 3, $$
Thai Food

Gösser's, Adolf-Pichler-Platz 3, $$
Traditional Austrian Food

Das Schindler, Maria-Theresien-Straße 31, $$$
Traditional Austrian Food

Hard Rock Cafe, Maria-Theresien-Straße 16, $$$
American Style

Oishi Innsbruck, Maximilianstraße 33, $$
Japanese and Thai kitchen

Klein und Fein, Herzog-Siegmund Ufer 1-3, $
Local organic food

Al Pacino Pizzeria, Innrain 109, $
Italian and Turkish kitchen

Glorious Bastards, Egger-Lienz-Straße 118, $$
American Style

$ … to 10 €
$$ … 10 to 18€
$$$ … from 18€