Friday, March 18, afternoon, Session V

L19

MOLECULAR DYNAMICS SIMULATIONS OF THE TRANSPORT OF LIGANDS THROUGH PATHWAYS OF HALOALKANE DEHALOGENASES DhaAwt AND DhaA31

Zuzana Dunajova, Sergio M. Marques, Jan Brezovsky*, Jiri Damborsky

Loschmidt Laboratories, Department of Experimental Biology and Research Centre for Toxic Compounds in the Environment RECETOX, Faculty of Science, Masaryk University, Brno
**brezovsky@mail.muni.cz*

DhaA31 is a five-point mutant of the haloalkane dehalogenase DhaAwt, with 32-fold improvement of the overall catalytic rate towards the anthropogenic substrate 1,2,3-trichloropropane (TCP). The higher activity of DhaA31 was achieved by introducing bulky residues and thus narrowing the tunnels that connect the active site with the bulk solvent, what demonstrated the importance of the access pathways for the catalytic efficiency [1]. To understand the performance of enzymes at the molecular level, molecular dynamics (MD) simulations proved to be very beneficial because of the possibility to analyse details of the catalytic cycle. Previous computational studies that were focused on the ligand transport were performed with DhaA31 before solving its crystal structure, and were also significantly limited by the accessible time-scales [1, 2]. Now, with the availability of high quality crystal structures [3], GPU enabled computation [4], and generic accelerating methods [5], a more realistic study of individual steps of the catalytic cycle can be performed.

In this study, MD simulations of DhaAwt and DhaA31 were carried out with either the substrate TCP, or the products 2,3-dichloropropan-1-ol (DCP) and Cl^- ion. The purpose was to monitor binding and release steps of the catalytic cycle, respectively. These simulations revealed that the substrate and the products strongly influence the tunnel opening in both enzymes and the main tunnel (known as p1) seems to be relevant for the transport of studied ligands in both enzymes. The binding and release of ligands was much slower in DhaA31, what is in agreement with the product release being the rate-limiting step. The TCP release from the active sites of both enzymes was much slower compared to DCP, which matches its more hydrophobic nature. Interestingly, several TCP molecules were observed to bind the active site of DhaAwt simultaneously, suggesting higher substrate inhibition of this enzyme. Simulations of TCP bound to the active site sampled more than three times larger amount of reactive positions with DhaA31 than with the DhaAwt, which could explain the better performance of this variant. The Tyr176 and Phe245 residues introduced in the DhaA31 notably contributed to the binding of TCP in the reactive positions. In conclusion, our MD simulations provided a mechanistic description of ligand transport in DhaAwt and DhaA31. This knowledge could help with designing the next generation of TCP-degrading enzymes.

1. M. Pavlova, M. Klvana, Z. Prokop, R. Chaloupkova, P. Banas, M. Otyepka, et al., *Nat. Chem. Biol.*, **5**, (2009), pp. 727–733.
2. M. Klvana, M. Pavlova, T. Koudelakova, R. Chaloupkova, P. Dvorak, Z. Prokop, et al., *J. Mol. Biol.*, **392**, (2009), pp. 1339–1356.
3. M. Lahoda, J. R. Mesters, A. Stsiapanava, R. Chaloupkova, M. Kuty, J. Damborsky, et al., *Acta. Cryst.*, **70**, (2014), pp. 209–217.
4. M. S. Friedrichs, P. Eastman, V. Vaidyanathan, M. Houston, S. Legrand, A. L. Beberg, et al., *J. Comput. Chem.*, **30**, (2009), pp. 864–872.
5. D. Hamelberg, J. Mongan, J. A. McCammon, *J. Chem. Phys.*, **120**, (2004), pp. 11919–11929.

L20

COMPUTER SIMULATIONS OF NUCLEOSOME POSITIONING AND ALLOSTERIC EFFECTS IN DNA

T. Dršata[1] and F. Lankaš[1,2]

[1]Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo nám. 2, 166 10 Prague, Czech Republic;
[2]Laboratory of Informatics and Chemistry, University of Chemistry and Technology Prague, Technická 5, 166 28 Pragure, Czech Republic

The positioning of a nucleosome with respect to DNA is an important factor influencing the regulation of gene expression. It has been shown that particular sequences, like A-tracts (i.e. A_nT_n or A_{2n}), may facilitate gene activation by excluding nucleosomes, which can be used to fine tune gene expression [1]. The affinity of a DNA oligomer for formation of a nucleosome is given by its sequence dependent structural and mechanical properties.

These properties also play an important role in DNA-mediated allosteric effects. DNA allostery is a phenomenon analogous to the extensively studied allosteric coupling in proteins. Binding of a small ligand or a protein to the DNA can cause changes in the DNA structure and flexibility that affect the binding affinity of a subsequent ligand. Examples include minor groove binders such as pyrrole-imidazole polyamides [2] or heterocyclic diamidines [3], potential gene expression regulators. However, allosteric coupling between proteins bound to the DNA has also been demonstrated experimentally [4].

A viable approach to probe sequence dependent structural and mechanical properties of DNA are computer simulations. We employed a coarse-grained model of DNA to investigate unique properties of A-tracts and their implications for the nucleosome formation [5]. The model was further extended to describe DNA-mediated allostery involving minor groove binders [6] and pairs of bound proteins [7]. Parameters of the model were inferred from standard explicit solvent simulations of molecular dynamics.

Our work elucidates the seemingly contradictory experimental stiffness data of A-tracts and exposes the differences in properties of symmetric (A_nT_n) and asymmetric (A_{2n}) A-tracts, with possible implications for gene expression manipulation. Our extended model predicts structural changes of the DNA upon minor groove ligand binding that are in quantitative agreement with experiment. Furthermore, it provides a mechanistic explanation of the experimentally observed allosteric coupling between proteins bound to DNA.

1. Segal, E. and Widom, J., Curr. Opin. Struct. Biol., 2009, 19, 65-71.
2. Chenoweth, D. M. and Dervan, P. B., Proc. Natl. Acad. Sci. USA, 2009, 106, 13175-13179.
3. Hunt, R. A. et al., Nucleic Acids Res., 2011, 39, 4265-4274.
4. Kim, S., et al., Science, 2013, 339, 816-819.
5. Dršata, T., Špačková, N., Jurečka, P., Zgarbová, M., Šponer, J. and Lankaš, F., Nucleic Acids Res., 2014, 42, 7383-7394.
6. Dršata, T., Zgarbová, M., Špačková, N., Jurečka, P., Šponer, J. and Lankaš, F., J. Phys. Chem. Lett., 2014, 5, 3831-3835.
7. Dršata, T., Zgarbová, M., Jurečka, P., Šponer, J. and Lankaš, F., Biophys. J., http://dx.doi.org/10.1016/j.bpj.2015.12.039.

L21

CATION TRANSLOCATION IN HUMAN ORAI CHANNELS: MODELING AND SIMULATIONS

Vasilina Zayats,[1] Irene Frischauf,[2] Saurabh Kumar Pandey,[1,3] Isabella Derler,[2] Christoph Romanin,[2] Rainer Schindl,[2] Rüdiger H. Ettrich[1,3]

[1]Center for Nanobiology and Structural Biology, Institute of Microbiology, Academy of Sciences of the Czech Republic, Zamek 136, CZ-373 33 Nove Hrady, Czech Republic, ettrich@nh.cas.cz
[2]Institute of Biophysics, JKU Life Science Center, Johannes Kepler University Linz, Gruberstrasse 40, 4020 Linz, Austria
[3]Faculty of Sciences, University of South Bohemia, Zamek 136, CZ- 373 33 Nove Hrady, Czech Republic

Ca^{2+}-release-activated Ca^{2+} channels, encoded by Orai channels, form an ubiquitous cellular Ca^{2+} entry pathway, and control diverse signalling processes including gene expression, cell proliferation and T-cell activation. The human genome contains three Orai isoforms; however it remains unknown if their sequence variations are required for specific Ca^{2+} signals. Orai1 senses the amount of cholesterol in the plasma membrane and apparently the interaction of Orai1 with cholesterol inhibits its activity, thereby limiting store-operated calcium entry [1]. High affinity Ca^{2+} binding to the pore entrance of Orai channels creates a local extracellular calcium accumulating region CAR and provides fundamental insight into the unique mechanism of Ca^{2+} permeation of Orai channels [2]. The combination of computational modeling of Orai channels and molecular dynamics simulations provided by the team in Nove Hrady, and functional patch clamp, site-directed mutagenesis and experimental biophysical experiments performed by the Linz Team allows to propose that the Orai1 channel architecture with a close proximity of CAR to the selectivity filter, which enables Ca^{2+}-selective ion permeation, enhances the local extracellular Ca^{2+} concentration to maintain Ca^{2+}-dependent gene regulation even in environments with relatively low Ca^{2+}concentrations [2].

1. Isabella Derler, Isaac Jardin, Peter B Stathopulos, Martin Muik, Marc Fahrner, Vaselina Zayats, Saurabh Pandey, Michael Poteser, Barbara Lackner, Marketa Absolonova, Klaus Groschner, Rüdiger Ettrich, Mitsu Ikura, Christoph Romanin (2016) Cholesterol binding modulates Orai1 channel function *Science Signaling* 9 (412): ra10. Jan 26.
2. Irene Frischauf, Vasilina Zayats, Michael Deix, Anna Hochreiter, Isaac Jardin Polo, Martin Muik, Barbara Lackner, Barbora Svobodova, Teresa Pammer, Monika Litvinukova, Amrutha Arumbakam Sridhar, Isabella Derler, Ivan Bogeski, Christoph Romanin, Rüdiger Ettrich, Rainer Schindl (2015) A calcium accumulating region, CAR, in the Orai1 channel enhances Ca^{2+}- permeation and SOCE-induced gene transcription *Science Signaling* 8 (408): ra131. Dec 22.

L22

LONE-PAIR- INTERACTIONS IN NUCLEIC ACIDS AND PROTEINS: PHYSICAL ORIGIN AND SIGNIFICANCE

Jan Novotný,[1] Sophia Bazzi,[1,2,3] Radek Marek,[1,3] Jiří Kozelka[2,4]

[1]CEITEC – Central European Institute of Technology, Masaryk University, Kamenice 5/A4, CZ-625 00 Brno, Czech Republic
[2]Department of Condensed Matter Physics, Faculty of Science, Masaryk University, Kotlářská 2, CZ-611 37 Brno, Czech Republic
[3]National Center for Biomolecular Research, Faculty of Science, Masaryk University, Kamenice 5/A4, CZ-625 00 Brno, Czech Republic
[4]Université Paris Descartes, UMR 8601 CNRS, 45, rue des Saints-Pčres, 75270 Paris, France, kozelka.jiri@gmail.com

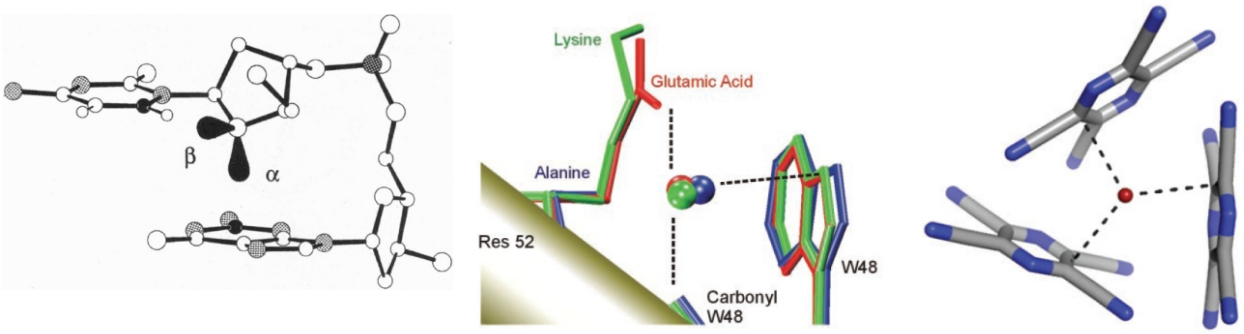
The lone-pair- interaction is a still relatively unexplored bonding between a lone-pair (lp) of electrons of an electronegative atom and a -system. In 1995, Egli and Gessner identified in the d(CpG) steps of Z-DNA an interaction between an oxygen lp of the cytidine deoxyribose and the guanine base, which they classified as n * hyperconjugation and related to the stability of Z-DNA [1]. A similar type of interaction was suggested to occur in many protein crystal sturctures where water molecules were observed to contact a tryptophan or a histidine residue along the normal to the ring plane through the endocyclic N atom [2].

A subclass of lp- interactions are anion- interactions, where the interacting lp is a part of an anionic residue. For instance, electron-deficient arenes such as 1,2,4,5-tetracyanobenzene (TCB) or tetracyanopiperazine (TCP) form with halide ions in solution and in solid phase complexes

whose charge-transfer absorption bands are indicative of a weakly covalent interaction. Since the halides interact with the -face of the arene in a similar manner as does water with tryptophan in proteins and deoxyribose with nucleobases in nucleic acids (see figure below), on may ask to which extent charge transfer, evidently operating in the halide-arene complexes, contributes to the binding of the lp- interactions in the biopolymers. Asking more generally: What are the energy components stabilizing lone-pair- interactions and how does their balance depend on the molecular properties of the interacting partners? Do force-field calculations portray such interactions properly?

The talk will attempt to provide the answer.

1. Egli, M. and Gessner, R., Proc. Nat. Acad. Sci. USA, 1995, 92, 180-184.
2. Stollar, E. J., Gelpi, J. L., Velankar, S., Golovin, A., Orozco, M. and Luisi, B. F., Proteins, 2004, 57, 1-8.
3. Rosokha, Y. S.; Lindeman, S. V.; Rosokha, S. V.; Kochi, J. K., Angew. Chem. Int. Ed., 2004, 43, 4650-4652.



Left: lp- interaction stabilizing Z-DNA [1]. Middle: lp- interaction suggested to operate between a conserved water molecule and tryptophan W48 in the Engrailed homeodomain and its mutants [2]. Right: lp- interaction observed in the X-ray structure of the charge-transfer complex between Br^- and TCP [3].

Friday, March 18, afternoon, Session VI

L23

STRUCTURAL INSIGHTS AND IN VITRO RECONSTITUTION OF MEMBRANE TARGETING AND ACTIVATION OF HUMAN PI4KB BY THE ACBD3 PROTEIN

Martin Klima, Daniel Toth, Rozalie Hexnerova, Adriana Baumlova, Dominika Chalupska, Jan Tykvart, Lenka Rezabkova, Nivedita Sengupta, Petr Man, Anna Dubankova, Jana Humpolickova, Radim Nencka, Vaclav Veverka, Tamas Balla, Evzen Boura

Ústav organické chemie a biochemie AV ČR, v.v.i., Praha

Phosphatidyl inositol 4-kinase beta (PI4KB) is one of four human PI4K enzymes that generate phosphatidyl inositol 4-phosphate (PI4P), a minor but essential regulatory lipid found in all eukaryotic cells. To convert their lipid substrates, PI4Ks must be recruited to the correct membrane compartment. PI4KB is critical for the maintenance of the Golgi and trans Golgi network (TGN) PI4P pools, however, the actual targeting mechanism of PI4KB to the Golgi and TGN membranes is unknown. Here, we present an NMR structure of the complex of PI4KB and its interacting partner, Golgi adaptor protein acyl-coenzyme A binding domain containing protein 3 (ACBD3). We show that ACBD3 is capable of recruiting PI4KB to membranes both *in vitro* and *in vivo*, and that membrane recruitment of PI4KB by ACBD3 increases its enzymatic activity and that the ACBD3:PI4KB complex formation is essential for proper function of the Golgi.