XX Discussions in Structural Molecular Biology and 7th User Meeting of the Czech Infrastructure for Integrative Structural Biology

Annual Meeting of the Czech Society for Structural Biology

Conference Centre of the Czech Academy of Sciences, Nové Hrady, March 21 - 23, 2024

Organisers:

Jan Dohnálek, Jarmila Dušková, Jan Stránský, Tereza Skálová, Kristýna Adámková, Radek Kužel

The event is organized by the Czech Society for Structural Biology, the Czech Infrastructure for Integrative Structural Biology, and the Institute of Biotechnology of the Czech Academy of Sciences.

Thursday, March 21, Session I

L1

FIRST TWENTY YEARS OF DISCUSSIONS IN STRUCTURAL MOLECULAR BIOLOGY

Bohdan Schneider

Institute of Biotechnology of the Czech Academy of Sciences, CZ-25250 Vestec, Czech Republic

I will tell you what are my memories of twenty years of Discussions in Structural Molecular Biology. I will try to explain the greatest mysteries of this conference: why in March, why in Saturday, why in Nové Hrady? I will also show programs, lists of participants, abstracts from the historically first meetings. These existed as html pages (Internet, yes!) and I found most of them beginning with the files from the first year in 2002. I also have pictures from some years and selected ones will be presented in spite of GDPR. PDB permitting, I plan to show (surely growing) numbers of experimental structures Made in Czech Republic and comment on changing focus from a lot of computer modeling at the beginning to mostly experimental structural biology in recent years.

PhD Thesis Award

L2

STRUCTURAL CHARACTERIZATION OF INFLUENZA A POLYMERASE PA SUBUNIT DOMAINS IN COMPLEX WITH NOVEL INHIBITORS

K. Radilova[1], M. Kral[1,2], T. Kotacka[1,2], R. Reiberger[1], J. Brynda[1], V. Zima[1,3], J. Hejdanek, J. Konvalinka[1,4], A. Machara[1], M. Kozisek[1]

[1]Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, CZ
[2]First Faculty of Medicine, Charles University, CZ
[3]Department of Organic Chemistry; Faculty of Science, Charles University, CZ
[4]Department of Biochemistry, Faculty of Science, Charles University, CZ

Influenza RNA-dependent RNA polymerase is a heterotrimeric complex and has an essential role in the virus life cycle. It is responsible for viral replication and transcription. One of its subunits, the PA, interacts with the PB1 subunit via a crucial protein-protein interaction at its C-terminus. This 3_{10} helix-mediated intersubunit interaction is required for the whole heterotrimer assembly (**Fig. A**) [1, 2]. The 14 amino acids long peptide from the PB1 N-terminus was identified as a nanomolar inhibitor of the PA-PB1 protein-protein interaction [3]. Furthermore, the peptide array identified several introduced mutations as beneficial for peptide binding, though without an X-ray structure [4]. We have structurally and thermodynamically characterized the PA C-ter interacting with optimized minimal peptide-based inhibitors derived from the PB1 N-terminus (**Fig. B**). Additionally, the N-terminal domain of PA contains the active site for endonuclease. A key characteristic of endonuclease inhibitors is their ability to bind to Mn^{2+} and Mg^{2+} ions that are embedded in the catalytic site of the enzyme. We have explored the inhibitory potency of flavonoids and their derivatives against the endonuclease domain [5, 6]. Using X-ray protein crystallography, we have described the binding modes of those inhibitors in the PA endonuclease active site (**Fig. C**). Using two different assays and structural analysis, we have been able to identify and characterize the molecular mode of action of flavonoids in cells infected with the influenza virus. We have successfully identified the target protein of these compounds, which are commonly used as supplements during influenza viral infections.

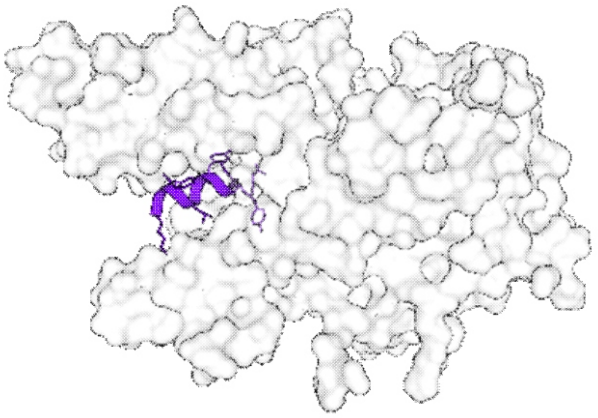

Figure A.

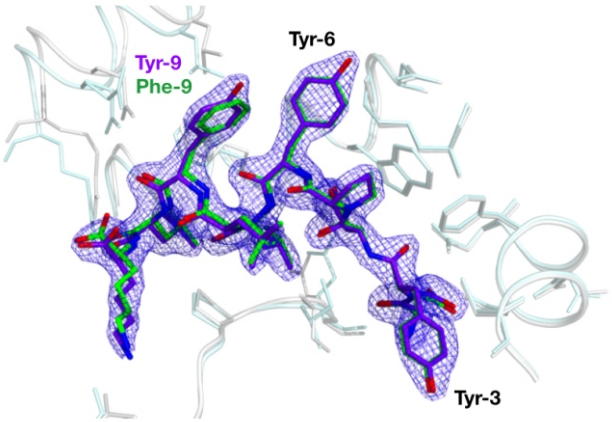

Figure B.

1. He, X.J., Zhou, J., Bartlam, M., Zhang, R.G., Ma, J.Y., Lou, Z.Y., Li, X.M., Li, J.J., Joachimiak, A., Zeng, Z.H., Ge, R.W., Rao, Z.H., Liu, Y.F. (2008). *Nature* **454**, 1123–1126.
2. Obayashi, E., Yoshida, H., Kawai, F., Shibayama, N., Kawaguchi, A., Nagata, K., Tame, J. R., Park, S.Y. (2008). *Nature* **454**, 1127–1131.
3. Wunderlich, K., Mayer, D., Ranadheera, C., Holler, A.S., Manz, B., Martin, A., Chase, G., Tegge, W., Frank, R., Kessler, U., Schwemmle, M. (2009). *PLoS One* **4**, e7517.
4. Wunderlich, K., Juozapaitis, M., Ranadheera, C., Kessler, U., Martin, A., Eisel, J., Beutling, U., Frank, R., Schwemmle, M. (2011). *Antimicrob. Agents Chemother.* **55**, 696–702.
5. Zima, V., Radilova, K., Kozisek, M., Albinana, C.B., Karlukova, E., Brynda, J., Fanfrlik, J., Flieger, M., Hodek, J., Weber, J. (2020) *Eur. J. Med. Chem.* **208**, 112754.
6. Reiberger, R., Radilova, K., Kral, M., Zima, V., Majer, P., Brynda, J., Dracinsky, M., Konvalinka, J., Kozisek, M., Machara, A. (2021) *Int. J. Mol. Sci.* **22**, 7735.

This work was supported by the European Regional Development Fund, OP RDE, Project: "Chemical biology for

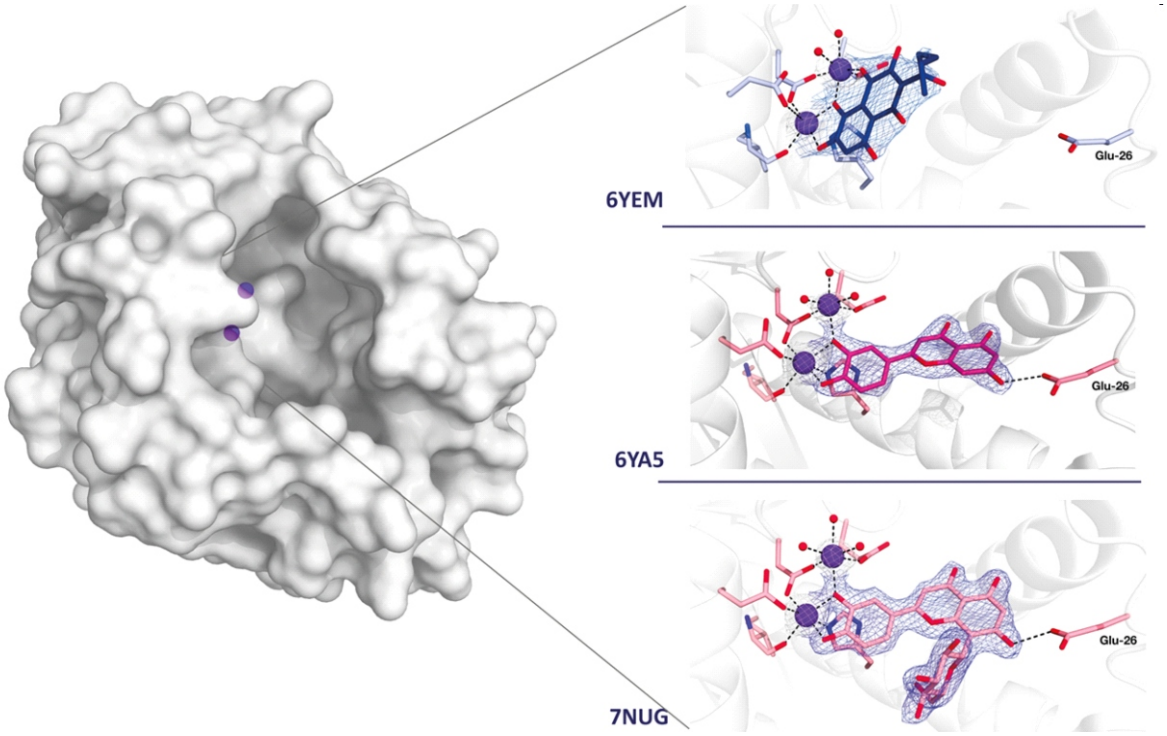

Figure C.

drugging undruggable targets (ChemBioDrug)" (No. CZ.02.1.01/0.0/0.0/16_019/0000729) and the European Union - Next Generation EU, The project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103).

Thursday, March 21, Session II

L3

X-RAY STRUCTURES OF (*S*)-ENANTIOSELECTIVE HALOALKANE DEHALOGENASE DmmarA FROM *MYCOBACTERIUM MARINUM* REVEAL A NEW MODE OF HOMODIMERIZATION THAT IS ATYPICAL FOR THIS ENZYME FAMILY

K. Šnajdarová[1], S. M. Marques[1,2], J. Damborský[1,2], D. Bednář[1,2], M. Marek[1,2]

[1]Loschmidt Laboratories, Department of Experimental Biology and RECETOX, Faculty of Science, Masaryk University, Kamenice 5, Bld. A13, 625 00 Brno, Czech Republic
[2]International Clinical Research Center, St. Anne's University Hospital Brno, Pekarska 53, 656 91 Brno, Czech Republic
517805@mail.muni.cz

Haloalkane dehalogenases (HLDs) are a family of -hydrolase fold enzymes that employ S_N2 nucleophilic substitution to cleave the carbon-halogen bond in diverse chemical structures, the biological role of which is still poorly understood. Their most important biotechnological applications include (i) biodegradation of pollutants such as 1,2-dichloroethane or 1,2,3-trichloropropane, (ii) decontamination of the warfare agent yperite, (iii) pollutant biosensing and (iv) HaloTag cell imaging [1, 2]. Atomic-level knowledge of both the inner organisation and supramolecular complexation of HLDs is thus crucial to understand their catalytic and non-catalytic functions.

Recently, database mining searches identified a new haloalkane dehalogenase, DmmarA, encoded in the genome of a waterborne pathogenic bacterium *Mycobacterium marinum* M. In our work, crystallographic structures of this (*S*)-enantioselective enzyme were determined at 1.6 and 1.85 Å resolution. The structures show a canonical -sandwich HLD fold with several unusual structural features. Mechanistically, the atypical composition of the proton-relay catalytic triad (aspartate-histidine-aspartate) and uncommon active-site pocket reveal the molecular specificities of catalytic apparatus that exhibits a rare (*S*)-enantiopreference of this enzyme family. Additionally, the structures reveal a previously unobserved mode of homodimerization, which is predominantly mediated through unique L5-to-L5 loop interactions. This homodimeric association in solution is confirmed experimentally by data obtained from small-angle X-ray scattering.