Laboratory of Structural Biology

Protein crystallography, HIV protease, antibody engineering

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Our laboratory carries out structural studies on various proteins of biological or medicinal interest using protein crystallography. Among our targets, proteases from pathogenic organisms [2-4], antibody fragments as well as other human enzymes [1] take a prominent position.

HIV protease [HIV PR] research is focused on development of novel potent inhibitors as well as on understanding the structural basis of drug resistance. More than two decades into the global HIV epidemic, HIV PR still remains an attractive target for structure-based rational drug design. Although nine inhibitors targeting HIV PR are currently approved for clinical use, their therapeutic efficiency is hampered mostly by resistance development. Understanding PR resistance at the structural level and development of new PIs acting by an alternative mode of inhibition is thus essential for successful treatment of HIV-positive patients. Our recent contribution to the field comprises structural studies of the enzyme-inhibitor complexes [2, 3].

Several recombinant antibody fragments of potential diagnostic and/or immunotherapeutic use [e.g. against human carbonic anhydrase IX, CD44, and CD3] have been prepared and characterized in our laboratory with the aim to improve their radionuclide labelling or to introduce further useful properties.

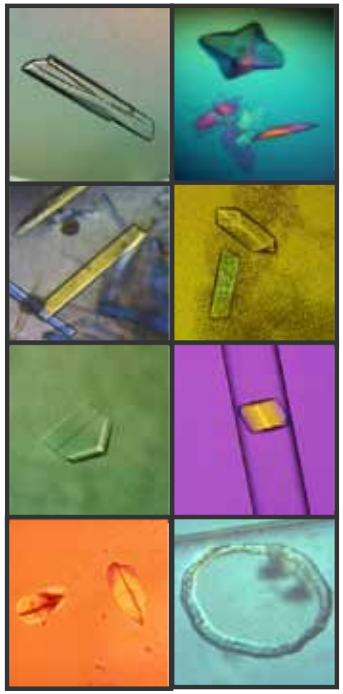

Fig. 1. Examples of protein crystals used for diffraction analysis and structure determination.

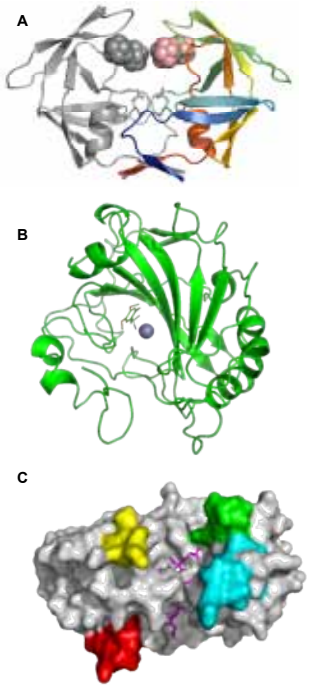

Fig. 2. Examples of crystal structures published in 2009-2010: (A) HIV protease in complex with metallacarborane inhibitor (see reference 2), (B) human carbonic anhydrase II in complex with inhibitor (see reference 1), (C) secreted aspartic protease from *Candida parapsilosis* in complex with pepstatin (see reference 4).

- Ministry of Education, Youth and Sports of the Czech Republic, 1M0505 – Centre of Targeted Therapeutic Drugs, 2005-2011, M. Fábry
- Ministry of Education, Youth and Sports of the Czech Republic, OE 210 – Engineering radionuclide-labelled antibodies, 2006-2009, J. Sedláček
- FP6 EU, 37693 HIV PI RESISTANCE – HIV protease inhibitor resistance by enzyme-substrate coevolution, 2007-2010, J. Sedláček
- GA CR, GA301/07/0600 – Intersubunit complementation studies and characterization of genotype – phenotype correlations in adenylosuccinate lyase deficiency, 2007-2009, J. Brynda
- Ministry of Industry and Trade of the Czech Republic, 2A-2TP1/076 – Generic therapeutic antibodies, 2007-2011, J. Sedláček
- GA CR, GA203/09/0820 – Structure based drug design of specific nucleotidases inhibitors, potentially pharmacologically important compounds, 2009-2013, J. Brynda

1. Gitto R, Agnello S, Ferro S, De Luca L, Vullo D, Brynda J, Mader P, Supuran CT, Chimirri A. Identification of 3,4-dihydroisoquinoline-2[1H]-sulfonamides as potent carbonic anhydrase inhibitors: synthesis, biological evaluation, and enzyme-ligand X-ray studies. **J Med Chem 2010** 53[6]: 2401-2408.
2. Řezáčová P, Pokorná J, Brynda J, Kožíšek M, Cígler P, Lepšík M, Fanfrlík J, Řezáč J, Grantz Šašková K, Sieglová I, Plešek J, Šícha V, Grüner B, Oberwinkler H, Sedláček J, Krausslich HG, Hobza P, Král V, Konvalinka J. Design of HIV protease inhibitors based on inorganic polyhedral metallacarboranes. **J Med Chem 2009** 52[22]: 7132-7141.
3. Šašková KG, Kožíšek M, Řezáčová P, Brynda J, Yashina T, Kagan RM, Konvalinka J. Molecular characterization of clinical isolates of human immunodeficiency virus resistant to the protease inhibitor darunavir. **J Virol 2009** 83[17]: 8810-8818.
4. Dostál J, Brynda J, Hrušková-Heidingsfeldová O, Sieglová I, Pichová I, Řezáčová P. The crystal structure of the secreted aspartic protease 1 from Candida parapsilosis in complex with pepstatin A. **J Struct Biol 2009** 167[2]: 145-152.
5. Řezáčová P, Kožíšek M, Moy SF, Sieglová I, Joachimiak A, Machius M, Otwinowski Z. Crystal structures of the effector-binding domain of repressor Central glycolytic gene Regulator from Bacillus subtilis reveal ligand-induced structural changes upon binding of several glycolytic intermediates. **Mol Microbiol 2008** 69[4]: 895-910.

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