Session IV - Friday, September 25 - afternoon

L22

ANTI-INFLAMMATORY DRUGS INTERACTIONS WITH OVINE AND CAPRINE SERUM ALBUMINS

Julita Talaj, Anna Bujacz

Institute of Technical Biochemistry, Faculty of Biotechnology and Food Sciences, Lodz University of Technology, Stefanowskiego 4/10, 90-924 Lodz, Poland
julita.talaj@dokt.p.lodz.pl

Serum albumin is a heart-like shape, three-domain protein with mostly alpha-helical structure. High flexibility of albumins and presence of three domains, possessing high capacity for binding a wide variety of ligands, makes serum albumins universal carriers of different molecules in organism.

Among mammalian serum albumins, only a few serum albumins' structures from different organisms have been known, most of them were determined by the X-ray Analysis Laboratory at Lodz University of Technology [1]. Recently, we obtained crystal structures of ovine serum albumin (OSA) and caprine serum albumin (CSA) in complexes with number of anti-inflammatory drugs molecules. These structures allow analysis of ligand binding sites of various serum albumins.

OSA and CSA, next to BSA (bovine serum albumin) belong to the even-toed ungulates' serum albumins. Their sequential identity is as high as 92-98.5%, but even these small differences influence on capacity of albumin binding pockets and their affinity to ligands.

The most frequently investigated compounds carried by albumins are drug molecules. Presented complexes of OSA and CSA with popular anti-inflammatory drugs, diclofenac, diflunisal and naproxen (Fig. 1), are compared with analogous complexes of albumins from different species. It is interesting that albumins from such a closely related organisms show differences in binding the same drugs molecules.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are ones of the most commonly used medicaments. They are widely applied in treatment of various inflammatory diseases, associated with the aches and sustained pain, such as arthritis and rheumatism [2].

Crystal structures of albumin-drug complexes give precise information about number and localization of drug binding sites and also about the character of these interactions. This knowledge, considering drugs affinity to serum albumins, contributes to the understanding of the mechanism of drug delivery.

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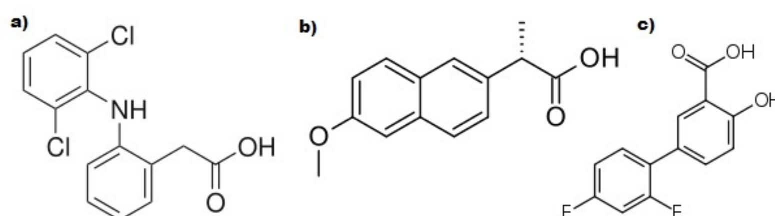
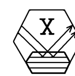


Figure 1. Structural formulas for diclofenac (a), naproxen (b) and diflunisal (c).



L23

CRYSTALLOGRAPHIC FRAGMENT-SCREENING AT THE HZB MX-BEAMLINES

F. U. Huschmann[1,3], M. Gerlach[1], R. Förster[1,2], A. Heine[3], M. Hellmig[1], G. Klebe[3], J. Linnik[1], P. H. Malecki[1,4], A. Metz[3], N. Radeva[3], J. Schiebel[3], K. Sparta[1], M. Steffien[1], M. Ühlein[1], P. Wilk[1,5], M. S. Weiss[1] & U. Mueller[1]

[1]*Macromolecular Crystallography (HZB-MX), Helmholtz-Zentrum Berlin für Materialien und Energie, Albert-Einstein-Str. 15, D-12489 Berlin, Germany*
[2]*Freie Universität Berlin, FB Chemie-Pharmazie-Biologie, Takustr. 3, D-14195 Berlin, Germany*
[3]*Philipps-Universität Marburg, Marbacher Weg 6, D-35032 Marburg, Germany*
[4]*Max Delbrück Zentrum für Molekulare Medizin, Crystallography group, Robert-Rössle-Str. 10, D-13125 Berlin, Germany*
[5]*Humboldt-Universität zu Berlin, Institute of Biology, Philippstr. 13 (Haus 18), D-10099 Berlin, Germany*
franziska.huschmann@helmholtz-berlin.de

In the last decade, fragment-based lead discovery has evolved into a widely applied technique in drug development. While originally pre-screening fragment binding investigations by biophysical methods were mandatory, nowadays complete fragment libraries can be screened by X-ray crystallography, owing to the ever increasing level of automation in diffraction data collection using synchrotron radiation and processing. In this context, it is essential to use high throughput methods, to have good diffraction quality target protein crystals and to work with a high quality fragment library. Thorough crystallographic analysis of protein-fragment complexes and their binding modes reveal detailed structural knowledge to develop fragments (100-200 Da) into new potential lead structures (300-500 Da).

Recently, we have started to establish an experimental facility optimized for high throughput fragment screening at the BESSY II storage ring [1, 2].

We have validated our assembled library of 96 fragments against two target proteins. These initial results revealed that this library can identify binding partners at a hit rate of about 10%.

In addition we are currently testing several novel techniques to simplify and accelerate sample preparation.

The ultimate aim is to make our library in combination with a highly automated beamline [3] available for academic and industrial users. This unique facility for screening experiments and evaluation of bound fragments will enable efficient fragment screening on a much broader basis.

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L24

STRATEGIES FOR DIFFICULT TARGETS: PRODUCTION OF READILY CRYSTALLIZABLE GLYCOPROTEINS IN HEK293S GnTI- CELL LINE, A CASE STUDY OF HUMAN LYMPHOCYTE RECEPTORS LLT1 AND NKRP1

Ondřej Vaněk[1], Jan Bláha[1], Petr Pachl[2], Petr Novák[1,3], Tereza Skálová[4], Jan Dohnálek[4]

[1]Department of Biochemistry, Faculty of Science, Charles University in Prague, Hlavova 8, Prague, 12840, Czech Republic
[2]Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo nám. 2, Prague, 16610, Czech Republic
[3]Institute of Microbiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, Prague, 14220, Czech Republic[4] Institute of Biotechnology, Academy of Sciences of the Czech Republic, Vídeňská 1083, Prague, 14220, Czech Republic
ondrej.vanek@natur.cuni.cz

Recombinant protein expression can be a costly enterprise, especially for proteins that are not easily expressed in prokaryotic cells and are sometimes labeled as „difficult targets“. Here we would like to show a case study of a recombinant expression of such a difficult target – human natural killer cell receptor protein 1 (NKRP1; gene *klrb1*) and its binding partner lectin-like transcript 1 (LLT1; *clec2d*).

Human embryonic kidney 293 cell line deficient in N-acetylglucosaminyltransferase I (HEK293S GnTI-) is well known tool for expression of proteins with homogeneous and deglycosylatable N-glycosylation, a feature crucial especially for protein crystallography [1]. However, production protocol using this cell line based on transient transfection of adherent cell culture is costly to scale-up and has reportedly lower expression yields [2]. In this work we have adapted HEK293S GnTI- cell line to growth in suspension and optimized its transient transfection. While transfection at standard cultivation cell density proved very little success we have found out that concentrating the cells to high cell density substantially increases transfection efficiency, greatly enhancing protein yields and creating fast and scalable production process.

We demonstrate this on the production of soluble LLT1 [3] naturally present on natural killer and T-lymphocytes, but upregulated in glioblastoma cells, one of the most lethal tumors, where it acts as a mediator of immune escape. The prepared soluble domain of LLT1 with homogeneous glycosylation was readily crystallized and following optimization of crystal conditions this protein preparation ultimately led to the first structure determination of this receptor described so far [4]. In order to improve on the productivity for hNKRP1 we are now using a stably transfected HEK293S GnTI- cell pool with a tenfold yield improvement.

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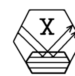

L25

PROTONATION AND GEOMETRY OF His RESIDUES

M. Kowiel[1], M. Malinska[2], M. Dauter[3], M. Jaskolski[4,5], Z. Dauter[2]

[1]Department of Organic Chemistry, Poznan University of Medical Sciences, Poznan, Poland
[2]Synchrotron Radiation Research Section, MCL, National Cancer Institute, Argonne National Laboratory, USA
[3]Leidos Biomedical Research Inc, Basic Science Program, Argonne National Laboratory, USA
[4]Department of Crystallography, Faculty of Chemistry, A. Mickiewicz University, Poznan, Poland
[5]Center for Biocrystallographic Research, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, Poland
mkowiel@ump.edu.pl

Protonation and tautomerism at the histidine side chain N atoms (Fig. 1) affect the geometry of the five-membered imidazole ring. We have analyzed and compared the geometry of the imidazole moieties found in atomic-resolution protein crystal structures in the Protein Data Bank (PDB) and in small-molecule structures in the Cambridge Structural Database (CSD).

The different protonation states lead to significant geometry variations of the imidazole moiety, both in the bond lengths and valence angles within the ring. We show that linear discriminant analysis [1] can be used for the recognition of the protonation state of imidazole moieties with sufficiently accurate geometry. Based on the CSD structures, we defined and trained two polynomial functions, corresponding to linear combinations of the four most sensitive stereochemical parameters: two bond lengths (ND1–CE1 and CE1–NE2) and two endocyclic valence angles (–ND1– and –NE2–). The results obtained from the structural information available in the CSD have been confirmed by a corresponding analysis of the best PDB entries.

Moreover, we propose to revise the currently used [2] geometrical restraints for histidine, and to distinguish, wherever possible, between the ND1-, NE1- and double-protonated histidine side chains for use in macromolecular refinement. We recommend to use the revised restraint targets and weights when the protonation state can be figured out from the hydrogen-bonding or metal coordination pattern, or when the structure is at near atomic (or higher) resolution.

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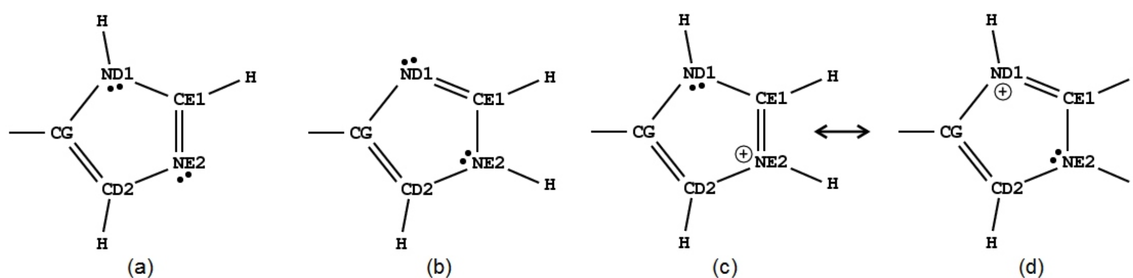

Figure 1. Imidazole ring tautomeric forms with ND1 protonated (a) and NE2 protonated (b), and the principal mesomeric forms of the imidazolium cation with both N atoms protonated (c-d).

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A NEW THERMOFLUOR DESIGN: SCREENS BASED ON FUNDAMENTAL AND SPECIFIC VARIABLES

Christin Reuter, Larissa Consani-Textor, Mathias Grün

Jena Bioscience GmbH, Loebstedter Str. 71, 07749 Jena, Germany

The success of protein purification, crystallization and further downstream processes greatly depends on the identification of a buffer environment that increases protein's **stability and homogeneity in solution** [1, 2]. The thermal shift-based **JBS FUNDAMENT** and **JBS SPECIFIC screens allow identification of protein-stabilizing buffer** conditions using the concept of strictly categorizing buffer components into

1. FUNDAMENTAL factors that influence the *whole* protein molecule such as pH and ionic strength and
2. SPECIFIC factors that affect energetically important *hot spots* on the protein such as cations, anions or small molecules.

Both screens are based on broad-range buffer systems [3] therefore screening the **pure pH effect** from pH 4.0 – 10.0.

While **JBS FUNDAMENT** allows screening the pure pH effect at different ionic strengths, the **JBS SPECIFIC** is designed for screening of high-scoring mono-, di- and trivalent ions [4].

JBS Thermofluor Screens are provided in ready-to-use 96-well PCR plate format compatible with standard qPCR machines and further contain a control protein and dye, thus serving as rapid approach for stabilizing the protein of interest *in vitro*.

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