Pro183 and Pro188, is accommodated in a specific binding pocket. Comparison with other similar kinase structures shows a 180° rotation of the loop and suggests a possible pro-active dimer formation, by which intermolecular phosphorylation may occur. The well-defined protein-ligand interactions further provide additional information for design of potent inhibitors.

Keywords: kinase structure; protein conformation; egulation and reaction mechanism of enzymes

FA1-MS07-P04

Is It Possible to Guess Potential Drug Activity from Its Crystal Structure? <u>Agnieszka Skórska-Stania</u>[a], Magdalena Śliwa[a], Barbara J. Oleksyn[a], Kamil Musilek[b,c], Kamil Kuca[b,c], Josef Jampilek[d,e], Robert Musiol[f], Jiri Dohnal[d,e]. *[a]Faculty of Chemistry, Jagiellonian University, Kraków, Poland. [b]Faculty of Military Health Sciences, Hradec Kralove, Czech Republic. [c]Faculty of Science, University of Jan Evangelista Purkyne, Czech Republic. [d]Zentiva a.s., Prague, Czech Republic. [e]Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic. [f]Institute of Chemistry, University of Silesia, Katowice, Poland.*
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Design of new reactivators of acetylcholinesterase (AChE) gained more interest recently [1]. We have studied crystal structures of two bis-pyridinium oximes (K075 and K282), which can be used as detoxifying agents in case of poisoning with organophosphorous compounds, e.g. sarin, soman, insecticides [2]. Based on the molecular structures of well known AChE reactivators, obidoxime and HI-6, the new potential agents (K075 and K282) were proposed. Their molecules differ in configuration in respect to the double bond between carbon atoms in the linker between two pyridines. The activity of K075 has been determined [3]. It is interesting if the activity of K282 can be predicted by comparison of the crystal structures of both potential reactivators.
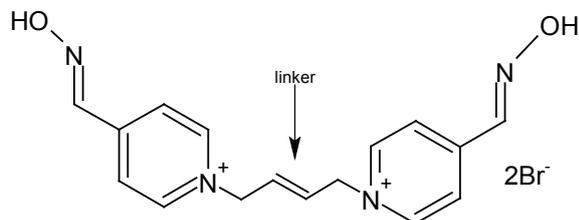
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Keywords: drug structure-activity relationships; drug interactions; stereochemistry

FA1-MS07-P05

Structural Studies of the Acetylcholine Binding Protein in Complex with Novel Compounds. <u>Line Aagot H. Thomsen</u> [a,b], Thomas Bale [a], Marianne L. Jensen[b], Philip K. Ahring[b], Jette S. Kastrup[a], Michael Gajhede[a]. *[a]Department of Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Copenhagen, Universitetsparken 2, DK-2100, Copenhagen, Denmark. [b]NeuroSearch A/S, Pederstrupvej 93, DK-2750 Ballerup, Denmark.*
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Cys-loop receptors form essential ligand-gated ion channels. In the central nervous system, the Cys-loop receptors mediate neurotransmitters signalling and are involved in fast communication between neurons. The receptors are formed by one to four different subunits in a pentameric complex. The Ligand binding domain is located extracellular with the ligand binding site located in the interface between the subunits and the ion channel pore region is located in the cell membrane of the neuron. Upon binding of neurotransmitter, the receptor undergoes conformational changes, which course the ion channel to open and allow ions to enter the cell. The Cys-loop receptors are implicated in several brain diseases including Parkinson's disease, schizophrenia, depression, Alzheimer's' disease, anxiety and epilepsy [1-3]. Detailed understanding of receptor structure and function is essential for providing a rational basis for the design of new drug allowing new therapeutic strategies for treating such disorders. Threedimensional structures of Cys-loop receptors have proven to be extremely difficult to obtain as the receptors are very

difficult to crystallize. However, a soluble protein forming a very similar pentameric structure, the Acetylcholine Binding Protein (AChBP) from the water snail *Lymnaea stagnalis*, has successfully been expressed and co-crystallized with different ligands [4]. Using the AChBP as a model system for Cys-loop receptors, the main objective is to co-crystallize AChBP with various novel compounds selected by binding affinity.

AChBP has been expressed in *Pichia pastoris* as a soluble protein and subsequently purified using ion exchange chromatography. Crystallization experiments are in progress and the current status of the project will be presented.

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Keywords: cys-loop receptors; AChBP; novel compounds

FA1-MS07-P06

How to Design Aurora Kinase A Selective Inhibitors. <u>Magda Kosmopoulou</u>[a], Amir Faisal[b], Chongbo Sun[b], Vassilios Bavetsias[b], Butrus Atrash[b], Nathalie Bouloc[b], Mizio Matteucci[b], Julian Blagg[b], Spiros Linardopoulos[b,c], Richard Bayliss[a]. *[a]Section*

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Aurora kinase A belongs to the serine/threonine subclass of kinases (the other two members are named B and C) and is involved in the regulation of mitosis. More specifically, Aurora kinase A localizes to the centrosomes and to spindle poles and it is implicated in centrosome maturation, centrosome separation and bipolar spindle assembly [1]. The human Aurora-A gene localizes to a chromosomal region (20q13.2) that is amplified in a variety of human cancers [2]. Concomitantly, Aurora-A is overexpressed in the corresponding tumours, and recent studies have identified that Aurora-A is a low-penetrance tumour susceptibility gene in both mice and humans [3]. Excess Aurora-A activity has been shown to confer tumourigenic properties to cells [4] and it is plausible that this reflects the ability of overexpressed Aurora-A to produce failure of cytokinesis, leading to centrosome amplification and polyploidization [5]. Given that aberrant expression and activity of Aurora kinase A is associated in tumorigenesis the inhibition of its kinase activity is an attractive approach for the treatment of tumours. Several inhibitors of Aurora-A have been developed up to date. These inhibitors target the ATP-binding site of Aurora A, which is identical among all members of this family, so they inhibit not only Aurora-A but also Aurora-B and Aurora-C. It is desirable to develop inhibitors that can discriminate between these kinases to ensure a properly targeted therapy, to reduce the likelihood of patient side-effects, and to produce more precise tools for cell biology studies. This presents a significant challenge for structure-based drug design. In this study we present the crystal structure of Aurora-A bound to several small molecule inhibitors and we describe the design principles underpinning the production of Aurora-A selective inhibitors. These principles can be applied to other systems amenable to protein crystallography. The binding modes of the inhibitors suggest the residues around the ATP binding pocket that can be successfully exploited in order to design small molecules inhibitors with high potency and selectivity. Selectivity can then be introduced by iterative cycles of structural biology, hypothesis-based chemistry and validation using selectivity assays.

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Crystal Structures of Actinohivin, an Anti-HIV Protein from an Actinomycete, and its Complex with Mannobiose. Masaru Tsunoda[a], Kaoru Suzuki[a], Sagara Tsubasa[a], Atsushi Takahashi[a], Junji Inokoshi[b], Satoshi Omura[b], Takeshi Sekiguchi[a], Haruo Tanaka[a], Akio Takenaka[a]. *a*Iwaki Meisei University, Iwaki, Japan . *b*Kitasato University, Tokyo, Japan.
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Actionohivin (AH) isolated from actinomycete *Longispora albida* K97-0003[T] inhibits HIV viral entry to human susceptive cells. As compared with cyanovirin-N, AH binds more specifically to the high mannose-type saccharide chains (HMTG) of HIV gp120 protein. This lectin is a small protein of 114 amino acid residues, being arranged in a unique sequence repeated by three times tandemly. To reveal the structure of the specific binding, X-ray analyses of the *apo*-form and its complex with mannobiose Man^{1}α(1-2)Man^2 have been performed at 1.19 and 1.65 Å resolutions, respectively. In the *apo*-form crystal, the protein structure is composed of the three modules similar to each other, as speculated from the tandem repeats in the sequence. Each module consists of a β-sheet of four β-strands, a long loop and a π-helix. The three modules are associated with a pseudo three-fold symmetry, in which the three β-sheets form a triangular barrel. Inside of the barrel, hydrophobic residues form a stable core. On the outer surface, a long loop with a π-helix in each module is running from the top to the bottom of the barrel. This loop and the preceding two β-strands containing the carbohydrate-binding motifs LD-QXW form a valley with a pocket for carbohydrate-binding. In the three sites of the complex-form, three mannobioses are bound, respectively. In the first pocket of the module 1, the carboxyl group of Asp15 bridges between the two hydroxyl groups (OH^3 and OH^4) attached to the C^3 and C^4 atoms of Man^2, through hydrogen bonds. At the same time, OH^3 forms another hydrogen bond with the amino group of Asn28 and OH^4 forms another hydrogen bond with the hydroxyl group of Tyr23. In addition, the mannose ring of Man^2 is sandwiched between Leu25 and Tyr32 by hydrophobic interactions. To stabilize the pocket formation, Gln33 forms double hydrogen bonds with the main chain amide group at Asn28 and the carbonyl group at Pro26. In the second and the third pockets, the mannobioses are also bound in the similar ways. The structural features are completely consistent to the results of mutation experiments of these amino acid residues. The Man^1 group which is largely bent by the α(1-2) bond is protruded into the solvent region. In this pocket, the mannobiose end of D1 branch of HMTG could be accommodated in a way similar to the geometry described above, and the end of D2 or D3 branch might contact with a valley. Therefore, it is possible to speculate that each pocket accepts the D1 and D2/D3 branches of HMTG. The three pockets are located to form an almost regular triangle at a distance of 17 Å between the