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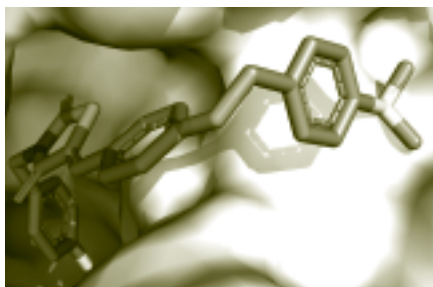


Figure 3:
The HA155 inhibitor bound to ATX

STRUCTURAL BIOLOGY

This year was marked by two breakthroughs on our major long-term in-house collaboration projects: determining the structure of Autotaxin (with Wouter Moolenaar, Division of Cell Biology I, and Huib Ovaa, Division of Cell Biology II) and of JBP1 (with Piet Borst, Division of Molecular Biology). Concurrently, our work on DNA replication licensing and on mitotic progression advanced and our adventures in X-ray crystallography model building and refinement are under-marked by a change of focus towards structural bioinformatics.

Structural studies of Autotaxin The role of the signaling phospholipid lysophosphatidic acid (LPA) and Autotaxin (ATX), the protein that produces LPA from lysophosphatidylcholine (LPC), was established over the last three decades, largely owing to the efforts of the group of Wouter Moolenaar at the NKI. LPA and ATX have been shown by numerous studies to be involved in cancer metastasis and other pathogenic situations, such as inflammation and neuropathic pain. ATX is the protein ecto-nucleotide phosphodiesterase 2 (ENPP2), a 100 kDa glycoprotein, capable of both lysoPLD and nucleotide pyrophosphatase activities. Over the last few years we have engineered a human cell line (HEK293 derivative) that is stably expressing glycosylation-deficient mutants of rat autotaxin, which allowed us to crystallize ATX, and determine its structure this year.

Autotaxin hydrolyzes different species of LPC (with different alkyl chain lengths) and some other lipids, as well as nucleotide substrates. Our structure shows that both nucleotides and lipids partially share the same binding pocket, but the alkyl-chain of lipid substrates form additional hydrophobic contacts with ATX. This model implies that the LPA product likely has higher affinity for ATX than any nucleotide substrate, a hypothesis consistent with the previous finding that LPA acts as an inhibitor of ATX activity against a variety of substrates, but not of LPC hydrolysis. Thus, LPA formed by the enzyme may act as a “substrate specifying factor” effectively inhibiting the hydrolysis of nucleotides because it can only be displaced by lysophospholipid substrates such as LPC. This mechanism could dictate ATX activity *in vivo*, because the spectrum of available LPA species could define ATX activity against specific lysophospholipid substrates. Our findings also suggest that further analysis of structural determinants of substrate discrimination could lead to the identification of molecules that inhibit the hydrolysis of specific substrates, *e.g.* long-chain rather than short-chain LPC species.

The group of Huib Ovaa has developed a series of small-molecule boronic acid inhibitors of ATX-mediated LPC hydrolysis, that lower plasma LPA levels in mice following intravenous administration. We also determined a structure of ATX in complex with one of these inhibitors, HA155, which enables us to correlate the activity of this inhibitor with its binding mode. We show that the boron atom on one end of the inhibitor forms a reversible covalent bond with the nucleophile hydroxyl group of the active site threonine nucleophile. The other end of HA155, a hydrophobic fluoro-benzene, is pointing directly into the deep hydrophobic pocket, oriented perpendicular to the protein surface.

Our results will allow us to provide feedback to inhibitor development studies, which are pursued in-house in collaboration with CR-UK, but also by our collaborators in Pfizer and by Merck-Darmstadt. Importantly we have now formed the basis for understanding how autotaxin sequesters its lipid substrates and we formulated testable hypotheses for how ATX can specifically present the hydrolysis products to the cell-surface LPA receptors, to elicit a variety of important responses. This will allow us how Autotaxin is related to processes such as inflammation, neuropathic pain, obesity, and most importantly in cancer metastasis.

Structural studies of JBP1 The JBP1 protein, discovered by Piet Borst and colleagues, binds to DNA that contains base J (-D-glucosyl-hydroxymethyluracil). JBP1 has been shown to be essential for survival in many major protozoa human pathogens such as *T. brucei* (sleeping sickness), *T. cruzi* (Chagas' disease) and *Leishmania* species (various types of Leishmaniasis). Last year, it has been shown that there exists a partial mammalian homologue of JBP1, which is localized in a region of the TET proteins that are involved in myeloid leukemia and

Publications

- Littler DR, Brown LJ, Breit SN, Perrakis A, Curmi PM. *Structure of human CLIC3 at 2 Å resolution*. *Proteins*. 2010;78:1594-600
- Littler DR, Alvarez-Fernandez M, Stein A, Hibbert RG, Heidebrecht T, Aloy P, et al. *Structure of the FoxM1 DNA-recognition domain bound to a promoter sequence*. *Nucleic Acids Res*. 2010;38:4527-38
- Hausmann J, Christodoulou E, Kasiem M, De Marco V, van Meeteren LA, Moolenaar WH, et al. *Mammalian cell expression, purification, crystallization and microcrystal data collection of autotaxin/ENPP2, a secreted mammalian glycoprotein*. *Acta Crystallogr Sect F Struct Biol Cryst Commun*. 2010;66(Pt 9):1130-5
- Brooks MA, Gewartowski K, Mitsiki E, Letoquart J, Pache RA, Billier Y, et al. *Systematic bioinformatics and experimental validation of yeast complexes reduces the rate of attrition during structural investigations*. *Structure*. 2010;18:1075-82

necessary for the synthesis and maintenance of the important epigenetic marker hydroxymethylcytosine.

We showed that JBP1 recognizes J-containing DNA through a 160-residue domain, DB-JBP1, with ten thousand-fold preference over normal DNA. The crystal structure of DB-JBP1 revealed a novel helix-turn-helix variant fold, a “helical bouquet” with a “ribbon” helix responsible for DNA binding. Mutation of a single residue (Asp525) in that helix abrogates specificity towards J-DNA, rendering mutated JBP1 unable to rescue the targeted deletion of endogenous *JBP1* genes in *Leishmania*. Based on mutational analysis and H/D-exchange mass-spectrometry data, a model of JBP1 bound to J-DNA was constructed, and validated by small-angle X-ray scattering. Our results open possibilities for utilizing JBP1 as a drug target and a tool for detecting the important mammalian epigenetic marker hydroxymethylcytosine.

Geminin and its homologues in DNA replication licensing A new twist to our research on the role of Geminin in replication licensing was provided by the discovery of two homologues of Geminin: Idas and Lygeas (in Greek mythology, ‘ and ‘ are the cousins of the two Gemini). Lygeas (who unfortunately made its first appearance in scientific literature under the more mundane name GemC1) directly mediates replication initiation through TopBP1- and Cdk2-dependent recruitment of Cdc45 onto replication origins. Idas, has a much more tissue-specific localization (predominantly in the mouse forebrain), interacts with Geminin, and inhibits the capacity of Geminin to sequester the pre-replication origin component Cdt1. We have recently determined the structure of the Geminin:Idas complex and have shown both in *Xenopus* and with biophysical methods, how Idas binding reduces the inhibitory effect of Geminin to replication initiation.

Structural studies of proteins involved in mitotic progression Our focus this year has been on understanding the role of the regulatory domain of Mps1 kinase. The MPS (MonoPolar Spindle) family of kinases was shown to be a dual specificity protein kinase in vitro, capable of autophosphorylation on serine, threonine and tyrosine residues. Mps1 is essential for maintaining chromosomal stability by allowing resolution of merotelic attachments and to ensure that single kinetochores achieve the strength of checkpoint signaling sufficient to prevent premature anaphase onset and chromosomal instability. We have determined the structure of the N-terminal domain of Mps1, which adopts the TPR fold, also found in the Bub1 and BubR1 family of kinases.

In collaboration with the group of Geert Kops (UMC Utrecht), we have shown with site directed mutagenesis and domain deletion and swapping studies, that the TPR domain of Mps1 is important for localization of Mps1 to the kinetochores. Pull-down experiments and peptide binding analysis, suggest that Mps1 is recruited to kinetochores through the combined action of Aurora B and Ncd80, via interactions with the TPR domain. We are currently analyzing how these interactions regulate the biological roles of Mps1.

We are continuing our work on the FoxM1 transcription factor that is implicated in a cell's proliferation potential and in cancer. Our goal remains to extend our work on the binding of the forkhead domain to DNA promoter sequences, towards understanding the structure of full-length FoxM1 and deciphering how phosphorylation regulates transcriptional activity.

Methods for X-ray crystallography The crystallographic models in the Protein Data Bank (PDB) were deposited there over several decades, and were created using the methods and software available at the time. We decided it is timely to consider updating these models by using state of the art software, to improve their accuracy and correct errors. This was addressed by PDB_REDO, a procedure to refine PDB models for which experimental data are available. This year, we transferred some functionality of our crystallographic model-building software ARP/wARP to extend PDB_REDO to rebuild protein models. These new improved models are freely available to a user community of tens of thousands of biologists, chemists, bioinformaticians and drug designers.