concave surface. The 2-5A molecule is accommodated in the concavity and interacts with ankyrin repeats 2 to 4. Two structurally equivalent 2-5A binding motifs are found at repeats 2 and 4. The structural basis for 2-5A recognition by ANK is essential for designing stable 2-5As with a high likelihood of activating RNase L.

[1] Tanaka N., Nakanishi M., Kusakabe Y., Goto Y., Kitade Y., Nakamura K.T., *EMBO J.*, 2004, **23**, 3929.

Keywords: 2-5A system, ankyrin repeat, interferon

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Crystal Structures of Autocrine Motility Factor Complexed with Inhibitors

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Autocrine motility factor (AMF), a tumor-secreted cytokine, stimulates cell migration *in vitro* and metastasis *in vivo*. AMF is identical to the extracellular cytokines neuroleukin and maturation factor and, interestingly, to the intracellular enzyme phosphoglucose isomerase. Cytokine activity of AMF is inhibited by carbohydrate compounds, as they compete for AMF binding with the carbohydrate moiety of the AMF receptor, which is a glycosylated seven-transmembrane helix protein. Crystal structure analyses and site-directed mutagenesis studies of human AMF revealed that the regions important for the enzymatic function of AMF/PGI overlap those for the cytokine function of AMF [1].

Here we have determined the crystal structures of the various length of inhibitor-bound AMF at high resolution and assayed the inhibitory activities of the various inhibitors. These data provide an insight into the lead compound design of more effective AMF inhibitors.

[1] Tanaka N., Haga A., Uemura H., Akiyama H., Funasaka T., Nagase H., Raz A., Nakamura K.T., *J. Mol. Biol.*, 2002, **318**, 985.

Keywords: cancer, metastasis, tumor

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LTB_4 12-hydroxydehydrogenase/15-oxo-PG 13-reductase and Indomethacin Complex

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The bi-functional leukotriene B_4 12-hydroxydehydrogenase/15-oxo-prostaglandin 13-reductase (LTB_4 12-HD/PGR) is essential for eicosanoid inactivation. It catalyzes the first irreversible reactions in each eicosanoid inactivation and the activity may be regulated by a protein with SH3 domain. LTB_4 is oxidized to 12-oxo-LTB_4, and 15-oxo-PGE_2 are reduced to 13,14-dihydro 15-oxo-PGE_2. Some none steroidal anti-inflammatory drugs inhibit LTB_4 12-HD/PGR activity. Here we report the structure of LTB_4 12-HD/PGR with $NADP^+$ and indomethacin. The indomethacin binds to the 15-oxo-PGE_2 binding site, indicating that the indomethacin competitively inhibits LTB_4 12-HD/PGR. The chloro-benzene moiety of indomethacin enters into the hydrophobic pore that is the recognition site of ω-chain of 15-oxo-PGE_2, and the carboxyl group of indomethacin interacts with Arg56 and Tyr262. The architecture is identical to those of indomethacin and cyclooxygenase complexes. The result may be useful for further development of cyclooxygenase inhibitor.

Keywords: anti-inflammatory compounds, dehydrogenases, protein-inhibitor binding

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Automated Structure Refinement for High-throughput Ligand Detection with BUSTER-TNT

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The use of crystallography for the discovery of lead compounds often involves a large number of experiments with different soaking or co-crystallization trials. The subsequent refinement and analysis of the resulting datasets can be time-consuming and tedious. Since the crystallographic parameters (resolution, space group, cell parameters) are quite often similar, this task is ideally suited for automation.

We present a method (**autoBUSTER**) that automates the refinement, solvent model update, ligand detection and analysis. Centered around the BUSTER-TNT program [1,2], it requires a minimal amount of user input. Although it can be used at any resolution and for any kind of macromolecular structure, it is tuned to the refinement of protein structures at better than 2.8 Å resolution.

The knowledge of any (possibly) bound ligand can be given (a) explicitly by supplying a PDB file of dummy atoms that describes the assumed binding site, or (b) by letting the system automatically analyze the residual density of difference Fourier maps. A unique feature of BUSTER-TNT is used, where the various masks describing the known fragment, the bulk solvent and the missing part can be given independently from each other. The results show that this can greatly enhance the capability of uniquely defining any bound ligand.

[1] Bricogne G., Irwin J., *Macromolecular Refinement: Proceedings of the CCP4 Study Weekend*, Warrington: Daresbury Laboratory, 1996, 85-92. [2] Blanc E., Roversi P., Vonrhein C., Flensburg C., Lea S. M., Bricogne G., *Acta Cryst*., 2004, **D60**, 2210-2221.

Keywords: refinement, ligands, automation

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Crystallographic Studies of Novel Inhibitors of β-Lactamases

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Bacterial expression of β-lactamases is the most widespread resistance mechanism to β-lactam antibiotics. There is a pressing need for novel, non-β-lactam inhibitors of these enzymes [1]. Our efforts to overcome bacterial resistance mechanisms have been directed towards novel, non β-lactam inhibitors of AmpC β-lactamase, a class C enzyme responsible of resistance to antibiotics treatment in gram-negative bacteria.

Through a structure-based approach, we discover novel inhibitors for this enzyme, with covalent mechanism of action such as boronic acid derivatives and with no-covalent, competitive mechanism of action, such as thiophene-2-carboxylic acid derivative [2].

In one case we were able to extend the inhibitory activity towards class A β-lactamases, obtaining a broad spectrum, highly potent inhibitor.

Some inhibitors were active in cell culture, reversing resistance to the third generation cephalosporin ceftazidime in bacterial pathogens expressing AmpC and did not up-regulate β-lactamase expression in cell culture.

The structure-based design, synthesis, biological evaluation and the crystallographic studies of such novel inhibitors will be described.

[1] Cosgrove S., Carmeli Y., *Clin. Infect. Dis.*, 2003, **36**, 1433-1437. [2] Tondi D., Morandi F., Bonnet R., Costi M. P., Shoichet B. K., *J. Am. Chem. Soc.*, 2005, **127(13)**, 4632-4639.

Keywords: enzyme inhibition, drug resistance, X-ray complexes