in silico model, based on the structure of a 93% sequence identical protein from *Streptococcus pneumoniae*. Constructs were evaluated by use of circular dichroism spectroscopy, mass-spectrometry (MS), ion mobility MS, surface plasmon resonance, and enzyme activity assays. Results indicate that lysine residue at positions 252, 255, 434, and 435 interact with plasmin(ogen) in a concerted fashion. Other point-mutations either destabilized the octamer (K344E), or caused SEN to lose enzymatic activity (K334A).

The current study aims at further exploring these results by X-ray crystallography. Diffraction data has been collected from all SEN constructs of interest, including the wild type, and is presently being refined and analyzed. These structures will be instrumental for proposing mechanistic models based on the data from the many functional studies, as well as provide detail for further experiments.

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Keywords: complexation, bacterial, disease.

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Structures of N-Acetylmannosamine kinase in complex with ADP/ATP for drug design

Jacobo Martinez,[a] Long Duc Nguyen,[b] Werner Reutter,[b] Hua Fan,[b] Wolfram Saenger,[a] Sebastien Moniot[b] [a]*Institut für Chemie und Biochemie-Kristallographie, Freie Universität Berlin, Berlin (Germany).* [b]*Institut für Biochemie und Molekularbiologie, Charité-Universitätsmedizin Berlin, Berlin (Germany).* E-mail: martinez@chemie-fu-berlin.de

Sialic acids are essential components of glycoconjugates in the cell membrane. As a terminal negatively charged sugars on cell surface glycans, they are responsible for the interaction, structure and functionality of all deuterostome cells. Pathogens and many malignant tumor types present abnormal quantities of sialic acid on the cell surface, which helps them avoid the host immune response. The key enzyme of the biosynthesis of sialic acid is UDP-N-acetylglucosamine-2-epimerase/ N-acetylmannosamine kinase (GNE). This bifunctional enzyme catalyzes the rate limiting step in the transformation of UDP-N-acetylglucosamine to N-acetylmannosamine-6-phosphate and has a direct impact on the sialylation of the cell surface. Distinct mutations on GNE have been found to cause hereditary inclusion body myopathy (HIMB), but the molecular basis of the pathophysiology are still unknown.

Here, we present the crystal structure of the Nacetylmannosamine Kinase (MNK) domain in complex with N-acetylmannosamine (ManNAc) at 1.65 Å resolution as well as the structures of the complexes MNK/ManNAc/ADP (1.8 Å) and MNK/ManNAc-6-Phosphate/ADP (2.1 Å). The V-shaped MNK monomer adopts a close configuration upon ManNAc binding. Located in a groove formed between the two domains of MNK, ManNAc is strongly coordinated in a network of 17 hydrogen bonds and interacts with eight residues distributed among the two MNK domains. This strong network correlates with the high MNK specificity for ManNAc.

Our findings offer a clearer understanding on the action of the key enzyme for sialic acid biosynthesis and serve as the necessary structural basis to design agonists and antagonists opening novel ways on glycan bioengineering and cancer therapy research.

An inhibitor of hMNK would be a valuable tool for sialic acid research and for an alternative approach in cancer treatment. Based on the here presented hMNK/ManNAc and hMNK/ManNAc/ADP structures, we hypothesize that a modification of the C6-position of ManNAc with a relatively small polar group could potentially be a good hMNK inhibitor. In a first approach to verify this idea, we synthesized ManNAc-6OAc and tested its inhibitory effects on hMNK compared with other previously published inhibitors of hMNK. Indeed ManNAc-6OAc showed a more efficient inhibition, demonstrating the value of the here presented structural information.

Keywords: sialic-acid, glycobiology, kinase

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Structural and functional determinants of protein kinase CK2α

Graziano Lolli, Roberto Battistutta, *Venetian Institute for Molecular Medicine (VIMM), via Orus 2, 35129 Padova, (Italy) and Department of Chemical Sciences, University of Padua, via Marzolo 1,35131 Padova, (Italy).* E-mail: graziano.lolli@unipd.it

Ser/Thr protein kinase CK2 is involved in several processes affecting and regulating cellular life, such as cell cycle progression, gene expression, cell growth and differentiation and embryogenesis.

In several cancer types CK2 shows elevated activity associated with conditions that favour the onset and the maintenance of the tumor phenotype. This lead to the identification of compounds able to inhibit the catalytic activity of this oncogenic kinase, in particular of ATP-competitive inhibitors.

The regulation of CK2 has been instead poorly explored. The many available crystal structures indicate that this enzyme owns some regions of remarkable flexibility that were associated to important functional properties. Of particular relevance is the flexibility, unique among protein kinases, of the hinge region and the following helix αD.

The structural bases of this characteristic flexibility are discussed. Some controversial issues concerning the functional interpretation of structural data on maize and on human CK2 are also analysed, pointing out what is reasonably established and what is still unclear about this enzyme. This analysis can be useful to outline some principles at the basis of the development of effective ATP-competitive CK2 inhibitors.

Keywords: kinase, flexibility, cancer

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Structural characterization of D-Phe-Pro-D-Arg-derived thrombin inhibitors

Pedro J. B. Pereira,[a] Ana Carvalho Figueiredo,[a] Sandra Macedo-Ribeiro,[a] Manfred Philipp,[b] Cristina C. Clement,[b] [a]*IBMC-Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto (Portugal).* [b]*Department of Chemistry, Lehman College & Biochemistry Program, CUNY Graduate School, New York, NY 10036 (USA).* E-mail: ppereira@ibmc.up.pt

Thrombosis-related disorders such as myocardial infarction, stroke, and pulmonary embolism remain a major cause of mortality worldwide. Physical damage to the vascular system triggers the coagulation cascade, a series of activation reactions of circulating precursor proteins and regulating factors that ultimately prevent blood loss without compromising blood flow through either the uninjured or the damaged vessels. The central role played by the serine proteinase thrombin in this process has driven the interest on thrombin inhibitors as potential anti-thrombotic drugs [1].

Rational design of peptide-based thrombin inhibitors led to

synthetic peptides with the general sequence D-Phe-Pro-D-Arg-X-$CONH_2$. These compounds are non-cleavable, reversible thrombin inhibitors and potent anticoagulants, as demonstrated by isothermal titration calorimetry and structure-activity relationship studies [2]. In order to characterize their molecular interactions with human thrombin, we solved the three-dimensional structure of the enzyme in complex with peptides with Ile (p3), Cys (p4) or D-Thr (p6) at the P1' position [3].

All the inhibitors bind in a substrate-like manner to the active site of thrombin. Although the contacts established by the P1 D-Arg with the enzyme are similar to those observed for the L-isomer in other substrates and inhibitors, it also allows for a deeper insertion of the P2-P3 segment into the respective selectivity pockets, and gives the cleavable bond an unfavorable geometry for nucleophilic attack by thrombin's Ser195 side chain. The latter occupies its canonical position in the thrombin-p3 complex, but in the p4 and p6 complexes it is rotated away from the catalytic histidine and hydrogen-bonded to the carbonyl oxygen of the P1' residue.

The side chain of Lys60F is thought to limit the size of thrombin's S1' pocket contributing to the frequent occurrence of small residues at this position in natural protein substrates. In the thrombin-p6 and -p4 complexes, the side chain of Lys60F is found in an extended conformation similar to that observed in the unliganded enzyme. However, in the thrombin-p3 complex, accommodation of the P1' isoleucine side chain implies the displacement of Lys60F, which is found in a different conformation similar to that observed in complexes of thrombin with bivalent inhibitors with a bulky residue (Nle or Thi) at the P1' position.

In brief, peptides with the general formula D-Phe-Pro-D-Arg-X-$CONH_2$ act as anti-coagulants by binding to thrombin in a substrate-like orientation. However, the presence of a D-Arg residue at position P1 impairs cleavage by the proteinase, preserves the interactions with the non-primed specificity subsites S1 to S3, and allows for the establishment of additional interactions by the residue in position P1', contributing for the observed high affinity of the peptides towards thrombin.

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Keywords: anticoagulant, peptide, thrombin

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X-Ray crystallographic studies of rationally designed dihydroorotate dehydrogenase inhibitors

Paul Acklam,[a] Paul Bedingfield,[a] Fraser Cunningham,[b] Deborah Cowen,[b] Glenn McConkey,[a] Colin Fishwick,[b] Peter Johnson,[b] Mark Parsons.[a] [a]*Faculty of Biological Sciences, University of Leeds, (UK);* [b]*School of Chemistry, University of Leeds, (UK).* E-mail: bspa@leeds.ac.uk

The *de novo* pyrimidine biosynthesis pathway represents an attractive and validated drug target in a variety of organisms, including the malaria parasite *Plasmodium falciparum*, the bacterium *Helicobacter pylori*, and in certain human conditions such as rheumatoid arthritis and cancer. Dihydroorotate dehydrogenase (DHODH) catalyses the rate-limiting reaction in this pathway, and the enzyme has been well characterised as a target for chemotherapeutic intervention. Various *in silico* design and screening methods have been used by our colleagues in the School of Chemistry at the University of Leeds to identify potential inhibitors of both human and *P. falciparum* DHODH, and *in vitro* testing of these compounds has revealed that many of them bind to their target with high affinity. X-ray crystallography has been used to investigate the binding of some of these compounds to both human and *P. falciparum* DHODH, and the resulting high resolution structures have enabled us to rationalise the potency of our inhibitors, as well as faciliating the design of a second generation of compounds. *Helicobacter pylori* DHODH has been successfully expressed, purified and crystallised, and optimisation of the crystallisation condtions is ongoing.

Keywords: rational drug design, antimalarial, anticancer

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Two-phase behavior of crystalline β-hematin: link to hemozoin (Malaria Pigment)

Tine Straasø,[a] Sergey Kapishnikov,[b] Jens Als-Nielsen,[a] Leslie Leiserowitz,[b] [a]*Niels Bohr Institute, University of Copenhagen, Copenhagen, (Denmark).* [b]*Weizmann Institute of Science, Rehovot, (Israel).* E-mail: straasoe@fys.ku.dk

Formation of crystalline hemozoin (HZ) is one of the main mechanisms in several blood-feeding organisms to detoxify free heme released upon digestion of host hemoglobin. Among these is the malaria parasite *P. Falciparum*, the most prevalent and most fatal among species affecting human beings. Although once considered eradicated, malaria has in recent times reemerged mainly due to parasitic resistance to commonly used quinoline drugs. These and other well-known antimalarials are anticipated to inhibit nucleation and crystal growth of HZ by binding to its surface[1], [2], [3]. Clearly, the crystallization process represents a vulnerability of the parasite, but the mechanism remains not fully understood.

Based on published spectroscopic and X- ray powder diffraction (XPRD) data, HZ is believed to be very similar to the synthetic compound β-hematin, which consists of heme moieties (ferriprotoporphyrin IX (Fe(3+) PPIX)) coordinated via Fe-O bonds to form cyclic centrosymmetric dimers [2]. However, acknowledging the enantio-facial symmetry of Fe(3+) PPIX, not only one, but four different Fe-O cyclic stereoisomers, two centrosymmetric and two chiral, of opposite handedness, should be formed in the crystallizing solution of β-hematin.

We address the question of the fate of the three other isomers and suggest the four isomers may all be present in different phases of β-hematin. A low-temperature (100 K) X-ray powder diffraction (XRPD) study of β-hematin was undertook and revealed the presence of not only the published phase, but also of a minor phase. Based on Rietveld refinement and DFT+vdW computations [4], we propose the minor phase consists mainly of the other centrosymmetric dimer in a crystal structure similar to that of the major phase. On symmetry grounds the two enantiomeric chiral isomers may be occluded into the growing crystals, introducing disorder. This occlusion may also explain the observed sub-micron size of the crystals; when the chiral dimers are adsorbed on the crystal faces, they would act as tailor-made additives, retarding crystal growth.

The existence of two phases in β-hematin stands in contrast to HZ, which according to published data crystallizes in only one phase. This finding may be essential for a better understanding and more complete determination of the crystal structure of HZ. From structural considerations the formation of a centrosymmetric dimer would imply the formation of the three other dimers. The formation of a chiral dimer of single-handedness is on the other hand unique. We therefore propose a bias towards the formation of one of the chiral dimers and thus HZ to consist primarily of chiral dimers. Such biasing could come about if the free heme was