exogenous recombinant 'truncated' Galectin-3 (analogous to the MMP-processed form), but not full length Galectin-3, dramatically increases migration of the human breast cancer cell line BT-549. These results suggest that the MMP-cleaved Galectin-3 and the resulting structural changes are responsible for pro-metastatic properties of Galectin-3. We have obtained crystallographic data for CRD of Galectin-3 in complex with a pentasaccharide to which Galectin-3 has higher binding affinity compared to other galectin family members. This structural information may be utilized in the design of Galectin-3 specific inhibitors targeting the carbohydrate-binding site. We have also explored the structural differences resulting from MMP cleavage of Galectin-3 using SAXS.

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Keywords: carbohydrate binding, crystal structure, SAXS

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Hinge-loop mutation can be used to control domain swapping of human cystatin C

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Human cystatin C (hCC) is a low molecular mass protein (120 amino acid residues, 13,343 Da) that belongs to a family of single chain, reversible inhibitors of papain-like (C1 family) and legumain-related (C13 family) cysteine proteases [1]. In pathophysiological processes, which nature of is not understood, hCC is codeposited in the amyloid plaques of Alzheimer's disease or Down's syndrome. The amyloidogenic properties of HCC are greatly increased in a naturally occurring L68Q variant, resulting in fatal cerebral amyloid angiopathy in early adult life [2]. At physiological conditions wild-type hCC is a monomeric protein, but under crystallization conditions (pH 4.8) forms a domain-swapped dimer [3]. The dimerization process is facilitated by the presence in the hCC structure of a flexible region created by the loop L1 (55-59, QIVAG) connecting protein subdomains undergoing the exchange process. This loop is the only part of hCC which undergoes significant structural changes during the dimerization process and, according to experimental [4], [5] and theoretical [6], [7] studies, these changes are driven by the conformational constraints attributed to the located near the top of the loop Val residue (Val57 for hCC).

With the aim to check implications of grater or decreased stability of this loop on dimerization and aggregation propensity of human cystatin C, we designed and constructed hCC L1 mutants with Val57 residue replaced by Asp, Asn [8], Gly (residues favored in this position of β-turns) or Pro, respectively. By applying this rational mutagenesis approach we were able to obtain hCC variants stable in the monomeric form both in solution and in the crystal (V57G, Figure 1), monomeric in solution but dimeric in the crystal (V57D) and dimeric in solution and oligomeric in the crystal (V57P). The results of structural studies of hCC L1 mutants will be presented.

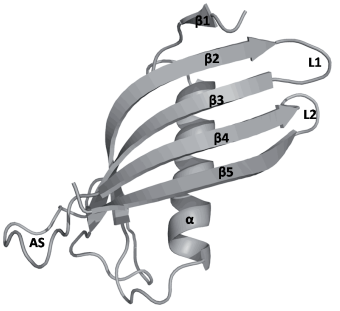

Figure 1. Crystal structures of monomeric hCC mutant V57G

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Keywords: proteases inhibitor, mutant, X-ray crystallography

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How group A streptococcus alpha-enolase interacts with human plasmin(ogen)

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The bacterium *Streptococcus pyogenes* causes a wide range of diseases in humans. More commonly known as Group A Streptococcus (GAS), this pathogen spans a huge spectrum of clinical conditions; from trivial to life threatening. Very common, non-invasive diseases like pyoderma and pharyngitis aside, this is also a causative agent of toxic-shock like syndrome, rheumatic heart disease, and necrotizing fasciitis. A prerequisite for these invasive conditions is that GAS has to migrate to and proliferate in areas of the body that are normally sterile.

It has been shown that the ability of GAS, and other bacteria, to interact with human plasminogen, a zymogen that circulates in our plasma at high concentrations, plays a major role in the invasive process. Plasmin, the active form of plasminogen, is a broad spectrum serine protease implicated in breaking down extracellular matrix and blood clots. The conversion of plasminogen to plasmin is normally under tight control; however this is subverted by GAS which activates this conversion by secreting the virulence determinant streptokinase. By forming a stable complex with either plasminogen or plasmin, streptokinase makes either complex display plasmin activity. GAS can bind the plasmin(ogen)-streptokinase complex to its surface via three known receptors; one of which is the streptococcal surface enolase (SEN).

SEN contains 435 residues per polymer and takes the form of an octameric ring, with a central 4-fold symmetry axis, and is found on the surface of GAS. Recent work [1] has focused on exploring the proposed plasmin(ogen) binding motifs in both wild type SEN, and in a range of point mutated residues. Choice of mutants was aided by creating an

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

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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

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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

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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