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MD-1 is a 162 amino-acid glycoprotein that associates with a B-cell-specific RP105 protein on the cell surface and has a low sequence identity of 16% to MD-2 that associates with Toll-like receptor 4 and recognizes endotoxic lipopolysaccharide. MD-1 and RP105 are supposed to mediate lipopolysaccharide recognition, however, little is known about their structures and functions. To obtain structural insights into the RP105 and MD-1 system, we have determined the crystal structure of mouse MD-1.

Mouse MD-1 was expressed in yeast *Pichia pastoris*, and was purified to homogeneity. Crystallization was performed with the sitting-drop vapor-diffusion method. Two crystal forms, hexagonal and tetragonal, were obtained. The crystal structure of the hexagonal form was initially determined at 2.4 Å resolution with the single isomorphous replacement method, and then the structure was further refined to 1.65 Å resolution against the tetragonal form.

MD-1 is folded into a single domain consisting of two antiparallel β-sheets in the β-cup fold; one sheet consists of three strands, and the other of six strands. A deep hydrophobic cavity of 1,915 $Å^3$ is formed between these sheets as is MD-2. No charged residues are located on the cavity entrance. Continuous electron-densities attributable to bound phosphatidylcholine were observed in the cavity. It is likely that phosphatidylcholine is an endogenous ligand of MD-1 and plays a role in retaining the MD-1 structure since the bound phosphatidylcholine from *P. pastoris* is copurified with MD-1. Together with the binding assay with tetra-acylated lipid IVa, MD-1 is shown to be a lipid-binding coreceptor.

Keywords: receptor, immune, lipid

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Structure and mechanisms of SIGN-R1, pneumococcal and sialylated protein receptor on macrophages

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The intricate system of serum complement proteins provides resistance to infection. SIGN-R1 is a transmembrane receptor found in Spleen macrophages, providing resistance against blood-borne S. Pneumoniae, and that can also recognize C. albicans, HIV and M. tuberculosis. SIGN-R1 extracellular moiety comprises a neck region formed by six repeats and a carboxyl-terminal calcium-type carbohydrate recognition domain (CRD). Pneumococcal recognition by SIGN-R1 activates the classical complement pathway independent immunoglobulins, as well as promotes phagocytes processes [1]. Furthermore SIGN-R1 has been probed to be required in anti-inflammatory activity of sialylated Fc fragments [2]. In order to better understand these processes we have performed the structural determination of SIGN-R1 CRD by X-Ray crystallography. The three-dimensional structures of the SIGN-R1 CRD in complex with a pneumococcal polysaccharide analogue (dextran sulphate) and sialic acid have been obtained at 2.5Å and 2.6Å of resolution respectively. SIGN-R1 CRD structure displays the typical long-form C-type lectin-like domains (CTLDs) fold [3] with two Ca2+ sites mediating carbohydrate binding. Binding site is altered by the unusual orientation of the long loop region, moreover two additional secondary structures elements are present: a 3_{10} helix and a small β-sheet arising from the extended β-sheet 2. Unexpectedly, crystal structure reveals five sulfate binding sites not been observed previously. In addition, docking experiments with models of pneumococcal CSP (CPS14) and *Staphylococcus aureus* "poliribitol phosphate" ligands have been done. We found that sialylated-glyco-proteins could bind to the classical lectin polysaccharide recognition zone while CPS seems to have a new secondary recognition region. This two different recognition sites will allow SIGN-R1 to bind both glyco-proteins and CPS simultaneously. Structural description and carbohydrate recognition are in deep described in the poster.

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Structural basis of carbohydrate recognition by calreticulin

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The calnexin cycle is a process by which glycosylated proteins are subjected to folding cycles in the endoplasmic reticulum (ER) lumen via binding to the membrane protein calnexin (CNX) or to its soluble homolog calreticulin (CRT). Defects in the calnexin cycle and ER protein folding in general are important for a number of diseases and conditions, ranging from cystic fibrosis to malfunctions of the ER stress response due to aging, genetic mutations, or environmental factors. Components of the calnexin cycle additionally play key roles in the assembly of major histocompatibility complex (MHC) class I molecules where CRT associates with protein disulfide isomerase ERp57, tapasin, the heavy chain/β2-microglobulin heterodimer and the TAP peptide transporter to form the peptide-loading complex. The previous crystal structure of CNX revealed two main structural components, a globular lectin domain and an extended arm-like domain, called the P-domain [1]. CNX/CRT specifically recognize monoglucosylated $Glc_1Man_9GlcNAc_2$ glycans, but the structural determinants underlying this specificity were unknown.

Here, we determined a 1.95 Å crystal structure of the CRT lectin domain in complex with the tetrasaccharide fragment from the glucosylated arm of the $Glc_1Man_9GlcNAc_2$ glycan [2]. The lectin domain shows a jelly roll fold similar to leguminous lectins and largely consists of a beta-sandwich formed by two curved beta-sheets. It also contains a single high-affinity calcium-binding site that plays an important role in stabilizing the protein but does not participate in carbohydrate recognition. The tetrasaccharide binds to a long channel on CRT formed by a concave beta-sheet. All four sugar moieties are engaged in the protein binding via an extensive network of hydrogen bonds and hydrophobic contacts. The structure explains the requirement for glucose at the non-reducing end of carbohydrate; the oxygen O2 of glucose perfectly fits to a pocket formed by CRT side chains while forming direct hydrogen bonds with carbonyl of Gly124 and the side chain of Lys111. The structure also explains a requirement for the Cys105-Cys137 disulfide bond in CRT/CNX for efficient carbohydrate binding. The Cys105-Cys137 disulfide bond is involved in intimate contacts with the third and fourth sugar moieties of the Glc_1Man_3 tetrasaccharide. Finally, the structure rationalizes previous mutagenesis

of CRT and lays a structural groundwork for future studies of the role of CNX/CRT in diverse biological pathways.

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Fluorogen-activating proteins

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Using phage display technology, antibody V_L or V_H domains have been selected for their binding to non-fluorescent dye molecules. The dye molecules (fluorogens) then become fluorescent [1] while bound within a dimer of the antibody fragments. These fluorogen-activating proteins (FAPs) are being developed for use as tags for the fluorescent imaging of proteins. Antibody-based FAPs can utilize different arrangements of immunoglobulin variable light and heavy domains (V_L-V_H, V_L-V_L, or V_H-V_H), with different combinations of V_L and V_H domains causing significant differences in fluorescence output. Crystal structures have been determined for the FAP L5-MG(L89S), in the unliganded and malachite green-bound forms, to 1.93 and 2.45Å, respectively. The structure of the malachite green-bound L5-MG(L89S) reveals an unusual homodimeric arrangement of human lambda V_L domains with the malachite green fluorogen sandwiched in between V_L subunits. The dimeric arrangement of the V_L domains is different from any seen thus far for V_L dimers. A similar homodimer is found for in the crystal of the unliganded L5-MG(L89S), although this V_L domain exists as a monomer in solution. The structural results are being used as a guide for design and improvement of future FAP constructs.

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Crystal structure of an aminopeptidase important in antigenic peptide processing

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Two highly homologous endoplasmic reticulum aminopeptidases, ERAP1 and 2, trim antigenic peptide precursors down to the mature epitopes for binding onto nascent MHC class I molecules. The two enzymes operate in concert and have to process a vast variety of different epitope precursor sequences. In this study we report the structure of ERAP2 at 3Å resolution and compare it to recently solved structures of ERAP1. The structure of ERAP2 resembles the structure of ERAP1 in "closed" conformation [1]. Domain IV in ERAP2 is in close proximity to domains I and II, forming a closed large internal cavity. This cavity extends away from the active site and is large enough to accommodate even the longest of antigenic peptide precursors. Amino acid side chains lining the interior of this cavity form shallow specificity pockets with distinct patterns, which can account for different specificity patterns between ERAP1 and 2. A crystallographic dimer formed through the interaction of the N-terminal domains may constitute a model for a proposed ERAP1/2 heterodimer. Overall, the ERAP2 structure provides insights on how two homologous aminopeptidases have evolved to be able to cooperate in order to trim a very large variety of peptidic sequences of relatively long peptides, a function fundamental to their biological role in antigen processing.

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Oppermann *Proc. Natl. Acad. Sci. USA* **2011**, doi/10.1073/pnas.1101262108

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Structure-Energy Relationship of Biological Halogen Bonds: Development of Anisotropic Force Fields

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Halogen bonds (X-bonds) result from an electrostatic attractive interaction between the electropositive crown of a polarized halogen, X, and an electron-rich Lewis base or accepting atom, A, resulting in an X—A distance closer than the sum of their traditional van der Waals radii. X-bonds have been shown to direct protein ligand recognition and binding [1] as well as the conformation of biological molecules [2].We have demonstrated via high-resolution x-ray crystallography the ability of X-bonds to direct the isomeric conformation of DNA Holliday Junctions [2]. The stacked-X junctions can isomerize between two conformations; an X-isomer stabilized by X-bonding at the junction crossover, or the H-isomer stabilized by a hydrogen bonding (H-bonding) at the junction crossover leaving the halogen on the outside strand. The structures of DNA Holliday junctions incorporating fluorine (F), chlorine (Cl), bromine (Br), or iodine (I) halogenated uracil were determined by single crystal x-ray diffraction from 1.6 to 2.2 Å resolution. DNA junctions that formed the X-isomer were found to have a junction stabilizing X-bond between the C5 halogenated uracil and a phosphate oxygen. The angle of approach of the oxygen towards the halogen was near linear with respect to the halogen sigma bond, consistent with current halogen polarization and sigma hole theory. These structures show X-bonding interactions primarily with the π-orbital electrons of the phosphate oxygen, similar to interactions seen with proteins. The ratio of each isomer observed in the crystal structure was determined via occupancy titration calculations in which the halogen occupancy are varied to determine the percent of junction in the X- vs. H-isomer. We have shown that this ratio is correlated with the isomeric concentrations present in solution and therefore an indication of stabilization energy provided by either the X- or H-bonding. We have supported this conclusion by differential scanning calorimetry of identical junctions in solution. We observe that halogen polarization, which increases from F > Cl > Br > I, affects both the X-bond structure