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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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