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DIVISION OF BIOCHEMISTRY

STRUCTURAL BIOLOGY

Development of cancer is generally due to errors that occur in cellular pathways. Understanding mechanisms of the underlying processes will help to determine where the errors occur and how they can be treated. We use X-ray crystallography as a tool to provide three-dimensional structures and we interpret the structural data using a variety of biochemical and biophysical techniques. These studies provide more insight in the molecular processes and they can also provide targets for drug design studies. In our group we focus mainly on proteins involved in ubiquitin conjugation in chromatin regulation, on DNA mismatch repair and nicotinic receptor signaling.

DNA mismatch repair DNA mismatch repair plays a crucial role in ensuring genomic stability. Defects in the mismatch repair cascade in humans predispose to hereditary non-polyposis colorectal cancer and are associated with a variety of sporadic cancers. DNA mismatch repair is initiated by the protein MutS (in Escherichia coli) or its MSH homologs. MutS recognizes and binds to mismatches or unpaired bases that have escaped the proofreading capacity of the DNA replication machinery or are present in the genome during recombinatorial events between non-fully complementary DNA strands.
We make use of variants of MutS that specifically maintain the dimer or tetramer state of these proteins in order to be able to provide quantitative data on the mismatch recognition cycle. In collaboration with the groups of Terence Strick (Paris), Peter Friedhoff (Giessen) and Joyce Lebbink (Rotterdam) this allows a precise analysis of the steps involved in mismatch recognition and the initiation of repair. These mutants allowed novel crystallographic analysis of the proteins with and without DNA, leading to new insight into the state of the protein prior to DNA binding.
Our detailed analyses of the steps following mismatch recognition are building up to a model that reveals the details of the complex ATPase cycle of the mismatch repair proteins.

Figure 1: Novel crystal form of E.coli MutS in complex with 16-mer DNA containing an AA mismatch revealed that crystal packing did not affect protein-DNA interactions, since all contacts were conserved between the two crystal forms (Lebbink et al, 2010)

Publications

Lebbink JH, Fish A, Reumer A, Natrajan G, Winterwerp HH, Sixma TK. *Magnesium coordination controls the molecular switch function of DNA mismatch repair protein MutS. J Biol Chem. 2010;285:13131-41*

Shanmugham A, Fish A, Luna-Vargas MP, Faesen AC, El Oualid F, Sixma TK, Ovaa H. *Nonhydrolyzable ubiquitin-isopeptide isosteres as deubiquitinating enzyme probes. J Am Chem Soc. 2010;132:8834-5*

El Oualid F, Merkx R, Ekkebus R, Hameed DS, Smit JJ, de Jong A, Hilkmann H, Sixma TK and Ovaa H. *Chemical Synthesis of Ubiquitin, Ubiquitin-based Probes and Diubiquitin Angewandte Chemie 2010;49:10149-53*

Ubiquitin and SUMO conjugation Ubiquitin conjugation is critical for signaling in almost all cellular processes, including DNA repair, apoptosis, cell cycle, chromatin regulation and endocytosis. Since these processes are important for cellular stability, deregulation of ubiquitin-dependent processes often leads to cancer. Structure analysis of the proteins involved in ubiquitin and SUMO conjugation could help the development of novel drugs inhibiting critical pathways in ubiquitin conjugation.

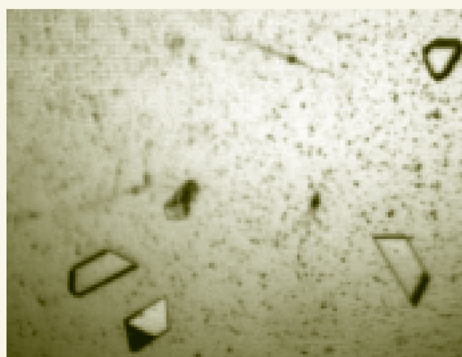
The process of conjugation by ubiquitin-(like) proteins involves covalent linking of one or more 76-amino-acid ubiquitins to a target protein by an E1/E2/E3 cascade of enzymes. Correct ubiquitination requires the complex spatial arrangement of ubiquitin, E2, E3 proteins and the target simultaneously in a precise but flexible manner. Although the overall mechanism has been defined, the atomic details have been lacking and the specificity determining factors are unclear. We study several E2/E3 complexes involved in conjugation of ubiquitin and the related modifier SUMO as well as proteins involved in de-ubiquitination.

Deubiquitinating enzymes are found in five classes, and there are many less than there are E2/E3 combinations. This indicates that regulation may well be taking place at a different level. In order to understand this we have undertaken a comparison of a number of ubiquitin specific proteases. In collaboration with Huib Ovaa we are making use of novel tools to study these enzymes. Thus we can incorporate the analysis of all diubiquitin pairs for this type of analysis.

In terms of regulation we are studying the role of the ubiquitin-like Ubl domains in a number of DUB enzymes. Although these domains have a similar fold to ubiquitin, their sequence properties are very different. Sofar our studies have included the Ubl domain that is internal to the Usp4 catalytic domain and we also analyzed the role of the C-terminal domain of Usp7 and its activating properties for catalytic activity.

In collaboration with Rolf Boelens in Utrecht we studied the chain formation activity of the Rad6 E2 enzyme and how this is modulated by the presence of Rad18. We studied the PCNA modification by Rad6/Rad18 and showed that Rad18 binding inhibits the chain forming activity of Rad6 (Hibbert et al, submitted for publication.

Figure 2: Crystals of catalytic domain of Usp4



Nicotinic Acetylcholine receptor homolog AChBP The acetylcholine binding protein (AChBP) is a homolog of the extracellular domain of the nicotinic acetylcholine receptor, and a representative of the cys-loop receptor family. It remains the best model for atomic resolution structures of ligand binding to this pharmaceutically important family of ion channels.

In collaboration with the group of Iwan de Esch (VU) novel interactors for AChBP have been identified, using a fragment growing approach, into a specific binding pocket (Edink et al, submitted for publication). Using a combination of different thermodynamic analyses and structural studies we were able to explain the behavior of the different ligands that were studied as part of this procedure. Interestingly the comparison of surface plasmon resonance and isothermal calorimetry indicated very similar results and provided an explanation for the observed variations in binding mode. Structural studies of a series of reference compounds including toxins and anti-smoking compounds provide insight in the details of subunit specificity of the nicotinic receptor homologs.