38.1

Structural Proteomics of *Mycobacterium tuberculosis*; Structure-based Research on Potential Targets for New Anti-TB Drugs

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Tuberculosis (TB) kills more people each year than any other single infectious agent. The impact of this disease increases with the spread of HIV/AIDS. Its treatment is complicated by widespread drug resistance. New therapeutics are needed. The German *Mycobacterium tuberculosis* Structural Genomics Consortium (XMTB) determines 3D structures of MTB target proteins and identifies lead compounds for potential drug development. The XMTB Consortium until now has selected nearly 200 targets mainly on the basis of comparative proteome analysis and RNA gene expression studies, without taking feasibility aspects into account. The Consortium has been solving high-resolution crystal structures at a rate of more than one new target structure per month. In addition, crystal structures of complexes of the target proteins with ligands have been determined. Subsequently, the structure-function relationships have been analyzed, and the targets have been screened against large compound libraries. The XMTB Consortium consists of three regional competence centres in Hamburg, Berlin and Munich, which carry out basic and applied research in academic institutions and industrial companies. The partners develop and apply high-throughput techniques of sample production, synchrotron X-ray diffraction, and NMR spectroscopy. Most of these techniques are of relevance also to possible human structural proteomics applications.

38.2

Interaction of Kinetochore Proteins with Tubulin

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Chromosomes segregation requires linkage between microtubules and the chromosomes. This linkage is established by kinetochores, a structure mainly constituted by proteins and localized in centromeric DNA. Kinetochores are large complex protein structures that assemble at the centromeric regions of each sister chromatid and perform three key functions (Nicklas, R. B. 1997 *Science* 275: 632). First, kinetochores attach chromosomes to the spindle. Second, kinetochores co-ordinate microtubule dynamics to allow chromosomes movement along the spindle. Third, kinetochores generate the "wait" signal that prevents anaphase onset until all the chromosomes are correctly aligned on the spindle. Biomolecular interation analysis by surface plasma resonance, a biosensor technology that detects binding events between two or more biomolecules, has become one of the most important and versatile approaches to study protein interactions, especially if combined with mass spectrometry. Mass spectrometric analysis has increasingly become the method of choice for analysis of complex protein samples, also allowing results of primary sequence, post-translational modifications and protein-protein interactions. MS-based proteomics is a discipline made possible by the availability of gene and genome databases and technical and conceptual advances in many areas, most notably the discovery and development of protein ionization methods (Mann, M. *et al.*, 2001, *TIBS*, 26, 54). We used biotinylated tubulin linked to a streptavidin coated Biacore chip to monitor the interactions between tubulin and the proteins of an enriched fraction of Drosophila kinetochore. The experimental results suggest the existence of specific interactions with tubulin. Similar conditions were applied to isolate the interacting proteins using streptavidin coated magnetic beads. The isolated proteins were identified by database search using monoisotopic masses of tryptic peptides acquired by MALDI TOF TOF, after separation of the protein extract by SDS-PAGE. One of the tubulin intercting proteins is Clip190, known as an outer kinetochore structural protein. This result strongly supports the adequacy of the developed experimental strategy to study protein organization in kinetochore.

38.3

Proteomic Investigations Suggest Conformational Equilibria Cause Charge Heterogeneity of 2DE Gel-separated Wool Intermediate Filament Proteins

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Genomic studies have shown that there are four abundant type I and type II Intermediate filament proteins (IFPs) in wool. When separated using two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) the type I IFPs separated into four clearly defined main rows, with 11 major spots. The type II IFPs separated into two distinct staggered rows, with 13 major spots.

The large number of additional spots seen on a 2D-PAGE gel could be due to post-translational modification of the protein. Several common post-translational modifications of proteins produce charge heterogeneity including phosphorylation and glycosylation. Analysis of wool IFPs by 2D-PAGE techniques and mass spectrometry revealed an absence of phosphorylation or glycosylation modifications.

Conformational equilibria as a cause of protein charge heterogeneity has recently been reported (Berven *et al*., *Electrophoresis* 2003, 24, 757–761). Investigations with both the type I and type II IFPs have shown that when single protein spots from a 2D-PAGE separation are eluted, re-focused and re-electrophoresed several spots are formed on both the acidic and basic side of the original spot. Amino acid analysis, mass spectrometry and Ellman's assay results support the hypothesis that the proteins have the same sequence but vary in isoelectric charge due to differences in exposure of charged residues on the molecular surface.

The cause of IFP charge heterogeneity is concluded from these results to be a conformational equilibrium between several different forms of the same protein in the rehydration solution used for the first dimension.

38.4

Proteomic Data Evaluation of Caseins from Milk and Mammary Gland

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Milk is an excellent protein food that provides essential amino acids, calcium, phosphate, casein, lipids and organic nitrogen for humans and animals of all ages. Caseins account for 80% of the total protein in bovine milk, and exist primarily as calcium phosphate stabilized micelle complexes. Caseins are a heterogeneous family of proteins predominated by alpha s1-, alpha s2-, beta and k. Individual casein proteins are small molecules with a molecular mass of 20 to 30 kDa, and primary amino acid sequences that are high in proline content. Classical proteomic approaches to identify complexes mixtures of proteins involves typically four steps: separation, digestion, MS analysis and PMF search in databases. Working with proteins which are secreted usually brings further complications to the last of the four step: PMF databases search. Usually these databases are built using mRNA or cDNA databanks of various organisms which obviously translate for proteins that often have to be physiologically processed. Aim of this work is to compare experimental MS data coming from two dimensional electrophoresis of milk and mammary gland with the deposited proteins sequences. Caseins are a good model for this kind of approach: they are synthesized by epithelial secreting tissue of the mammary gland in a immature form which has to be cleaved at the N-terminal in order to be secreted. This work shows that the subtraction of the weight of the signal peptide to the *in silico* tryptic digest permits to identify experimental peptides which would not be recognised.

38.5

HIV-1 Co-receptor CXCR4; Structural Mapping in the Mammalian Cellular Context

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We have used the chemokine receptor CXCR4, which is also a HIV-1 co-receptor and the cognate ligand Stromal cell Derived Factor 1 (SDF-1) as a model system to study the intermolecular interactions involved in ligand-receptor recognition and other protein-receptor complexes. Previously, specific docking and activation sites on the ligand were mapped; however, the actual binding site(s) on CXCR4 have not been characterized and a high resolution structure will not be likely available in the near future due to the intrinsic difficulty involved in overexpressing and purifying G-protein coupled receptors. Here, we propose an alternative, sensitive, sequence-based approach to map the receptor surface in the natural cellular context. The sensitivity of the approach has allowed for the implementation of a mammalian system for receptor expression with the physiologically relevant post-translational modifications.

SDF-1 analogs containing photoactive benzophenylalanine and a biotin tag for complex isolation were designed and chemically synthesized. We have photocrosslinked with a series of analogs onto whole cells expressing CXCR4 to footprint and model the binding site. The complex was isolated by avidin and immunoaffinity chromatography then subjected to proteolysis and mass spectrometry. The complex purifies as two different sized species suggesting that there is possible modification of the receptor upon ligand stimulation. The individual components of the receptor-ligand complex were identified in western blots and mass spectrometry. We are in the process of determining the specific receptor-ligand contact sites. Three different isoforms of CXCR4 and a novel interacting protein has been identified upon specific elution conditions which may play a key role in receptor modulation.