have been shown to control MT dynamics by local influences on microtubule assembly and to be responsible for 'search-and-capture' of microtubule targets, such as the cell cortex and chromosomes. However, the molecular mechanisms by which +TIPs perform these functions are not clearly understood. +TIPs include structurally unrelated proteins such as EB1, cytoplasmic linker proteins (CLIPs), the dynein-dynactin complex. Here we report the crystal structure of the C-terminal zinc-binding motif of CLIP-170 in complex with the microtubule-binding (CAP-Gly) domain of p150 Glued solved by multiple-wavelength anomalous diffraction using the zinc atom. The structure reveals that CLIP-170 binds p150Glued via the same surface as another +TIP, EB1. Using biochemical and biophysical approaches, we investigated protein interactions involving CLIP-170 and found that an intramolecular interaction area overlaps with EB1- and microtubule-binding sites in an analogous manner to p150Glued.

Keywords: cytoskeleton, crystal structure, NMR

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Topological classification of protein

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Classification of the protein crystal structure is one of the important studies in molecular biology. SCOP [1], CATH [2], and FSSP [3] are known as some of the data base where the classification is based on the amino acid sequence, the secondary structure, and the protein structure. The classification of the graph with respect to the genus obtained by embedding the graph into two dimensional surface is a fundamental problem in the topological combinatorics. The genus is a measure to distinguish the graph complexity. The method is applied to classify the crystal [4]. One of the most interesting applications is the classification of RNA structure by the pseudo-knot [5]. In this study we apply the method to protein classification. The diagram of genus versus hydrogen-bond plot shows a rich structure. The fine structure of the motif can be distinguished well. In the classification of the protein mutant, which is defined as single or several amino-acid structural polymorphism, even the "zero amino-acid structural polymorphism" can be classified in several categories. In the analysis of the disulfide-bond formation facilitator (human), the data can fit well by the straight line in the diagram of genus versus hydrogen-bond plot. The result shows that the mutant can be classified further families. The method opens to construct the periodic table of protein.

[1] SCOP, http://scop.mrc-lmb.cam.ac.uk/scop.
[2] CATH, http://www.cathdb.info/latest/index.html.
[3] FSSP, http://www2.ebi.ac.uk/dali/fssp/fssp.html.
[4] M. Yamanaka and A. Tanaka, cond-mat/0510161.
[5] M. Bon, G. Vernizzi, H. Orland, and A. Zee, q-bio/060732.

Keywords: protein structure and folding, topological aspects of structure, biological macromolecules

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Structural insights into the mitochondrial import complex, TIM9.10

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Over 99% of human mitochondrial proteins are synthesised from nuclear DNA and must be imported as immature precursors *via* a coordinated series of specific, tightly regulated events. Encoded topological signals ensure nascent proteins are ushered to their correct mitochondrial destination. Proteins destined for the inner or outer mitochondrial membranes contain internal targeting information. After transfer through the outer membrane's general import pore, preproteins encounter TIM9.10, a hetero-hexamer of two homologous polypeptides, Tim9 and Tim10. TIM9.10 mediates preprotein passage across and within the intermembrane space. Inner membrane carrier proteins (*e.g.* metabolite carriers) are transferred to the inner membrane translocase, Tim22, for insertion, whereas β -barrel proteins of the outer membrane are transferred to the sorting and assembly machinery, SAM. We have determined the structure of the hTIM9.10 assembly to 3.5 Å resolution and can now verify key contacts in our 2.5 Å structure from *Saccharomyces cerevisiaea*. The molecular assembly has a unique α -propeller topology in which alternating Tim9 and Tim10 subunits alternate about a central pseudo-hexad. Each subunit of the hexamer has a helix-loop-helix topology, and a highly conserved twin CX3C motif present in both forms two intra-chain disulfides that define a central loop. The six loops come together to to form one face of the core assembly, below which tentacle-like helices emanate. Helical protrusions in the structures of other chaperones have been implicated in substrate binding so we are now combining structural and biochemical approaches to investigate chaperone-substrate interactions in our system.

Keywords: membrane trafficking, mitochondria, chaperone

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Structural basis for peroxisomal localization of tetrameric carbonyl reductase

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Peroxisomal matrix proteins are nuclear-encoded and synthesized in the cytosol. There are two well-characterized classes of peroxisome targeting signals (PTS), known as PTS1 and PTS2. Proteins carrying one of these signals are recognized in the cytosol by soluble PTS receptors, Pex5p for PTS1 and Pex7p for PTS2. The PTS1 is a carboxy-terminal sequence ending in the tripeptide Ser-Lys-Leu (SKL) or some conservative variant. All proteins bearing the PTS1 do not undergo cleavage of the targeting sequence upon transport into the peroxisome. Pig heart peroxisomal carbonyl reductase (PerCR) is a 100 kDa homotetrameric enzyme and exhibits NADPH-linked reductase activity towards alkyl phenyl ketones, alpha-dicarbonyl compounds, and all-trans-retinal. It belongs to the short-chain dehydrogenase/reductase (SDR) family, and its sequence comprises a C-terminal SRL tripeptide, which is a variant of the PTS1, SKL. PerCR is imported into peroxisomes of HeLa cells when the cells are transfected with vectors expressing the enzyme.

Interestingly, however, PerCR does not show the specific targeting when introduced into the cells with a protein transfection reagent. To resolve the structural basis for peroxisomal localization of PerCR, we have determined the crystal structure of PerCR at 1.5 A resolution [1]. The structure revealed that the C-terminal PTS1 of each subunit of PerCR was involved in intersubunit interactions and was buried in the interior of the tetrameric molecule. These data indicate that the monomeric form of PerCR whose C-terminal PTS1 is exposed will be recognized by the PTS1 receptor Pex5p in the cytosol and then, is targeted into the peroxisome and thereby forms tetramer.
[1] Tanaka *et al.*, *Structure* **16**, 388-397 (2008).

Keywords: carbonyl reductase, PTS1, SDR

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Advances in the structural elucidation of *Clostridium difficile* toxin B using SAXS and MX techniques

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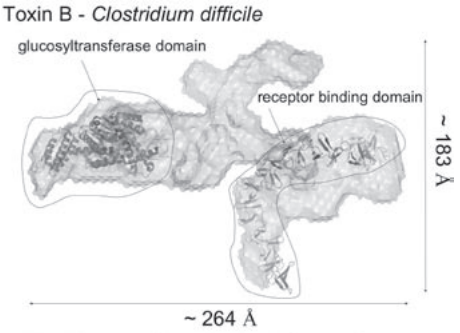
Clostridium difficile is an anaerobic bacterium that is present in the gut of up to 3% of healthy adults and 66% of infants. *C. difficile* can however cause serious gastrointestinal disease, ranging from severe diarrhoea to pseudomembranous colitis. Disease is particularly evident in elderly patients who have undergone antibiotic therapy. Two toxins: A and B [1], can be produced by *C. difficile*. These toxins are members of the Large Clostridial Cytotoxin family and are high molecular weight glucosyltransferases (toxin A: 308 kDa; toxin B: 270 kDa). These two toxins exert their cytopathic action from within the cytosol after receptor-mediated endocytosis. In the growing effort to fully understand the mechanism of action of these toxins, we are carrying out their structural characterization by macromolecular crystallography and SAXS techniques. Current progress will be presented, including the first low-resolution SAXS structure obtained for toxin B and a high-resolution structure of the receptor binding domain [2].



High resolution structure of the recombinant glucosyltransferase domain (Reisert, D.J., *et al.* (2005) *J. Mol. Biol.* 351: 973-981) and model of the receptor binding domain of *C. difficile* toxin B fitted into the low resolution SAXS structure of the full length native toxin.

1. von Eichel-Streiber, *et al.* (1996). Trends Microbiol. 4(10) : 375-82.
2. David Albesa-Jove, *et al. in preparation*.

Keywords: Clostridium difficile, SAXS: Small Angle X-ray Scattering, MX: Macromolecular Crystallography

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Activities and structure of beta toxin

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Beta toxin is a virulence factor of Staphylococcus aureus that catalyzes the cleavage of sphingomyelin(SM) in biological membranes to ceramide and phosphorylcholine causing lysis of erythrocytes. Crystals of beta toxin were found to be fully merohedrally twinned. The structure was solved via molecular replacement using SmcL (SMase C from Listeria ivanovii) as the search model and refined to 2.4 Å resolution. Beta toxin belongs to α/β protein family and is arranged in a 4-layer sandwich. Assays of native and structure suggested site-directed mutants of beta toxin demonstrate that the lysing of sheep erythrocytes and the killing of proliferating human lymphocytes is linked to the SMase activity of beta toxin. These data are the first to show a direct effect upon human tissue and provide a rationale for the importance of beta toxin in virulence. A C-terminal β hairpin has been proposed to penetrate the lipid bilayer and aid in substrate binding and positioning. Our analysis shows this involved in the observed twinning. Three variations of the β hairpin were created, crystallized and solved via molecular replacement and refined. The β hairpin mutations did not significantly perturb the structure of beta toxin, but do affect toxicity towards human cells. A partial lipid was found in one of the structures. SM has been co-crystallized with Beta toxin, and the structure solved and refined to 1.65 Å resolution. The β hairpin has an important role in the SMase activity and cytotoxicity. Current experiments are aimed at elucidating the role of the β hairpin using liposome disruption assays and co-crystallization of the mutants with SM.
[1]www.cdc.gov/ [2]Huseby et al. J Bac, 2007. [3]Openshaw et al. JBC, 2005.

Keywords: sphingomyelinase, toxin, staphlococcus aureus

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Structure and function of C-terminal catalytic region of *Pasteurella multocida* toxin

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Pasteurella multocida toxin (PMT) is one of virulence factors responsible for the pathogenesis in some *Pasteurellosis*. We determined the crystal structure of the C-terminal region of PMT (C-PMT), which carries an intracellularly active moiety. The overall structure of C-PMT displays a Trojan horse structure, composed of three domains arranged in feet, body and head subunits with each linker loops, which were designated C1, C2, and C3 domains from the N- to C-terminus, respectively. The C1 domain showing marked similarity in steric structure to the N-terminal domain of Clostridium difficile toxin B, was found to lead the toxin molecule to the plasma membrane. We found in the C3 domain the Cys-His-Asp catalytic triad that is organized only when the Cys is released from a disulfide