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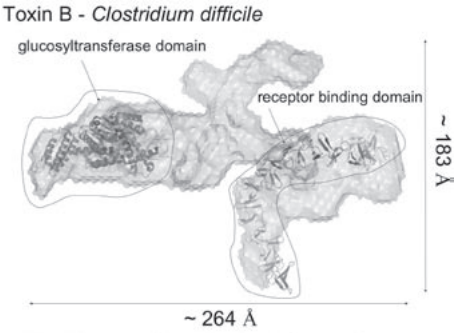
Advances in the structural elucidation of *Clostridium difficile* toxin B using SAXS and MX techniques

David Albesa-Jove[1], Thomas Bertrand[1], Katherine A Brown[1], Dmitri I Svergun[2], Christoph von Eichel-Streiber[3], Neil Fairweather[1]
[1]Imperial College, Centre for Molecular Microbiology and Infection, Flowers Building, Room 5.40, South Kensigton Campus, London, Greater London, SW7 2AZ, UK, [2]European Molecular Biology Laboratory, Hamburg Outstation, D-22603 Hamburg, Germany., [3]Johannes-Gutenberg Universitat Mainz, Institut fur medizinische Mikrobiologie and Hygiene, Hochhaus am Augustusplatz, 55131 Mainz, Germany., E-mail：d.albesa-jove@imperial.ac.uk

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

Medora J Huseby, Ke Shi, Andrew C Kruse, Patrick M Schlievert, Douglas H Ohlendorf, Cathleen A Earhart
University of Minnesota, BMBB, 312 Church St, MINNEAPOLIS, MN, 55455, USA, E-mail：huse0050@umn.edu

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

Kengo Kitadokoro[1,2], Shigeki Kamitani[3], Aya Fukui[3], Hiromi Toshima[3], Masami Miyake[3], Yasuhiko Horiguchi[3]
[1]Kyoto Institute of Technology, Department of Biomolecular Engineering, Matsugasakigosyokaidou-cho, Sakyo-ku, Kyoto, Kyoto, Kyoto 606-8585, Japan, [2]LTM Center, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan, [3]Research Institute for Microbial Diseases, Osaka University, Yamada-oka 3-1, Suita, Osaka 565-0871, Japan, E-mail：kengo@kit.ac.jp

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