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Putting an N-terminal End to the *Clostridium Thermocellum* Xylanase Xyn10B Story: Structure of the CBM22-1-GH10 Modules Complexed with Xylohexaose. Shabir Najmudin[a,b], Benedita A. Pinheiro[a], José A.M. Prates[a], Maria J. Romão[b], Carlos M.G.A. Fontes[a]. [a]*CIISA - Faculdade de Medicina Veterinária, Universidade Técnica de Lisboa, Avenida da Universidade Técnica, 1300-477 Lisboa, Portugal.* [b]*REQUIMTE, Departamento de Química, FCT-UNL, 2829-516 Caparica, Portugal.*
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Xylan, the most abundant hemicellulose in plant cell walls, is a chemically and structurally complex polysaccharide, whose complete degradation requires the action of a dedicated enzymatic consortium. Endo-xylanases are implicated with the random hydrolysis of the xylan backbone that can be decorated with acetyl, glucuronyl and arabinose residues. Xylanase Xyn10B from Clostridium thermocellum is a highly active multimodular bifunctional xylanase that belongs to the extracellular multi-enzyme complex of cellulases and hemicellulases termed the cellulosome. Xyn10B is one of the largest cellulosomal modular enzymes (1077 amino acid residues; Mr = 120 kDa) and comprises two family 22 carbohydrate-binding modules (CBM22-1 and CBM22-2), which flank the glycoside hydrolase GH10 catalytic module, a dockerin sequence and a C-terminal family 1 carbohydrate esterase catalytic module. The structure of the N-terminal bi-modular CBM22-1-GH10 moiety of the xylanase Xyn 10B from Clostridium thermocellum has been determined using SeMet derivative by SAD to 2.5 Å [1]. The data was extended to 2.0 Å for the non-SeMet mutant complexed with xylohexaose. It is a 60 kDa bi-modular protein with an E337A mutation to render the GH10 subunit inactive. Three of the six xylose subunits of the xylohexaose substrate are shown to be bound in the inactivated catalytic site, with the other three presumably disordered in the solvent channel. The protein is a dimer in the asymmetric unit with extensive surface contacts between the two GH10 modules and the CBM22-1 and GH10. However, the two CBM22-1 are only held together by two cadmium ions from the crystallisation buffer salt-bridging between the highly acidic loop in the CBM22-1 (containing three tandem glutamate residues). Currently, we are using SAXS to analyse the arrangement of CBM22-1-GH10-CBM22-2 and extending this study to the complete xylanase Xyn10B structure.

[1] Najmudin, S., Pinheiro, B. A., Romao, M. J., Prates, J. A. & Fontes, C. M., **2008**. *Acta Crystallogr* Sect F S 64, 715-718.

Keywords: cellulosome; xylanase; carbohydrate binding modules

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Crystal Structures of Complexes of the IgG1 Fc Fragment with Peptidomimic Ligands. Anna Bujacz[a], Izabela Redzynia[a], Grzegorz Bujacz[a]. [a]*Institute of Technical Biochemistry, Technical University of Lodz, Lodz Poland.*
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Monoclonal antibodies (MAb), due to their high selectivity to target-specific receptors, are recently being used as main anticancer drug components, as well as in many diagnostic tests. Their production is very expensive because of high costs of the purification process. Our research is a part of the European AIMs Project, the goal of which is to develop innovative, interactive materials, which could optimize cost-effective processes of MAb purification. The current methods of MAb purification are based on chromatography columns with protein A or G agarose. These materials are universal for a wide range of antibodies; however, they are expensive and impractical for use in purification on an industrial scale.
As a source of Fc we used an anticancer drug - Herceptin, in which monoclonal antibody IgG1 is the active component. We performed the cleavage of IgG1 using papain to obtain the isolated Fc fragment, which after purification, concentration and buffer exchange was crystallized with ligands. Crystals were measured on the EMBL synchrotron, using R,R-2,3-butanediol as a cryoprotectant.
We determined a number of crystal structures of the Fc complexes with ligands from two leading groups: "cauliflower" peptidomimic and triazine derivatives. All structures were solved in the $P2_12_12_1$ space group. In the asymmetric unit, there are 2 monomers (A,B) creating a homodimer, the shape of which is reminiscent of a twisted horseshoe. The interactions of chains A and B in the crystal lattice are not identical. Chain A is involved in more crystal contacts and chain B has a better access to the water channels. The unit cell contains four homodimers, related by three perpendicular two-fold screw axes. The domains of each monomer (CH_2, CH_3) possess predominantly β architecture. The CH_2 domains are naturally glycosylated and a proper distance between them is maintained through the polysaccharide chains interactions. The "cauliflower" peptidomimic ligands interact with the CH_2 domain of chain B close to the linker region with CH_3, but the triazine derived ligands have fewer binding sites.
The structural information on the Fc-ligand complexes can lead to the designing of a new generation of active components for chromatographic columns, which will significantly reduce the overall costs of monoclonal antibody production.

Keywords: Fc fragment of IgG; monoclonal antibody purification; peptidomimic ligands

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The Chemical Basis of the Colour of Lobster Shell; Structures of Carotenoids. Madeleine Helliwell[a], Giuditta Bartalucci[a], Stuart Fisher, John R. Helliwell[a], Synnøve Liaaen-Jensen[b], John E. Warren[c], James Wilkinson[a]. [a]*Department of Chemistry, University of Manchester, UK,* [b] *Norwegian University of Science and Technology, Trondheim, Norway;* [c]*STFC,*