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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,*

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There has been considerable interest and speculation as to why, when a lobster is cooked, it changes from a dark slate blue colour, to bright red. It is now known that the chromophore responsible for the colour of the lobster is the carotenoid astaxanthin, AXT (3,3'-dihydroxy -β,β-carotene-4,4'-dione), which is found in the protein α-crustacyanin (α-CR). The crystal structure of β-CR has been determined [1] allowing a number of theories to be put forward to explain the bathochromic shift between the unbound and protein bound forms of AXT. The crystal structures of a number of unbound carotenoids have also been determined, including that of AXT [2]. Most of these adopt the 6-s-*cis* conformation, unlike the protein bound molecule, which is 6-s-*trans*. Recently, however, we have determined the crystal structures of the 6-s-*cis* and 6-s-*trans* isomers of the diacetates of astaxanthin (AXT), **s-*cis*-1** and **s-*trans*-1,** and those of 7,8-didehydroastaxanthin ((3S,3'S)-3,3'-dihydroxy-7,8-didehydro-β,β-carotene-4,4'-dione), **(2),** and 7,8,7',8'-tetradehydroastaxanthin ((3S,3'S)-3,3'-dihydroxy-7,8,7',8'-tetradehydro-β,β-carotene-4,4'-dione), **(3)** [3]. The conformations of these four molecules vary in particular, with the angle of twist of the end rings out of the plane of the polyene chain; for **s-*cis*-1**, the end rings are bent out of the plane of the polyene chain by an angle of -49.0(5)°, and the conformation is therefore similar to that found for unesterified AXT as well as for the carotenoids, canthaxanthin and β,β-carotene. For **s-*trans*-1**, the end rings are coplanar with the polyene chain and its conformation is much more similar to that of the protein bound AXT in crustacyanin. In **(2)** and **(3),** the end rings are also almost coplanar with the polyene chain with the end rings in **(2)** in the s-*cis* conformation, and in **(3)** in the s-*trans* conformation. Thus, an extensive ensemble of the possible β end ring conformations has been determined [2,3]. These structures are compared with one another as well as unbound, unesterified AXT and protein bound AXT. Also, the effect of the end ring conformations on the colour and UV/Vis spectra of the crystals was established, and showed that the s-*trans* conformation provides part, but not the entire bathochromic shift seen in the AXT in crustacyanin.

[1] Cianci, M., Rizkallah, P. J., Olczak, A., Raftery, J, Chayen, N. E.,. Zagalsky, P. F & Helliwell, J. R. (2002). *PNAS,* **99**, 9795-9800. [2]Bartalucci, G., Fisher, S., Helliwell, J.R., Helliwell, M., Liaaen-Jensen, S., & Fisher, S. (2007). *Acta Cryst.* B**63**, 328- 337. [3] Bartalucci, G., Fisher, S., Helliwell, J.R., Helliwell, M., Liaaen-Jensen, S., Warren, J.E. & Wilkinson, J. (2009). *Acta Cryst.* B**65**, in press.

Keyword: carotenoids; protein; colour

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Structural Investigations of the Proteolytic Complexes Triggering the Complement System. Christine Gaboriaud[a], Nicole Thielens[b], Gérard Arlaud[b]. [a]*LCCP or* [b]*LEM, Institut de Biologie Structurale, Grenoble, France.*
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The complement system is a first line of defence against infection, and is also involved in immune tolerance, allograft rejection and various pathologies. Several large protein complexes (> 500 kDa) can specifically trigger this system: the classical pathway C1 complex, and the MBL-MASP and ficolin-MASP complexes involved in activation of the lectin pathway. These complexes are assembled from two kinds of subunits: an oligomeric pattern recognition protein (C1q, MBL, L- or H-ficolin), and a protease component, which is either a tetramer (C1s-C1r-C1r-C1s) or a dimer ((MASP)(2)). These modular proteases (C1r/C1s, MASPs) trigger the proteolytic cascade of the complement system as a consequence of the binding of their associated recognition protein to the target. The main objective was to decipher the main structure-function relationships involved in these complex activation processes.
We have used a dissection strategy to define the main functional protein fragments involved in this process: the protease catalytic domains, the recognition domain of C1q and ficolins, the protease interaction domains, which mediate both the protease dimerization and their association to their cognate recognition proteins. We have solved by X-ray crystallography the structure of these domains, including several complexes with ligands for the recognition domains [1-2]. Site-directed mutagenesis was also used to probe the main binding sites in the proteases interaction domains [3]. Recent functional and 3-D structural investigations have revealed that C1r/C1s and the MASPs associate through a common mechanism involving their N-terminal CUB1-EGF region. Moreover, they use homologous binding sites in their CUB module to associate to their cognate recognition protein [3]. New structures of protein-ligand complexes have shed lighton the structural bases of the various recognition properties of C1q and ficolins. Regarding the proteolytic cascade involved in C1 activation, C1r autoactivation was shown to be the first step. The structure of the C1r protease, has revealed that this first step of activation requires a mechanical stress transmitted from the C1q stems to the C1r catalytic region through the C1q–C1r–C1s interfaces [1, 4].An overview of these investigations, including the most recent structural studies, will be given.

[1] Gaboriaud C, Thielens NM, Gregory LA, Rossi V, Fontecilla-Camps JC, Arlaud GJ, **2004**, *Trends Immunol* 25, 368. [2] Garlatti V, Belloy N, Martin L, Lacroix M, Matsushita M, Endo Y, Fujita T, Fontecilla-Camps JC, Arlaud GJ, Thielens NM, Gaboriaud C, **2007** *EMBO J* 26, 623. [3] Teillet F, Gaboriaud C, Lacroix M, Martin L, Arlaud GJ, Thielens NM, **2008** *J Biol Chem* 283, 25715. [4] Gaboriaud C, Juanhuix J, Gruez A, Lacroix M, Darnault C, Pignol D, Verger D, Fontecilla-Camps JC, Arlaud GJ, **2003**, *J Biol Chem* 278, 46974.

Keywords: macromolecular complex; complement protein; pattern recognition

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ββα-Me Restriction Endonuclease Hpy99I in Complex with Target DNA. Honorata Czapinska[a,b], Monika Sokolowska[a,b], Matthias Bochtler[a,b,c]. [a]*IIMCB, Trojdena 4, 02-109 Warsaw, Poland.* [b]*MPI-CBG, Pfotenhauerstr, 108, 01309 Dresden, Germany.*