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FCT FACULDADE DE CIÊNCIAS E TECNOLOGIA UNIVERSIDADE NOVA DE LISBOA

Chemistry Department

The Cellulosome, by X-rays & NMR

Macromolecular Crystallography Group

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requimte rede de química e tecnologia

FACULDADE DE MEDICINA VETERINÁRIA

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Objectives

In anaerobes, the degradation of the plant cell wall is carried out by a high molecular weight multifunctional complex termed the **cellulosome** (Fig.1), whose architecture and efficiency are defined by high affinity protein-protein interactions between **cohesins** and **dockerins** (Fig.2) and by the presence of highly specific **carbohydrate-binding modules** (CBMs – Fig.3), respectively.

Our objective is to identify the molecular determinants of ligand specificity in carbohydrate binding-modules and to understand the mechanisms of molecular recognition between cohesins and dockerins.

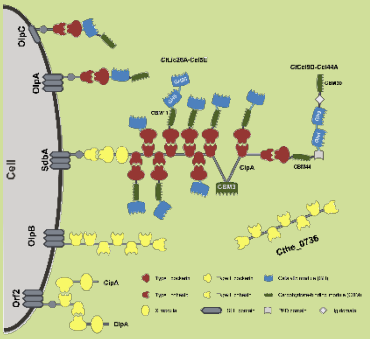

Figure 1: The cellulosome of *C. thermocellum*.

Methodology

In order to study this mega Dalton complex and understand how it works we used an **X-ray crystallography**, **NMR** and **Molecular modeling** approach.
We used **X-ray crystallography** to determine the 3D structure of CBM11 and two Type II cohesin-dockerin modules (Fig.2).
We used **NMR** also to determine the solution structure of CBM11, to study the interaction of CBMs target ligands and to study the influence of temperature and ligand binding in the internal dynamics.
Molecular modeling was used to obtain docking models of the studied proteins with the target ligands in order to better rationalize the experimental results (Fig. 3).

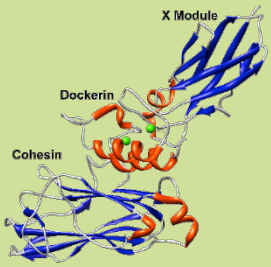

Figure 2: Crystal structure of the *Ct*Coh-XDocII complex

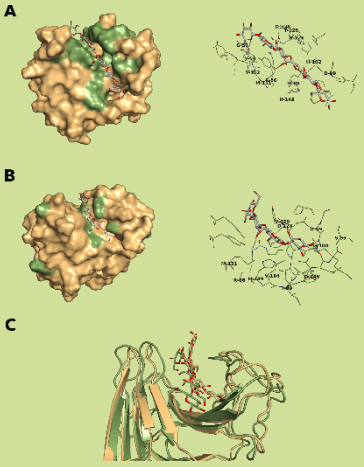

Figure 3: Molecular Dynamics models of *Ct*CBM11 with cellohexaose (A) and cellotetraose (B) at 25 ºC and superposition of both structures (C).

Expected Results

- Identification of the residues of *Ct*CBM11 involved in binding
- Identification of the atoms of the ligands involved in binding
- Rationalization of mechanism by which *Ct*CBM11 is able to distinguish and select its ligands
- Determination of the 3D structures of CtCBM11 (NMR) and two type II cohesin-dockerin complexes (X-ray Crystallography)
- Contribute to the understanding of the atomic interactions that mediate the cellulosome architecture assembly

Funding: We thank the beamline scientists at ID14-EH4 and ID14-EH1, European Synchrotron Radiation Facility (ESRF) and Fundação para a Ciência e Tecnologia for funding and support through projects PTDC/QUI-BIQ/100359/2008 and PEst-C/EQB/LA0006/2011 and grants SFRH/BD/35992/2007 and SFRH/BD/38667/2007. The NMR spectrometers are part of the National NMR Network (RNRMN) and are funded by FCT