SCIENCESPRINGDAY

FACULDADE DE CIÊNCIAS E TECNOLOGIA UNIVERSIDADE NOVA DE LISBOA

Chemistry

"Looking" at protein interactions

Macromolecular Crystallography Group

xtal Crystallography Group @ REQUIMTE — http://xtal.dq.fct.unl.pt/

requimte rede de química e tecnologia

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Post-Doc Fellow since 2010

- 2004 – Graduate in Biochemistry at FCUL
- 2010 – PhD in Animal Science and Technology at FMV/UTL (FCT Fellow)
- 7 publications and 1 oral communication
- PI of 1 project funded by FCT
- Supervisor: Professor Maria João Romão

Objectives

- To understand the structure-function role of a **novel membrane anchored protein involved in *N*-glycosylation pathway, Malectin,** which binds with high selectivity to one of key intermediate *N*-glycan players of this pathway in the endoplasmic reticulum (Figure 1).
- Obtain a better understanding of the organization of a large extracellular enzyme called the **cellulosome**, capable of degrading plant cell walls (Figure 2). We aim to do this by making a detailed characterization of the **cohesin-dockerin interaction**, responsible for the assembly of the multienzymatic complex.
- Develop a **protein microarray to manner protein-protein interactions** between novel cohesins and dockerin pairs which will allow the rational discovery of novel cohesin-dockerin complexes (Figure 3). The structural studies will permit to understand, at a molecular level, the flexibility of the cellulosome complex.

Methodology

- **X-ray crystallographic characterization** of the protein involved in the *N*-glycosylation in complex with its glycan ligands.
- Assessment of the different cohesin-dockerin specificities and affinities through the use of **functional protein microarrays and Isothermal Titration Calorimetry (ITC)**.
- **Heterologous co-expression**, crystallization, mutagenic studies and characterization by X-ray crystallography of cohesin-dockerin complexes .
- Use of **novel high-throughput cloning and purification techniques** for fabricating microarray slides and performing the experiments.

Expected Results

- To **solve the first crystal structure** of malectin in complex with its selected di-glucosylated high mannose *N*-glycan oligosaccharides to derive structural information of the interaction at molecular and atomic levels (Figure 3).
- To **discover the structural specificities** of the various cohesin-dockerin pairs in order to understand the reason behind the differences in affinity between them which may be responsible for the flexibility of the cellulosome.
- To **generate microarrays** containing both known cohesins, our own system control, and novel cohesins recently identified in the genomes of cellulolytic organism. To **screen cohesin-dockerin specificities** in novel organisms, selecting cohesin-dockerin pairs for posterior structural characterization.

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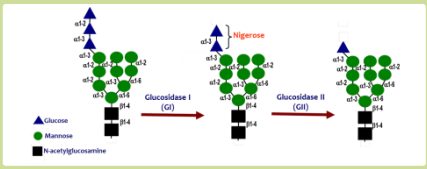

Figure 1 – The three key glucose terminating glycan intermediates of the *N*-glycosylation pathway of the type that occurs in the ER. Malectin selectively binds to the di-glucosylated high mannose *N*-glycan (middle sequence).

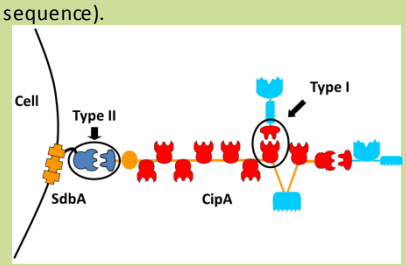

Figure 2 – A schematic representation of the modular arrangement in the *Clostridium thermocellum* cellulosome, highlighting the two type of cohesin-dockerin interactions.

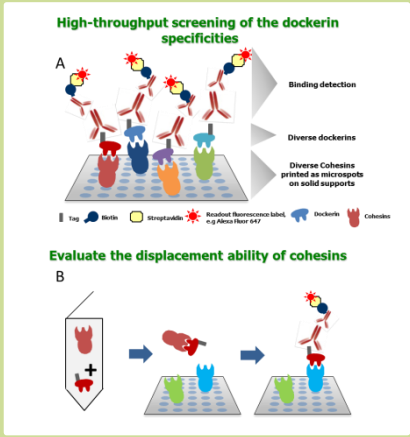

Figure 3 – A schematic representation of the cohesin microarray and the experiments performed.

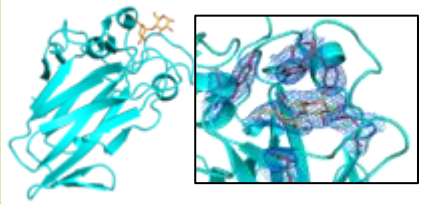

Figure 4 – Crystal structure of the malectin carbohydrate recognition domain complexed with one of its glycan ligands.