LABORATORY OF BIOCHEMISTRY
GROUP OF BIOLOGICAL CHEMISTRY AND BIOTECHNOLOGY,
BIOENGINEERING DEPARTMENT

RESEARCH PROJECT

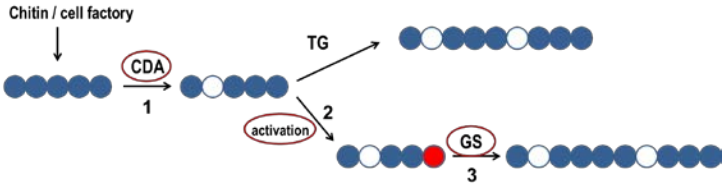
***Positions offered* (2017):** 1 Master research project (6-9 month), 1 Undergraduate project (2 to 6 month).

Engineering synthases and deacetylases: towards the enzymatic production of chitosans with defined deacetylation patterns for biomedical applications

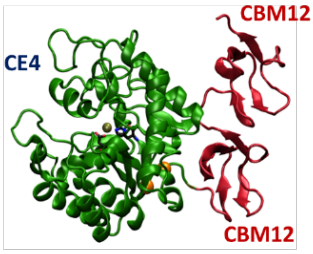
One of the most promising and most advanced functional bio-polymer is the polysaccharide **chitosan**. The term chitosan refers to a family of polysaccharides obtained by partial de-N-acetylation from one of the most abundant renewable resources on earth, chitin, a linear polysaccharide of β-1,4-linked *N*-acetylglucosamine (GlcNAc) residues. Chitin is found in the exoskeletons of insects and crustaceans such as shrimp and crab, in the endoskeletons of mollusks such as squid, in many invertebrates as in e.g. the egg shells of nematodes, and in the cell walls of fungi and some diatom algae.

Chitin processing, mainly in the form of depolymerization and de-*N*-acetylation reactions by chitin-modifiying enzymes (chitinases and deacetylases), generates a series of derivatives including chitosan and chitooligo-saccharides (COS), which play remarkable roles in Nature. COS are particularly involved in molecular recognition events, including the modulation of cell signaling and morphogenesis, the immune response, and host-pathogen interactions. Chitosans and COS are also attractive scaffolds for the development of bionanomaterials for drug/gene delivery and tissue engineering applications. Most of the biological activities associated with COS seem to be largely dependent on the degree of polymerization and the specific acetylation pattern, which define the charge density and the distribution of GlcNAc and $GlcNH_2$ moieties in chitosan and COS.

Our group is engaged in the structure/function analysis and protein engineering of chitin-modifying enzymes to build up biocatalytical approaches towards the production of tailor-made chitosans and chitooligosaccharide derivatives with defined deacetylation patterns. The general approach involves: a) biotechnological production of chitooligosaccharides (COS) by a cell factory approach, b) specific partial deacetylation of COS (paCOS) by engineered chitin deacetylases, and c) enzyme-catalyzed polymerization of defined paCOS by engineered chitinases acting as glycosynthases.



Chitin de-*N*-acetylases (CDAs) catalyze the hydrolysis of the acetamido group in GlcNAc residues of chitin, chitosan, and COS. The deacetylation pattern exhibited by CDAs and related carbohydrate esterase (CE4) enzymes active on COS is diverse, some being specific for a single position, others showing multiple attack. *A major challenge is to understand how CDAs specifically define the distribution of GlcNAc and $GlcNH_2$ moieties in the oligomeric chain.* By means of structural and biochemical studies, we have proposed a *subsite capping model,*[1] which is guiding enzyme engineering approaches (rational and directed evolution) towards the production of paCOS with defined and novel deacetylation patterns.



Chitinases (Chi) are glycoside hydrolases that catalyze the hydrolysis of chitin and chitosans to short oligosaccharides. Family 18 chitinases (GH18) operate by a double displacement mechanism by substrate assisted catalysis. *We are engineering GH18 chitinases to introduce synthase activity* for the controlled polymerization of paCOS with the aim of producing polymeric sequence-defined chitosans and their evaluation as novel biomaterials.

[1] E.Andrés, et al. Structural basis of chitin oligosaccharide deacetylation, **Angewandte Chemie Int. Ed. 5**3, 6882–6887 (2014). doi: 10.1002/anie.201400220
[2] S.N.Hamer,et al., Enzymatic production of defined chitosan oligomers with a specific pattern of acetylation using a combination of chitin oligosaccharide deacetylases. **Scientific Reports, 5**, article 8716 (2015) doi:10.1038/srep08716

***Funding*:** European project NANO3BIO (http://www.nano3bio.eu); MINECO project GLYCOZYMES

***Researchers*:** Dr. Antoni Planas (PI), Dr. Xevi Biarnés, Postdoc: Dr. Enea Sancho, PhD students: Hugo Aragunde, Cristina Alsina, Laia Grifoll, Sergi Pascual.

Contact: Dr. Antoni Planas (antoni.planas@iqs.edu)