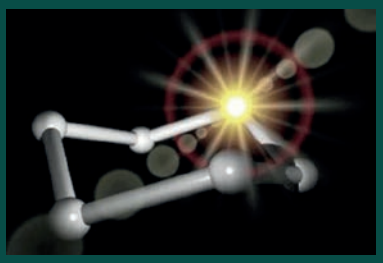
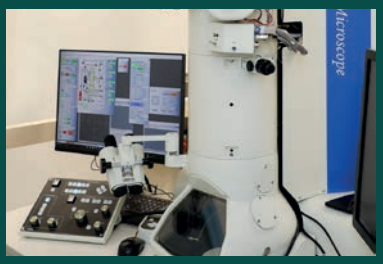
IDEX project
Cross Disciplinary Program





Glyco@Alps

Université Grenoble Alpes

Annex:
Reports from funded projects

Report 2017-2021

UGA
Université
Grenoble Alpes

Molecular diversity of hemicelluloses oligosaccharides from wood

PROJECT LEADERS

Christine Chirat (LGP2)
Claire Boisset (Cermav)
Bertrand Toussaint (TIMC-IMAG)
TheRex

WP1

One of the main directions of this work is the separation and characterization of small molecular weight oligosaccharides extracted from wood. A part of the project has been done in partnership with Jahan Golestani (PhD student at LGP2) who is working on the enzymatic hydrolysis of wood pulp for hemicelluloses oligomers recovery. The aim of our partnership was to develop a method for the fractionation and identification of Jahan's samples, by means of size exclusion chromatography and Electrospray Ionization Mass Spectroscopy (ESI MS).

Funding provided by Glyco@Alps did allow for the purchase of Bio-Gel P2 and P4 (polyacrylamide beads from Bio-Rad) and thus the design of a low pressure liquid chromatography system with glass columns. The new set-up is now part of the Plateau PCANS semi-preparative purification systems at Cermav. After several configurations, looking at the influence of the eluting temperature, the eluent ionic strength and the Bio-Gel fractionation range, we managed to observe a good separation of one of Jahan's samples, see Figure 1. ESI MS performed on F1 reveled the clear isolation of three charged xylan structures with polymerization degrees of 3, 4 and 5, each functionalized with one 4-O-Methylglucuronic acid. F2 and F3 were composed of neutral xylo-oligosaccharides with respectively three and two xylose units.

Furthermore, this grant from Glyco@Alps enabled me to present a part of my work during an oral conference (International Symposium on Wood Fiber and Pulping Chemistry, 20th ISWFPC) taking place at the University of Tokyo in Japan, from the 9th to the 11th of September 2019. This oral presentation, entitled "Oligosaccharides from wood autohydrolysates: a multi-step purification technique", was giving to expose some results relating to the first line of work of the project and led to the publication of an article in Holzforschung journal in June 2020.

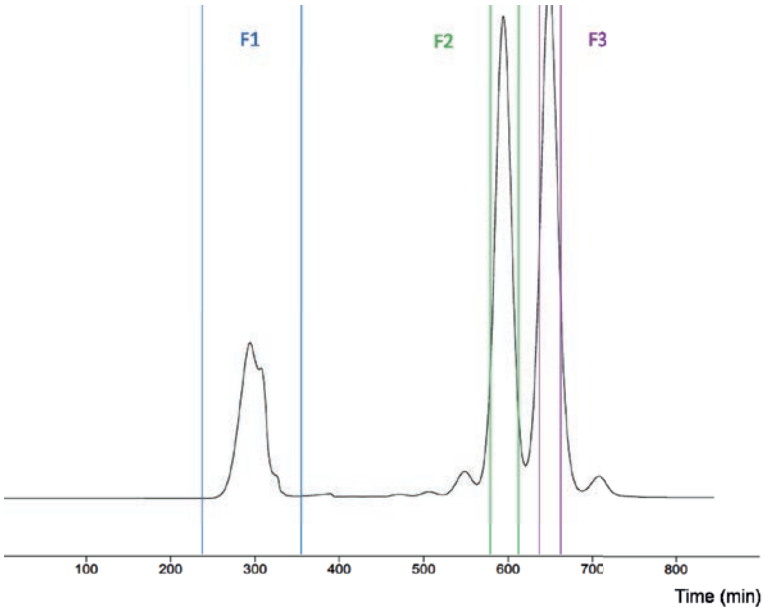


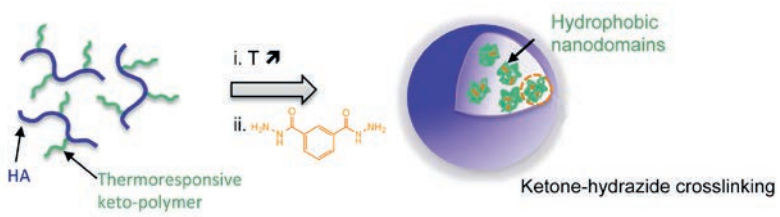
Figure 1 Size exclusion chromatogram of water-soluble hemicelluloses oligosaccharides enzymatically extracted from hardwood pulp

Since wood chips autohydrolysis process triggers the solubilization of hemicelluloses, it also implies the chemical degradation of lignin in wood and its depolymerization, as well as the formation of sugars degradation products (F, 5-HMF, levulinic acid, acetic acid, formic acid). The study was aiming at removing impurities from hydrolysates using:

- Granulated activated carbon adsorption
- Ultrafiltration

Ultrafiltration allows for the total removal of the monomeric part of extracted hemicelluloses, as well as low molecular weight impurities, but at the expense of great sugars loss (up to 50% at 1kDalton for softwood). As a comparison, activated charcoal detoxification is incomplete, F and HMF are eliminated but organic acids are not adsorbed. Nevertheless, the acid soluble lignin to oligosaccharides ratio obtained from activated charcoal treated samples was higher than for ultrafiltration retentates. Since then, further purification steps have been performed combining both techniques to reach satisfying oligosaccharides rates.

Biodistribution and blood kinetics of nano-glycogels in rodents



PROJECT LEADERS

Lucie Sancey (IAB)
Rachel Auzély (Cermav)

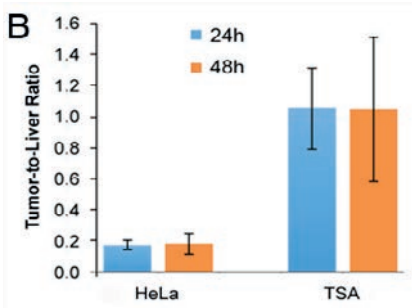
WP3

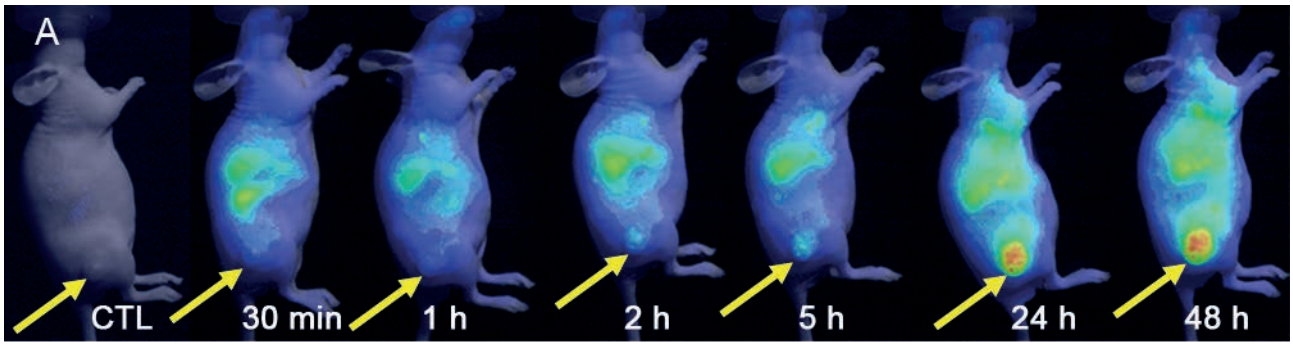
Amongst the development of nanocompounds loaded with contrast agents or drugs, polysaccharide-based nanogels are of great interest due to their strong biocompatibility, mastered production cost and high chemical flexibility that allow their application in various fields. In this project, we aimed at understanding the cell uptake and the in vivo biodistribution in tumor-bearing mice of different nanogels containing hyaluronic acid (HA).

We demonstrated that despite the presence of HA, a natural ligand of the CD44 receptor, the cell internalization of the HA-based nanogel was not only driven by the cell expression of this CD44 receptor, as the nanogels were also internalized in CD44-negative cells. Similar results were also demonstrated in vivo, in mice-bearing tumor models. The next figure (A) demonstrated the tumor uptake of the HA-nanogel, with a strong accumulation at 24/48h post injection. The figure (B) reveals that CD44-negative tumor TSA accumulate 5 times more the nanogel as compared to the CD44-positive tumor HeLa.

This nanogel was enriched in boron, and evaluated. In addition to a high cell uptake in various cell lines, the boron-enriched nanogel was very safe for cells. Due to the presence of Boron atoms, this nanogel has been tested for boron-neutron capture therapy (BNCT), an innovative method of radiotherapy, with promising results on cells. In vivo, preliminary biodistribution data were obtained, and should be optimized before next in vivo therapy assay.

Lastly, amongst the experiments performed on mice, a new polysaccharide nanogel based on Heparonan appears very interesting and will be evaluated in the next months.





Virtual reality to explore sugars

PROJECT LEADERS

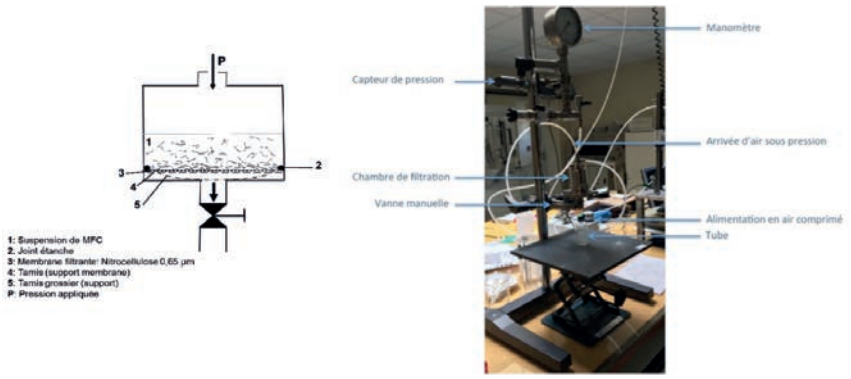
Alain Rivet (Cermav)
Serge Perez (Cermav)
Marc Baaden (Institut de Biologie Physico-Chimique, Paris)

WP4

In the framework of the collaborative work with the Laboratoire de Biochimie Théorique, CNRS (Institut de Biologie Physico-Chimie, Paris) the development dealt with a novel interactive approach to molecular visualization (*). Special attention is given to the world of complex glycans and polysaccharides taking advantage of recent progress in the field of virtual reality. The platform for Virtual Reality has been implemented at Cermav during the period 2017-2018, and has been open to a large public. The platform is equipped with an appropriate portable computer and ad hoc equipment for Virtual Reality. As such it has been used on several occasions, out the premise of the Cermav ; e.g. national meeting of the French Glycoscience Group, week sciences,…. There is a regular usage by glycoscientists who are eager to visualize some of their favorite objects of research ; nanocrystals of cellulose, carbohydrates interacting with proteins, glycolipids and glycoproteins embedded in complex membranes decorated. A special « Virtual promenade in Sugar Land » was organized in the context of the visit of 30 teachers of physics and chemistry. Access to this platform is on demand, as is the quest for partnership to develop innovative educational illustrations and tools.

(*) Three-Dimensional Representations of Complex Carbohydrates and Polysaccharides. SweetUnityMol: A Video Game Based Computer Graphic Software. S Pérez, T Tubiana, A Imberty, M Baaden, Glycobiology 25 (5), 483-491

Fractionation of suspensions of cellulose nanocrystals obtained in subcritical water by ultrasonic assisted membrane separation



PROJECT LEADERS

Nicolas Hengl (LRP)
Evelyne Mauret (LGP2)

WP3

Figure 1: Descriptive diagram of the CNC filtration pilot.

The objective of this exploratory work is to propose an alternative to conventional processes for the production of Cellulose NanoCrystals (CNC) used in the biorefinery field, as additives in bio-sourced materials to confer gas barrier properties, mechanical properties but also optical properties. This new production process, which consumes less water and chemicals, is based on the use of sub-critical water (T=120°C, P=200atm, t= 60 minutes). By using sub-critical water, all the washing steps are simplified and, even with a slightly lower yield, this technique makes it possible to divide the production cost by 10 to 100. Another advantage of this process is the possibility of directly functionalizing the CNCs, using a gas such as CO2 or carboxylic acids. On the other hand, this process has the disadvantage of producing a heterogeneous final mixture composed of both CNCs and micrometer-sized particles. Membrane separation would allow to answer this problem by separating the CNCs present in the final mixture. Used on a large scale in many industrial sectors, this operation based on physical separation allows to concentrate or fractionate products/elements of different sizes. The coupling of a sub-critical water reactor and a membrane process could therefore allow a continuous and homogeneous production of CNCs. The objective of this project is to determine, through an experimental approach, the filtration and fractionation properties of heterogeneous suspensions of cellulose nanocrystals obtained from a conventional process.

Starting at 0.2µm, we immediately notice a retention of CNC on the membrane (figure 2). This deposit is a factor limiting the performance of the filtration because it causes a decrease in the flow of material through the membrane. Therefore, membranes with larger pore diameters will have to be used to allow the passage of CNCs. We were able to observe that CNCs pass through membranes with pore diameters 8µm, 3µm and 0,65µm.

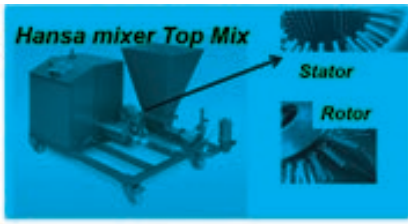
First results of filtration of mixtures containing microcrystalline cellulose and CNCs have been obtained on 0.65µm membranes. It shows that cellulose (coarse particles) is retained on the membrane while part of the CNCs in the mixture pass through. Fractionation of these complex mixtures is therefore possible using membrane separation.
Further results have to be obtained and filtration kinetics will have to be implemented. The next step would be to perform filtration experiments on the mixtures obtained in subcritical water. Contacts have been made with the IFS (Institute of Supercritical Fluids) in Valencia to start a collaboration or to carry out preliminary tests. The search for financial resources is underway to advance this unique and innovative research project in the field of biorefinery.

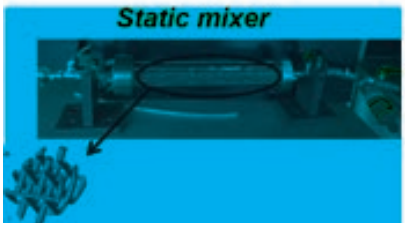
Figure 2: Image of a 0.2µm PVDF membrane after filtration of 1% CNC suspension at a pressure of 3.5 bar.

3D foams bio-based material : In line-process, formulation, structure and end-used properties links of highly porous alveolar cellulosic materials

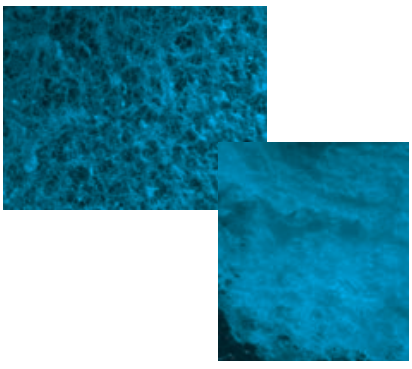
Human society has benefited tremendously from the use of synthetic plastic foams. For instance, expanded polystyrene (EPS) foams are extraordinarily manufacturable and offer a wide range of mechanical properties. However, these petroleum-based plastics deplete fossil fuels, produce wastes and degrade the environment. Bio-based counterpart materials owing to their renewable, sustainable and biodegradable capacities are good candidates to replace petroleum-based plastics. Up to now, no suitable solution has been proposed to produce bio-based foams in large quantity at acceptable costs with adaptable structural and mechanical properties.

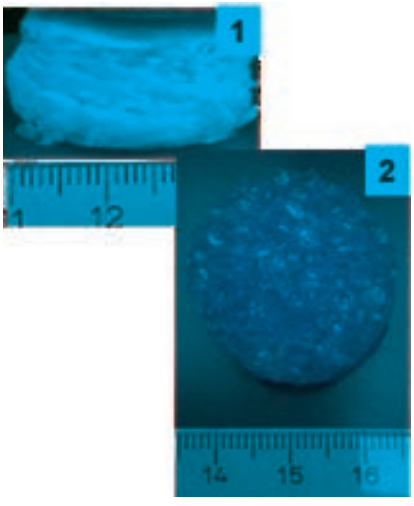
In this context, the main objective of this project is to perform a process of cellulose-based foams with controllable and adapted formulation, structure and end-used properties. A second objective is to upscale an in-line whipping technology without losing the aerated and controlled morphology of the final cellulose-based materials. For these purposes, we used two foaming pilot loops developed and instrumented during the ANR PolyNat Carnot (2015-2017) projects (figures beside).





The CDP Glyco@Alps grant allowed improvement of the pilot loops: (i) in-line foam characterization thanks to the void fraction evaluation based on pressure drop measurements and (ii) bubble size distribution characterization based on optical tools.
First productions of cellulose-based foams were carried out and compared to synthetic benchmark (see below), specifically concerning mechanical properties via compression tests (see table, right). Whereas results are encouraging in terms of adaptation of foaming processes at a pilot scale and foam density targeted, structure and mechanical properties remains to be improved.

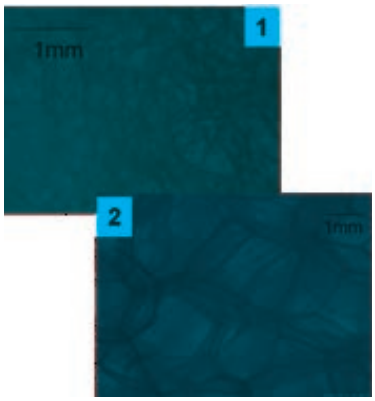




PROJECT LEADERS

Emeline Talansier (LRP)
Denis Roux (LRP)
Davide Beneventi (LGP2)

WP3



	Density (g/L)	Young's Modulus (Pa)
1	26-31	2082
2	21	115341

Macroscopic, millimeter, density and mechanical properties comparison between cellulose foam (1) and synthetic benchmark (2)

New aldehyde functionalization of oxidized cellulose microfiber

Cellulose is an abundant, renewable and biodegradable natural polymer produced by plants with unique structure and properties, used to manufacture a number of technical products. The use of oxidation-modified celluloses makes it possible to develop original biosourced and biocompatible substrates for controlled release of active molecules in the field of the smart medical devices. Up to now, several cellulose oxidation processes have been developed, including the commonly used TEMPO or periodate catalysed processes, but both are expensive and polluting. The ozonation of cellulose, proposed to mainly form aldehydes, appears as a sustainable alternative. The aim of the project was to evaluate the relevance of ozonation to functionalize cellulose microfibers (MFC).

To do so, we designed and synthesized dansyl based fluorescent reagents able to specifically react with aldehydes, and studied their reactivity with ozonized-MFC. The choice of fluorescent probes allowed easier monitoring and follow-up of the reactions using various techniques such as fluorimetry and hplc. The results obtained so far were not those expected as most of the fluorescence was found in the water phases and not on the oxidized fibers. Therefore, we tested the reactivity of the probes with the well-known and well-characterized TEMPO-oxidized MFC and the results were very encouraging. The purification steps were optimized by monitoring the fluorescence of the washing solutions used to remove the excess of reagents. Ultimately, fluorescent films were obtained and characterized by IR and elemental analysis.

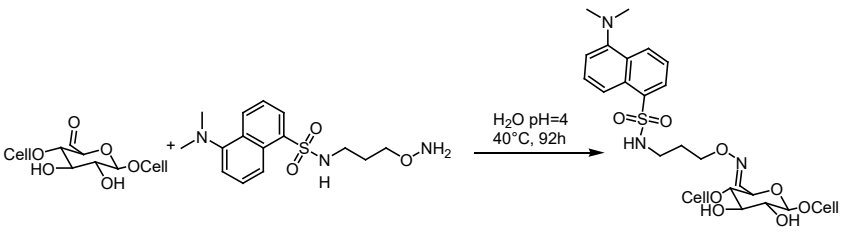
PROJECT LEADERS

Isabelle Baussanne (DPM)
Elisa Zeno (CTP)
Julien Bras (LGP2)

WP3

Efforts now focus on the characterization of the mode of binding (adsorption vs covalent binding) by nmr and its quantification.

on the left, MFC-TEMPO, on the right, Dansylated MFC-TEMPO



Reference: The advantages and challenges raised by the chemistry of aldehydic cellulose nanofibers in medicinal chemistry, I. Baussanne, J. Bras, B. watbled, M. Demeunynck. Future Medicinal Chemistry 2018, 10, 2679-2683. DOI : 10.4155/fmc-2018-0277

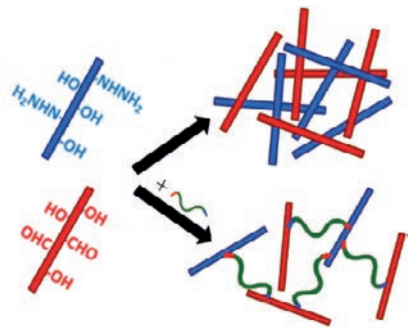
CROSSNANO - Improving Nanocellulose Foams through Crosslinking for Biomedical Release Applications in wet condition

Project summary

The proposed research aims to develop value-added applications for nanocellulose as biomedical materials with specific release capabilities by controlling material structure and function. This will be achieved through new strategies to produce interconnected nanocellulose materials (i.e., through crosslinking) that will ultimately provide biocompatible foam-like materials with uniform distribution of components and enhanced physical/mechanical properties. Nanocellulose is a promising family of renewable nanomaterials and this project will use mainly nanofibrillated cellulose (NFC). These nanoparticles have advantages including low cost, non-toxicity, biodegradability, high aspect ratio and chemically modifiable surfaces which can be useful for crosslinking. Meanwhile lipidot have been developed with success as nanoscaled drug carrier.
The proposed work will build on past research from Cranston's group at McMaster that produced flexible 3D foams from CNCs that could support functional nanoparticles for energy storage and water purification devices and previous collaboration between LGP2 and Mac master on new crosslinking CNF aerogels or previous collaboration between CEA and LGP2 on Nanocellulose lipidot aerogel. In each project, preliminary results have shown the positive impact of nanocellulose crosslinking or the possibility to mix nanocellulose and lipidot in aerogel.

Main results

Two internships have been funded thanks to this ticket (Justine Sollier, M1 & Mitul Patel, M2) during first semester of 2018.
The aim of Sollier's project was to produce aerogels of crosslinked nanofibrilated cellulose (NFC) using the Diels-Alder reaction between furan and a maleimide functionalized NFC. These aerogels are designed for biomedical applications such as drug delivery and tissue engineering. Because of this biomedical use, the process used to obtain aerogels have to be mostly green. The experiments done need to be more analyzed but high level characterization(13C NMR , elemental analysis and XPS) have been performed and conclude with the successful crosslinking of our biopolymers. All these results are promising and pave the way to other grafting methods and crosslinking.
Main objective of the Patel's internship was to improve Nanocellulose Foams through Crosslinking for Biomedical Release Applications



in wet condition and investigate the ability for the "sponges" to take up and release various active and non-active biopharmaceuticals and drugs. Crosslinking is required so that the materials do not redisperse in liquid or wet environments. We tried to achieve it through new three strategies to produce interconnected nanocellulose materials (i.e., through crosslinking) that will ultimately provide biocompatible foam-like materials with uniform distribution of components and enhanced physical/mechanical properties.
Firstly, crosslinking with PAE was tried by many researcher but the applications was different then biomedical. The aerogel was quite yellow in colour and the mechanical strength was not achieved up to the level. Strategy with Hydrozone crosslinking works extremely well. The research was done with Nanocrystal (NCC) at Macmaster university. However, we have tried with NFC with small change (reaction time) in protocol. The results i.e. mechanical properties in water and drug release works well. Even though these experiments need to be more analyzed like 13C NMR , elemental analysis and XPS signals, which might be useful to conclude with the successful crosslinking of our biopolymers.
Finally the last strategy, crosslinking with Diamine with and without using EDC-NHS coupling agent was also quite successful. To conclude, Hydrozone crosslinking and diamine crosslinkinng of NFC system for crosslinking is a green and effective way to obtain aerogels which has to be developed and improved.

PROJECT LEADERS

Julien Bras (LGP2)
Isabelle Texier (CEA)
Emily Cranston (McMaster University, Canada)

WP3

Break-up of self-assembled chitosan / surfactant microcapsules for controlled drug delivery

The proposal was based on a recent publication of LRP and Cermav on a system of microencapsulation1 based on the self-assembling of chitosan, a carbohydrate polymer, with a surfactant that is comprised of a commercial lecithin (Palsgaard 4448, food-grade, E442). This system is cheap, green and biocompatible. The elasticity of these microcapsules has been extensively studied in our recent publications1 and preliminary results have been obtained concerning the break-up properties in extensional flow (see Figure 1).

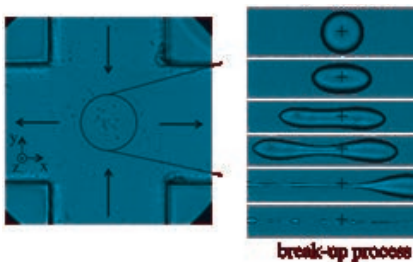


Figure 1: Left: Extensionnal flow chamber. Right : Dynamics of break-up of a microcapsule in an extensionnal flow.

The objective of this proposal was to characterize the break-up properties of self-assembled chitosan / surfactant microcapsules in extensional flow.

The results of this project has been obtained by Kaili Xie, a PhD student in LRP who was in charge of microcapsules preparation and characterisation by AFM and Majid Rodgar (M2 in Grenoble INP) who did the experiments on break-up in extensional flow.

We have observed three different break-up morphologies according to the properties of the microcapsules and the strength of the flow: (i) 'tip-streaming', as already observed for droplets, (ii) detachment of two lobs or (iii) break-up by 'folds'. The break-up process by the detachment of two lobs of the microcapsule has been already observed in our previous publication (Figure 1-right). The two other morphologies are new and never published for microcapsules at our knowledge.

Figure 2: Tip-streaming

In the tip streaming regime, the break-up is localised at the tips of the microcapsules (Figure 2). The break-up by 'folds' happens when the microcapsule present wrinkles that merge into folds at high stress. When the stress increases again, the break-up happens into a folds and the microcapsule is damaged (Figure 3).

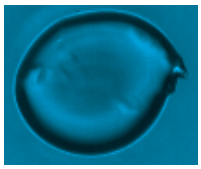


Figure 3: Break-up by folds

We have been able to make a phase diagram of break-up morphologies as a function of the Capillary number and the surface elasticity of the microcapsule. The phase diagram shows that 'tip-streaming' and 'detachment of two lobs' are observed at low surface elasticity, whereas that 'folds break-up' has been observed at high surface elasticity. Moreover, we have shown by AFM that the surface elasticity of microcapsules is proportional to the membrane thickness.

In conclusion, we have shown that break-up morphology of self-assembled microcapsule depends on their elasticity and membrane thickness. Supplementary experiments are required to finalize the results and will be then submitted in Physical Review Letter.

PROJECT LEADERS

Clément de Loubens (LRP)
Frédéric Dubreuil (Cermav)

WP3

Reference
1. Xie, K. et al. Interfacial rheological properties of self-assembling biopolymer microcapsules. Soft Matter 13, 6208–6217 (2017).

DNP enhanced solid-state NMR applied on lignocellulosic materials: a feasibility study

PROJECT LEADERS

Gaël De Paëpe (MEM-INAC)
Christine Chirat (LGP2)
Marie-Christine Brochier (LGP2)

WP1&4

Thanks to the support from Glyco@Alps, a new collaboration between two teams (LGP2 and MEM) was initiated in 2018. The goal of the project was to evaluate the relevance of developing a hyperpolarization technique called Dynamic Nuclear Polarization (DNP) for the study of wood. The long-term challenge is to detect and observe lignin in situ in the lignocellulosic material. This will help to better understand its structure and its interweaving with hemicelluloses, and then adjust and optimize delignification processes.

As a feasibility test, several biomass-derived samples (prepared at LGP2) were studied using the DNP technique developed in the MEM laboratory. [2]. Using DNP, we were able to hyperpolarize raw material, as well as samples subjected to auto-hydrolysis treatment, and to perform solid-state NMR experiments with improved sensitivity. The 13C NMR spectra presented below reveal the presence of hemicellulose, lignin as well as cellulose the main constituent of wood. In addition, one can prove that the auto-hydrolysis treatment results in the removal of the hemicellulose content. This is illustrated in Figure 1 where the substantial reduction in hemicellulose signal is highlighted by dashed ellipses. In Figure 2, we compare the spectra of lignin extracted from wood with native lignin present in wood. Interestingly, we note that the relative signal intensity of the two peaks highlighted by dashed lines (in the 145-160 ppm range) is different in the two cases. This suggests that the chemical composition of lignin after extraction from wood is not the same as the native lignin inside the wood.

These preliminary results highlight the importance to develop DNP as an in situ characterization technique for an improved understanding of wood and its constituents.

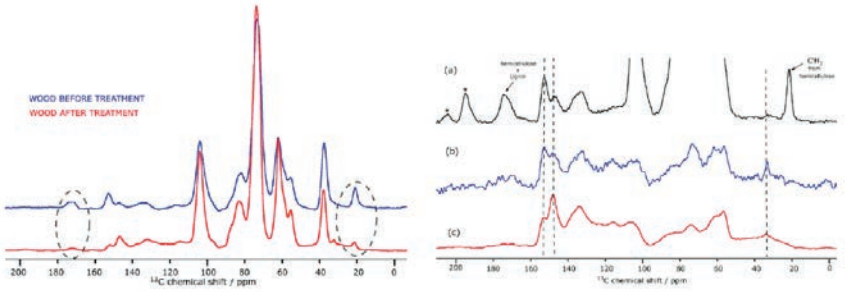


Figure 1. (left) DNP-enhanced 13C-CPMAS NMR spectra of wood before treatment (blue) and wood after treatment (red). Dashed ellipses in the figure highlight the difference in hemicellulose content in the two wood samples. A strong reduction of signals from hemicellulose can be clearly observed. (right) 13C-CPMAS SSNMR spectra of (a) wood before treatment with DNP enhancement, (b) lignin extracted from wood before treatment and (c) lignin extracted from wood after treatment without DNP enhancement. Dashed lines highlight the lignin peaks with different relative intensities.

Vectorization of imaging agents to the brain

The development of therapeutic or diagnostic agents able to reach the brain in significant amounts to exert an efficient biological action is a real challenge for the treatment and diagnosis of many neurological diseases.

This project in the field of medicinal chemistry aims to explore the targeting of the glucose transporter Glut-1 widely and selectively expressed at the blood-brain interfaces to improve brain penetration of diagnostic agents.

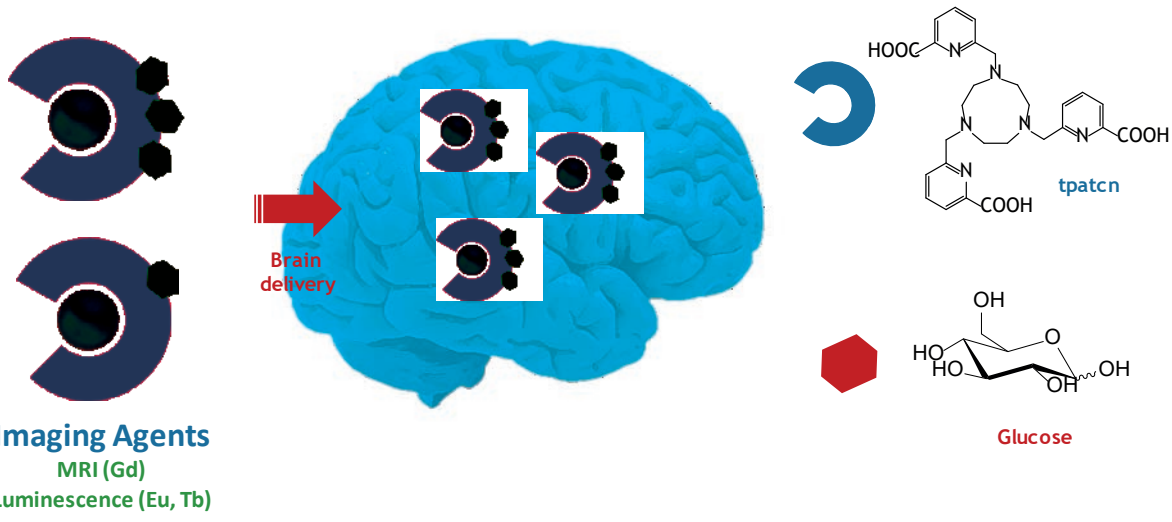
During this project, the synthesis of some vectors, which combine one or more glucose residues to known lanthanide complexes as MRI and optical imaging agents was undertaken. Thanks to the support funded by Glyco@Alps, several key intermediates were obtained: glucose targeting units and lanthanide complexes bearing one to three reactive groups to graft the glucose-based targeting units. The coupling of these units was attempted with the synthesis of a lanthanide complex appended with one glucose unit, with a moderate yield. Unfortunately, due to COVID interruption we could not optimize this last step. Nonetheless, the biological evaluation of these targeted complexes in the future should allow a better understanding of the transport systems to the brain and propose new diagnostic and therapeutic approaches dedicated to other brain diseases.

PROJECT LEADER

Christelle Gateau (SyMMES)
Pascale Delangle (SyMMES)

WP2

Project funded in 2018 and 2020.



Construction of a permanent magnet with variable field and coupling with an electric field

PROJECT LEADER

Laurent Heux (Cermav)

WP3

The purpose of the ticket was to fund the visit at Cermav of Bruno Frka-Petesic, postdoctoral researcher in the Bio-inspired Photonics group of Silvia Vignolini in Cambridge University for two weeks from January 8th to January the 19th 2018. As a postdoctoral researcher in Cermav in 2013 and in Cambridge University ever since, he has developed a new research theme on the orientation of cellulose nanocrystals by using electric and magnetic field alignments. His work resulted in publications in Advanced Materials in 2017, one of the biggest impact factor journals in the field of materials
chemistry1.

The visit of Bruno Frka-Petesic in Cermav had two main objectives :
- Training Axel Fouques (PhD student in the group since October 2017 funded by Glyco@Alps and Cambridge University) to the theory of cellulose nanocrystals field alignments and to get him started with the experimental setups Bruno built up several years ago for his PhD project. It helped him saving months of work to catch up on these topics.
- Achieving novel results during and after his stay in Cermav : the first magnetic field
alignment of organic suspensions of cellulose nanocrystals and exploring other configurations of electric field alignment.

In the scope of aligning with magnetic fields, a magnetic pinch has been conceived prior to his arrival and assembled on the first days of his stay but was found not to be effective enough. Later in another geometrical design for the magnets, it resulted in a 0.65 T field and the first reported magnetic alignment of cellulose nanocrystals helices in organic solvents. Magnetic field has been shown to be a very straightforward way of optimizing the optical response of cellulose nanocrystals helices, and is also planned to be used along with electric fields for materials-making (PhD project of Axel Fouques).

Regarding the alignments in electric fields, we have completed the work started by Bruno Frka-Petesic in 2013. We acquired experimental data consistent with the current theory on electric field alignments, showing that electric field alignments result in a plane of possible helix orientations in the suspensions leading to various consequences such as the loss of iridescence at higher thicknesses. Moreover, competition experiments with both electric and magnetic fields showed how efficient is electric field at aligning compared to magnetic fields.

Bruno Frka-Petesic's visit in Cermav thus allowed efficient skills transfer to the group, leading to new advances in the area of external fields alignment. We do think that this will contribute to Cermav keeping up at the cutting-edge of this area of research in the coming years, leading to valuable contributions and publications.

Références :
1 : Bruno Frka-Petesic et al, Adv. Mater. 2017, 29, 1606208

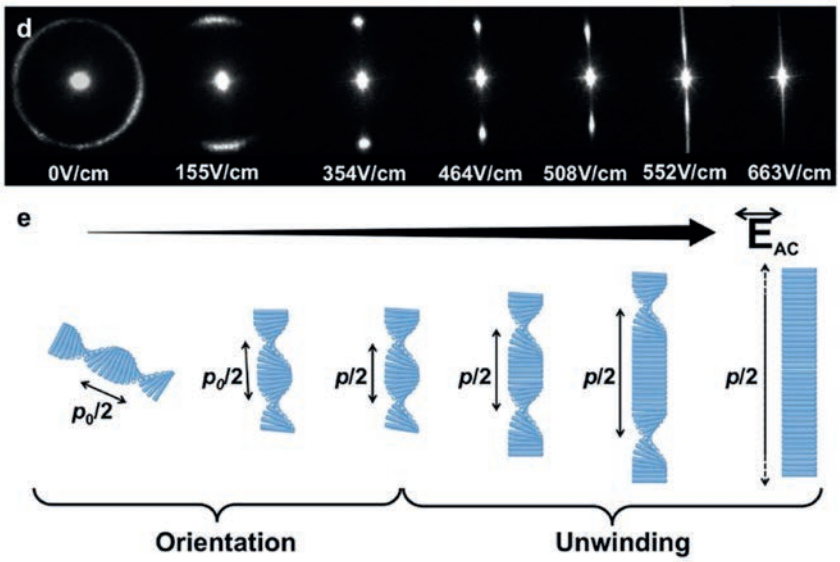
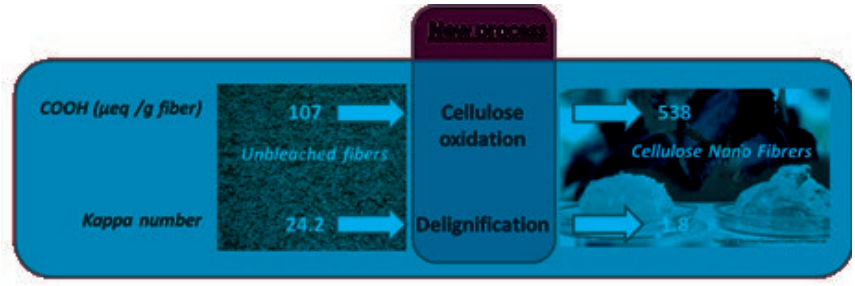


Figure 1 : Pioneering work of Bruno Frka-Petesic on the alignment of cellulose nanocrystals helices in electric fields, published in Advanced Materials in 2017.

Development of an innovative chemical-pretreatment for Cellulose Nano Fibers (CNF) production from unbleached lignocellulosic fibers



PROJECT LEADERS

Gérard Mortha (LGP2)
Nathalie Marlin (LGP2)

WP3

Lignocellulosic biomass valorization into added value bio-products is a highly topical issue. Among possible products, cellulose nano fibers (CNF) are of great interest for biomaterial formulation. CNF, initially present in the fiber structure, are individualized and separated through fiber wall deconstruction, before being used as nanomaterials, usually as additives in another polymer matrix. CNF possess singular mechanical and chemical properties (high strength properties, biodegradability character, transparency and barrier properties …) valuable for various applications such as material, packaging, cosmetics…

CNF are classically obtained from bleached fibers (free of lignin) after intensive mechanical grinding. This operation consumes a lot of energy limiting the possible CNF production at industrial scale. To decrease the energy demand, chemical or enzymatic pretreatments are carried out before grinding. In particular the cellulosic fiber deconstruction may be facilitated by the pre-oxidation of cellulose using the TEMPO/NaClO/NaBr system. After the cellulose C6 oxidation, the fiber wall is weakened, leading to an easier separation into individualized nano fibers. However this treatment is highly polluting and costly, partly due to the use of NaBr as a catalyst in TEMPO oxidation. In this context, Lucas Dollié developed during his PhD work at LGP2 (2015-2019) a chemical-pretreatment for CNF production suited to unbleached lignocellulosic fibers, without the use of NaBr. This work has been supported by the LabEx tec 21 and by Glyco@Alps. This new pre-oxidation process combines bleaching of unbleached Kraft fibers and carboxylation of the substrate in a single stage, using TEMPO as catalyst but free of NaBr (confidential results protected by an "envelope SOLEAU"; patent under study). CNF of same quality as those produced from bleached fibers pre-oxidized by the classical TEMPO/NaClO/NaBr system have been obtained. The energy demand is also similar.

The innovation is thus (1) the choice of the starting substrate, unbleached Kraft fibers, (2) the replacement of NaBr by a conventional bleaching chemical widely employed in paper pulp bleaching lines, and (3) the development of a process combining both delignification and cellulose oxidation in one single operation. This process may be easily implemented in paper pulp mills, in strong interaction with unit operations already existing in the production line, since some chemicals and effluents may be re-used or recycled.

Innovation potentials triggered by glycoscience research results

PROJECT LEADERS

Mireille Matt (GAEL)
Frédéric Corolleur (GAEL)
Serge Perez (Cermav)

WP5

Glycoscience is an interdisciplinary field, which leads to different industrial applications derived from physicochemical and/or biological properties of carbohydrates. The aim of our research is to understand the different innovation potentials based on glycoscience research. By adopting an interdisciplinary approach through the combination of economics and glycosciences, we pave the way to the definition of "glycoeconomics". We consider three value-chains related to the two properties of carbohydrate molecules and analyze the main innovation drivers and bottlenecks along the value-chains.

The regional biomass (sugar, starch, wood) value-chain exploits the physicochemical properties of carbohydrates. It involves low and high-tech mature industries, which are developing new processes and products at various steps of the value chain (e.g. nanocellulose, hydrophobic paper based on chromatogeny). The quality and quantity of intermediary bio-products, the price/performance ratio of functionalized and processes products, the low demand for these new products and the unstructured value chain constitute the main challenges to switch from a fossil-based economy to a glycoeconomy.

The glycomics explores the biological functions of carbohydrates and involves high-tech mature industries and emergent start-ups. The main upstream innovations concern kits, enzymes, reagents (for example, Automated Glycan Assembly) and instruments and lead to disruptive innovations such as Tests to estimate the biological age of a patient. The innovators face many challenges related to the creation of new business models, metrology, glycobioinformatics, and intellectual property issues.

We show that glycosciences play an important role in the emergence of niches at different steps of these three value-chains. These niches are at an emerging stage. They are characterized by a techno-scientific push approach aimed at developing high value-added products with new functionalities, new bioactive glycans, and new enabling technologies that will lead to new applications and possible novel therapies and diagnostics not enabled by the non-glycan regime.

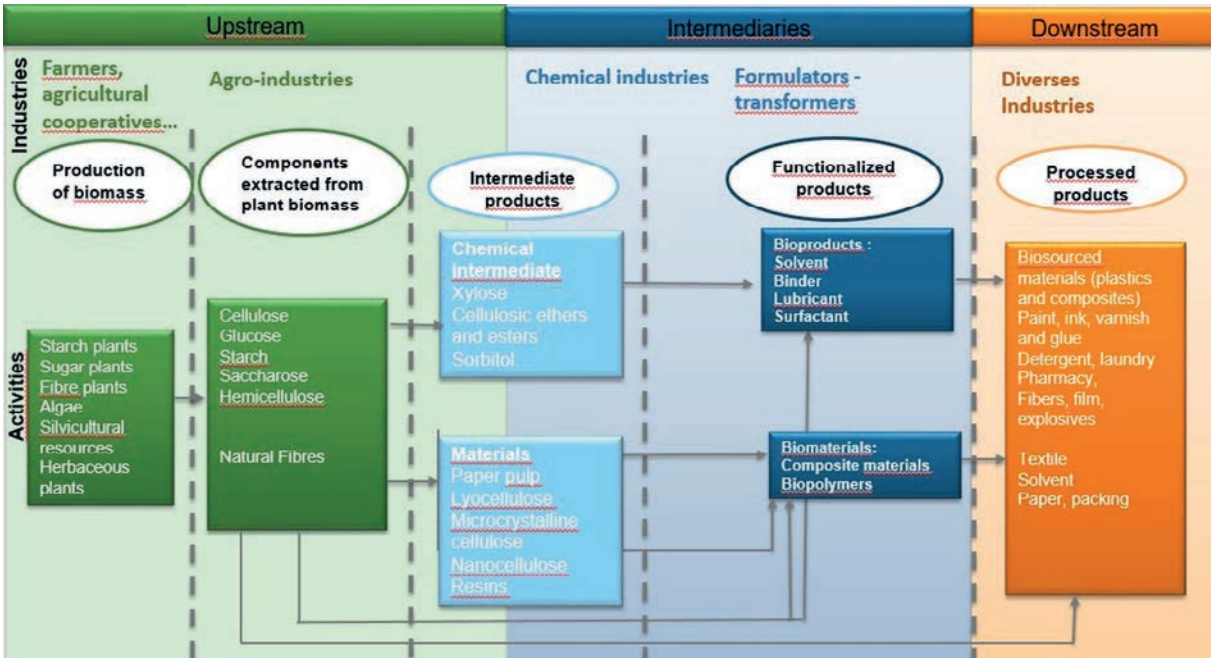


Figure 1: The regional biomass value-chain

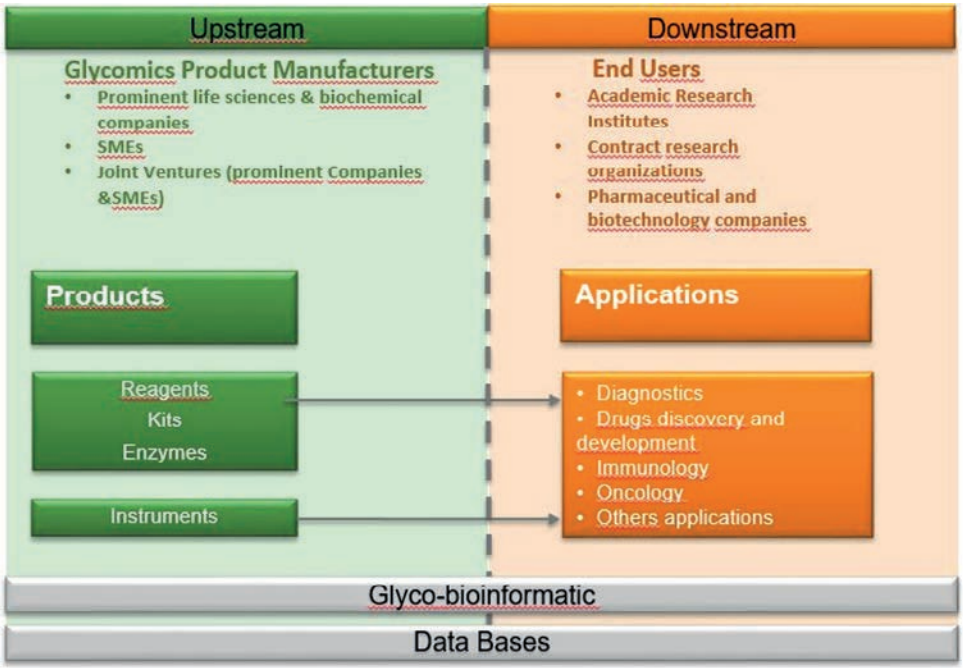


Figure 2: glycomics value-chain

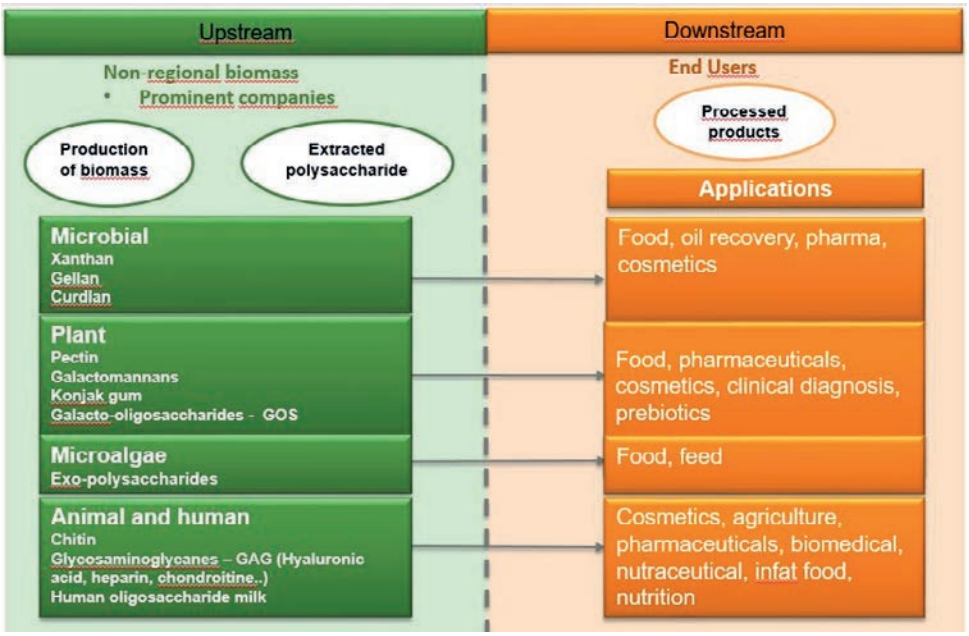


Figure 3: the non-regional biomass value-chain

Structural adaptation of snow algae to extreme variations of their life conditions: focus on the metabolism of starch

PROJECT LEADERS

Jean-Luc Putaux (Cermav)
Denis Falconet (BIG CEA)

WP3

Context and objective of the study

Snow is the home of an ecosystem that participates to the biological diversity of the Alps. Several algal species thrive in this ecosystem, such as Chlamydomonas, Chloromonas, Trebouxia or Stichococcus. These algae meet extreme conditions during their life cycle. These abiotic stresses include, at different timescales (in particular diurnal and seasonal), i) variations of temperature (day/night cycle, sunshine period, etc.), ii) variations of light intensity, the radiating light being rich in ultraviolet at the surface but of very low intensity under the covering of snow, and iii) an environment poor in nutrients. The snow algae use light, CO2 and mineral nutrients to produce their primary organic compounds that are essential to other organisms in the ecosystem. In this respect, these algae play a major role in the alpine ecology. Chlamydomonas nivalis has become the model alga to study this ecosystem and its life cycle is well documented. In particular, the stresses to which the algae are subjected induce accumulations of carbonated chains (starch and/or lipid droplets) [Holzinger et al. Micron 37 (2006) 190].

This project aimed at associating structural modifications of the snow algae with their adaptation mechanisms to the extreme variations of their life conditions, focusing on the metabolism of starch. We identified the internal structures of C. nivalis using various electron microscopy techniques. In particular, we have studied the localization and size of starch granules in cells subjected to different stresses (illumination and composition of the culture medium). In addition, the starch granules have been extracted and purified, facilitating the characterization of their morphology and the determination of their supramolecular organization by X-ray diffraction (XRD).

Methods

The strain of C. nivalis that we have studied was purchased from the Culture Collection of Algae at the University of Texas and is referred to as UTEX-2765 (Fig. 1a). It has been cultivated at LPCV during 15 days, at 20 °C, in continuous illumination with white or blue light, in tris-phosphate (TP) medium with or without acetate (A) in the culture medium and with or without nitrogen (N). The cells have been fixed with glutaraldehyde, post-fixed with Os2O4, contrasted with tannic acid and embedded in Epon resin. Ultrathin sections were prepared using Leica UC6 (at Cermav) and RMC PowerTome (at BIG / CEA) ultramicrotomes. In addition, starch granules have been extracted and purified using the following protocol: lysis of the cells with a cell disruptor, centrifugation of the lysate to take out the white pellet containing the granules, centrifugation of the pellet after suspension in a Percoll density gradient to separate the remaining cell fragments, and extensive washing with water.

The resin blocks in which the cells were embedded have been cut with a diamond knife and the surface was observed with a FEI Quanta FEG 250 scanning electron microscope, collecting backscattered electrons that are sensitive to the atomic number of the staining heavy atoms. Since the resin was non-conductive, the blocks were observed under a low pressure of air in order to cancel the charging effects. The thin sections of resin-embedded cells have been observed with a JEOL JEM-1200EX transmission electron microscope (TEM) operating at 80 kV (at GIN) and a FEI-Philips CM200 operating at 200 kV (at Cermav). Purified starch granules were observed by SEM as well, under high vacuum after metal coating and in secondary electron imaging mode. The purified granules have been centrifuged and the pellet was poured into a 0.7 mm (outer diameter) glass capillary that was flame-sealed and probed with X-rays (XRD) using a Philips PW3830 generator (λ = 0.1542 nm). Two-dimensional diffraction patterns were recorded on Fujifilm image plates.

Results

By comparing electron microscopy images of resin-embedded cells grown in various conditions, some general tendencies can be summarized: in -N media, both with or without acetate, white light favors lipid accumulation whereas blue light favors starch accumulation. There appears to be a slightly higher lipid accumulation in the TAP-N culture compared to the TP-N culture, due to the presence of a carbon source (Figs. 1b,c). Therefore, in order to promote a large cell density, significant starch accumulation and extract a sufficient amount of granules for the various characterization techniques, we have selected the «blue light / TAP-N» culture conditions. A few hundred milligrams of purified starch granules were obtained. The SEM images of

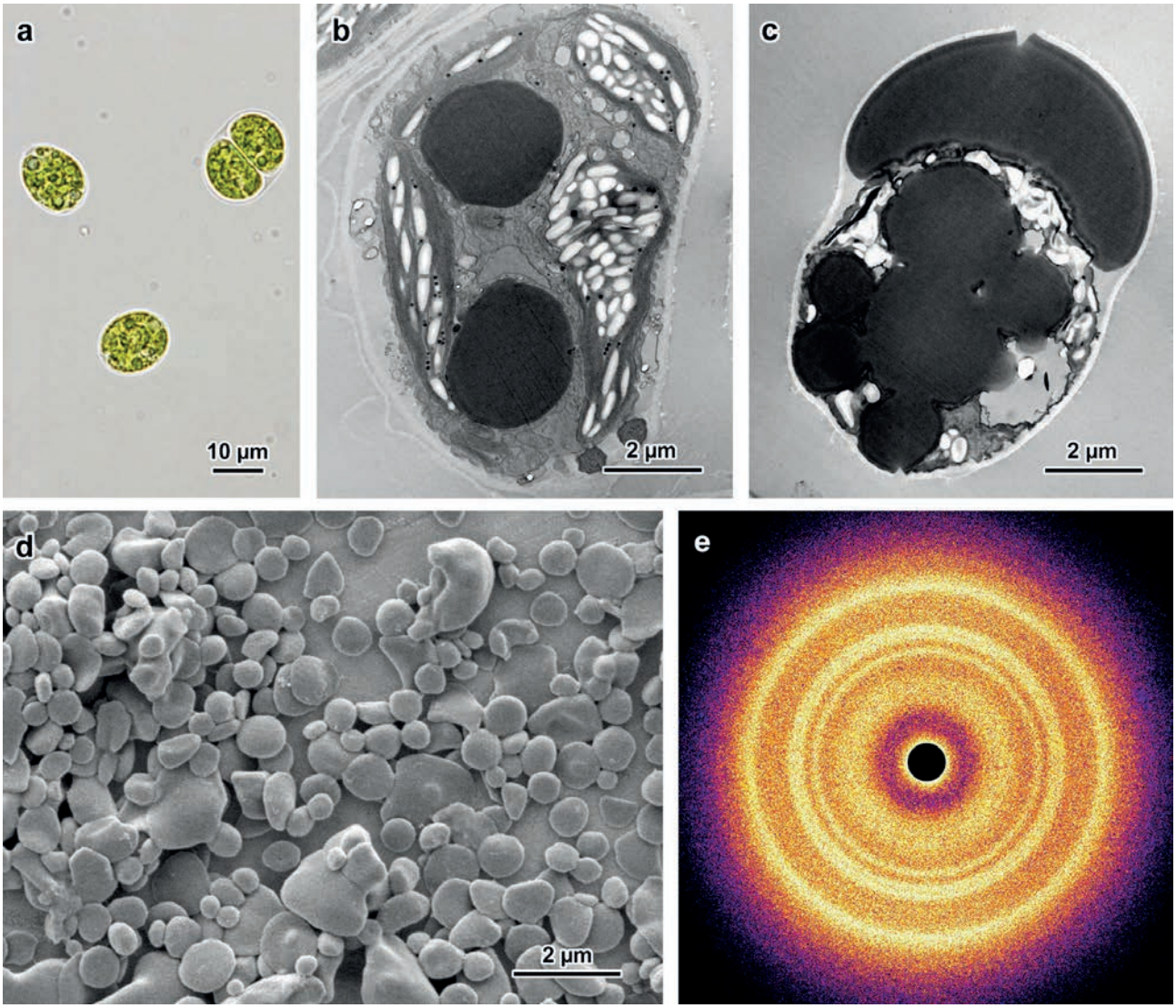


Figure 1. a) Chlamydomonas nivalis cells (optical microscopy); b,c) TEM images of ultrathin sections of C. nivalis cells grown in two sets of conditions: blue light TAP-N (b) and low white light TP-N (c). The white regions correspond to starch granules while the larger dark regions correspond to lipid compartments; d) SEM image of the starch granules extracted from C. nivalis grown in blue light TAP-N conditions; e) X-ray diffraction pattern of hydrated starch granules.

the granules revealed two types of particles (Fig. 1d): discoidal granules with a diameter ranging from 500 nm to 1 µm, as well as larger particles with irregular shapes that may correspond to the fusion of granules growing in more confined regions of the cell. As revealed by their XRD pattern, the hydrated starch granules are indeed semi-crystalline and correspond to the allomorph A (Fig. 1e), also observed in the case of wild-type Chlamydomonas reinhardtii cells and also typical of cereal starch. Finally, to complement the results obtained during our study, the fraction of purified starch has been sent to Unité de Glycobiologie Structurale et Fonctionnelle (Université de Lille) where the amylose/amylopectin composition and the chain length distribution will be determined.

Synthesis of iminosugar probes for SPR selective detection of α-glucosidases

PROJECT LEADERS

Sandrine Py (DCM)
Aurélie Bouchet-Spinelli (SYMMES)

WP4

Glycoside hydrolases (glycosidases, GHs) are responsible for the hydrolytic cleavage of a wide range of oligo- and polysaccharides, glycoproteins, and other glycoconjugate substrates. The Carbohydrate-Active enZymes (CAZy) database references over 600,000 DNA sequence reports for GHs. However less than 3% of the latter have been more or less characterized in their function and only 0.23% are of known structure. The vast biodiversity of GHs thus constitutes an important reservoir of biological targets to explore for future advances in disease control (GHs are important targets for the treatment of viral infections, lysosomal storage disorders, cancer and diabetes, …), but also in biocatalysis for selective transformation of bio-sourced glycopolymers (i.e. for biomass and food transformation industrial sectors). The ultimate goal of our project is to implement new methods to detect and identify such enzymes in biological extracts based on their ability to bind iminosugar-based probes specifically designed for their selectivity against α-glucosidases.

The team in DCM has recently designed a new class of iminosugars exhibiting excellent affinity for α-glucosidases (with Ki values in the nanomolar range) and an unprecedented selectivity for these enzymes compared to other GHs.
A new project was set up with the team at SyMMES to use this class of iminosugars to build up sensors for Surface Plasmon Resonance (SPR) detection and characterization of α-glucosidases in solutions and, in further prospects in complex biological samples (Fig. 1). Surface Plasmon Resonance imaging (SPRi) is a powerful technique to monitor biochemical interactions allowing real-time observation of the interactions with only small amounts of chemical and biological materials. SPRi, which is well mastered at SyMMES, has not yet been applied to glycosidase detection and could allow the isolation and identification of yet unknown α-glucosidases.

The CDP Glyco@Alps ticket funded one Master student (G. Gauthier de Lahaut, Univ. Paris-Sorbonne, Feb-Jul 2018) to implement the synthesis of novel iminosugars bearing the key structural elements for selective recognition by α-glucosidases (represented in red and blue) and a functional handle for anchoring on an SPR-chip through adequate spacers (Fig. 2). His results fostered the obtention of a Labex Arcane PhD grant to pursue this project.

In addition, thanks to the CDP Glyco@Alps support, a new international collaboration was started with the group of Pr Herman S. Overkleeft for asserting the α-glucosidase selectivity of new iminosugars from DCM using a fluorescence polarization (FluoPol) activity-based assay recently developed at the Leiden University. The Glyco@Alps support allowed us to host Prof Overkleeft in Grenoble in July 2018 (he was the special guest of a Glyco@ Event) and also funded a one-month internship of a PhD student from DCM (A. Vieira Da Cruz) at the Leiden University (NL) to learn how to use the Fluopol assay and evaluate the DCM iminosugars as inhibitors of a human α-glucosidase.

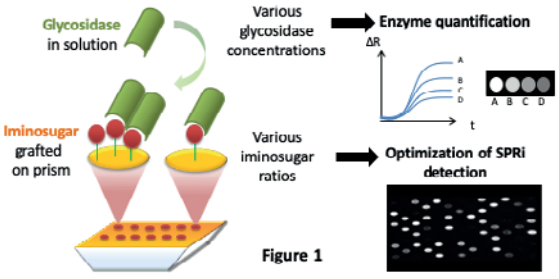
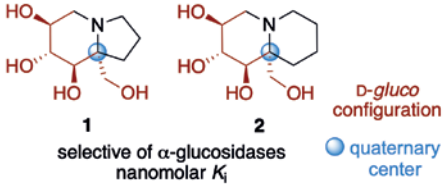
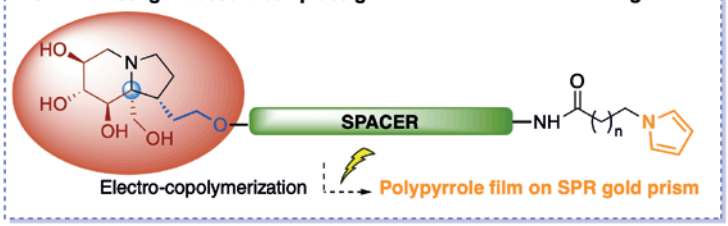


Figure 1

Examples of iminosugars synthesized in DCM



SPR iminosugar-based biochip design **Figure 2**



Development of injectable hyaluronic acid (HA) hydrogels for stroke cell therapy

PROJECT LEADERS

Claire Rome (GIN)
Olivier Detante (GIN)
Rachel Auzély (Cermav)

WP3

Stroke is the second cause of mortality in the world and the leading cause of adult disabilities. Currently, there is no treatment for patient after the critical phase excepted specialised re-education. Cell therapy has been proposed as a potential source of new cells to replace lost cells due to central nervous system injury and promote endogenous neuroprotection and neural repair. The stroke cavity can be an ideal target for transplantation because it is a compartmentalized region of necrosis. In ischemic lesion, the extensive cell death and dramatic inflammatory response make it a more hostile environment for cell transplantations resulting in a severe loss of grafted cells. These problems could be avoided by the use of a material able to protect the cells from the aggressive medium while maintaining the therapeutic effects as HA hydrogels.
Numerous HA hydrogels cross-linked with boronate ester, a dynamic bond, and with β-sheet-forming peptide were developed in order to tune their stiffness to the extracellular matrix of the brain. Their rheological and injection properties have been studied.

The most promising candidates have been selected for further biological studies such as biocompatibility in vitro (Figure 1) and with sub-cutaneous injections on mice.

Furthermore, HA have been modified with a contrast agent, to monitor the hydrogel by Magnetic Resonance Imaging (MRI) (Figure 2).

Figure 1 : Stem Cell viability in hydrogel

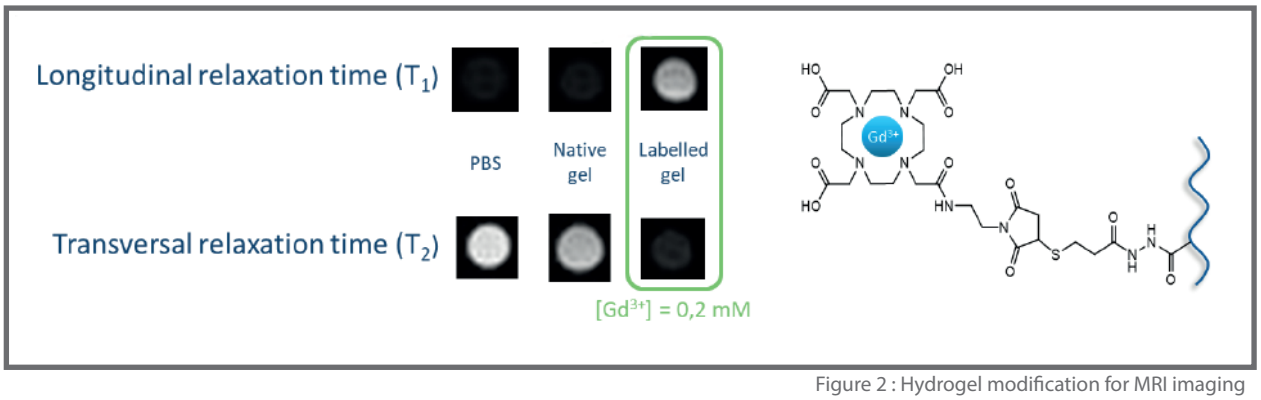


Figure 2 : Hydrogel modification for MRI imaging

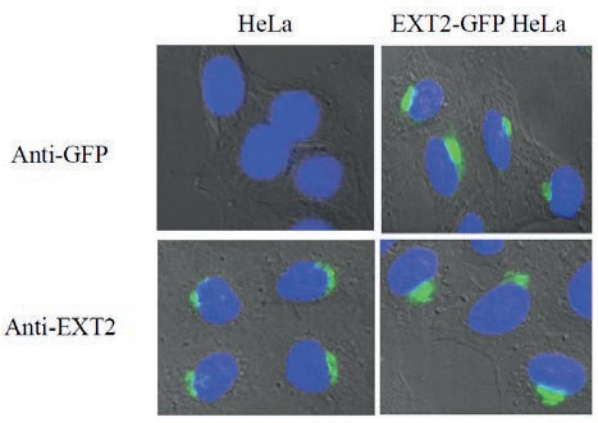
Identifying EXT2 interaction partners involved in Heparan Sulfate biosynthesis using immunoaffinity purification and mass spectrometry

PROJECT LEADERS

Rabia Sadir (IBS)
Yohann Couté (BIG CEA)

WP2

Heparan Sulfate (HS) is an important and highly sulfated linear polysaccharide and belongs to the family of glycosaminoglycans (GAG). HS is ubiquitously present on the cell surface and in the extracellular matrix, predominantly in basement membranes, of both vertebrate and invertebrate cells. HS occur as a proteoglycan (PG) in which two or three HS chains are attached in close proximity to cell surface or extracellular matrix proteins, known as "core proteins". HS binds to a variety of protein ligands (growth factors, cytokines, receptors,...) and regulates many biological activities which gives a great importance and specificity to these molecules. HS has a wide range of biological functions including embryonic development, inflammatory response, cell adhesion and motility, regulation of blood coagulation and tumour metastasis. This extensive functional repertoire reflects the important structural complexity of HS. Functional activity of HS directly depends on its structure, which is determined by a complex system of HS biosynthetic enzymes. HS structure is based on the disaccharide repeating unit consisting of N-acetylglucosamine (GlcNAc) and glucuronic acid (GlcA) carrying sulfate groups in different positions. The HS patterns are created during biosynthesis which is a complex non-template driven process involving several enzymes. This biosynthesis takes place in the Golgi compartment, where the polysaccharide is polymerized on a linkage tetrasaccharide attached to a serine residue of a core protein. The chain polymerization is then catalyzed by exostosin-1 and -2 (EXT1 and EXT2) glycosyl-transferases. As the chain polymerizes, HS undergoes a series of modification reactions including N-deacetylation/N-sulfation (NDST enzymes family), epimerization (C5-epimerase) and subsequently O-sulfation (2-O, 6-O and rarely 3-O-sulfation) through O-sulfotransferases enzymes. The action of the biosynthetic enzymes gives rise to regions of distinct sulfation patterns in the HS chain. This confers to these polysaccharides a unique molecular organization. Although the biosynthesis of HS is not genetically coded, the changes within these HS chains are strictly regulated and depend specifically on the cell type and its environment. The cells are thus able to produce HS-specific saccharide sequences that will modulate protein binding and influence biological processes. It has recently been suggested that the enzymes involved in the biosynthesis of HS could form enzymatic complexes within the Golgi apparatus constituting a "GAGosome", where the enzymes act in a close and concerted manner.



In this study, we seek to characterize and identify the nature of our target interaction partners by the combination of immunoaffinity purification (co-immunoprecipitation) and mass spectrometry (MS). For this, we develop and optimize the conditions for the preparation of these resident Golgi enzyme complex samples compatible with MS analysis. The sample preparation step remains a very challenging task due to the transmembrane nature of enzyme targets and the unique structure and dynamics of the Golgi apparatus. In the current study, we target the EXT2 enzyme and we used different strategies with parental HeLa cells and the transfected HeLa cells with EXT2 fused to GFP (EXT2-GFP). To facilitate the capture of weak and transient enzyme interactions that occur in the Golgi compartment, we used a chemical in-cell cross-linking. In the present study, we describe a novel method for identifying enzymes complex, involved in HS biosynthesis, using in-cell cross-linking coupled to immunoaffinity chromatography and mass spectrometry-based protein identification. This method allowed us to confirm some enzyme interactions (EXT1-EXT2) and we also identified two other enzymes of interest, EXTL3 and Xylk. Further studies will be needed to improve the sample preparation step (and more in an endogenous system).

Evaluation of the labeling of tumor associated carbohydrates antigen with fungal lectins

PROJECT LEADERS

Annabelle Varrot (Cermav)
Jean-Luc Coll (IAB)

WP4

The work has been done by Aurore Cabanettes, PhD at Cermav (Defense 15/10/2019) and Maxime Henry at IAB. It was focused on the determination of the potential of three fungal lectins and their mutants to recognize specific glycanic epitope associated with cancers (TACAs) such as TF antigen (Galβ1-3GalNAc) and 6-core fucosylation. Using the purified recombinant lectins labelled with Alexa680, FACS experiment were performed on a series of cancer cells such as MINO and Z138 (peripheral blood/mantle), HT-29 (colon), MCF7 (breast) and A549 (lung). Inhibition with ligands or specific inhibitors confirmed that the labelling was depending only on the antigen recognition. In the case of TrfbL2 lectin, the labelling was only dependent on TF recognition and not on GlcNAc recognition from its secondary binding site. This explains why mutation in the GlcNAC binding site such as Y111A shows little impact compared to the wild-type, (Fig. 1)

The lectins were then tested for their potential in immunolabelling on tissue sections from tumor obtained in mice models. The lectins were either peroxidised for direct labelling or biotinylated for indirect labelling when peroxidation inactivated the sugar recognition. For all lectins, we could see labelling of the cells and in our case, the labelling was more intense on tumor sections than on section from healthy liver, Fig. 2. The labelling was inhibited after addition of exogenous antigen confirming again the dependency of the labelling on the recognition of the desired glycanic pattern, Fig. 2. Mutations have improved the recognition of 6-core fucosylation. All the lectins tested show very good labelling both on cells or tissue sections and could become tools in cancer research. Complementary experiments will be necessary to complete this study.

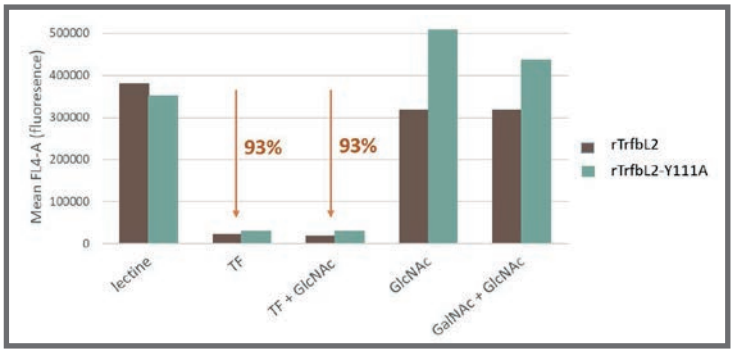


Figure 1: Study of the inhibition of TrfbL2 binding on MCF7 cancer cells by FACS.

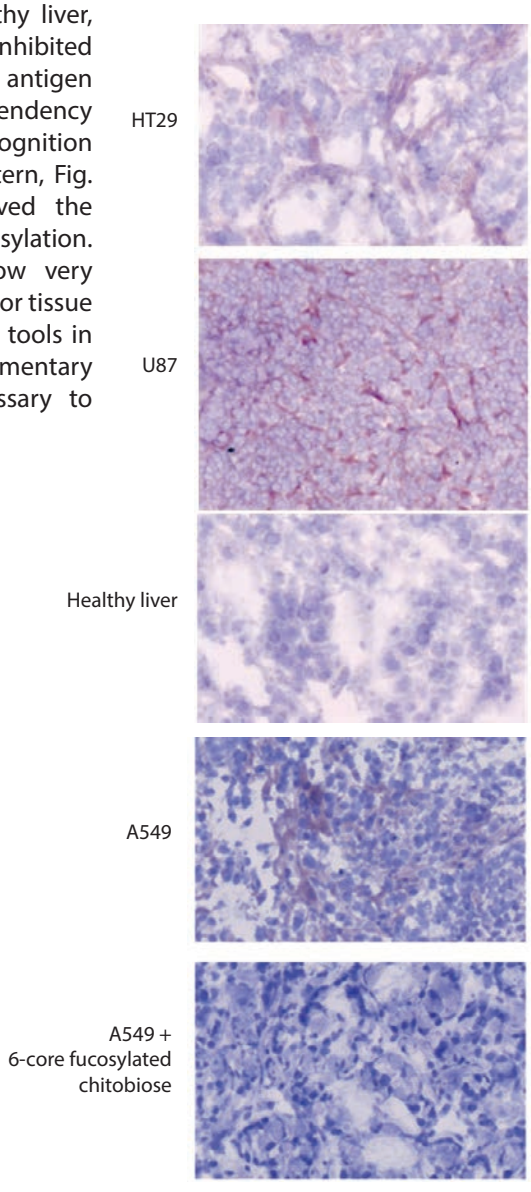


Figure 2 : Labelling of tumor tissue sections with rPhoSLm peroxidated à 20 µg.mL-1. Inhibition with 25 mM 6-core fucosylated hitobiose.

Gelation of hybrid iota-/alpha-Carrageenan as an introduction to the study of new carrageenan polysaccharide

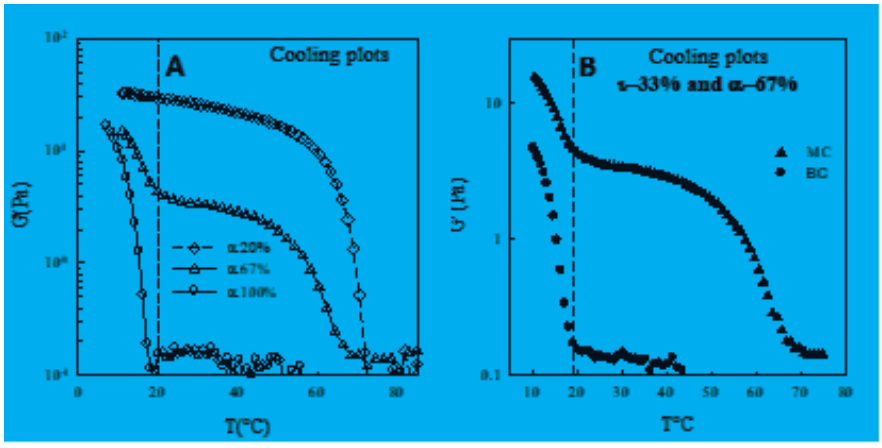
Carrageenan is a name of the familly of many polysaccharide species, which are extracted from wild marine algae to be used as thickeners, gelling agents, stabilizers of viscosity, etc. Their properties are function of the molecular structure of their subunits and some of physical characteristics namely the amount of charge and the length of the polymers. Therefore, only some of the carrageenan types are appropriated for application in food, cosmetic and pharmaceutical products. For example, in presence of calcium and potassium ions, iota- and kappa-carrageenan form respectively fine and coarse gel whereas lambda-carrageenan does not form gels regardless the concentration of the ions. Kappa-carrageenan is characterised by an alternating disaccharide unit of α-(1-3)-D-galactose-4-sulfate and β-(1-4)-3,6-anhydrose-D-galactose. The difference between iota type and kappa type is the additional sulfate group on the o-2 of the 3,6-anhydro-D-galactose group in iota-carrageenan. This additional sulfate group yield a new behavior of the polysaccharide. In this work, we have started to study the gelation behavior of iota-carrageenan, of which the sulfate in the α-(1-3)-D-galactose-4-sulfate is removed. The removal reaction is performed enzymatically. By doing so, we produced different type of iota/alpha-carrageenan hybrid characterized by the percentage of alpha-disaccharide unit in the iota-polysaccharide. The 100% alpha-carrageenan is then composed of alterning α(1,3)-D-galactose and β(1,4)-3,6-anhydro-D-galactose-2-sulphate. The following figures reported some of our result on the gelation temperature for different percentage of alpha-carrageenan (figure A). We show on figure B that the samples made of hydbrid iota/alpha of 33%/67% (figure B-plot MC) is totally different from a mixture of 33% iota-carrageenan and 67% alpha-carrageenan (figure B-plot BC). The total concentration of the solutions is 6g/L in presence of 20mM of CaCl2. A picture of the iota-carrageenan is shown on figure C.

PROJECT LEADERS

Sophie Matthieu (Cermav)
Komla Ako (LRP)
William Helbert (Cermav)

WP2

This work is performed in the frame work of the project, rheological characterization of new polysaccharides, and is granted by Glyco@Alps. The enzymatically modification of the iota-carrageenan is performed at Cermav and the rheological measurement is performed at LRP.



C

Cellulose oxidation assisted by low and high frequency ultrasound

PROJECT LEADERS

Stéphane Baup (LRP)
Sonia Molina-Boisseau (Cermav)

WP3

Ultrasound processes are known to be a promisingway to achieve intensification both for (heat and mass) transfers and chemical reactions whereas cellulose is an abundant and biodegradable natural polymerwith uniqueproperties, likely to be used in various domains. So, at low frequency, ultrasound physical effects may increase cellulose accessibility, its fragmentation and its fibrillation. At high frequency, hydroxyl radicals generated during cavitation bubble implosion improves the chemical reactivity.Within this framework, this collaborative study associated the ultrasonic skills of LRP with the proven expertise of Cermav in the field of cellulose and its characterization. The main objective of the study is to have a better understanding of low and high frequency ultrasound effects on reactions carried on to make chemical modifications of cellulose.

SoTEMPO oxidationof paper pulp was achieved in a new device operating at 18 and 400 kHz (Fig. 1), in a batch configuration, from 5 to 12 liters, with a 200 to 400 L.h-1 flowrate range. Ultrasound reactors were chemically (KI formation rate) and physically (calorimetry) characterized before TEMPO oxidation were performed. Paper pulp came from Centre Technique du Papier (CTP) and diluted at 1%. Once the TEMPO-oxidation of paper pulp is achieved, the obtained suspension was treated to isolate and purify the cellulose micro(nano)fibrils. The techniques of centrifugation and dialyse were classically used. Results were given by solid matter balances and oxidation degrees. The first objective of the project was to optimize the steps of post treatment of the cellulose suspension.

In the first part, an ultrafiltration separation method was developed in order to replace a dialyse step. As a result handling time was reduced by three for the same cellulose mass yield. In a second part, we have studied the effect of cellulose mass fraction (from 0.25% to 1%) during the step of centrifugation. The results obtained are not clear and need to be continued.

TEMPO-oxidation was carried out without ultrasound (WUS), with low frequency ultrasound (LUS), high frequency (HUS) or simultaneously low and high frequencies (LHUS). Comparative results show the highest oxidation degree of cellulose fibrils is obtained for LHUS conditions. Supplementary experiments are required to optimise the process TEMPO-oxidation under ultrasound and the post treatment of the cellulose suspension.

Fig. 1 : Low and high frequencies ultrasound device.

Study of multivalency interaction by BLI between lectin and glycan

PROJECT LEADER

Jérôme Dejeu (DCM)

WP4

The study of complex multivalent carbohydrate–protein interactions remains highly complicated and sometimes rendered impossible due to aggregation problems. In this study, we demonstrate that bio-layer interferometry (BLI) is an excellent complementary method to standard techniques such as SPR and ITC. This apparatus is available on the ICMG platform (ICMG FRE 20607) Characterization of the Interaction (PCI). Different lectins (Anne Imberty, Cermav) and multivalent glycoconjugates (Olivier Renaudet, DCM), with various linkers and/or platform, were tested. First, tetra- and hexadecavalent GalNAc glycoconjugates and the Helix pomatia agglutinin (HPA) lectin were selected because the evaluation by ITC failed due to aggregation problems. We were able to measure reliable kinetic and thermodynamic parameters of multivalent binding events with binding affinity going from the micro to the nanomolar range. The results are in good agreement with previous studies by micro-array, thus confirming the reliability of BLI for studying multivalent interactions. Moreover, we could highlight significant differences in kinetic association constants depending on the ligand flexibility.

We next studied the interaction of three other lectins: Lec A, Lec B and BambL immobilized on the streptavidin sensor (BLI sensor) and respectively Galactose and Fucose as selective ligands. The results obtained were concordant with the previous ITC measurement (manuscript in preparation). Finally, we decided to evaluate whether the immobilization of the glycoconjugate on the sensor and the lectin in solution could provide comparable results. We indeed obtained similar result whatever the molecule immobilized on the sensor. The results suggest that BLI could be used to catch lectin which interacts specifically with the glycan immobilized on the sensor. After dissociation of the complex, the solution could be analyzed by mass spectroscopy to determine the lectin captured.

Besides determining both kinetic and thermodynamic parameters, BLI experiments offer the several advantages. The analysis is fast, can be operated at low cost and requires a lower quantity of both lectin and ligand compared to other analytical techniques. While low affinity ligands could not be evaluated, we believe that BLI represents an excellent alternative technique to SPR and ITC to gather binding parameters and understand multivalent interactions in deeper details.

(E. Laigre, D. Goyard, C. Tiertant, J. Dejeu, O. Renaudet, The study of multivalent carbohydrate–protein interactions by bio-layer interferometry, Organic & Biomolecular Chemistry, 2018, 16, 8899-8903, E. Laigre, D. Goyard, J. Dejeu, O. Renaudet, Efficient screening by biolayer interferometry (BLI) of multivalent glycoconjugates towards lectins, 1/2 journée des utilisateurs Rhône-alpins des systèmes SPR et BLI, Grenoble, 14 juin 2018)

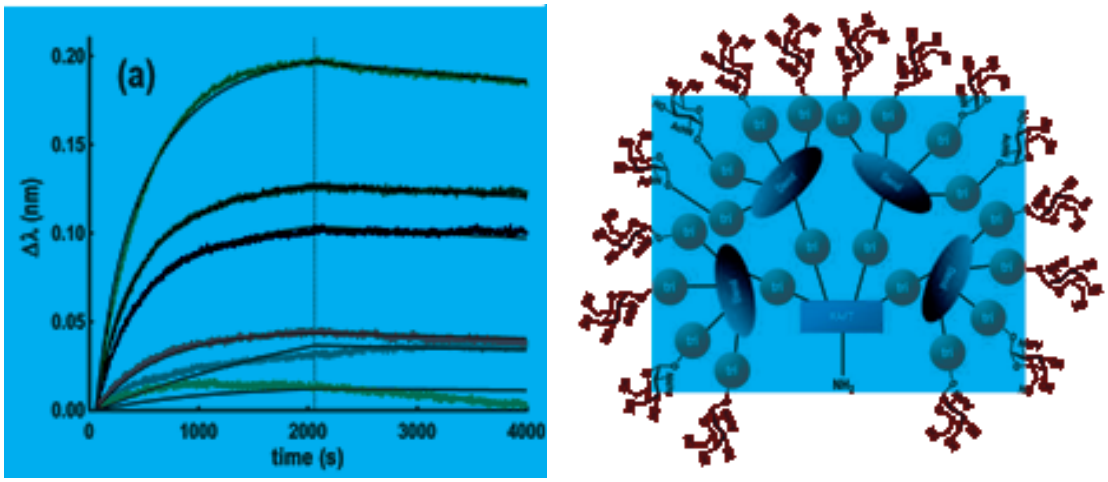


Figure 1: a) BLI sensorgramm between HPA and hexadecavalent GalNac.

Versatile biosensor for deciphering glycosyltransferase activity

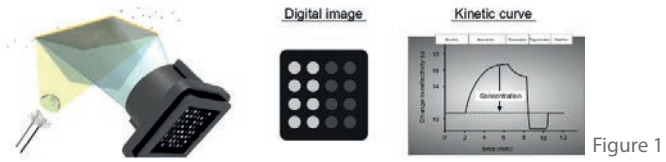


Figure 1

PROJECT LEADERS

Didier Gasparutto (SyMMES/CREAB)
Aurélie Bouchet-Spinelli (SyMMES/CREAB)
Olivier Lerouxel (Cermav)

WP4

This multidisciplinary Ph.D project is well-integrated in the Glyco@Alps framework funding from University Grenoble Alpes (UGA) as it aims to the development new analytical tools in Glycobiology. It requires both chemistry and biochemistry pathways focused on the preparation of original SPRi-based glycochip adapted for characterizing all single objects such as glycomolecules, glycoconjugates, lectins, glyco-enzymes, possibly provided by the Glyco@Alps consortium.

The power of this biochip lies on the ability of monitoring biological interactions in real time without any labels. To that purpose, surface plasmon resonance imaging or SPRi has emerged as an optical and analytical technique to explore the affinity (KD) and binding kinetic (ka and kd) events with high selectivity and sensitivity (figure 1)

This study focuses on SPRi tool as its main platform to rationalize the characterization of an important family of enzymes in Nature called glycosyltransferases (GTs) yet to be deciphered. They play a crucial role in living organisms catalysing the stereo- and regiospecific transfer of an activate donor sugar to an acceptor moiety. This process is named glycosylation and relies upon these enzymes to produce in vivo biomass to build up complex oligosaccharides onto the cell surface. In particular, this project is inspired to study a model of glycosyltransferase crucial in the biosynthesis of plant cell walls called fucosyltransferase 1 from Arabidopsis thaliana (AtFUT1). This specific enzyme contributes to the physical cell wall structure adding a fucose sugar moiety regiospecifically on the xyloglucan (XG) side chain in the Golgi complex.

In this regard, the Ph. D project tackles the original strategy for grafting XG building-blocks onto the SPRi via DNA directed immobilization approach (DDI) as depicted in figure 2.

This approach enables both chemical strategies to functionalize large moieties of ligand to graft it on the gold surface and interaction-monitoring platform giving access to the sugar specificities of each GT. Finally, it drives to detect transferase activities either on-chip or, after cleavage, using MALDI-Tof mass spectrometry

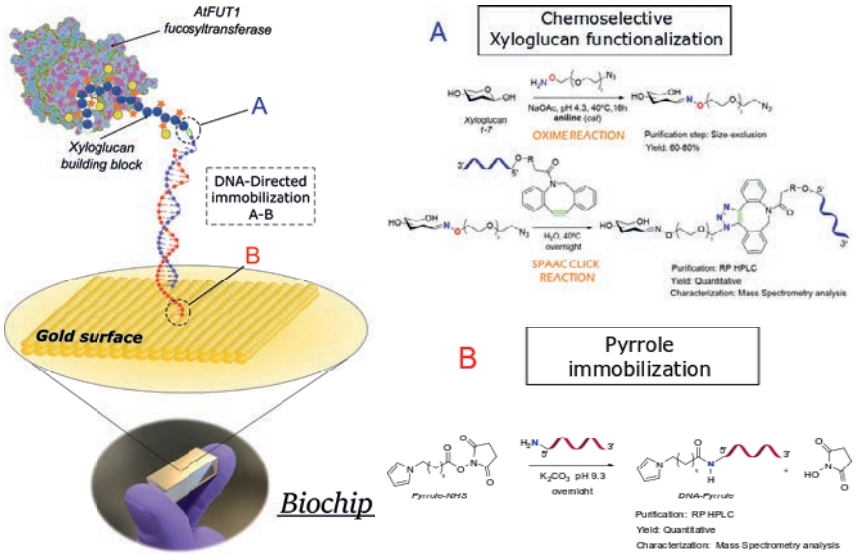


Figure 2. Original strategy of using DNA-Directed immobilization to anchor XG building-blocks onto the gold surface for SPRi monitoring interactions with AtFUT1.

Cellulose nanofibrils with high consistency produced via twin screw extrusion

Context and aims

During the last two decades, the production of nanosized cellulose nanofibrils commonly known as CNF or NFC (nanofibrillar cellulose) has emerged as one of the most promising nanomaterials derived from renewable natural resources and has aroused considerable academic and industrial interest. Although methods for the production of CNFs such as high pressure homogenizer (HPH), microfluidize and Masuko grinding were effective in breaking down cellulose to nanoscale and despite the commercial availability of the microfluidizer, grinder or the HPH at different scales, the widespread use of CNFs is still limited and below expectations. Three main obstacles are facing the large-scale production of CNFs: (i) firstly is the high energy consumption involved in the production of CNFs, (ii) secondly is the low consistency of the CNFs produced via these disintegration modes. Typically, CNFs are produced at a solid content between 0.5 up to 2.5 wt% at maximum, which led to a large dilution effect when CNFs are used as an additive, and (iii) thirdly is the high cost of the high-pressure homogenizer or the microfluidizer and their frequent clogging during the disintegration process. Twin screw extrusion (TSE) might be interesting alternative for the production of CNFs with high solid content ranging from 10 up to 25%. Although, this approach is promising for the high consistency production of CNF with low energy demand. The aim of the project is to investigate the production of CNFs at high solid content (between 10 to 15w.%) using twin screw extrusion for the disintegration of the pulp. Within this project, we would like to go further in exploring how the chemical pretreatment and the extrusion parameters in terms of the screw speed, the temperature and the presence of additive are likely to affect the extent of fibrillation and the rheological properties of the ensuing CNFs suspension. This research has been conducted in collaboration with Prof. S. Boufi of the Laboratory of Materials and Environmental Sciences of the Faculty of Sciences of Sfax (Tunisia). The LABEX TEC XXI has granted financial support for a stay of Professor Boufi in Grenoble in 2018.

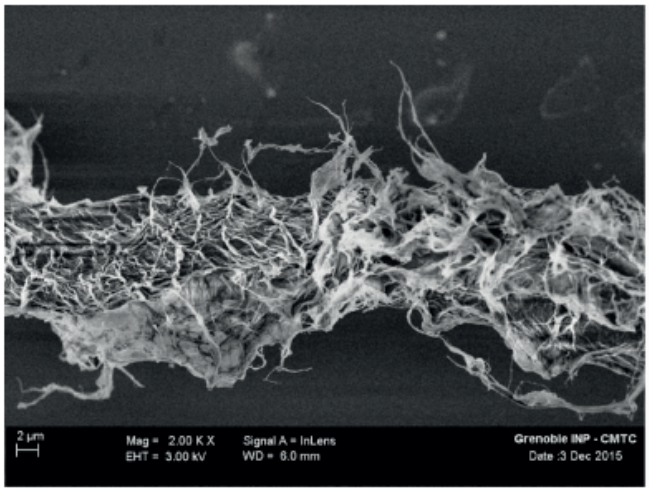


Fig. 1 : FE-SEM observation of the partially fibrillated fibers after TSE

Results

The aid granted by Glyco@Alps has led to the establishment of new results that have been published (R. Baati, A. Ben Mabrouk, A. Magnin, S. Boufi, CNFs from twin screw extrusion and high pressure homogenization : A comparative study, Carbohydrate Polymers, Volume 195, September 2018, Pages 321-328)
In this study, TSE and HPH have been selected in order to produce CNFs at a respective solid content of 10 and 1% starting from never dried Eucalyptus pulps pretreated through a TEMPO-mediated oxidation. In TSE, the pulp was continuously extruded by recirculation during 30 min, while a pulp suspension at 1% was forced to pass through HPH for 6 passes at a pressure between 300 and 600 bar (Fig. 1). After dilution of CNFs gel from TSE at 1%, the morphological, rheological and reinforcing potential of the two CNFs were studied. The transparency degree of CNFs from HPH was much higher than from TSE due to the lower yield in NF in the later. A comparative study has been performed by using a twin-screw mini compounder. However, despite the difference in their fibrillation extent, both types of CNF exhibited solidlike behaviour with shear elastic modulus G' being were one order of magnitude higher than shear viscous modulus G' , and nearly frequency-independent. The reinforcing potential of CNFs has been studied by DMA and tensile-strain test using nanocomposites films prepared by mixing the CNF with a waterborne polymer dispersion and film-formation process at a temperature around 40 °C. In DMA, the inclusion of CNF

PROJECT LEADERS

Albert Magnin (LRP)
Naceur Belgacem (LGP2)
Julien Bras (LGP2)

WP3

has led to a steady enhancement of the elastic modulus E' in the rubbery domain.

The tensile strength as well as the tensile modulus have been strongly enhanced for the ductile nanocomposite films, which is in line with the well-known high reinforcing potential of CNFs. However, the reinforcing effect is about 80 100% higher for CNFs from HPH (Fig. 2). The lower reinforcing effect of CNFs produced from TSE is explained by the difference in the nanosized fraction and by the difference in the morphological features of the two CNFs.

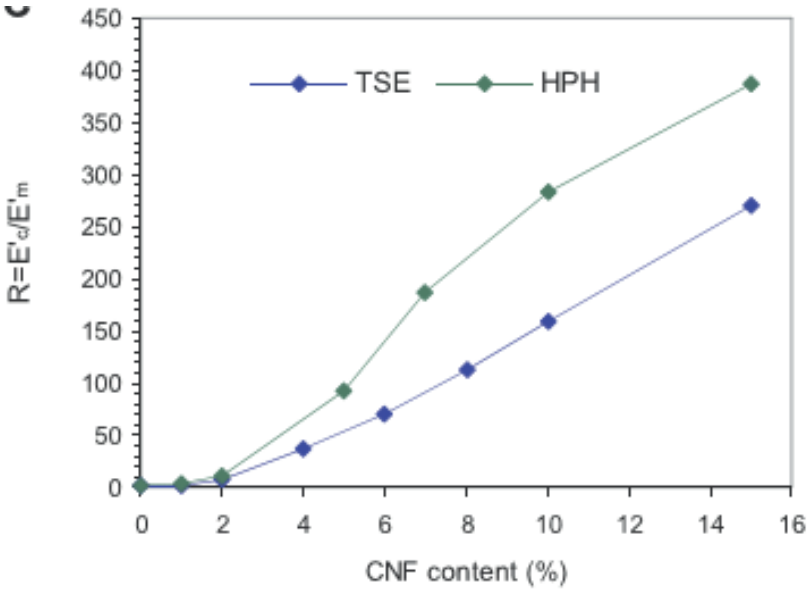
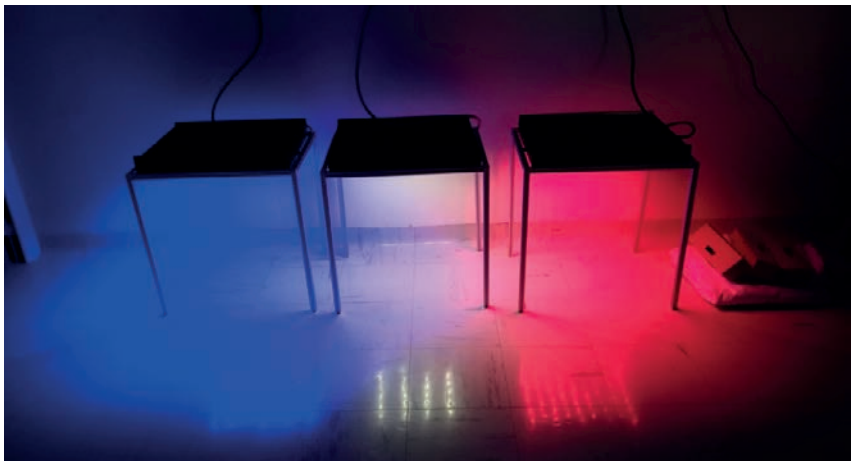


Fig. 2 : Evolution of the R=E'c/E'm versus CNF's content at 60°C (E'c and E'm are the respective moduli of the composite and matrix at 60°C)

Chromatic control of photosynthetic carbon partitioning in the snow alga *Chlamydomonas nivalis*



PROJECT LEADER

Dimitris Petroutsos
(BIG CEA)

WP1

We have recently shown that in C. reinhardtii, model photosynthetic organism and close relative of C. nivalis, acclimation to high irradiance stress is mediated by the blue-light photoreceptor phototropin (1). The same bluelight photoreceptor has been suggested to play a role in guard-cell starch and neutral lipids degradation in Arabidopsis plants (2, 3). In the light of this information and taking into consideration that the natural habitat of C. nivalis is enriched in the blue portion of the visible spectrum, we hypothesized that carbon partitioning into starch and lipid synthesis in C. nivalis might be blue-light regulated.

We have gathered data showing a role of the blue-light photoreceptor phototropin in the acclimation of C. reinhardtii to low-nitrogen stress and to lipid accumulation under these conditions (Petroutsos, unpublished). Our results from the two Chlamydomonas species, C. nivalis and C. reinhardtii, indicate that carbon partitioning into starch and lipid synthesis might be blue-light and phototropin dependent.

Within the proposed project, we aim to explore this further by combining biochemistry, molecular physiology, genetics and microscopy. C. nivalis and C. reinhardtii will be grown in N-replete and N-depleted media under white, blue and red illumination. This will be possible thanks to the very recent purchase of high intensity LED panels of white, blue and red colour, shown in the figure below.

References
1. Petroutsos et al. Nature. 537, 563 (2016)
2. Horrer et al.Curr. Biol. 26, 362 (2016)
3. McLachlan et al. Curr. Biol. 26, 707 (2016).

Editing (Crispr / Cas9) of the genes encoding the monogalactolipid transferases *(MGDs) of the diatom Phaeodactylum tricornutum*

PROJECT LEADERS

Félix Cicéron (CEA)
Eric Maréchal (LPCV)

WP1

Project summary

MonoGalactosylDiacylGlycerols (MGDGs) are major components of chloroplast membranes in most photosynthetic organisms. These galactolipids are essential for processes such as thylakoids expansion and stabilisation of the protein complexes that form the photosynthetic machinery. In the model diatom Phaeodactylum tricornutum, three MGDG synthases (MGDs) isoforms have been identified by sequence homology. The aim of this project is to determine the role of each MGD isoform in the synthesis of MGDGs, and consequently their influence on lipid profile and chloroplast biogenesis.

Objectives and results

In order to characterise the exact function of each MGD-like isoform (numbered 1 to 3) in P.tricornutum, we use the genome editing CRISPR-Cas9 technology to knock-out gene expression. Several mutants have already been generated for each MGD-like gene and we are currently analysing their lipid profile. In order to control the quality of our mutants, the absence of each MGD-like protein will be verified by Western Blot, using specific anti MGD-like antibodies. We therefore selected three peptides per MGD-like isoform, against which the Agro bio company is currently developing antibodies.

The Glyco@ Alps ticket has been entirely used to buy rabbits polyclonal antibodies raised against two of our three proteins of interest (the third one being funded from our lab). These antibodies will be used to assess the absence/presence of each PtMGD-like in our mutants. They will also be used to verify the amount and size of PtMGDs-like when produced recombinantly in vitro. Once these enzymes will be produced in vitro, their galactosyltransferase activity will be tested in close collaboration with the Cermav.

Polysaccharide Utilization Loci for Glycosaminoglycan degradation

PROJECT LEADERS

Marie Couturier (Cermav)
Romain Vivès (IBS)

WP2

Most studies on human gut microbiota (HGM) so far have focused on identification of bacterial populations. In-depth characterization of host-microbiota interactions at the molecular level are needed to understand how these bacteria play a role in human health, and particularly how they participate in nutrient and energy acquisition. In this project, we focused on host glycosaminoglycan (GAGs) degradation by the HGM. GAGs are a class of complex, sulfated polysaccharides and their degradation involves sequences of enzymes that remain to be characterized. In Bacteroidetes, these enzymes are encoded by Polysaccharide Utilization Loci (PULs).

The first step of the project was to rationally select targets of interest. A bioinformatic analysis of PULs potentially involved in GAG degradation was performed using the PUL database (http://www.cazy.org/PULDB). We were able to identify two promising PUL : One was identified in Bacteroides ovatus (Figure 1), a well known member of HGM. This PUL encodes predicted chondroitin lyases as well as several proteins of unknown function which might be involved in chondroitin degradation.

The other PUL was identified in Prevotella oris (Figure 2). Prevotella are an interesting group of HGM bacteria, for which the role is not clear yet. Whereas some studies have hypothesized that Prevotella bacteria were beneficial for human heath, others suggest that Prevotella are abundant in unbalanced microbiota. This second PUL encodes putative polysaccharide active enzymes as well as proteins with unknown function.

All the genes encoding predicted carbohydrate degrading enzymes and protein of unknown function were selected for in depth characterization. The bioinformatically selected gene synthesis was outsourced. Gene sequences were optimized for expression in E. coli and a histidine tag was added for easy purification of the recombinant enzymes. The resulting plasmids were transformed in E. coli and production of the target proteins was performed. In the next steps, our efforts are going to focus on identifying the substrate of each enzyme as well as the sequence of activities required for complete GAG degradation.

Figure 1 : Gene structure of the potential GAG-targeting PUL identified in Bacteroides ovatus

Figure 2: Gene structure of the potential GAG-targeting PUL identified in Prevotella oris

Composite materials based on cellulose and III-N nanowires

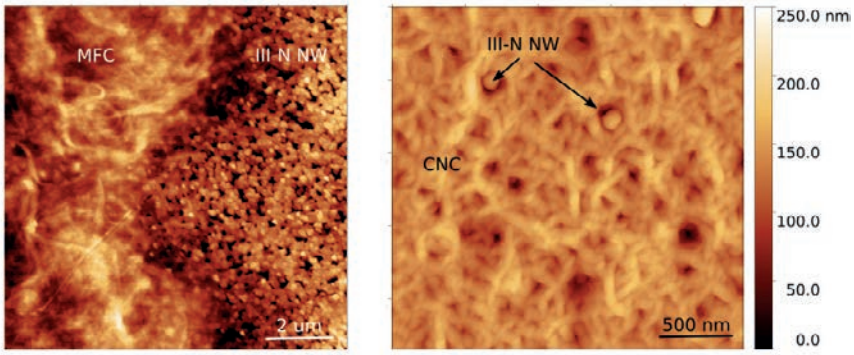


Figure 2: Topographic images obtained by scanning force microscopy to compare MFC (left) and CNC (right) deposition on the surface of III-N nanowires assembly.

PROJECT LEADERS

Franck Dalhem (Cermav)
Rudeesun Songmuang (Institut Néel)

WP3

III-Nitrite materials are wideband gap semiconductors which are studied and used for their optical properties (UV, blue LED) as well as their spontaneous and piezoelectric polarisations (sensors, actuators). III-N nanowires with their large aspect ratio have recently been applied for nanopiezotronics. On its side, cellulose is biopolymer which is an abundant and renewable resource, allowing the production of flexible and transparent films. A hybrid material based on III-N nanowires and nanocellulosic layers serving as support or host matrix would allow the development of actuator, sensor and energy harvesting systems that are biocompatible and recyclable. To manufacture such materials, the first step is to be able to incorporate or attach/link the semiconductor nanowires to the cellulosic material. Here we have carried out several preliminary analyses in order to test and select the best assembly method. Efforts have been made to set the manufacturing conditions: %m, size/shaped ratio, etc. The possibility of keeping the alignment of the nanowires in respect to each other was an important selection criterion.

By using cellulose nanocrystals (CNC) and cellulose microfibrils (MFC) on the one hand and III-nitride nanowires or microwires on the other, it is possible to test different types of mixture by playing with geometric form factors. The results presented here focus on small aspect ratio elements that is more suitable for incorporation, i.e. CNC in/on NW, or NW inside a networking of MFC. Several phase, i.e. solid, gel, colloidal, for incorporation were analysed (drawings in Figure 1). In each case, various cellulose concentrations, level of drying/casting were prepared.

As a result, the loading of III-N NW during MFC casting was not conclusive, revealing mainly pieces of semiconducting layers inside or at the surface of the cellulose layer. This is irrespective of the water content of the layer, i.e. the density of the microfibrilles. The direct mechanical deposition of NW on a cellulosic layer does not achieve sufficient concentration of active semiconductor material. As expected, the preferred strategy, which would have the advantage of leaving the piezoelectric nanowires aligned with each other, is to colloidally deposit the cellulose directly on the surface of the nanowire assembly. Figure 2 shows the distribution of MFC (left) or CNC (right) on top of vertically oriented NW in the case of a small concentration of cellulosic material.

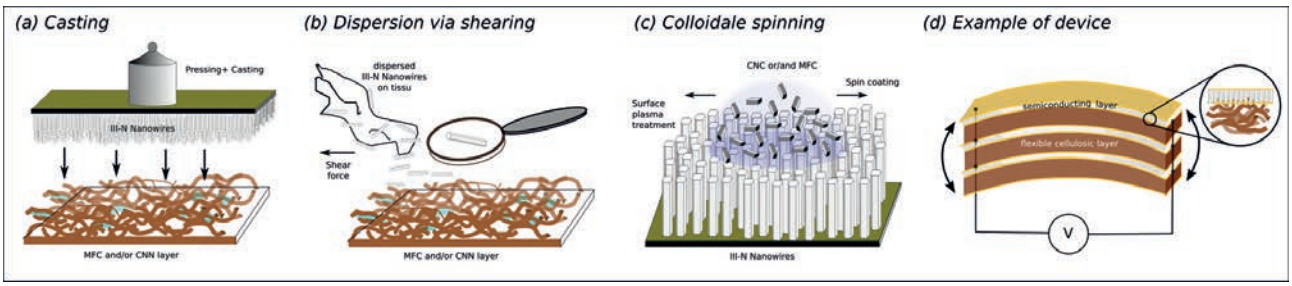


Figure 1 : a-c) illustration of the different techniques tested to incorporate III-N nanowires and cellulose together. d) Example of a device structure.

Framework for modelling the upscaling of emerging processes from an environmental perspective

PROJECT LEADERS

Damien Evrard (GSCOP)
Agnès Boyer (LGP2)

WP5

The ticket enabled to fund Tasnim Balgobin's master's internship and to publish this work in the CIRP Life Cycle Engineering Conference in May 2020. The purpose was to research a framework for modelling the upscaling of emerging processes from an environmental perspective. This subject came from the observation that upscaling emerging processes is a very uncertain topic due to the lack of information about the impacts of their dissemination in the industrial system and the environment. However, anticipating potential environmental impacts of novel technologies appears to be essential to reach sustainability. In this context, a framework was created in order to help give an insight of these impacts from an early stage, using laboratory data.

The main problem to do so is that the impacts of the industrial scale are not directly proportional to those of the lab scale. The answers to this issue encompass researches about upscaling of a process or, in general, of a system. Works on this aspect can be found in the literature and include the consideration of technical, economic and environmental aspects, with high uncertainties. However, no generic methods exist to assist in doing this upscaling. In this work, the main focus was the environmental impacts of an upscaled process with qualitative technical considerations. To do so, a process engineering software was used in order to perform an analysis of the lab process. Then, considering the thermodynamic characteristics obtained with this tool, industrial techniques were added to the process, based on the Best Available Techniques defined in the European regulation (Figure 1). The assumption here is that, if a novel process is to be industrialised, it should comply with the latest regulations.

The main limit to this approach was the impossibility to consider the differences of yield that can be expected to be found between the lab and industrial scales. Therefore, the integration of this aspect would be the next step. The difficulty on this is the dependency of this factor to the intrinsic characteristics of the reaction. Indeed, it seems that the prediction of the yield, either higher or lower, when upscaling may greatly vary from one case to another. Consequently, these would also need to be investigated in future researches to ensure consistency in data sources and accuracy, especially about localisation of processes or their age. An idea to consider a more realistic behaviour of the simulated installation would be to include in the framework, a connection to open databases to formalise the selection of techniques and to associate emission and consumption levels to the simulated industrial process. These data could be processed thanks to data mining and machine learning methods, such as the ones used in previous researches about Best Available Techniques.

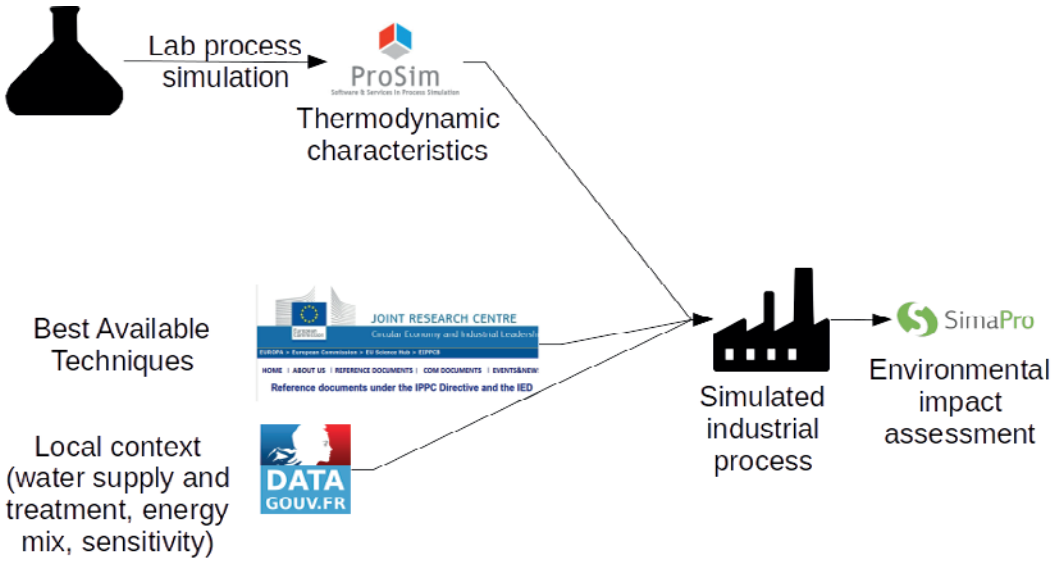


Figure 1: Framework proposed by Tasnim Balgobin.

Anti-cancer lectin cluster

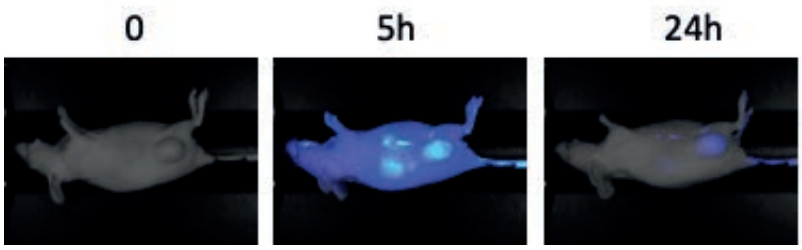


Figure 2 : Monitoring of in vivo fluorescence, by fluorescence reflectance imaging (2D-FRI), after injection of 200 µL to 50 µM of MGL ECD-Alexa 680 IV.

PROJECT LEADERS

Franck Fieschi (IBS)
Jean-Luc Coll (IAB)
Olivier Renaudet (DCM)

WP2

The Tn antigen (Thomsen-new antigen) is present in mucin-type O-glycans (a highly glycosylated protein, covering the cells in contact with the external environment, protecting the epithelia against all kinds of aggression). In healthy tissues the antigen is masked by other sugars, whereas it is accessible in 90% of carcinomas. The fact that this antigen is not present in healthy adult tissues makes it an interesting cancer marker for targeted therapy approaches.
It is within this framework that our work aims to develop a vector based on the recognition of this Tn antigen by a type-C lectin, the "galactose lectin macrophage (MGL)". Since protein/sugar interactions are not very strong, we must multiply the recognition modules in order to reach sufficient affinities. To do this, we want to place on a cyclic peptide support (RAFT), several sugar recognition domains (CRD) of the MGL and thus increase multivalence by forming a lectin cluster.

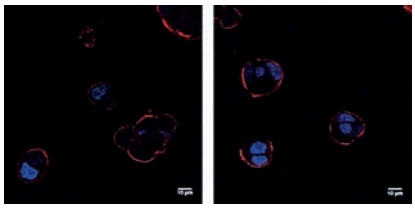


Figure 1 : Observation by confocal microscopy of the HT29 cell line after MGL ECD labelling (red) and DAPI, revealing the nuclei (blue).

We were able to show the targeting of human cancer cell lines expressing the Tn antigen by the extracellular domain (ECD) of the GLM (Figure 1). These experiments were performed using flow cytometry and microscopy.

We have also shown MGL targeting of subcutaneously implanted tumors in mice. MGL was injected intravenously.

The experiments show the persistence of fluorescence in the tumour because the fluorescent signal decreases more rapidly in all organs than in the tumour (Figure 2). Regarding the design of the cluster (Figure 3), we first optimized the production of the recognition domain in order to multiply the yield by 20, which was one of the necessities initially identified at the start of the project.

To build the cluster, we chose to explore 3 conjugation strategies. The first two involve an enzyme (sortase A), a pseudopeptide and Click chemistry while the last one based on oxime ligation requires the oxidation of the N-ter serine of the CRD into oxoaldehyde which will be able to react chemoselectively with the cyclopeptide platform carrying oxyamine groups. Their characterization by SPR is in progress. We will then be able to perform the same biological tests as those performed with the MGL ECD.

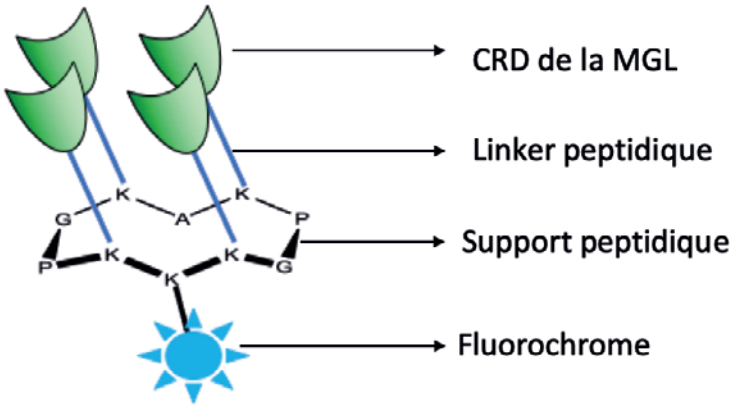


Figure 3 : Schematic representation of the cluster.

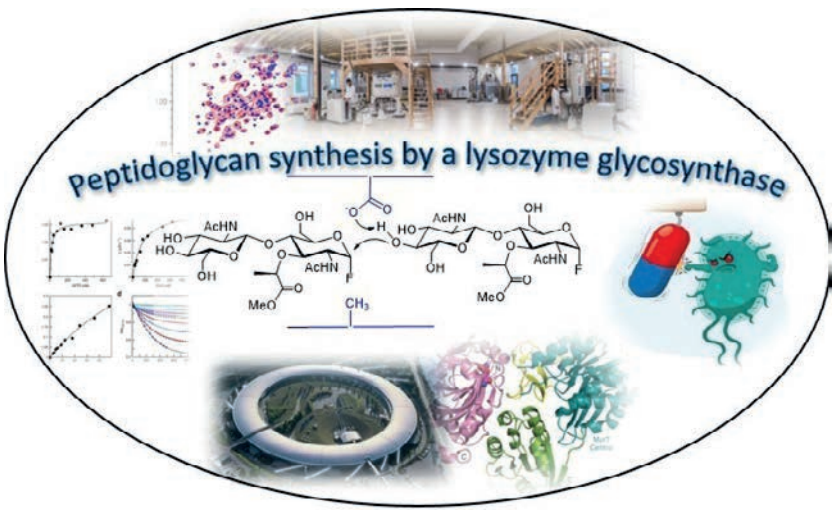
Synthesis of bacterial cell wall mimics (SWIM)

PROJECT LEADERS

Sébastien Fort (Cermav)
Catherine Bougault (IBS)

WP2

Antibiotic resistance is rising to dangerously high levels in all parts of the world, becoming one of the biggest threats to global health. According to the O'Neill report, this scourge could indeed lead to 10 million deaths per year by 2050. Most antibiotics used in human medicine inhibit the synthesis of peptidoglycan (PG) that is the main and essential component of the cell wall of all bacteria. PG is a complex macromolecule consisting of polysaccharide strands alternating ′ -1,4-linked N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc) units that are cross-linked by stem peptides. The peptidoglycan provides the cell with structural strength, protects the osmotically sensitive membrane, and preserves cell morphology throughout the bacterial life cycle. Disruption of its metabolism by antibiotics or its degradation by enzymes leads to rapid cell death or growth arrest. Major antibiotic resistance mechanisms are associated with mutations and structural changes in enzymes involved in PG metabolism. An in-depth structural and functional analysis of these proteins is therefore necessary. However, this work is hampered by the insufficient availability of well-defined PG substrates. SWIM aims at developing a new chemo-enzymatic methodology to access well-defined PG oligomers and to use these molecular probes in mechanistic and structural studies. We indeed designed a synthetic route in which elementary building blocks produced by hijacking the PG recycling pathway are oligomerized in a controlled manner by a lysozyme-derived glycosynthase. Metabolic engineering of E. coli was carried out to produce the GlcNAc-1,6-anhydro-MurNAc disaccharide, a key intermediate of PG metabolism. Further chemical modifications allowed introducing a fluorine atom at its reducing end, a prerequisite for glycosynthase-catalyzed reactions. In parallel, D52S mutant of hen egg-white lysozyme, the best-known PG degrading enzyme was expressed in yeast. We showed that the mutant is devoid of hydrolytic activity and efficiently polymerizes chitinbiosyl fluoride. In association with a chitin N-deacetylase, the glycosynthase also allowed performing monocondensation reactions. Chitin oligosaccharides with a controlled degree of polymerization were produced up to the octasaccharide. We are now exploring the ability of the glycosynthase to transfer fluorinated GlcNAcMurNAc disaccharide. At last, the decoration with stem peptides will provide PG oligomers that will be used for enzymology and structural studies.



Deciphering recognition patterns of glycan motifs of lipopolysaccharides by human lectins using NMR spectroscopy

PROJECT LEADERS

Jean-Pierre Simorre (IBS)
Antonio Molinaro (Univ. of Naples)

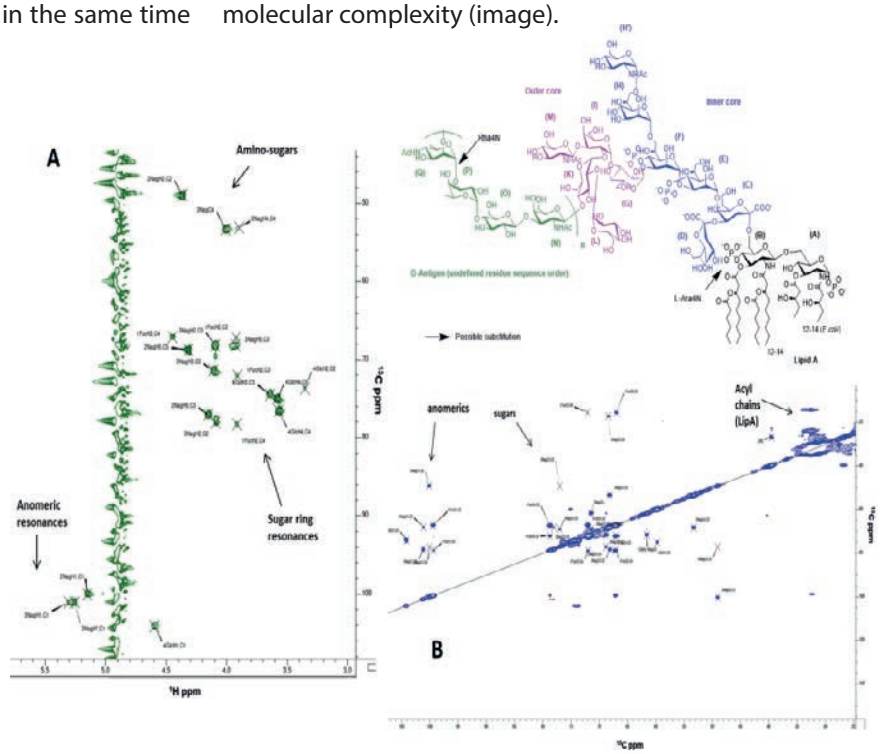
WP4

Within the frame of a collaboration with the university of Naples (Molinaro's group), this PhD project aim at shedding lights on the molecular details of the recognition between glycan motifs of pathogenic lipopolysaccharides (LPS) and human lectins using Nuclear Magnetic Resonance spectroscopy and biophysical approaches.

The molecules that we are dealing with possesses very diverse and heterogeneous structures which makes them difficult to explore experimentally but in the same time very interesting targets. A part of the LPS molecule consist of a chain of sugars which could be recognized by the immune cell receptors (lectins) during the immune response, however the mechanism of interaction have not been revealed yet.

Our first results demonstrated that the human lectin MGL could selectively recognize the outer core oligosaccharide of the Lipooligosaccharide(LOS) from E.coli pathogens, using Saturation Transfer Difference STD-NMR coupled to computational approaches. Furthermore, the interaction between the whole 13C-LPS structure isolated from another strain of E.coli pathogen is being investigated by solid state NMR. This technical approach, although it requires huge expenses, allows us to analyze molecules that are very difficult to study because of their molecular complexity (image).

Thanks to the funding from Glyco@Alps, we were able not only to carry out NMR experiments but also to buy expensive material required for the production of labelled molecules such as polysaccharides and proteins. Moreover, we established a new collaboration with Fieschi's group (IBS) who had contributed to the project by the purification of labeled proteins.



(A) ^{1}H-^{13}C hCH-INEPT and (B) ^{13}C-^{13}C hCC-DARR spectra of 2 mg of ^{13}C LPS *Sakai* alone in Tris buffer.

Highly concentrated suspensions of cellulose nanofibrils

Context

The support from Glyco@Alps enabled Mrs. K.Trigui to carry out her Master 2 internship in Grenoble in 2019. It actively supported the scientific collaborations of the laboratories of the Grenoble site (Laboratoire Rhéologie et Procédés, LGP, Cermav) with the Laboratoire des Matériaux et de l'Environnement (University of Sfax). Now, K.Trigui has started her thesis under joint supervision between the laboratories.

Summary of internship works

Commercial never-dried cellulose pulp was used as starting material to produce CNF gels at high consistency) by twin-screw extrusion ()TSE. Three approaches of chemical pretreatment consisting in a TEMPO- mediated oxidation at basic and neutral conditions and carboxymethylation with sodium chloroacetic acid were adopted to generate carboxyl groups at a controlled amount. The successful disintegration of cellulose fibers into CNF gels was observed only for samples with a carboxyl content exceeding 700 µmol.g−1, irrespective of the method of chemical pretreatment. Based on optical observation, it was proposed that the chemical pretreatment led to a swelling of the fibers turning them more flexible to be recirculated through TSE without any risk of clogging. The swelling of fibers facilitates also their breakdown by reducing the interfibrillar interaction through hydrogen bonding. The increase in carboxyl content led to an increase in the nanosized fraction and enhancement in the tensile modulus and strength of na nopaper from CNF gels. FE-SEM observation of CNF films revealed a random in-plane web-like network structure, exhibiting a layered structure when observed in cross-section. The transmittance and haze at 600 nm are shown in Figure. At a higher carboxyl content, the transmittance further increased up to 80 % at a 1300 µmol.g−1 carboxyl content and with a haze around 7. The drop in haze corresponds to a decrease in scattered light which is due to the lower porosity, enhancement in the nanosized fraction and reduction in surface roughness of CNF films.

The successful disintegration of commercial eucalyptus pulp into cellulose nanofibrils by TSE at a solid content of 10 % contributed to further develop this relatively new processing method to produce CNFs. This work has contributed to identify some of the parameters controlling the breakdown of cellulose fibers into nanoscale objects when a chemical pretreatment based on TEMPO-mediated oxidation or carboxymethylation. Although this chemical pretreatment was necessary to run the process, the high solid content of the produced CNFs without any risk of clogging and the use of conventional commercial TSE constitutes the main advantage of this processing route. The lower energy consumption of TSE is another merit of this method.

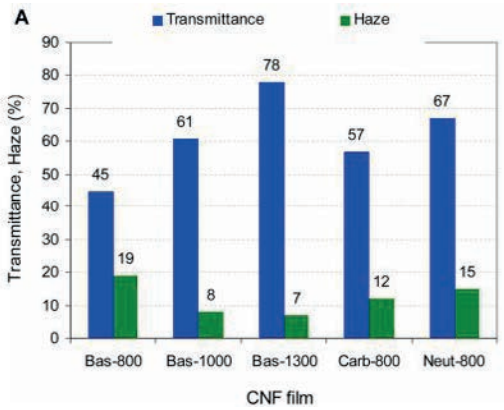


Figure : Transmittance and haze at 600 nm of CNF films extrusion of pretreated fibers at different carboxyl content K. (from Trigui et al., Carbohydrate Polymers, 2020, 242, 116342)

PROJECT LEADERS

Albert Magnin (LRP)
Naceur Belgacem (LGP2)
Julien Bras (LGP2)

WP3

Publications resulting from the work

Trigui K., Production des nanofibres de cellulose par extrusion à partir de la cellulose oxydée, Rapport de Master 2 Chimie, 2019. Université de Sfax

K.Trigui, C. De Loubens, A. Magnin, JL Putaux, S Boufi, Cellulose nanofibrils prepared by twin-screw extrusion: Effect of the pretreatment on the fibrillation effiency, Carbohydrate Polymers, 2020, 242, 116342

Reactive grinding of cellulose: properties and applicability

PROJECT LEADERS

Sonia Molina-Boisseau (Cermav)
Gérard Mortha (LGP2)
Jean-Luc Putaux (Cermav)

WP3

Grinding is the most widely used method for obtaining powders. The solid material is mechanically stressed which increases its free energy. This energy is mainly converted into elastic energy (that creates network defects and generates cracks) and surface energy (that propagates cracks and promotes fracture), resulting in the amorphisation of crystalline solids, polymorphic transitions, decrease in molecular weight of polymers, particle agglomeration or mechano-chemical reactions. Despite a broad range of applications of cellulose powders, few studies have addressed the influence of mechanical stress parameters on the properties of cellulose upon grinding.

We have studied the impact of dry ball milling on the structure and morphology of different cellulose samples as a function of milling time. Commercial microcrystalline cellulose derived from cotton linters (Whatman CF11) and wood pulp (Avicel pH105) were ground in planetary (Retsch) and tumbling (Faure) ball milling machines to yield ultrafine powders that were characterized by laser granulometry, optical microscopy, X-ray diffraction, solid-state-NMR and intrinsic viscosity measurement. After 20 h of milling, the size of Avicel pH105 particles, initially around 20 µm, did not significantly change whereas the fibrous CF11 particles (about 65 µm) were fragmented into significantly shorter elements, down to a size around 20 µm (Fig. 1a,b) that seemed to be the limit size reached under our operating conditions.

At a rotation speed of 100 rpm, the tumbling ball milling was more efficient that the planetary system. However, higher speeds were available in the latter. The influence of rotation speed in the planetary ball mill was evaluated by monitoring the evolution of the median size d50 and crystallinity index Ic of CF11 particles, and the degree of polymerization (DP) of the cellulose chains. The fragmentation rate was faster at higher rotation speeds, and the Ic and DP decreased upon milling. The variation of the ratios of Ic(t) and d50(t) at specific milling times divided by the initial values Ic(0) and d50(0) was studied as a function of rotation speed (Fig. 1c) and suggests that at any speed, the energy supplied by milling was first essentially used for particle fragmentation. As a size limit was reached, the amorphisation of cellulose became predominant.
Further studies will be needed to finalize the characterization of ground samples. We aim to understand and control the properties of cellulose during milling.

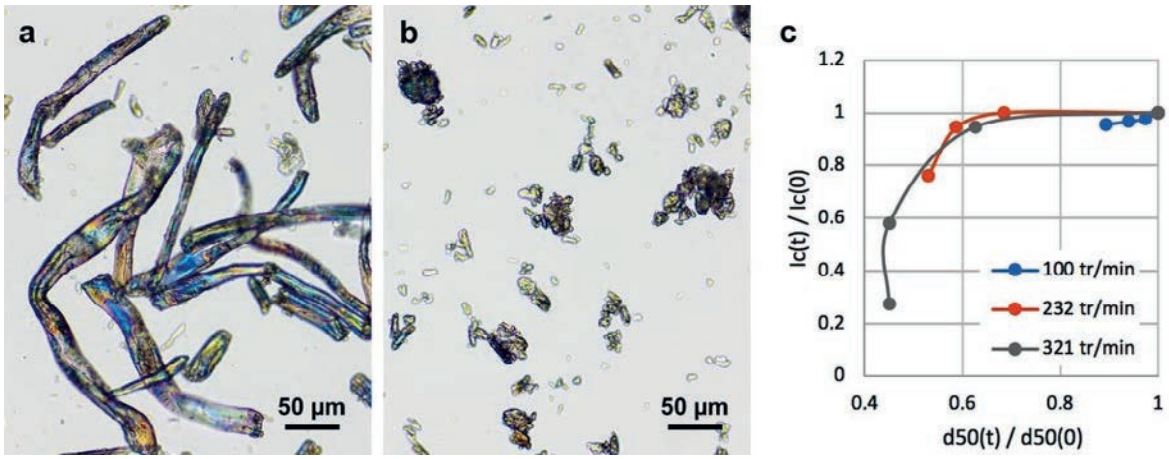


Fig. 1: Polarized light optical micrographs of initial fibrous CF11 cellulose particles (a) and powder (b) obtained after a 20-h planetary ball milling. c) Variation of the normalized crystallinity index with the normalized median size of CF11 for various rotation speeds of the ball mill.

Surface studies by DNP enhanced NMR of active pharmaceutical ingredients grafted on the surface of cellulose nano-fibrils

Cellulose nanofibrils (CNF) are renewable bio-based materials with high specific area, which makes them ideal candidates for multiple emerging applications including for instance on-demand drug release. However, in-depth chemical and structural characterization of the CNF surface chemistry is still an open challenge, especially for low weight percentage of covalent functionalization. This currently prevents the development of efficient, cost-effective and reproducible green synthetic routes and thus the widespread development of targeted and responsive drug-delivery CNF carriers.

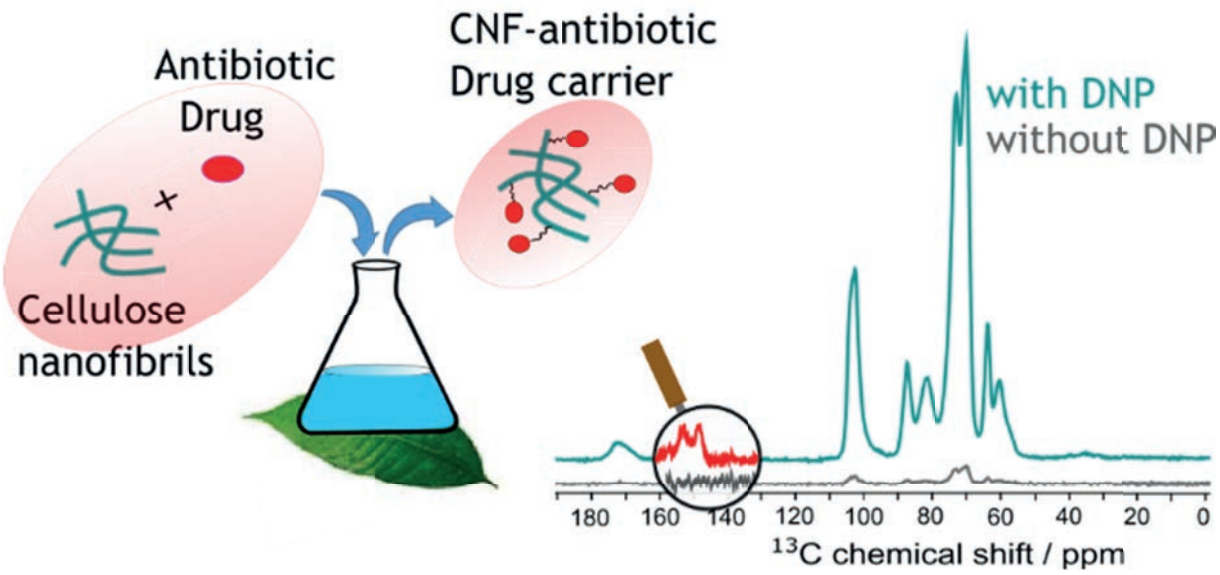
Thanks to the support from Glyco@Alps, we were able to show that dynamic nuclear polarization (DNP) can overcome the sensitivity limitation of conventional solid-state NMR and gain insight into the surface chemistry of drug-functionalized TEMPO-oxidized cellulose nanofibrils. The DNP enhanced-NMR data report unambiguously on the presence of trace amounts of TEMPO moieties and depolymerized cellulosic units in the starting material, as well as side-products issued from the coupling agents covalently linked on the CNFs surface. This enables a precise estimation of the drug loading while differentiating adsorption from covalent bonding (' 1 wt% in our case) as opposed to other analytical techniques such as elemental analysis and conductometric titration that can neither detect the presence of coupling agents, nor differentiate unambiguously between adsorption and grafting. The approach, which does not rely on the use of 13C/15N enriched compounds, will be key to further develop efficient surface chemistry routes and has direct implication for the development of drug delivery applications both in terms of safety and dosage.

PROJECT LEADERS

Gaël De Paëpe (MEM)
Martine Demeunynck (DPM)

WP3 WP4

Kumar et al., Chem. Sci., 2020, 11, 3868



Glyconanoparticules functionalization by enzymes and fluorescent compounds

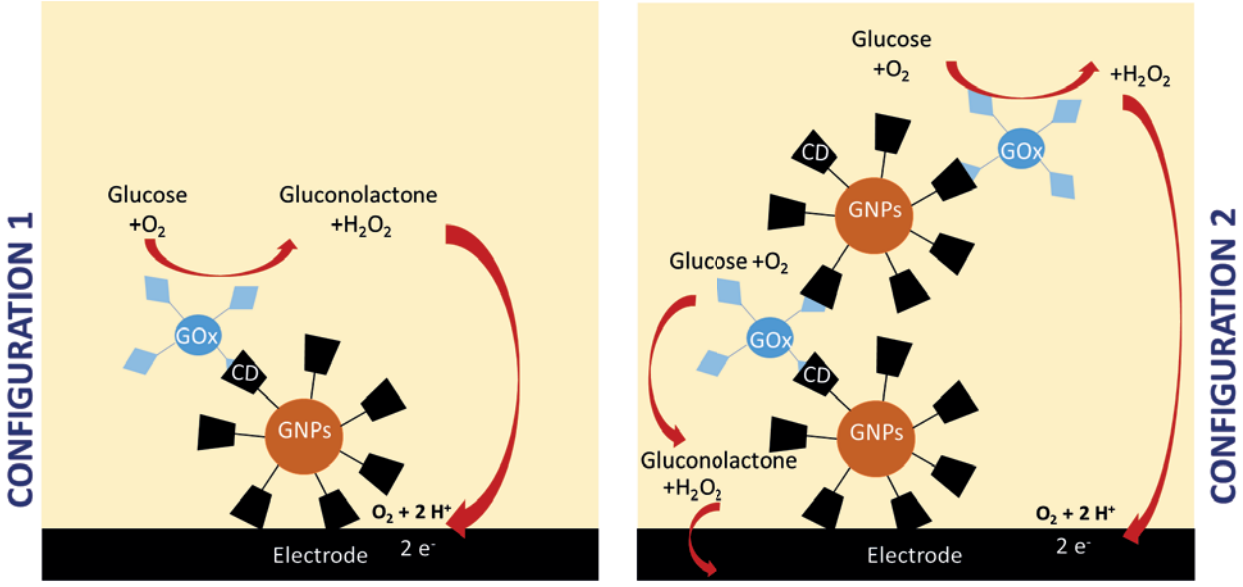
PROJECT LEADERS

Karine Gorgy (DCM)
Redouane Borsali (Cermav)

WP3

The project in collaboration between Cermav and DCM [1] was based on glyconanoparticules (GNPs) functionalization. The glyconanoparticles were obtained by self assembly of diblock copolymers composed of a polystyrene chain (PS) and a cyclodextrin (CD). By using direct precipitation, the obtained glyconanoparticules (GNPs) were characterized by DLS, MEB, TEM and were used firstly to get enzymatic glucose biosensors. The biosensor design is based on the immobilisation of glyconanoparticules by simple adsorption on platinum electrode (scheme 1). After drying, the electrodes were incubated in a second step by glucose oxidase modified by adamantane groups for 2 hours at room temperature allowing to get bioelectrodes via inclusion interactions between cyclodextrin and adamantane. By applying a potential of 0.7 V at the platinum electrode (scheme 1, configuration 1), the injection of glucose leads to an anodic current due to the oxidation of H2O2 produced by enzymatic reaction between immobilized enzyme and glucose. The detection limit, the linearity range, the sensitivity which is equal to the linear part slope of the calibration curve and the current density jmax, which is directly correlated to the immobilized enzyme amount on the electrode, were determined. These characteristics were compared to these obtained with a new biomolecular configuration (scheme 1, configuration 2) alternating GNPs and glucose oxidase modified by adamantane groups layers. The 70 % decrease of jmax value, can be explained by the fact that GNPs layer adsorption is not stable on Pt over time. Besides, we have developed new methods allowing to get homogeneous suspensions of multiwalled carbon nanotubes in association with GNPs that will be very useful in bioelectrochemistry for biofuel cells and biosensors design. In addition, our interest was focused on the assembly of GNPs with fluorescent properties by using ruthenium complexes modified by suitable hydrophobic ligands.

[1] M. Carrière, M. Mumtaz, C. Travelet, K. Gorgy, R. Borsali, S. Cosnier



Scheme 1 : Configuration 1 and 2 of bioelectrodes obtained by inclusion interaction between adamantane groups from enzymes and cyclodextrins from GNPs for glucose enzymatic glucose biosensors

Regioselectively modified cellulose-II nanocrystals as temperature-sensitive rheology modifiers

PROJECT LEADERS

Fangbo Lin (Cermav)
Bruno Jeau (Cermav)
Frédéric Pignon (LRP)

WP3

Cellulose nanocrystals (CNCs) are biosourced colloidal rods that are attracting increasing attention from both the academic and industrial communities due to their numerous properties such as renewability, high specific surface area, excellent mechanical properties, light weight, and non-toxicity. CNCs are thus considered as highly promising building blocks for the production of high performance biobased composites. The successive use of a mercerization treatment and a sulfuric acid hydrolysis allows the preparation of a special class of CNCs, referred to as cellulose II nanocrystals (Cel-II-NCs), which comprise nanorods of average dimensions equal to 100 nm in length, 20 nm in width and 6 nm in height. Made of the cellulose II allomorph, these nanorods possess reactive aldehyde groups on both ends but not along them. In this Glyco@alps project and in the framework of the PhD of Fangbo Lin at Cermav, we took advantage of this chemical specificity to graft thermoresponsive polymer chains on both ends of Cel-II-NCs. The regioselectively modified Cel-II-NCS were characterized by advanced experimental techniques such as transmission electron microscopy (TEM) and viscoelastic measurements as a function of temperature (performed at LRP for the latter). Below the LCST of the thermoresponsive polymer, TEM observations show that the derivatized Cel-II-NCs behave as repulsive nanorods (Fig. 1a). However, above the LCST, hydrophobic desolvated chains act as cross-linking agents between the nanocrystals ends, resulting in the spectacular formation of a Cel-II-NC network (Fig. 1b). The evolution of the storage and loss moduli (G' and G", respectively) evidenced that, below the LCST, the aqueous suspension of derivatized rods behaved as a viscous fluid, whereas above the LCST, a gel-like behavior characterized by a high G'/G" ratio of about 30 was obtained (Fig 1.c). Such a macroscopic behavior is in line with the network formation observed by TEM and other results obtained from dynamic light scattering. Results also show that such a temperature-induced gelation was fully reversible upon cooling of the samples. A deeper structural investigation of the phenomena is currently being carried out thanks to the implementation in the project of a new light scattering set-up. This collaboration between Cermav and LRP thus resulted in innovative smart glycomaterials able to feed the needs for biosourced and stimuli-sensitive rheology modifiers adapted to a wide range of industrial applications (e.g. paints and cosmetics).

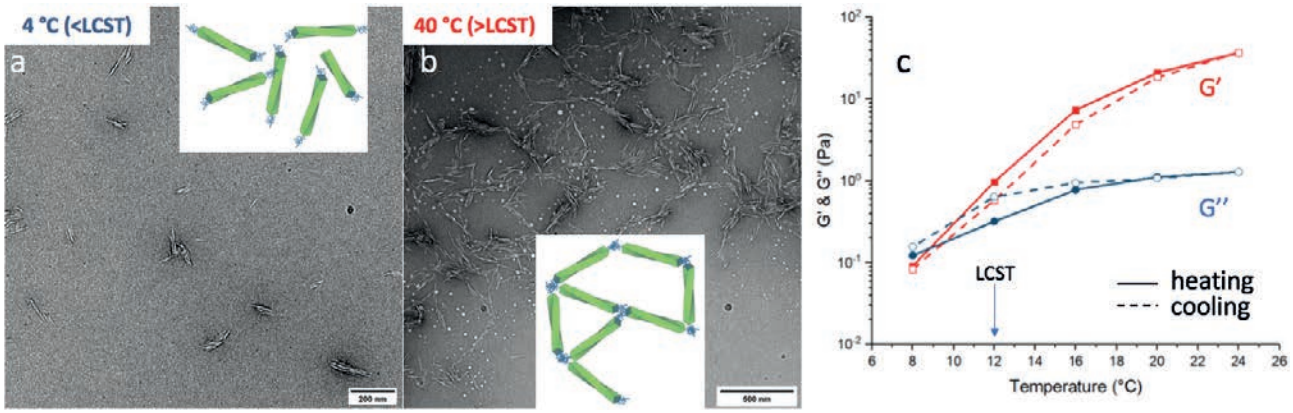


Figure 1: TEM observations of thermosensitive polymer-grafted Cel-II-NCs below (a) and above the LCST (b) and viscoelastic behavior as a function of temperature (c).

Biodegradable plastics based on mixtures of plasticized starch reinforced with cellulose nanofibers and polybutylene adipate terephthalate

PROJECT LEADERS

Albert Magnin (LRP)
Jean-Luc Putaux (Cermav)

WP3

Context

The aid granted by Glyco@Alps provided logistical support for two scientific stays by two Tunisian doctoral students. These stays are part of a collaboration between the Laboratoire Rhéologie et Procédés and Cermav with which the Laboratoire Matériaux et Environnement (University of Sfax) are associated.
Logistical support consisted of morphological and structural analyzes by X-ray diffraction, scanning and transmission electron microscopies, freeze-fracture, AFM, chemicals, consumables.

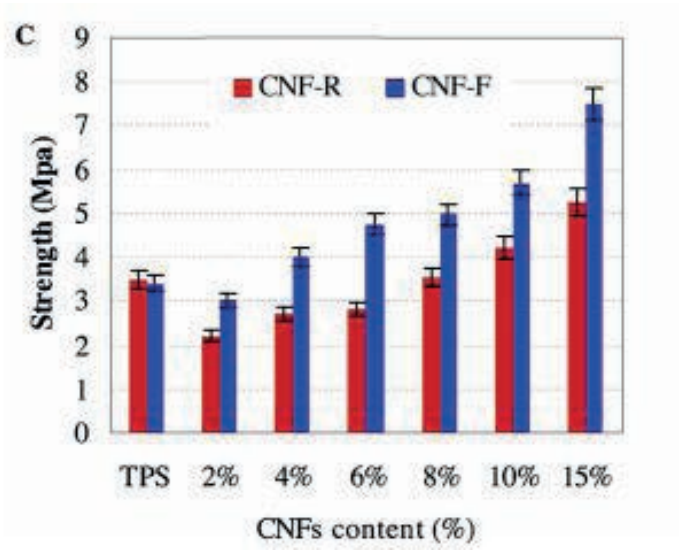
Summary of work

The first scientific stay, led by Yesmine Fourati was to produce TPS/CNF nanocomposites in a single step by twin-screw extrusion, using starch, glycerol and oxidized fibers as starting material. Unlike the conventional route for the processing of TPS/CNF nanocomposites where CNF suspensions were first prepared and then mixed with TPS or starch/plasticizer mixture, CNFs were generated in situ during the gelatinization of starch and the extrusion. This approach offers several advantages: (i) facilitate the processing of the nanocomposites, (ii) avoid the use of ready CNF suspension with low solid content, (iii) reduces the energy consumption during the generation of CNFs, (iii) reduction of the amount of water.
TPS/CNF nanocomposites with a similar composition were produced by processing ready prepared CNFs, starch and glycerol for comparison purpose. The mechanical testing of nanocomposites showed an increase in tensile strength (Figure) and modulus by the presence of CNFs becoming more marked as the CNF content exceeded 10 wt%. Moreover, better mechanical performance was observed when the nanocomposite was CNFs were generated in situ during the extrusion process. The increase in modulus of the material due to the incorporation of CNFs was also confirmed by DMA, especially over the glass transition of the starch-rich phase. In addition, the TPS/CNF nanocomposites kept a good transparency quality of up to 20 wt% CNFs with only an 11 % reduction in the transmittance in comparison of the unfilled TPS matrix, confirming the absence of micron-sized cellulose fibrils or aggregated CNFs. The effective disintegration of cellulose fibers during the extrusion of starch, glycerol and fibers was also confirmed by SEM and TEM. This new approach is the first report describing the single-route processing of TPS/CNF nanocomposites and the in situ disintegration of cellulose fibers into nanofibrils. This work resulted in an international publication in Carbohydrate Polymers.

The second scientific stay, led by Belgacem Chihaoui, was devoted to the production of nanocomposite based on NFC and polylactic acid (PLA) by mixing the NFCs with polyethylene glycol which will serve both as a plasticizer for PLA and as an agent which will prevent the agglomeration of NFC during the extrusion process. This work consisted of exploring the effect of the addition of NFC on the mechanical performance of PLA, on the one hand to increase its mechanical performance and on the other hand to make it more flexible. A publication is being drafted.

Publications resulting from the work
Y. Fourati , A. Magnin , J. Putaux , S. Boufi,, One-step processing of plasticized starch/cellulose nanofibrils nanocomposites via twin-screw extrusion of starch and cellulose fibers, Carbohydrate Polymers, 2020 , 229, 115554

Figure : Tensile strength of the of TPS/CNF-R and TPS/CNFfilms at different CNFs (from Fourati et al. Carbohydrate Polymers, 2020 , 229, 115554



Removal of residual hemicelluloses from cellulosic fibres by coupling mechanical treatment and chemical/enzymatic treatment

PROJECT LEADERS

Jean-Claude Roux (LGP2)
Dominique Lachenal (LGP2)

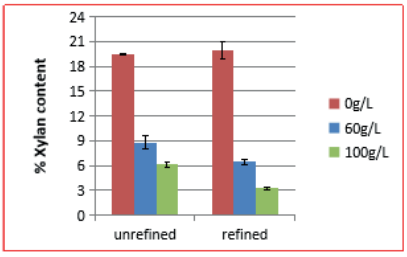
WP1

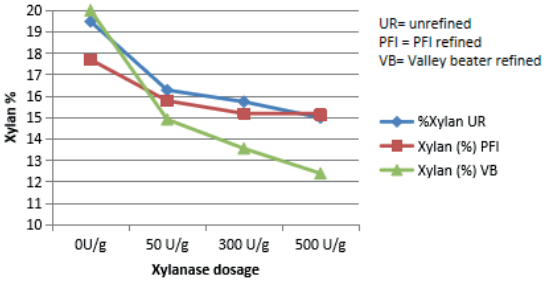
For a sustainable upgrade of paper pulp to dissolving pulp via processes involving alkaline extraction, limiting the conversion of native cellulose to cellulose II is essential. Firstly because cellulose II is formed at a high alkaline dosage which means increased chemical consumption and cost. Secondly, cellulose II is a more compact structure compared to native cellulose. It has been reported to inhibit pulp reactivity to derivatization particularly in the viscose process. Therefore, part of the objectives of our study was to improve the efficiency of the residual hemicellulose removal with minimal cellulose II formation. To achieve this, refining was coupled with alkaline extraction at various NaOH concentrations.

The 5000€ grant received from Glyco@Alps was primarily used for the quantification of Cellulose II in our study. The cellulose II content and crystallinity index of samples was quantified by the high-resolution solid-state 13C CP-MAS NMR spectroscopy. This method uses the unique chemical shifts generated by carbon-13 atom of the AGU to differentiate between the various cellulose allomorphs, polymorphs, and to quantify the crystallinity degree as well as cellulose II content in pulp samples. With the results obtained from the 13C CP-MAS NMR spectroscopy, we were able to determine the optimal NaOH concentration in order to limit cellulose II formation.

Furthermore, part of the grant was used to purchase enzymes (xylanase) which is useful for xylan removal without the risk of cellulose II formation. However, xylanase in itself was not as efficient in xylan removal as the alkaline treatment. Also, chemicals such as (dimethylacetamide (DMAc) for further analysis of the molecular weight distribution by gel permeation chromatography were also purchased along with some laboratory wares (lab coat, glassware).

Finally, we were able to print a poster of our study from the grant which was presented at one of the Glyco@ Alps club meetings.





sample	Cell II	CI	I/S(crystallites)
up_0% NaOH	33%	56%	1.54
rp_0% NaOH	37%	53%	1.54
up_6% NaOH	34%	51%	1.85
rp_6% NaOH	38%	51%	1.70
up_10% NaOH	56%	48%	1.94
rp_10% NaOH	**65%**	**39%**	**1.89**

up= unrefined pulp
rp= refined pulp

Figure 1. Some results obtained from the study

INTERFLAX

The INTERFLAX Project is integrated in the PhD of Estelle Doineau supported by Julien Bras (LGP2 Grenoble), Nicolas Le Moigne (PCH, IMT Mines Alès), Bernard Cathala (INRAe Nantes) and Jean-Charles Bénézet (PCH, IMT Mines Alès). This PhD deals with the concept of self-assembly of nanocellulose and biopolymers on natural fibres for their incorporation in biocomposites. The aim of the INTERFLAX Project was to better understand the interactions in the interphase zone flax fibre / polymeric matrix in the biocomposites by using the Atomic Force Microscopy (AFM).

Adhesion forces measurement by AFM using force curves

The PhD studies the adsorption of xyloglucan and cellulose nanocrystals on flax fibres and needs to go further in the interactions between these different compounds. The measurements of adhesion force have been performed by AFM via different methods. The AFM tips can be functionalized by dipping in suspension of xyloglucan (XG) or with polyethyleneimine PEI and then cellulose nanocrystals (CNC). Measurements of the adhesion forces from an approach-retract force curve obtained in AFM between these tips and different substrates, especially raw and treated flax fibres, were obtained in order to better understand the flax fibre – XG – CNC interactions, Figure 1.

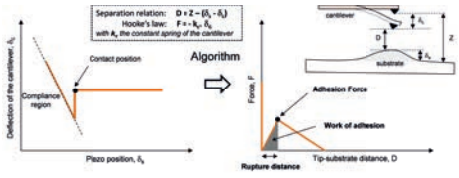


Figure 1: Algorithm employed to convert the raw data graph of the force measurements obtained by AFM to the force-distance curve

Results and discussions

We obtained interesting results with different configurations:

(a) neat-tip or XG-tip / CNC-surface

(b) XG-tip / flax-CNC or XG-tip / raw flax, where flax-CNC corresponds to a flax fibre surface adsorbed with CNC,

(c) CNC-tip / flax-XG or CNC-tip / raw flax, where flax-XG corresponds to a flax fibre surface adsorbed with XG.

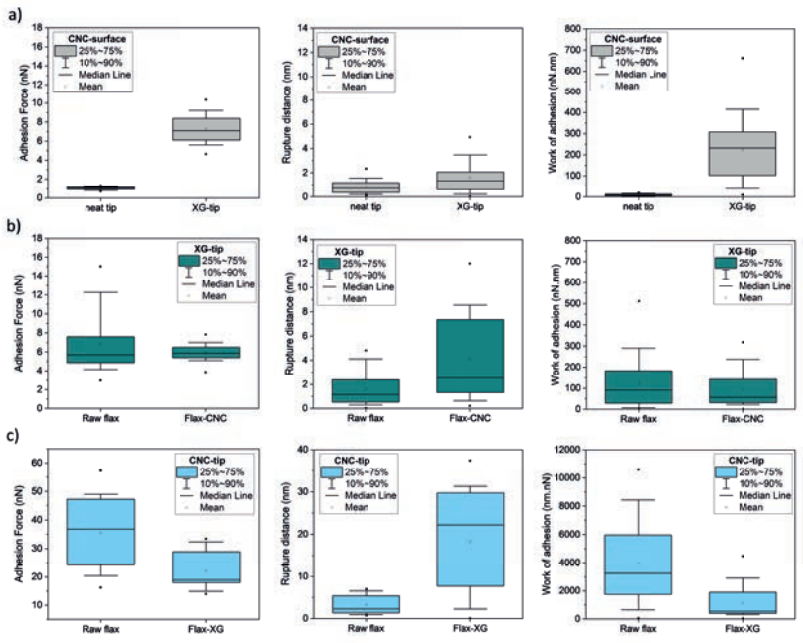


Figure 2: Adhesion force, rupture distance and work of adhesion obtained with force measurements by AFM in different tip / substrate configurations (a) neat-tip or XG-tip / CNC-2surface, (b) XG-tip / raw flax or flax-CNC, (c) CNC-tip / raw flax or flax-XG.

The adhesive force measurements show first a good affinity between CNC and XG. Then, it seems that CNC have a strong affinity with the neat flax woven fabric, without the presence of XG on the surface. However, it seems that the pre-adsorption of XG on the fabric increases the rupture distance with the CNC-tip. The combination of the two biobased building blocks XG and CNC on the surface of flax fibres appears to create an extensible network. This study is detailed in a scientific paper in submission to Carbohydrate polymers (Estelle Doineau et al, Adsorption of xyloglucan and cellulose nanocrystals on natural fibres for the creation of hierarchically structured fibres, 2020).

Some measurements have also been done with colloidal tips (Figure 3).

PROJECT LEADER

Cécile Sillard (LGP2)
Estelle Doineau (PCH, IMT Mines Alès)

WP3

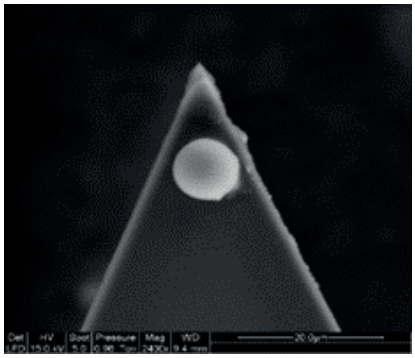


Figure 3: Optical microcopy image of a glass bead bonded to an AFM lever

A PEI + CNC functionalized glass bead has been prepared and measurements of adhesive forces with different substrates were carried out. We found high values of adhesive forces (around 800 nN)

In order to measure the interactions between a polypropylene (PP) matrix and different reinforcements we wanted to functionalize the AFM cantilever with polypropylene microbeads.
Polypropylene microbeads could be obtained by emulsion: A solution of PP in methylene chloride was prepared. The methylene chloride was evaporated at 60°C and the beads obtained have been filtered and washed with ethanol. This solution was added to a tetradecyltrimethylammonium bromide solution in water (Figure 4).

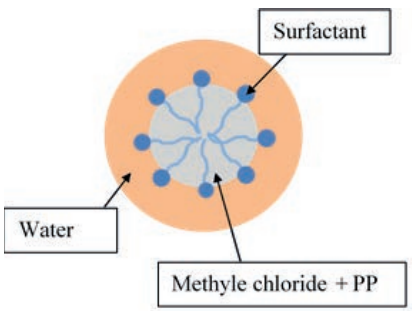


Figure 4: schematic of emulsion of a PP solution in water

The presence of the peaks between 2800 and 2950 cm-1 is a proof of the solubilization of the PP in this solvent (Figure 5).

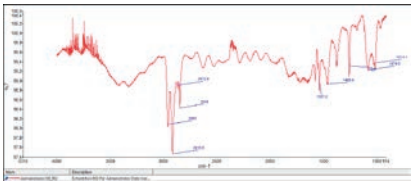


Figure 5: Infrared spectrum of PP solution in methylene chloride

The PP beads obtained were stuck on cantilevers (Figure 6).

Figure 6: Optical microcopy image of PP bead on AFM cantilever

Tests are still in progress to measure the adhesion forces of the bead on different substrates.
However, the bonding is still not effective: The bead came off the cantilever after a measurement (a publication is planned).

Young's modulus measurements by AFM Mode QNM (Quantitative Nano-Mechanics)

The purpose of these measurements was to identify through module measurements the interphase fibre/matrix zones in the biocomposites. We wanted to localize the CNC adsorbed on the flax woven fabric to improve the fiber-matrix compatbilization. In fact, CNC possess a very high Young's modulus between 100 to 150 GPa.
First, we managed to prepare a sufficiently flat surface with the microtomy to be able to perform modulus measurements and to identify the interphase zones.
On the Figure 7, we can observe an elementary flax fibre embedded in an epoxy matrix. The two corps are well defined with a modulus around 2 GPa for the epoxy resin and much higher moduli in the flax fibre (depending on the components). The CNC are not very visible so the results still need to be optimized and some additional tests have to be planned (a publication is planned).

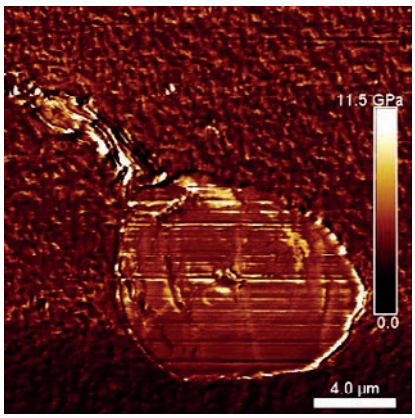


Figure 7: AFM image of Young modulus of a composite flax fiber and polypropylene

Characterization of the structure of cellular cellulosic materials

PROJECT LEADERS

Emeline Talansier (LRP)
Denis Roux (LRP)
Jean-Pierre Chevallet (LIG)
Lorraine Goeuriot (LIG)
Georges Quénot (LIG)

WP3

Foodstuffs and food packaging are just a few examples of the omnipresence of foams in everyday life. However, this type of material is still mostly petro sourced, as is expensed polystyrene (EPS). Alternative solutions coming from the use of polylactic acid or starch have been studied and even sometimes commercialized, but at prohibitive costs. In addition, their properties, in particular the mechanical ones, still remain insufficient and their manufacturing processes are still difficult to adapt to both massive and low added value production.

Thanks to ANR Carnot PolyNat (2014-2018) allocations, a foaming pilot loop was entirely developed and instrumented at the LRP (Laboratoire Rhéologie et Procédés). Alternative to the existing processes, its main advantage is to allow a perfect control of the inlet air fraction. Promising results have been obtained with the production of 100% cellulosic solid material. A PhD program (IMEP2, UGA) is underway at the LRP, supervised by D.C.D Roux (MCf HDR, UGA) and E Talansier (MCf, UGA). One of the objectives of this program is a deeper understanding of the foam dynamics via an in-line characterization of the processed liquid foams at the outlet, in particular in morphological terms, i.e. bubble type and distribution. Once achieved, a rigorous study about the formulation/structure/end used properties relation-ship will follow. For that, the improvement of our current in-line optical imaging technique is required, both in equipment and post processing terms.

In this context, the CDP Glyco@Alps grant allowed the improvement of the pilot lighting system (Figure 1), a key issue in terms of image quality.

This grant also allowed the concretization of an inter-disciplinary collaboration with the MRIM team of the LIG (Laboratoire d'Informatique de Grenoble, Modélisation et Recherche d'Information Multimédia). The co-supervision of two internships between May and August 2019 lead to the definition and the implementation of a software namely pyBubble that allows the post processing of the raw images and videos (Figure 2).

This collaboration initiated with the CDP Glyco@Alps grant is still active today thanks to a LIG dotation (Projet CAMAC, Emergence) obtained in November 2019 with the more ambitious goal regarding the use of Artificial Intelligence and more precisely Neuronal Deep Learning instead of the current algorithms of image processing.

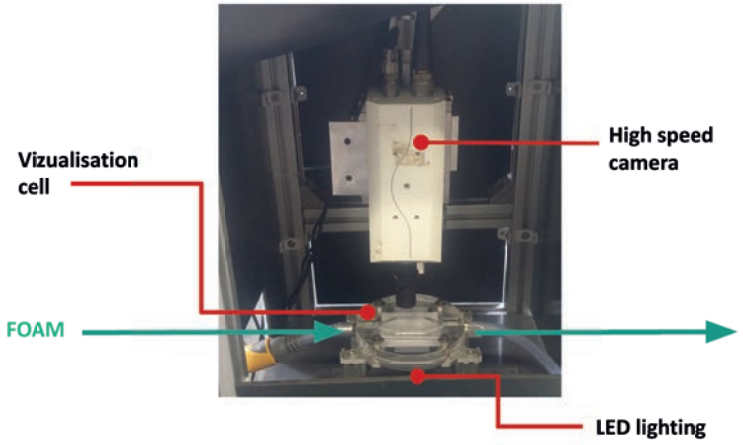


Figure 1: Visualization system for images acquisition with high-speed camera

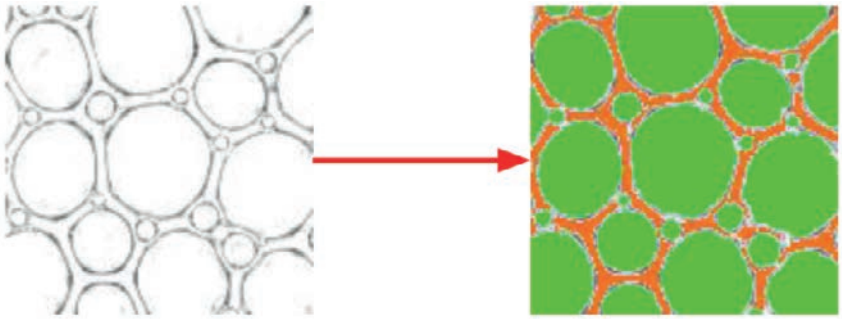


Figure 2: Example of results obtained with the pyBubble software

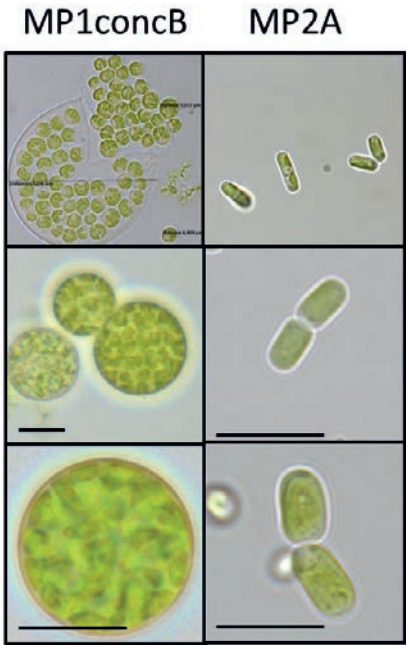
Sequencing of two snow algae genomes

PROJECT LEADERS

Eric Maréchal (LPCV)
Alberto Amato (LPCV)

WP1

The colour of the snow is due to clusters of ice crystals that reflect and scatter wave length in the visible light spectrum. Sometimes red swaths appear. Red snow is a phenomenon triggered by algal blooms occurring in the first centimeters of the snow layers. Such blooms are dominated by green algae that in certain conditions produce massive quantities of the carotenoid astaxanthin giving to the cells a brilliant crimson colour. The red snow algae biodiversity is not resolved at all and one of the aims of the present project is to populate a list of species that can be found in the red snow from the Alps around Grenoble and to compare the biodiversity of Alpine snow algae to that from other locations like Svalbard to assess whether Alpine snow algae are endemic or all the alpine environments contain the same biodiversity. In order to have a first broad list of species, a metabarcoding (mBC) approach was chosen. This choice was driven by the need of by-passing the cultivation step because not all the species can be grown in the laboratory. The mBC has several pros but the main drawback of this technique is the bias introduced by the differential DNA extraction and amplification efficiency showed by different species. For this reason, a cultivation approach was undertaken to isolate monoclonal snow algal cultures. In 2017 and 2018 snow algae were sampled at the Lautaret botanical garden in four different locations: Col des rochilles, col de Cerces, two stations at Mi Pons. Microscopic inspection of the initial snow samples showed mostly red cycsts and a few round-shaped green algae. After a long period of cultivation four different morphologies were recognised: filamentous rods, non-filamentous rods, round, and oval. To date 15 monoclonal strains are available, but species identification is still based on microscopy. Two of them, namely MP1concB and MP2A isolated at Mi Pons, were chosen on the basis of their phenotype in culture, for a hybrid genome sequencing approach. A next generation sequencing by Illumina and a third generation sequencing by PacBio were performed. Both genomes were successfully sequenced with high read quality. 23.5 Mbp and 51 Mbp were the estimated genome sizes for MP1concB and MP2A, respectively. Both strains are possibly haploid with an genome coverage (Illumina data only) of 270× and 125×, respectively. Preliminary bioinformatic analyses on both genomes shed some light on the taxonomic assignation of the strains. By means of a K-mer distribution of the reads on the 107 Chlorophyte genomes present in NCBI, MP1concB may be either identified as or classified as being very close to Auxenochlorella. Conversely, only 6% of MP2A reads found a correspondance with a known genome, namely Stichococcus. Further analyses will identify identify genes involved in interesting pathways, such as the genes putatively coding for Glycosyltransferases (GT) and glycosyl hydrolases (GH) which will be chracterised in the future.



Towards a structure of the EXT1-EXT2 complex involved in GAG biosynthesis

PROJECT LEADER

Rebekka Wild (IBS)

WP2

This project aims for the structural characterization of an important enzyme complex of glycosaminoglycan biosynthesis. Glycosaminoglycans (GAGs) are long linear polysaccharides that are found on the cell surface and in the extracellular matrix. They are covalently attached to serine residues of core proteins, e.g. perlecan, neurocan, syndecan, glypican or fibromodulin, thereby regulating their interaction with growth factors, signaling receptors, cytokines, adhesion molecules, matrix components and many others. Based on their involvement in many biological processes, malfunction of GAG biosynthesis has been linked to a range of diseases such as Alzheimer's disease, acute and chronic inflammation, tumorigenesis and diabetes.

To arrive at a better understanding of the GAG biosynthesis, our aim is is to characterize the enzymes EXT1 and EXT2 involved in polysaccharide chain elongation. EXT1 and EXT2 are Golgi-localized type II membrane proteins and thus challenging targets for in vitro functional and structural analysis. The Glyco@Alps ticket supported the initiation of this project. We have started to screen different protein expression constructs using various mammalian expression systems in order to recombinantly express the two proteins. One strategy aims for the secreted expression of the soluble, catalytic Golgi-luminal domains of EXT1 and EXT2. Our experiments demonstrated, that if the two proteins are expressed on their own, they are not properly processed in the ER and Golgi apparatus and remain trapped in the cell. Co-expression, however, leads to successful secretion of the protein complex to the cell medium as shown by western-blot experiments (Figure 1). These test-expression experiments set the stage for further optimization of protein expression and purification, which will finally enable us to study this important protein complex of GAG biosynthesis on a molecular level.

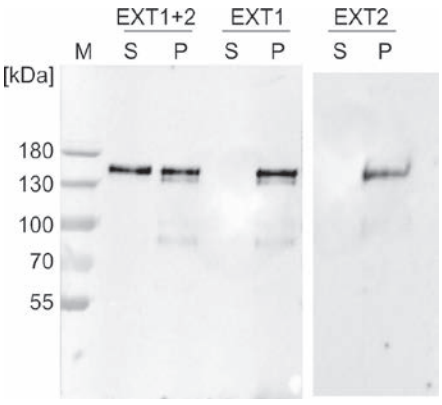


Figure 1: Western-blot analysis of EXT1 and EXT2 expression
Mammalian cells were transiently transfected with constructs encoding either EXT1 or EXT2 or with both plasmids at the same time. The supernatant (S) and the cell pellet (P) were analyzed by SDS-Page and western-blot analysis using an antibody that recognizes the purification-tag present in both constructs. The constructs encoding a secretion signal and the EXT1 and EXT2 protein have a molecular size of 145kDa and 140 kDa, respectively. Protein is only detectable in the supernatant when EXT1 and EXT2 are co-expressed.