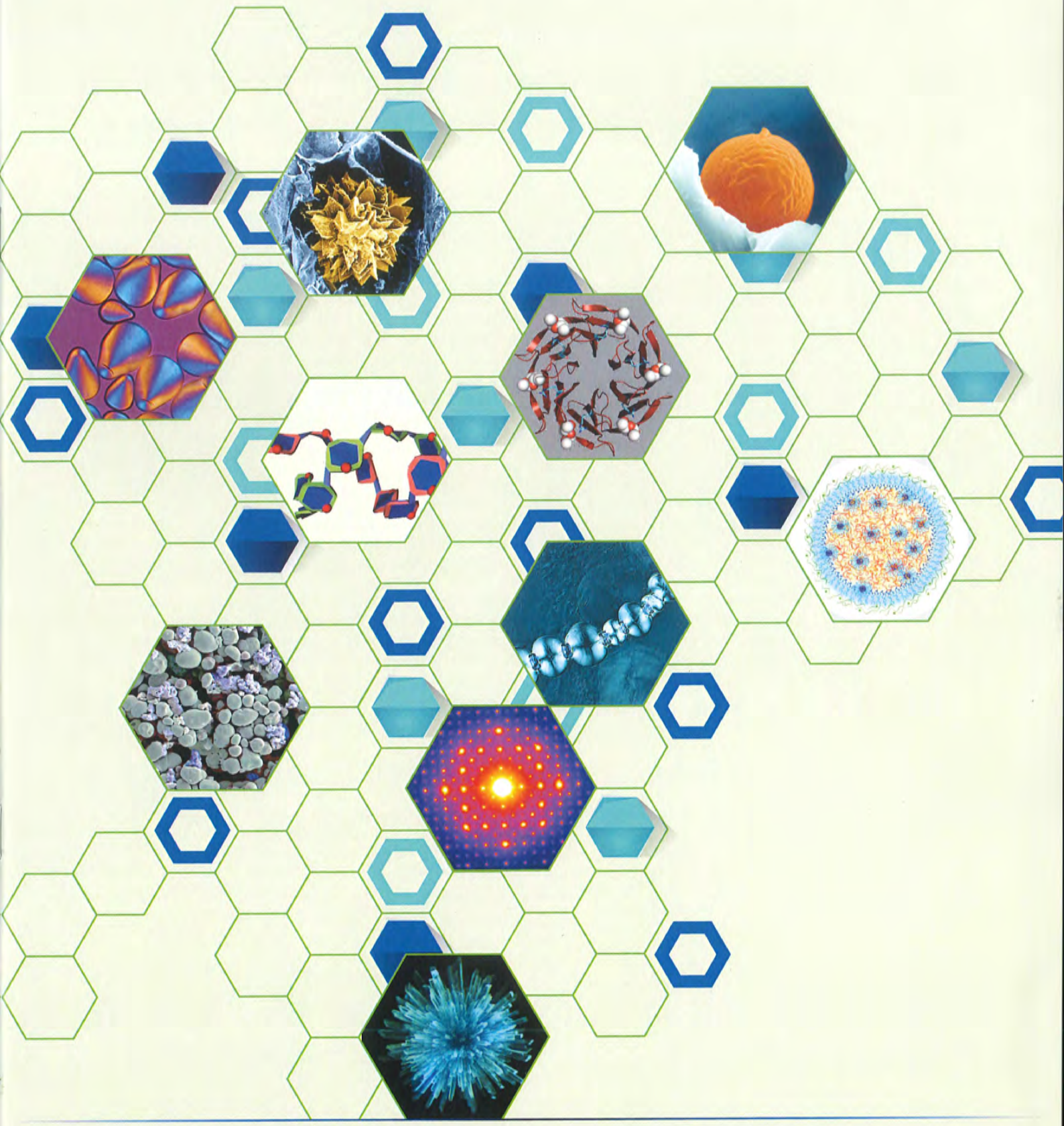
Highlights

Cermav



Centre de Recherches sur les Macromolécules Végétales

Why Glycosciences ?

Glycoscience aims at understanding the structures and functions of glycans, to synthetize them and to develop their use in wide range of domains, from human health to agriculture, from material science to nanotechnology. Glycoscience is a highly interdisciplinary field gathering researchers from all backgrounds.

From the biochemistry point of view, glycans, in the form of oligosaccharides or glycoconjugates encode biological information. Glycans are directly involved in the pathophysiology of every major disease, and their therapeutic potential is presently largely underestimated. They are key players in interactions among and between microbes and host cells, therefore involved in infections processes, but also in the establishment of symbioses between plants and microorganisms of the rhizosphere. At CERMAV, chemical approaches for developing new synthetic pathways and analytical methods for glycans mirror the efforts of biochemists to understand the glycan synthesis by glycosyltransferases, their modification by a large variety of carbohydrate-acting enzymes and their recognition by lectins.

From the structural point of view, polysaccharides are fascinating and ubiquitous molecules, playing a central role in the intrinsic constitution of a huge number of living organisms, from the most simple bacteria or yeasts to the delicate butterflies or giant sequoias. Human activity has used them since the dawn of mankind in their rawest form (fuel, food, housing, furniture, clothes, etc.) evolving to more transformed materials (derivatives, paper, additives, etc.) supporting an active industry. As our civilization turns to be more connected and technological, the need of tailored materials is a strong motor for innovation, and since its creation, CERMAV has naturally accompanied this evolution from the accumulation of fundamental knowledge on the main components of plant cell walls to the elaboration of dedicated smart material. In terms of chemistry and material science, the research relies on more sophisticated approaches with up-to-date characterization tools and the design of smart green building blocks, self-assembled structures, two or three-dimensions and stimuli-responsive biomaterials for reparative surgery or drug delivery, (nano)composites, etc. Researchers face exciting challenges and possibilities of inventing new materials for a more sustainable world.

Nature is an immense source of inspiration and our best supplier of an indefinitely renewable variety of molecules and materials.

Anne Imberty,
Head of CERMAV.

50 Years of Glycosciences at Cermav

The "Centre de Recherches sur les Macromolécules Végétales" (CERMAV) has celebrated its 50th anniversary in 2016 and on the occasion of this jubilee, we would like to share some of our key features and accomplishments with the glycoscience community.

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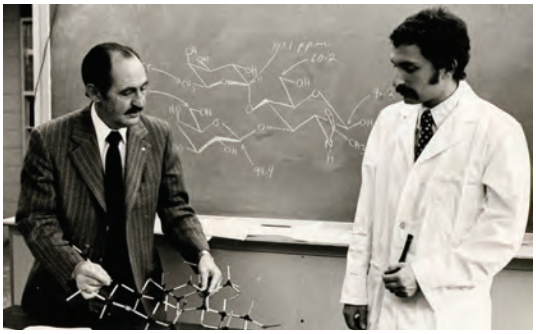

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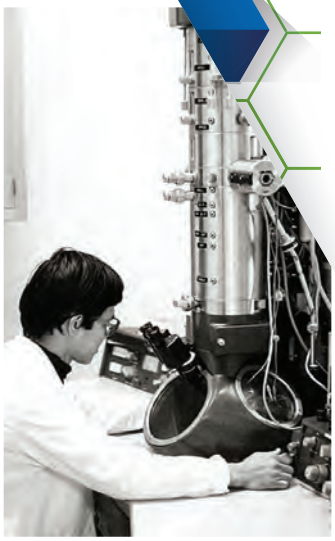

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CERMAV was created in 1966, and the institute was built on the young campus of Grenoble, close to the Chemistry and Biology buildings, neighboring the "Centre Technique du Papier" and later, the "Ecole Française de Papeterie". With Prof. Didier Gagnaire as first chairman, the institute initially aimed at conducting fundamental research on cellulose, lignin, and other plant compounds. With state-of-the-art equipment at the time (such as a 60 MHz NMR spectrometer !), the institute was a pioneer in the research on the chemistry and physico-chemistry of polysaccharides, and on the biosynthesis and degradation of cell walls. Appointed chairman in 1983, Prof. Marguerite Rinaudo introduced biotechnology developments in polysaccharide engineering and materials from biomass. In 1991, an extension to the main building that increased the surface of the institute to 3500 m², was opened. With the progressive incorporation of new researchers, the institute reached its present population of around 100 staff members and students. At an international level, CERMAV was on the frontline regarding emerging approaches in glycosciences with the development of electron crystallography, molecular modeling, rheology, nuclear magnetic resonance, etc. The research topics diversified, covering the structural characterization, chemical synthesis, biosynthesis and degradation, sol-gel characterization and the more recent biomass valorization of oligo- and polysaccharides. In 1996, Dr. Serge Pérez, the new chairman, promoted the development of molecular glycobiology and glycobiotechnology as well as a significant development of the crystallography approaches in collaboration with the European Synchrotron Radiation Facility in Grenoble. With an efficient utilization of networking funds provided by EEC, CERMAV was a leading force in glycosciences in Europe. The appointment of Dr. Redouane Borsali as chairman in 2007 promoted the re-introduction of synthetic polymers into glycosciences, and glyconanotechnology appeared as a key field of research in the institute.

Over the years, CERMAV has asserted its unique profile of "glycoscience" institute, while diversifying and including technical advances and novel fields of application. Since its creation, CERMAV has been closely associated with the University of Grenoble, first as Université Joseph Fourier, and now as Université Grenoble Alpes (UGA). In 2016, UGA has been granted the "Initiative of Excellence" (IDEX) label by the government. The presence of teaching-researchers in CERMAV and its geographical location on the campus of Saint-Martin-d'Hères are two essential factors which explain the strong involvement of the staff in academic training. The high-level education and training of students is stimulated within the framework of the doctoral schools and, in particular, the Doctoral School of Chemistry and Life Sciences to which CERMAV is strongly associated. In a world where the access to up-to-date equipment is crucial, CERMAV is part of "Institut de Chimie Moléculaire de Grenoble" (ICMG) that runs the NanoBio-Chemistry technical platform. With four other laboratories, CERMAV is also part of Institut Carnot PolyNat, a network dedicated to the eco-production of high-value-added functional biosourced materials, and LabEx Arcane, a partnership of seven laboratories in Grenoble promoting bio-driven chemistry.

1- Cermav building in 1966.

2- Prof. Raymond Lemieux with Dr. Hugues Driguez, 1973.

3- Roger Vuong, engineer, in front of the Transmission Electron Microsope, 1976.

4- Cermav building today.

Prof. Didier Gagnaire
1966-1983

Prof. Marguerite Rinaudo
1984-1995

Dr. Serge Pérez
1996-2006

Dr. Redouane Borsali
2007-2015

Dr. William Helbert, Dr. Anne Imberty & Dr. Laurent Heux
since 2016

Since 2016, CERMAV has been chaired by a new team consisting of Dr. Anne Imberty, Dr. Laurent Heux and Dr. William Helbert. The institute consists of five research groups that take advantage of a large panel of complementary characterization platforms either run by CERMAV (chromatography, rheology, mechanical tests, etc.) or incorporated in the larger Chemistry Platform run by ICMG (mass spectrometry, electron microscopy, NMR spectroscopy, computing). In addition, the institute relies on a significant staff support for administration, building maintenance, computing and information technology, etc. The five research teams are:

- Structure and Properties of Glycomaterials, with research focusing on multiscale approaches to study polysaccharides in the solid state (cellulose, starch, chitin, etc.), from the atomistic description to the structural and functional properties of both natural and man-made glycomaterials.
- Structure and Modification of Polysaccharides, with an interest in selective chemical modification of polysaccharides and the build-up of new materials for different applications.
- Physico-chemistry of Glycopolymers, with research projects on the production of new functionalized glyco-nanoparticles, thin films and surfaces for a variety of applications in molecular recognition, controlled release, drug delivery, new lithographic templates and flexible electronics.
- Chemistry and Biotechnology of Oligosaccharides, with an interest in carbohydrate-acting enzymes and synthesis of oligo- and polysaccharides and glycoconjugates by chemical, enzymatic and microbial combined approaches, resulting in applications in the domains of health, food and agriculture.
- Structural and Molecular Glycobiology, that focuses on structural and molecular glycobiology, aiming at unraveling the structure/function relationships of lectins and glycosyltransferases with an interest in the biosynthesis and recognition of complex glycoconjugates or polysaccharides.

CONTACTS
Anne Imberty, Head
William Helbert, Deputy Director
Laurent Heux, Deputy Director

Transverse research axes have recently been defined in order to answer important economy-related societal questions, combine the expertise of the research teams and stimulate fundamental investigations along three topics: i) *the design of functional smart materials* (coupling, functionalization, self-assembling of gels, nano-objects, surfaces), ii) *glycobiotechnology* (biologically-active oligosaccharides, synthesis and biosynthesis, enzymes and lectins), iii) *the structure of polysaccharides and the architecture of plant cell walls* (extraction, purification, characterization, valorization of natural polysaccharides). A fourth axis, *e-CERMAV*, aims at developing numerical tools to efficiently manage and organize the considerable amount of data on glycomolecules generated at the institute and maintain databases freely accessible to the international community through the internet.

With 290 PhD theses, 2 850 scientific publications and extensive collaborations with private companies, CERMAV is a world leading laboratory in Glycosciences. With many contracts with industrial partners and national and international public institutions, the institute fulfills its missions of fostering fundamental research, training by research, disseminating results, but also transferring technology and developing the economy. Without doubt, biomacromolecules, and more particularly glycomolecules, will play a key role in major areas such as cosmetics and health care products, food and environment-sustainable agriculture, energy production, nanotechnology and value-added functional materials.

4

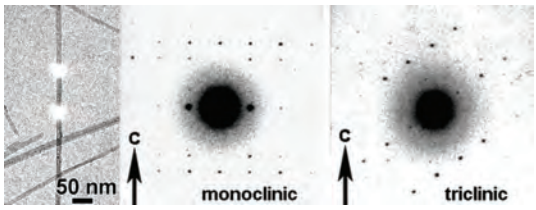
Fifty Years of Cellulose Research at Cermav

From 1966, cellulose research has been one of the main topics which has federated many researchers at Cermav. This summary highlights a few of the landmarks where our Institute wrote part of the advancement of cellulose science during the last fifty years.

CONTACT

Henri Chanzy

Polymorphism and crystallography of native cellulose. When Cermav was created in 1966, this domain was mysterious. To lift the mystery, our approach was based on the harvesting, extraction and purification of highly crystalline cellulose samples from various origins and their analysis by electron, X-ray and neutron diffraction together with solid-state NMR and FTIR spectroscopies.



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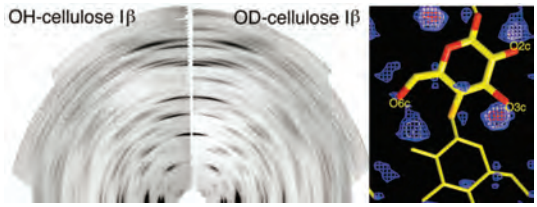
In this domain, our significant contributions have been (i) the identification of the two crystalline phases (Iα and Iβ) of native cellulose along one microfibril leading to the definition of the two unit cells [1], (ii) the identification of pure cellulose Iβ in tunicin [2], (iii) the refinement of the crystal structure and hydrogen bonding system in cellulose Iα and Iβ [3, 4].



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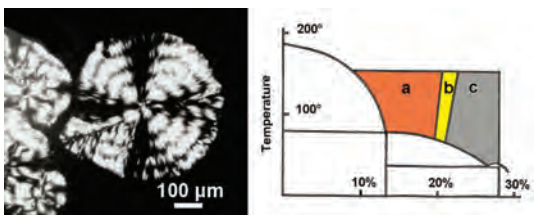
N-methyl morpholine N-oxide (NMMO) as a new organic solvent for cellulose. With the development of the NMMO technology for the dissolution and spinning of cellulose, our involvement was to characterize the main aspects of the three-component system cellulose/water/ NMMO and its implication for the dissolution and processing of cellulose. A fifteen year research program, conducted at Cermav by several teams, allowed to establish the fundamental aspects of this system before and during its industrialization [5, 6].



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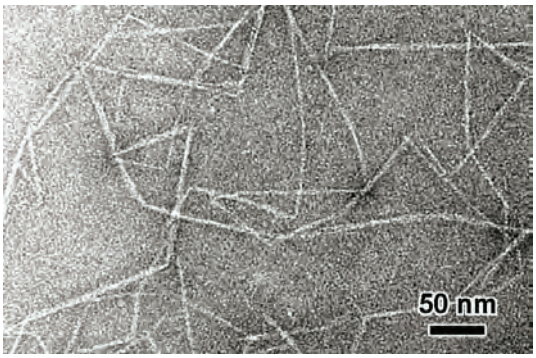
Topochemistry of nanocellulose. With the recent interest for the use of nanocellulose, the preparation and handling of cellulose microfibrils or nanocrystals (CNCs), homogeneously dispersed in various media, have been developed by using specific surface chemistry and physicochemistry. In this line, we can quote the TEMPO modification, which allows the preparation of non flocculating aqueous suspensions of elementary cellulose microfibrils and CNCs [7, 8].



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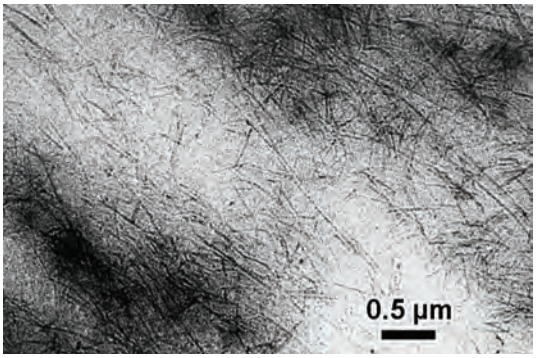
This development was recognized by the committee of the prestigious Markus Wallenberg Prize, shared in 2015 by Y. Nishiyama. Homogeneous suspensions of cellulose CNCs in apolar solvents were also produced by either using dedicated surfactants [9] or applying controlled surface chemistry (e.g., surface silylation [10]).

Cellulose nanocomposites. In 1991, Cermav initiated a program whereby non-flocculated suspensions of CNCs could be homogeneously (i) dispersed into water-soluble thermosetting resins [11], (ii) mixed with latex suspensions [12] or (iii) dispersed into hydrophobic matrices [13] to yield cellulose-reinforced nanocomposites.



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[19] J. Lai Kee Him et al., J. Biol. Chem. 177, 2002, 36931
[20] J. Phys. Chem B, 2003, 2394 (enlever le 107)
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These new materials showed dramatically improved physical properties. Our seminal work in this domain is enjoying a worldwide interest [12].

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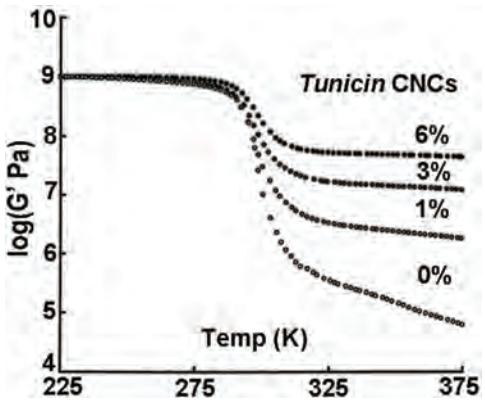

Orientation of cellulose nanocrystals by external fields: magnetic or electrical. In many products where CNCs are important, the control of their orientation is crucial. For this, external magnetic or electrical field can be used to drive the CNCs into specific orientations. With magnetic field, we could demonstrate that the CNCs in suspension became aligned with their long axis perpendicular to the direction of the field [14], an observation, which was particularly important for perfectly aligning chiral-nematic phases of CNCs [15]. With electrical field, the CNCS became aligned with their long axis parallel to the field direction [16]. This is due to the existence of a "giant" permanent electric-dipole moment along the CNc-Cs long axis [17].

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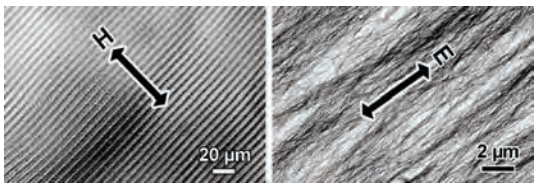

Biodegradation of cellulose. To understand how a multicomponent cellulase could digest cellulose crystals, we have used fractionated cellulase monocomponents for visualizing the digestion of well-characterized nanocelluloses. In a second step, the fractionated monocomponents were recombined to reveal the topology of their synergistic action [18].

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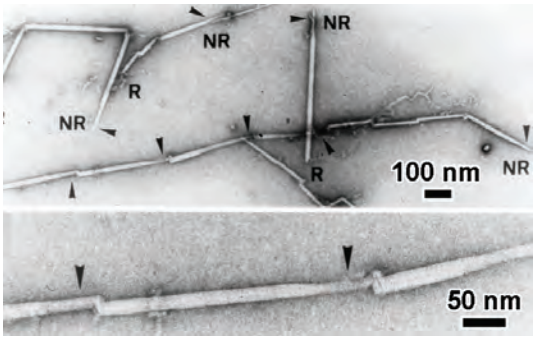

In vitro biosynthesis of cellulose was achieved by selectively extracting intact synthesizing terminal complexes from microsomal fractions from the wall of *Rubus fruticosus* cultured cells. When incubated in vitro with UDP-glucose, crystalline microfibrils of cellulose I grew in suspension from such preparations [19].

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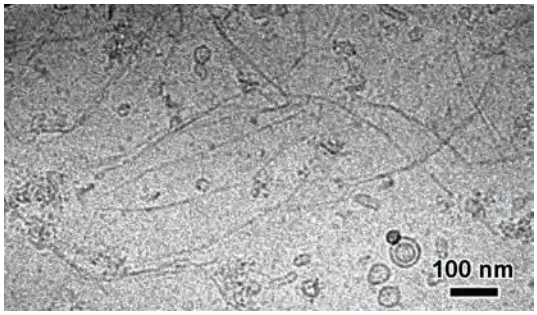

Cellulose modeling. Whereas the molecular organization of perfectly crystalline cellulose can be precisely described from the analysis of diffraction data, these measurements are ineffective to give access to the ultrastructure of paracrystalline or amorphous cellulose, which are important components in all cellulose materials. Molecular modeling, developed in particular at Cermav, has proven to be the choice technique to successfully describe the molecular structure of disorganized cellulose. Molecular dynamics simulations of amorphous cellulose [20] or of the partial disorganization of cellulose crystals during annealing [21] exemplify the outcome of such modeling in presenting a molecular rationale for poorly explained experimental phenomena.

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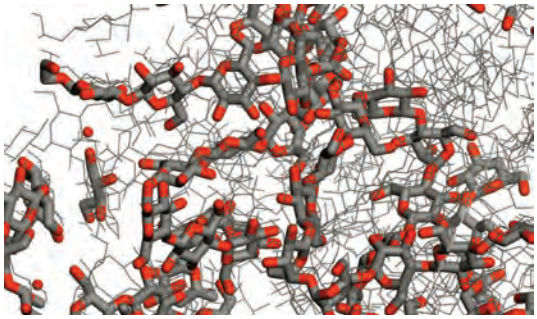

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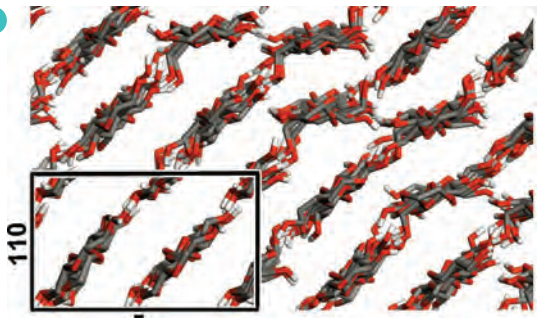


1- *Sequential electron microdiffractions on one microfibril of* Microdictyon *cellulose showing alternating patterns of monoclinic (cellulose Iβ) and triclinic (cellulose 1α) unit cells.*

2- *Neutron diffraction fiber patternsof hydrogenated and deuterated tunicin, leading to the precise geometry of the hydrogen bonds in Iβ cellulose crystal.*

3- *Left: Texturing of cellulose by co-crystallization with NMMO/ H20. Right: Phase diagram of NMMO/H20: dissolving cellulose (orange), swelling only (yellow), no action (gray).*

4- *Dispersion of individualized cotton microfibrils after TEMPO surface modification.*

5- *Homogeneous dispersion of tunicin CNCs into styrene butyl acrylate nanocoposite films.*

6- *Dramatic improvements of the mechanical properties of the reinforced nanocomposite films.*

7- *Left: A magnetic field will orient CNCs with their axis perpendicular to the field and accordingly will align chiral-nematic suspensions of cotton CNCs. Right: An electrical field will orient CNCs parallel to the field, as exemplified with tunicin CNCs.*

8- *Unidirectional digestion of* Valonia *CNCs by Cel6 cellulase from* T. reesei, *acting as 'pencil-sharpener' at the non-reducing ends of the crystals.*

9- *Cryo TEM image of in vitro cellulose microfibrils growing from terminal complexes extracted from* R. fruticosus *cell wall.*

10- *The shape of one cellulose chain within an amorphous cellulose matrix.*

11- *ab projection of a cellulose crystal equilibrated at 450K by molecular dynamics. Insert: The same, equilibrated at 300 K.*

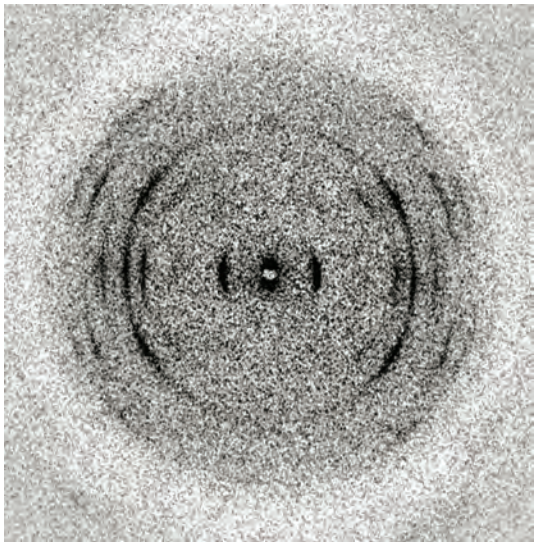
Polysaccharides Structure and Models

Polysaccharides form the most abundant and diverse family of biopolymers. With several hundreds of known examples, they offer a great diversity of chemical structures ranging from simple linear homopolymers to branched heteropolymers, having repeating units that consist of up to octasaccharides. Polysaccharides may also be branched, which is a unique feature among naturally occurring macromolecules. Despite their properties and functions the elucidation of the three dimensional structures of polysaccharides is lagging behind other macromolecules. It has been the honor of researchers at Cermav to lead the way,over a 50-year period, in the assessment of three- dimensional structures of polysaccharides, using continuously developing methodologies.

CONTACT

Serge Pérez

The most important method for the structure determination of crystalline polysaccharides is X-ray fiber diffraction. Diffraction from well-ordered three-dimensional single crystals provides the most detailed information; it should be recognized that fibers do have certain advantages over single crystals. It has been observed that linear polysaccharides prefer to exist as long helices rather than more convoluted structures. A fibrillar sample may be a more relevant environment for biological macromolecules, as it can accommodate, without loss of order, changes in polymer structure and conformational flexibility that would destroy order in a single crystal. Besides, the native organization of several polysaccharides occur in a fibrillar form and it is therefore quite relevant to investigate the structural results of concomitant biosynthesis and crystallization. As for other types of polysaccharides, samples can be produced after dissolution, in which helical macromolecules are aligned with their long axes, parallel or antiparallel. Further lateral organization may occur, but rarely to the degree of a three dimensionally-ordered single crystal.



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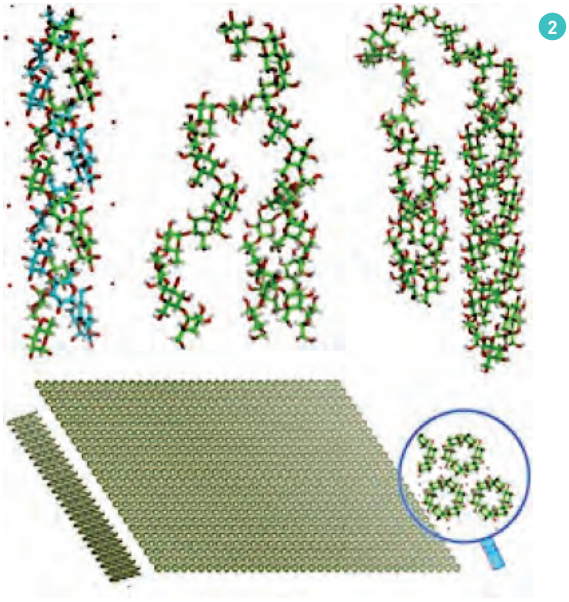
The development of new experimental tools, such as those provided by large scales facilities offering access to synchrotron and neutron techniques, was instrumental to obtain highly accurate structural descriptions. To achieve this, methods have been developed for deuteriation of the intracrystalline regions of native materials without affecting the overall structural integrity. The deuterated fibers give high-resolution neutron diffraction patterns with intensities that are substantially different from the intensities observed on neutron diffraction patterns obtained from hydrogenated fibers. Such a methodology has been applied to the resolution of the crystalline structures if allomorphs of native celluloses.

As with many other stereoregular polymers, simple linear polysaccharides, once dissolved and recrystallized, can yield single crystals. In other instances, crystallin arrangement of polysaccharides occurs as concomitant to biosynthesis, as in cellulose, starch, chitin, etc. In many cases, polysaccharides crystallize with the incorporation of water or other solvent molecules. In most cases, a well-defined morphology is obtained, the most common being plate-like. These thin lamellar surfaces have lateral dimensions of several micrometers for only a few tenths of an angstrom in thickness.



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Crystalline domains of such dimensions are well suited for examination by transmission electron microscopy in both imaging and diffraction modes. A problem frequently encountered when studying crystalline

FURTHER READING

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S. Perez et al., Biochimie, 2003, 109-121

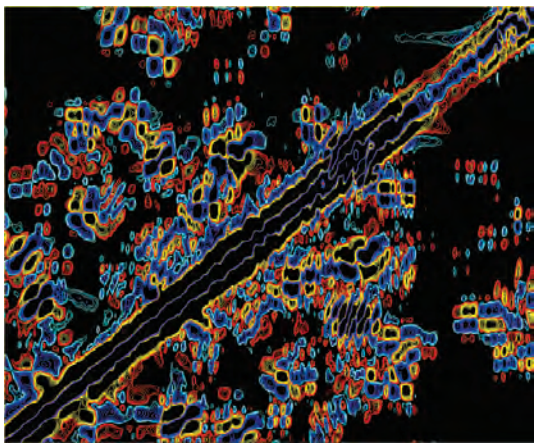
biopolymers with the electron microscope relates to the vacuum dehydration of the specimen when inserted in the instrument column. This is particularly critical when water or solvent is part of the crystalline structure. In such instances, total or partial decrystallization takes place, in a matter of minutes, accompanied by drastic distortion of the sample. Several methods have been developed, whereby the sample is either viewed inside a hydration chamber or quenched in a cryogenic bath prior to insertion into the electron microscope. In these instances, the observations are performed at a temperature close to that of boiling liquid nitrogen, where the water of crystallization is stable in high vacuum. With such a technique, frozen wet electron diffractograms are readily recorded.

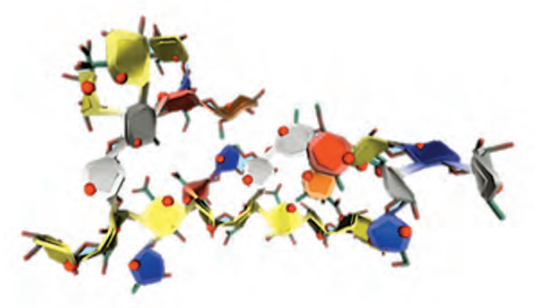
In contrast to other macromolecules, the diffraction data that can be obtained from polysaccharides are not sufficient to permit crystal structure determination based on the data alone. A molecular modeling technique must be used that allows the calculation of diffraction intensities from various models for comparison with the observed intensities. The joint use of molecular modeling and diffraction techniques has been invaluable in the quantitative elucidation of crystals and molecular structure.

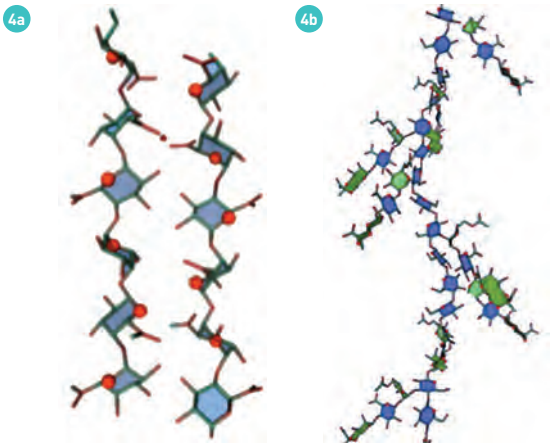
The mutual enrichment between experimental and modeling approaches has been accompanied by progress in algorithms and computational power allowing for the simuation of polysaccharides in their natural environment, that is solvated in water, in concentrated solution, in the binding site of protein receptor, in the context of complex cell wall components. The increasing interplay of computational methods with NMR and other biophysical methods is widening and strenghtening the all area of polysaccharide structures and interactions.

A molecular visualization program tailored to deal with the range of three-dimensional structures of complex carbohydrates and polysaccharides, either alone or in their interactions with other bio-macromolecules, has been developed using advanced technologies elaborated by the video games industry. All the specific structural features displayed by the simplest to the most complex carbohydrate containing molecules have been taken into account and can be conveniently depicted. This concerns the monosaccharide identification and classification, conformations, location in single chain or multiple branched chains, depiction of secondary structural elements, and the essential constituting elements in very complex structures.

Because of its unique contribution to the overall area of polysaccharide crystallography, the Cermav has taken the responsibility to create under an annotated database named PolysacDB (http://www.cermav.cnrs.fr) of a repository of all three-dimensional structures of polysaccharides which have been published. It provides information regarding the structure (determined by X-ray crystallography, electron diffraction, and molecular modeling), linkage, repeating units, diffraction patterns, and reference of the publication of each of the respective polysaccharide entries.







Next frontiers will deal with the exploration of more complex architectures, a step forward to understanding biosynthetic pathways and relating 3D structures to functional properties and biological roles.

1- X-ray fiber diffractogram of tuber starch

2- Different levels of structural organization in starch: from double-helical structure to ordered arrangements in micro-crystalline platelets.

3- RhamnoGalacturonan-II, a primary cell wall component of higher plants.
a) Fingerprint region of the 750 MHz spectrum
b) 3D representation

4- a) Depiction of the complexation of calcium ion by a gel forming plant polysaccharide: pectin
b) Molecular depiction of xanthan macromolecule, a highly viscous bacterial polysaccharide

A Long Way from Polysaccharide Structures to their Physicochemical Properties

From 1960, we started to investigate on physicochemical properties of polysaccharides. Our main interest was to be able to predict their properties as soon as the chemical structure is known. From that time, we especially studied the influence of the electrostatic interactions in charged polymers on their properties in collaboration with many researchers and PhD students, involving also international cooperations.

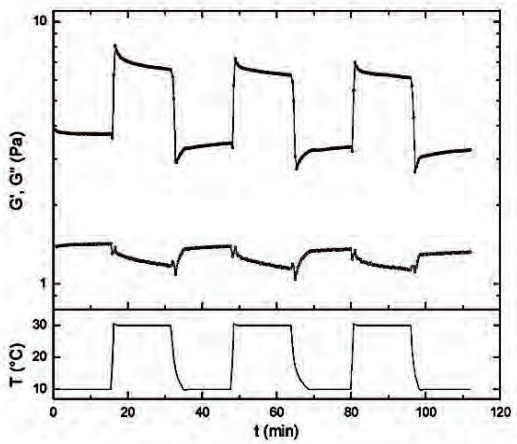
CONTACT

Marguerite Rinaudo

The first step was to establish the role of the charge density on aqueous solution behavior using a cellulose derivative: carboxymethylcelluloses prepared at different degrees of substitution up to DS=3 were obtained using an unsual technique. Secondly, the influence of the degree of polymerization (DP) was established using the synthesis of oligomers of α-L-glutamic acid or partial hydrolysis of α-D-polygalacturonic acid followed by chromatographic separation of the oligomers.

Having established the influence of charge density and degree of polymerization on the physicochemical properties of charged macromolecules in aqueous solutions, the different theoretical models for polyelectrolytes were examined and the experimental parameters were compared with theoretical prediction [1]. Later, the main characteristics of few polysaccharides such as radius of gyration and persistence length were determined for exploiting the potentialities of multidetection steric exclusion chromatography equipped with a multiangle light scattering detector: the experimental values were compared with success with data obtained by molecular modeling on chitosan, galactomannan and hyaluronan in cooperation with S. Perez and K. Mazeau [2]. In fact, semi-rigid character is one of the very specific and original properties of many steroregular natural polysaccharides.

Our knowledge on charged polysaccharides was then extended to natural polymers and the mechanisms of interaction in solution were studied. In that way, an original behavior of some of them was extensively analyzed from solution to physical gel state and the different types of physical gel were investigated.



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Firstly, thermoreversible gelation on κ- and ι-carrageenans was studied and the sol-gel transition as a function of temperature and ionic concentration was established and a phase diagram was proposed [3].

Secondly, the ionic crosslink of alginates and pectins was examined involving the roles of charged blocks and nature of divalent counterions [4]. Recently, new gelling copolymers were prepared by grafting randomly oligoguluronic acid with controlled DP on a synthetic polymeric backbone in cooperation with L. Albertin [5]. Thirdly, the gelation mechanism of amphiphilic polymers was studied using methylcelluloses with different molar masses, alkyl-chitosans and -hyaluronans and chitosan-g-N-isopropylacrylamide copolymers with different degrees of chemical modification and grafted chain lengths [6]. The thermoreversibility of this new copolymer is shown in figure 1. Finally, the mixed gel, formed by the specific interactions between two different non-gelling polysaccharides, was analyzed on the galactomannan-xanthan systems [7].

A third category of polysaccharides was introduced with the microbial biosynthetized polymers. The first tested polysaccharide was the xanthan proposed for tertiary oil recovery in concurrence with high molar masses partially hydrolyzed polyacrylamides.

We succeeded to relate the original properties of xanthan such as the stability of viscosity in presence of external salts, with its local stiffness or persistence length. For the first time, a nice cholesteric phase was observed on xanthan solution (figure 2).

Other microbial polysaccharides were extensively studied with, especially, hyaluronan (HA) for viscosupplementation in cooperation with ARD Cy France and rheumatologists [8], gellan in cooperation with Biomérieux, and later with Morris [9], succinoglycan with Shell Cy, and scleroglucan among others. By AFM on succinoglygan, a polysaccharide single chain was identified allowing to confirm its persistence length [10] (figure 3).

1- Mechanical moduli variations for chitosan-g-N-isopropylacrylamide in aqueous solution due to periodic stepwise changes in temperature between 10 and 30 °C

2- Cholesteric liquid crystalline phase obtained with xanthan in water

3- AFM image for a single chain of succinoglycan

The knowledge accumulated so long allows entering with success into the field of smart materials and biomaterials based on polysaccharides. In addition, it is clear that more and more attention was payed during these last fifty years to polysaccharides which were progressively recognized as important for their biological activities but also for applications in large domains such as pharmaceutical, biomedical, cosmetics, food, ...

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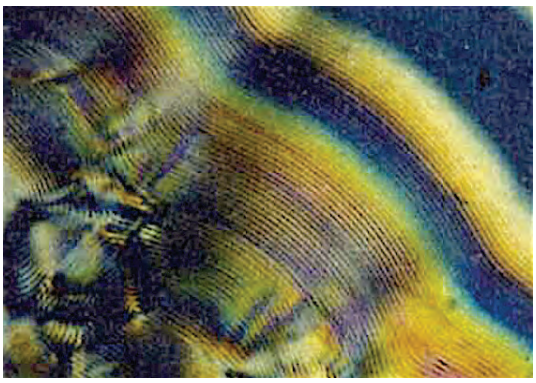

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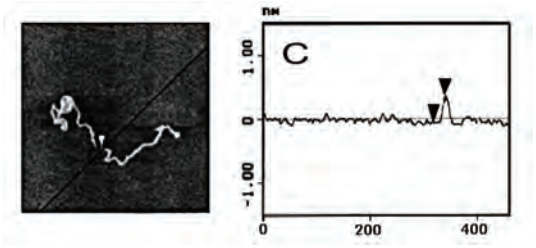

Two polysaccharides were particularly studied in this view: hyaluronan and chitosan. Firstly, the biological properties and applications of chitosan and derivatives were covered, taking into account their biocompatibility and biodegradability. For the first time, the roles of chitosan molar mass and degree of acetylation were clearly demonstrated on antimicrobial activities [11]. Then, examples of biomaterials were studied: 1) multilayers capsules were prepared based on electrostatic complexes of HA and chitosan; 2) liposomes were coated and stabilized against external stresses (pH, salt, glucose) with chitosan and with HA with different molar masses; the mechanisms of interaction were established [12].

This period of more than 50 years of work, in cooperation with many students and foreign groups, allows us to be able to propose convenient techniques to get information on the relationship between the chemical structure of polysaccharides, their physical properties in sol and gel states, and many possible applications of natural or modified polysaccharides.

On a fundamental basis, the physical behaviour of semi-rigid polymers merit to be developped especially to propose new scaling models allowing to relate solution viscosity, relaxation time and modulus in an extended range of polymer concentration.

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Studies of Glycanases by the Substrate Analog Approaches

It has been known for a long time that carbohydrate-enzyme recognition is crucial for the industrial development of natural oligo- and polysaccharides and, in recent years, it has also been considered that such interactions are the essential process for biological transfer of information into living organisms. There are essentially two approaches for these investigations: site-directed mutagenesis in the active site of proteins and use of natural substrates or the use of native proteins with substrate analogs.

CONTACTS

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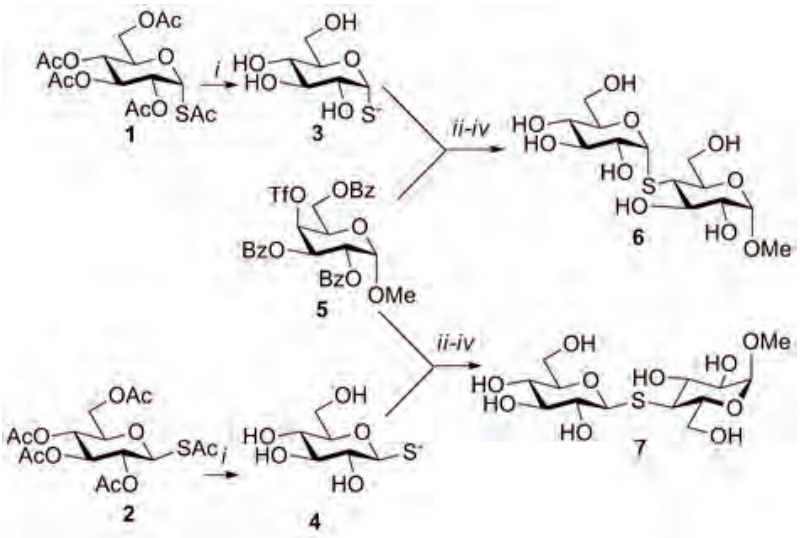
All over the years in Cermav, we developed methodologies for the syntheses of two series of non-natural oligosaccharides to analyze enzyme-substrate interactions. The first one deals with oligosaccharides which are recognized in the active site but are not hydrolyzed. This can be achieved by substituting the natural O-glycosidic linkages that should be split by sulfide linkages. Stepwise chemical syntheses have been used for the preparation of these molecules called thio-oligosaccharides. In the same time, we have in mind to developed sensitive and specific assay for glycoside hydrolases by fluorescence quenching. So the other set of molecules is made with oligosaccharides carrying non-carbohydrate substituents at both extremities like a fluorescent energy donor at the reducing end and an acceptor chromophore at the other end. After splitting of any glycosidic bond an increase of fluorescence was observed. The main task for the synthesis of such compounds was to avoid chemical glycosidic coupling with its low yield so to develop enzymatic approach using transglycosylation reactions. Compounds in both series have been used to elucidate the fundamental interactions by which some of glycanases recognize their substrates and how they catalyze their reactions.

Synthesis of thio-oligosaccharides as tools for structural studies of glycanases. In the late seventies a general method for the preparation of this class of non-natural compounds was developed . This method takes advantage of the fact that sulfur is less basic and more nucleophilic than oxygen. For instance deacetylation of peracetylated 1-thioglucoses 1 or 2 gave the corresponding 1-thiolates 3 or 4 respectively, which reacted by a S_N2 type mechanism with the acetylated acceptor 5 to give after *O*-acetylation/*O*-deacylation the expected thio-disaccharides 6 and 7 in fair yields (Scheme 1).

Later on, an improvement of this method was achieved by a selective *in situ* S-deacetylation by using cysteamine (cyst) and dithioerythritol (DTE). The versatility of this approach has been used to prepare several thio-oligosaccharides as substrate analogs for enzymes acting on cellulose and starch. This survey is only devoted to substrates for cellulases. Depending of the mechanism of recognition of enzymes, only the scissile bond of the substrate has to be a thio-linkage. The following Figure described the structure of some thio-cellodextrins which were synthesized.



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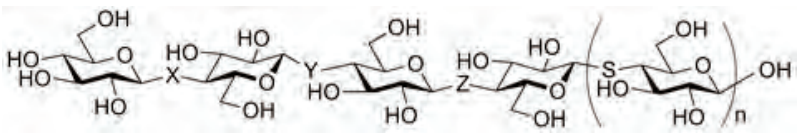

X = Z= O, Y= S, n =0; X = Y = Z = S, n = 0; ; X = Y = Z = S, n = 1

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Some of these compounds were found to be excellent inhibitors for cellulases and X-ray studies of the enzyme-substrate complex revealed for the first time that a molecule spanning the point of cleavage (between -1 and +1 subsites) has a conformation change of its sugar unit in -1 subsite: from $^4C_1$ chair to $^1S_3$ skew-boat or ^4E envelope with a quasi-axial orientation for the scissile bond. This distortion induces a movement of the sugar unit which has to fit into the +1 subsite and accommodate the kink found around the catalytic point in many glycanases. The consequences of this deformation for the mechanism of action of retaining β-glycanases have also favorable steric and stereoelectronic implications [1-3].

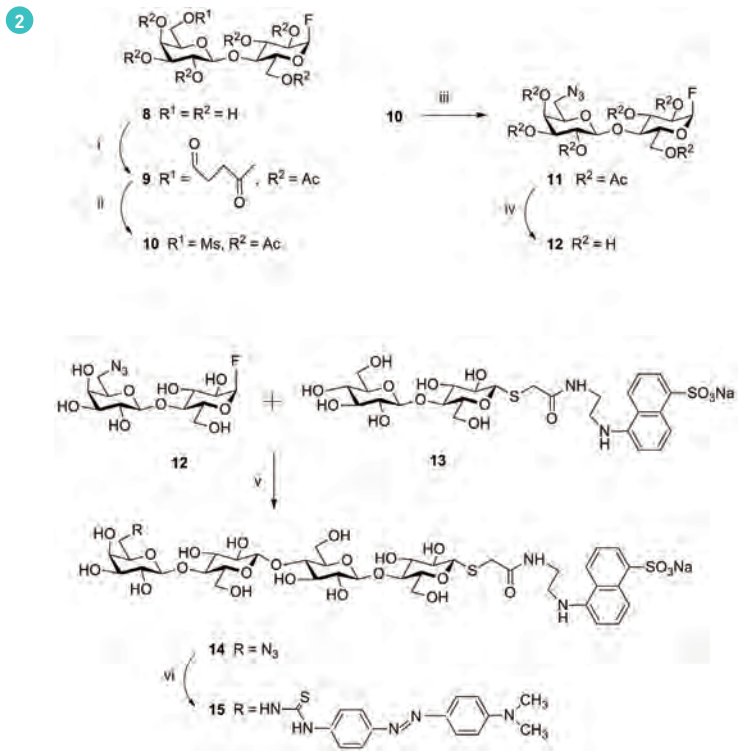
Syntheses of specific substrates for sensitive assays of glycanases by fluorescence quenching. Heterogeneity of polysaccharides and specially cellulose precludes to use them as substrates for biochemical studies of glycanases. Soluble oligosaccharides labeled at their reducing end have proven useful for kinetic studies but need endpoint monitoring of hydrolysis and therefore cannot permit continuous assay. These problems can be overcome by exploiting the fluorescence resonance energy transfer (FRET) on bifunctionalized fluorogenic substrates. In the 1.4-α-glucan series such substrate was useful for discrimination between the *exo*- and *endo*- types of action of starch-degrading enzymes. In the 1.4-α-glucan series, the comparison of catalytic constants with those obtained on reduced cellodextrins showed that the aromatics at both ends of the molecule did not hinder the binding even for cellulases classified as exo-enzymes. Scheme 2 described the methodology and the synthesis of a bifunctionalized cellotetraoside as a potent specific substrate for sensitive assay of cellulases. The efficiency of FRET depends of the distance of the two groups, their spectral overlap and the lifetime of the excited fluorophore. The following combination of the acceptor chromophore-donor fluorophore was chosen: 4-(4'-dimethylamino-benzeneazo)benzene derivative as acceptor chromophore and (2'-aminoethyl)aminonaphtalene sulfonic acid (EDANS) as donor fluorophore. The coupling of the two functionalized synthons was based on glycosynthase methodology that was well developed by us and other laboratories. Retaining α-glycoside hydrolases in which the carboxylate nucleophile was changed to alanine had no hydrolytic activity but were able to catalyze the quantitative transfer of α-glycosyl fluoride(which mimic the glycosyl enzyme intermediate) onto various acceptors. The Glu-197-Ala mutant of cellulase Cel7B from *H. insolens* was used once more in this study for coupling the two synthons. *Candida antartica* lipase allowed the selective protection/deprotection/functionalization of lactosyl fluoride 8 into the target donor 15 (Scheme 2).

Fluorescent cellobioside 13 and the fluoride 12 after incubation with Glu-197-Ala mutant of cellulase Cel7B gave the expected tetrasaccharide 14 in 79% yield. Mild reduction of the azide and coupling with commercially available 4-dimethylaminophenylazophenyl isothiocyanate gave the final compound in 69% yield. This compound was useful for biochemical characterization of a cellulose oxidizing enzyme lytic polysaccharide monooxygenase (LPMO) a metalloenzyme of considerable interest in the industrial utilization of biomass [4-6].

This work was funded mainly by CNRS and Biotech Programs of the European Union.

1- First synthesis of methyl 4-thio-α-maltoside 6 and cellobioside 7.
i) MeONa (1.1 equiv) in MeOH;
ii) HMPA;
iii) Ac_2O in pyridine;
iv) MeONa (catalytic amount) in MeOH. (6 and 7 34 and 52 % yield over three steps)

2- Synthesis of FRET cellotetraoside 15.
i) trifluoroethyl levulinate, Candida antarctica lipase, 2-methyl-2-butanol; Ac2O, DMAP, pyridine, 48%;
ii) hydrazine acetate, EtOH; methanesulfonyl chloride, DMAP, pyridine, 90% ;
iii) NaN3, 18C6, DMF, 80°C, 59%;
iv) MeONa (catalytic amount) in MeOH, 96%;
v) Cel7B Glu197Ala glycosynthase, sodium phosphate buffer (0.1 M, pH 7), 79%;
vi) H2S, pyridine-H2O; DABITC, NaHCO3, DMF, 69%.



The Family Classification of Carbohydrate-Active Enzymes

CONTACT

Bernard Henrissat,

currently at Architecture et Fonction des Macromolécules Biologiques (AFMB) Laboratory, overseen by the Centre National de la Recherche Scientifique & Aix-Marseille Université, Marseille, France

Photosynthesis has transformed carbohydrates into the most abundant biological commodity on Earth. In theory, carbohydrates can be linked to each other or to non-carbohydrate moieties in an astronomical number of stereochemical possibilities. Although not all possibilities exist, Nature has evolved a large variety of complex carbohydrate assemblies to perform functions as diverse as structure, defense, energy storage, and a myriad of intra and intercellular recognition events. These different functions can only be achieved *in vivo* if carbohydrates are assembled and broken down selectively. The assembly and breakdown of glycoconjugates, oligo- and polysaccharides is catalyzed by a large variety of enzymes collectively designated as carbohydrate-active enzymes (CAZymes). These enzymes, which are the molecular tools that Nature utilizes to harness the rich stereochemical diversity of carbohydrates, find biotech applications in the food, detergent and biofuels sectors as well as in nutrition and health. Degradative CAZymes comprise several classes, namely the glycoside hydrolases (GHs), the polysaccharide lyases (PLs), the carbohydrate esterases (CEs) and the recently added lytic polysaccharide monooxygenases (LPMOs). The assembly CAZymes are essentially glycosyltransferases (GTs), which utilize activated sugar donors to perform the glycosylation of almost all molecules of life, and of carbohydrates in particular.

In the late 80's, the only system that was available to classify enzymes was the EC nomenclature of the IUBMB, which classified enzymes by the reactions they catalyze, that is by describing the substrate and the product of the reaction. This classification, which proved useful to accompany the early characterizations of carbohydrate-active enzymes, did not integrate the amino acid sequence features of the enzymes nor their three-dimensional structures.

My work on the establishment of an alternative classification system for CAZymes started with the cellulases, whose detailed biochemical activity was incongruent with the simple endo vs. exo division that prevailed in the late 80's. Utilizing hydrophobic cluster analysis with 21 sequences available in 1989, I was able to delineate six families. After performing a similar work on chitinases, I generalized the sequence-based classification scheme to all known GHs in 1991. Not only did the new classification explicitly incorporate the sequence of the enzymes, but it incorporated *de facto* their three-dimensional structure since it was known that sequence similarity coincides with structural similarity. The first GH family classification, and what could be deduced from it, proved to be an ideal system to accompany the explosion of three-dimensional structures that resulted from the structural biology revolution. The success of the classification led to successive expansions to incorporate new families of GHs in 1993 and 1996, and to its application to the GTs in 1997. During a sabbatical stay with Martin Schülein at Novo-Nordisk (now Novozymes) in 1997, I developed the first prototype of an online version of my classification. The public online version, nicknamed the 'CAZy database', went online on September 1st, 1998, a few months after I moved from Cermav to join my current laboratory in Marseille.

My classification system was rapidly adopted by the glycoscience community as it was endowed with predictive power. The first feature that became predictable was the stereochemical outcome of the reaction. It was quickly established that all members of a given GH family performed their catalysis with the same catalytic residues and hence with the same mechanism. When the inverting or retaining mechanism was determined for a member of a family, it could safely be extended to all other members of the family. The number of counterexamples known 25 years after the classification paper is tiny (less than 0.1% of all GHs !). The structural biologists could utilize the classification to select their next target and then compare the structures in different families. This is how the concept of clans was developed.

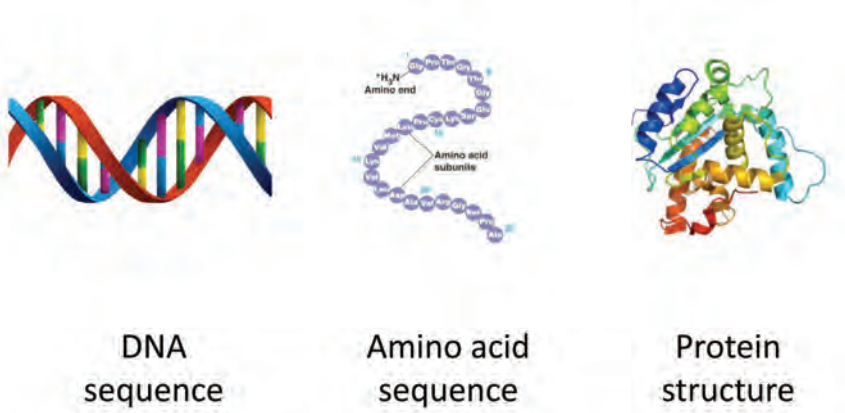
Most importantly, a key feature of my classification system is that it is stable despite a >1000 fold increase in the number of sequences since its inception. A testimony to the success of this classification is the >2100 citations that the original 1991 paper has attracted, making it the 4th most cited French paper of 1991. The public CAZy database (www.cazy.org) that resulted from the classification has been maintained, updated and upgraded ever since its launch. Approx. 2 million pages are downloaded each year by over 60,000 unique visitors. Importantly the classification has opened the door to collaborations with the top scientists in the field of CAZymes (I am happy to cite Professors Gideon J. Davies, Harry J. Gilbert, Tuula Teeri, Birte Svensson, Tony Warren and Steve G. Withers among many others) during the structural biology boom of the 90's.

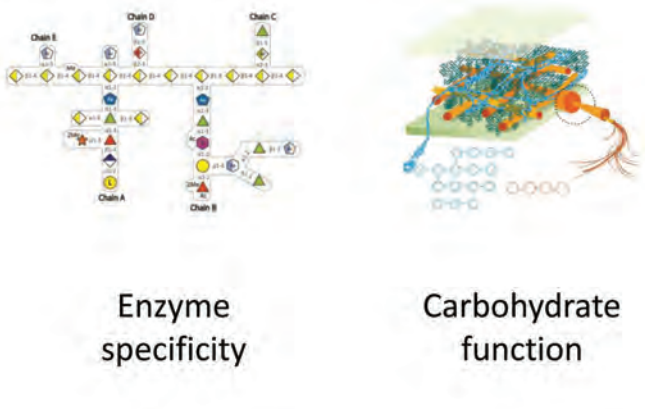
In the mid 90's, the first reports of completely sequenced genomes appeared. The functional predictive power of the family classification (that had since grown to incorporate the PLs, the CEs, and their appended

carbohydrate-binding modules) gave a new dimension to the classification. The drop in cost of sequencing technologies that took place in the first decade of the 21st century resulted in a tsunami of sequences to analyze. Having started early, we were able to streamline the classification process and to create new tools for the comparison of genomes. Glycogenomics was born !

Over the last 20 years, the family classification that I created at Cermav has been applied successfully to hundreds of eukaryotic and thousands of prokaryotic genomes, and has also been instrumental in the metagenomics analyses of the human gut microbiome. The classification is now central to the analysis of the carbohydrate-active enzyme profile of all organisms and we have shown that this profile is shaped by evolution and lifestyle of an organism. We are currently participating to numerous projects such as the 1000 fungal genomes initiative, the study of human and animal digestive microbiomes, of environmental microbes and communities thereof, etc.

While the specificity of other enzyme classes (DNAses, RNAses, kinases, proteases, esterases, lipases etc) is difficult and often impossible to predict based on their sole amino acid sequence, the classification system that I have created often allows a broad functional prediction by the simple assignment to a CAZy family. However, the presence in families of enzymes that act on different substrates is a problem that must be overcome if one wants to predict accurately the substrate and reaction product of CAZymes. We have shown that the subdivision of the multifunctional families into subfamilies based on the utilization of phylogenetic trees is a promising approach towards this goal. Our knowledge of the relations between the sequence and the specificity of CAZymes is, however, largely insufficient and ill-distributed for most CAZy families (some families count only one characterized member!). Here my classification reveals another useful facet: it can be used to select the CAZymes to characterize experimentally, by focusing on under-characterized families or on subfamilies that have not been characterized so far, or on very distant relatives, in other words where the substrate cannot be predicted. I am happy to collaborate with William Helbert of Cermav on this subject. Combined with the use of high throughput biochemistry, we should be able to massively improve the functional power of the classification. We are breaking the carbohydrate code !





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A 50-year Immersion in the Biosynthesis and Macromolecular Organization of the Plant Cell Wall

The team 'Biochemistry of the Plant Cell Walls' successively directed by Prof. Fernand Barnoud, Prof. Jean-Paul Joseleau, and Dr. Katia Ruel, had for objectives to describe the chemical structures of the cell wall macromolecular components, and the mechanisms underlying their variations during their incorporation in the primary and secondary wall layers. In this view, chemical, biochemical and ultrastructural results were always put together to propose a structural model of the macromolecular organization of the plant cell walls. A significant part of the results and concepts acquired by the Group along the 50 years of research has been transferred and applied to wood fiber and to pulp and paper industries.

CONTACTS

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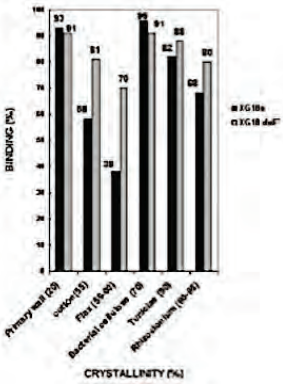
PLANT CELL WALL BIOGENESIS

Structural determination of Non-Cellulosic Cell Wall Polysaccharides. The plant cell is encased in a complex and dynamic macromolecular extracellular matrix organized around cellulose microfibrils. In the early years of Cermav, it was of crucial importance to acquire the techniques allowing the description of the chemical structures of the various polysaccharides from the cell walls. In this respect, bacterial polysaccharides with regular structures were taken as models to refine structural analysis methodologies.

Extracellular Polysaccharides from various serotypes of *Klebsiella*. Through the diversity of these polysaccharides, chemical methods of specific degradations and ^{13}C-NMR were assessed. The development of an original specific cleavage via bacteriophage enzyme technology proved to be a powerful approach to the sequence of the repeating-unit [1].

Hemicelluloses and pectic polymers from suspension-cultured *Rubus fruticosus* cells. Variation of their structure during primary wall biogenesis. These studies were previously initiated in 1966 with Prof. G.G. Dutton (Vancouver) on an *in vitro* cell culture isolated in the team (G. Hustache). Various sequential extraction schemes, use of cellulose solvent NMMO and extensive implementation of ^{13}C-NMR led to the structural identification of the complex polysaccharides from primary wall. Interestingly, multiple forms of xyloglucans, differing in the ratios of their mono-saccharide composition, were demonstrated along the primary wall maturation [2,3]. Most of the wall polysaccharides could be found in the extra-cellular medium due to autohydrolysis process.

Structural rearrangements of wall polysaccharides during the culture cycle of *R. fruticosus* cell suspension were shown to undergo a *turnover* by partial glycosidic bond cleavage induced by various wall-bound polysaccharidases. Thus, endogenous xyloglucan oligosaccharides with *elicitor* activity were shown to affect the cell wall development [4].



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A basic feature of the supramolecular assembly of the plant cell wall rearrangements during growth involves the particular interaction between cellulose and xyloglucan. Using various types of xyloglucans and celluloses with different degrees of crystallinity, we showed that the sorption of xyloglucans onto cellulose microfibrils was affected by their substitution patterns [5].

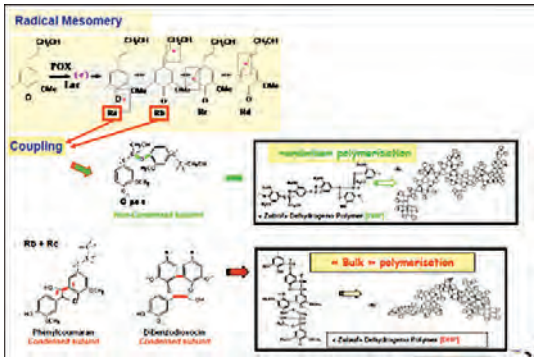
Such complementarity suggests that in the growing wall XGs adopt a cellulosic conformation. Studying the distribution of amorphous and crystalline cellulose, respectively, during cell wall formation underscored the particular influence of crystallinity along cell wall biogenesis [6].

Similarly, during secondary wall assembly, we showed by immunolocalization techniques the occurrence of two molecular kinds of xylans interacting differently with cellulose micro-fibrils depending on their side chain substituents, revealing their respective role in cross-linking the cellulose lamellae. Xylans metabolism, studied by antisense downregulation of xylosidase and UDP-glucu-ronidase decarboxylase genes in *Arabidopsis*, was shown to have a determining impact on secondary wall biogenesis [7].

DECIPHERING THE ULTRASTRUCTURE OF PLANT CELL WALLS THROUGH THE EYE OF TRANSMISSION ELECTRON MICROSCOPY

In our approach of the understanding of the plant cell wall macromolecular organization and its development during formation, we privileged the observation *in situ* by TEM in connection with chemical analyses. To this end, we decided to forge specific methodologies for the specific detection of polysaccharides and lignins. A few highlights due to the progresses obtained in this domain are briefly recalled below.

Validation of lamellated organization of lignified fiber wall by TEM / STEM [8]. This concept is still valid today. Divising new specific markers of cell wall macromolecules. To go beyond the general PATAg method for staining polysaccharides in TEM, we developed specific markers based on the interaction between *endo*-polysaccharidases and various hemicelluloses, complexed with colloidal gold to localize the corresponding polysaccharides within the cell walls [9]. This approach gave rise to an original method that we called ETAg [10]. However, the implementation of *immunological approach* that takes advantage of the high specificity of recognition of antibodies for their antigens was a real breakthrough for the specific *in situ* labeling of wall polymers. After raising antibodies against xylans and xyloglucans, we devoted efforts towards lignin structural characterization. Several lignin antibodies with specificities directed against the three



2

1- Binding capacity of native (XG18n) and de-fucosylated (XG18-deF) xyloglucan as a function of cellulose origin and crystallinity %. Data are the means of at least three different experiments with triplicate samples.

2 – Mode of formation of condensed and non-condensed lignin dehydrogenopolymers from basic monolignols of lignin

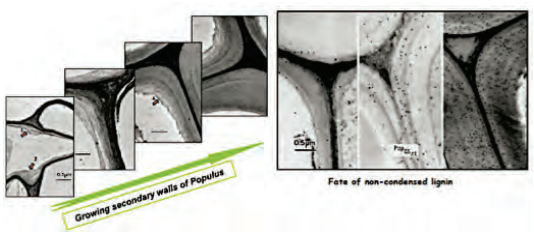
3- In planta immunolocalisation of non-condensed lignin (black dots) during primary and secondary cell wall formation in Poplar wood

4- Consequences of cinnamyl CoA reductase down regulation in Arabidopsis and visualization of the modification of the distribution of non-condensed lignin (black dots)

basic lignin monomers and against their *condensed* and *non-condensed* inter-unit linkages, respectively, were obtained [11].

These are still today the only lignin specific markers of the kind and they are largely provided to groups from various countries. In conjunction with FTIR and ^{13}C-NMR, they allowed us to establish for the first time a precise *topochemistry* of lignin types [12].

3



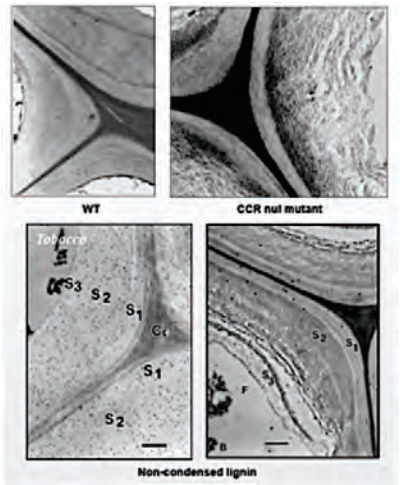
Such precise markers proved to be invaluable to explore the impact of genetic modifications of lignin biosynthesis by the anti-sense technique, as well as demonstrating for the first time, the presence of lignin in the intertidal red seaweed *Calliarthron* showing the convergent evolution of cell wall architecture in algae ant higher plants [13].

The specificity of Cellulose Binding Modules *(CBMs)* was exploited as probes for the detection of crystalline versus amorphous cellulose for the first time in TEM in the developing walls of *Arabidopsis*, showing the precocious deposition of crystalline cellulose in the incipient primary wall [6]. The same approach was applied to *Calliarthron* and confirmed the evolutionary convergence of cellulose deposition in the terrestrial plants and the red seaweed [*2016 under publication*].

LIGNIN BIOSYNTHESIS AND p-HYDROXYPHENYL PROPANOID PATHWAYS

Started in the team 'Plant Cell Wall Biochemistry' before Cermav's era, studies on the mechanisms of lignification were initiated in the 60s by Prof. F. Barnoud and Prof. T. Higuchi (Japan). Using the model of plant cell cultured *in vitro* under the form of calli in which wall secondarization is actively occurring. The roles of phenylalanine transaminase and tyrosine transaminase in the monolignol pathways were demonstrated. Lignin biosynthesis was later pursued in Cermav within the frame of European contracts with the objective of deciphering the *p*-hydroxyphenyl propanoid pathways leading to polyphenols and lignin via genetically-engineered model plants and trees. The consequences of down-regulation of several genes on cell wall biogenesis were investigated. In particular, repression of Cinnamoyl CoA reductase (CCR; EC1.2.1.44) was shown to dramatically impact the secondary wall assembly in *Populus* and Tobacco, as well as in *Arabidopsis* [14].

4



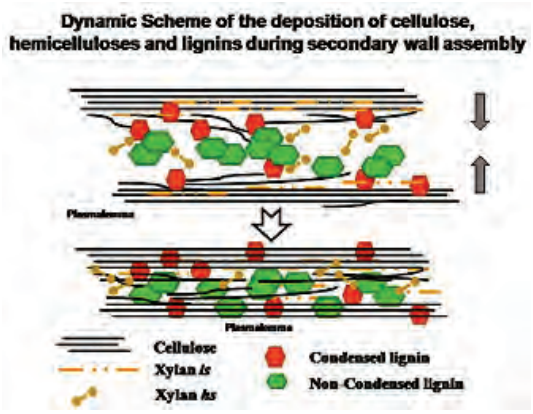
In situ analysis of condensed and non-condensed structures revealed a characteristic phenotype of the spatial lignin deposition at the cellular and subcellular levels, and an impact on cell wall degradability [15].

In situ visualization of the various monomer compositions and modes of linkage of lignins were possible thanks to the unique immuno-gold labeling probes developed in Cermav [11].

Lignin-carbohydrate linkages. In keeping with our interest in the macromolecular architecture of the lignified plant cell wall, the covalent linkages between hemicelluloses and lignin were investigated and the particular role of arabinose as cross-linking end group involved in a glycosidic bond was evidenced in poplar wood and further demonstrated to occur during peroxidase-catalyzed synthesis of a DHP in the presence of arabinose [16].

Lignin-degrading enzymes and mode of action of wood-rotting fungi. The mode of degradation of wood by the extracellular oxidative and hydrolytic enzyme systems of white rot fungi, generating OH. free radical, was investigated by combining immunolabeling of the enzymes [17] and characterizing the removal *in situ* of specific lignin and hemicellulose moieties [18].

On the basis of the lamellation model and of our biochemical and ultrastructural results, we integrated the concepts of lignifications of condensed and non-condensed lignin structures interacting with linear and substituted xylans, into a dynamic model of biogenesis of the lignocellulosic fibre wall showing the macromolecular assembly from primary to mature secondary wall [19].



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Tunable Assembling of Biosourced Nanoparticles: from Bioinspiration to Functional Materials

As Nature offers a great variety of nanoparticles as building blocks, which are assembled in particularly ingenious hierarchic architectures, a great choice is offered to the researcher for harvesting new ideas and concepts. Taking advantage of a long tradition in polysaccharide crystal science at Cermav and pioneering discoveries in the field, we have designed new 2D or 3D materials, which can be stimuli-sensitive, often based on cellulose nanocrystals (CNCs) as elementary bricks. These few hundred nanometer-long slender rods, ubiquitously spread in higher and lower plants as well as in other creatures can nowadays be referred to as the new Green Nanoparticle Eldorado.

CONTACTS

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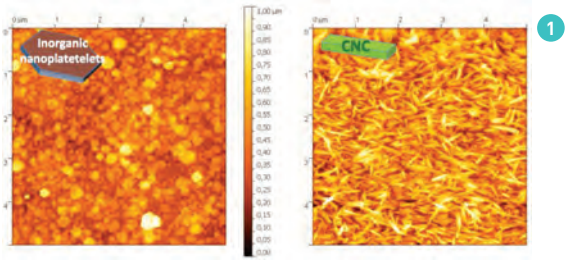
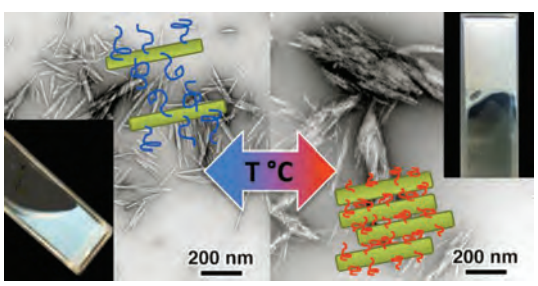
2D Assemblies. Nanostructured thin films incorporating CNCs in association with synthetic or natural polymers or inorganic nanoparticles can be designed using the versatile layer-by-layer assembly technique. The architecture of such films, which we precisely investigated using advanced surface-sensitive techniques like neutron reflectivity or AFM, is highly tunable and depends on the individual properties of the constituents, the interaction forces between the CNCs and the polymer/particle and the deposition method parameters. We successfully prepared films mimicking primary plant cell walls [1] or hybrid organic/inorganic all-nanoparticles multilayers exhibiting tunable mechanical, optical and water sensitivity properties. This strategy can be transposed from solid surfaces to 3D objects.



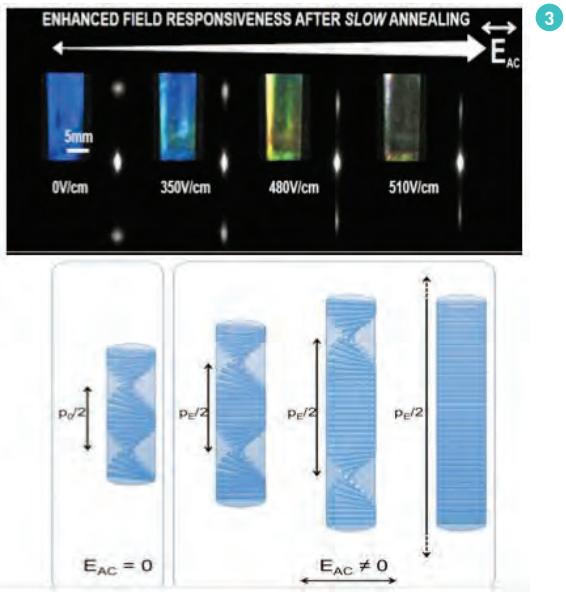
1

Switchable nanoparticles. A fine-tuning of the surface properties of CNCs is another way to control their colloidal assembling and macroscopic properties. For example, the successful covalent grafting of thermosensitive polymer chains onto CNCs enables their redispersion into aqueous as well as organic solvents and imparts fully reversible and reproducible temperature induced aggregation properties, that can be tuned by varying the ionic strength and/or the pH of the medium, thus making the suspensions multiresponsive [2]. Such a control of the assembling arises from the variations of the sign and range of the different types of interaction forces between the nanoparticles. Additionally, such polymer-decorated CNCs exhibit reversible thermally-induced gelation properties. These enhanced functionalities pave the way for the design of advanced CNC-based materials, benefitting from both the intrinsic characteristics of these biosourced particles and the new properties imparted by the stimuli-sensitive grafted chains.



2

3D assemblies. Discovered in the early 1990, another outstanding property of CNCs is the self-organization of their aqueous suspensions into cholesteric structures, reminiscent of their intrinsic chirality. We have reproduced this phenomenon by transferring the CNCs with surfactants into organic solvents to suppress the electrostatic interactions: the helical pitch of these structures strongly decreased and reached the limit of the wavelength of visible light, making them iridescent, similar to the cuticle of colored beetles. Based on our recent discovery that these nanoparticles also bear a giant electric dipole, we used electrical fields to unwind the whole cholesteric structure, allowing a fine control of the reflected colors, thus tuning a biomimetic version of photonic artificial coleoptera.



3

1- Colored CNC-Gibbsite platelets multilayered films and AFM topography image.

2 -Temperature-induced aggregation and gelation of polymer-decorated CNCs.

3- Tunable iridescence of CNC suspensions due to electric field-triggered unwinding of a cholesteric structure.

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Biosourced Materials: Plastics to Harvest?

Finding alternative to products from fossil origin is a major challenge of our century. In this line, the use of biosourced materials is a matter of common sense, since these are ubiquitously available and easily biodegradable: one could dream of a plastic bag harvested in the garden and when no longer in use, buried for humus restoration! More pragmatically, the development of biosourced materials requires great R and D efforts, essentially designed to overcome their water sensitivity and difficult processability, with the goal of proposing some of these as novelties in the Material Sciences field. For the last few years, we have developed such biobased materials under various forms, ranging from blends, to aerogels, nanocomposites, composite inclusion compounds and even unidirectional composites , often in direct collaboration with industrial partners (Solvay, Michelin, Rossignol...).

CONTACTS
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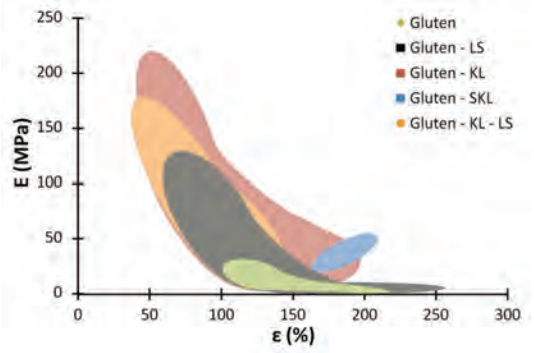
Sonia Molina-Boisseau

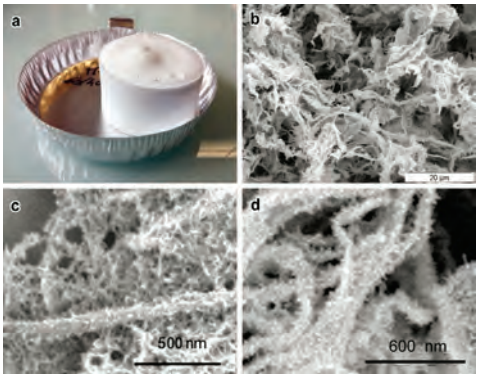
Blends of crop by-products. Lignin and gluten, by-products of the pulp and food industries, respectively, are available in huge amounts at low price. Independently, they exhibit poor mechanical performances and in the case of gluten, a detrimental sensitivity toward moisture. Playing with the constituents and elaboration processes allows the design of new wheat gluten-based materials with synergetic properties (coll. LGP2, Grenoble). On the one hand, mixing gluten with kraft lignin (KL) enhances the material rigidity and thermal properties, while reducing its water sensitivity. On the other hand, the addition of lignosulfonates (LS) to gluten results in a decrease of the required amount of glycerol plasticizer, with the result of better mechanical properties and reduced water sensitivity. A subtle formulation of these components or a combination of both lignin types has been implemented to produce materials with tailored-made properties: they offer a large choice of rigidity (E) and deformation (ε) suitable for targeted applications.

1



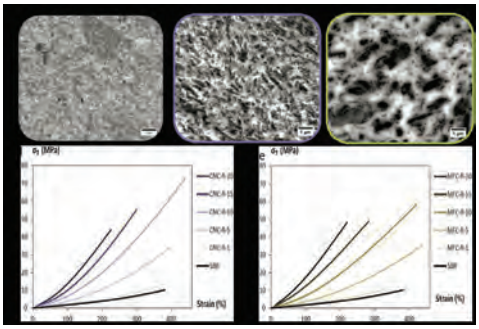
Nanocellulose aerogels. Despite the fast increasing interest in the so-called nanocellulose products, either cellulose nanofibrils (NFCs) or nanocrystals (CNCs), one bottleneck remains their use in a dry form, as they tend to collapse irreversibly. In this context, we have developed a method to prepare aerogels from wood pulp NFCs and cotton CNCs by solvent exchange with *tert*-butanol, preserving the initial porous network and thus leading to products with surface areas in the range of several hundred $m^2.g^{-1}$, comparable to silica or carbon black. We have further designed a solvent-free green process that leads to a controlled surface modification of these aerogels, turning them from hydrophilic to highly hydrophobic. Further modifications with metal oxides allow the development of hybrid inorganic aerogels, designed to function in innovative batteries or photocatalytic devices, prepared in processes with low pollution level. (Coll. ICG, Montpellier).

2



Filled elastomers. Interested in nanocelluloses that could be used as green reinforcing fillers, we codeveloped and patented with Michelin, the well-known tire company, a coupling agent able to transfer the stresses imposed to the elastomer to these rigid nanoparticles. Mixing these with the hydrophobic gum was another bottleneck, but thanks to the aerogel approach, functionalized nanocellulose could be successfully introduced into the elastomer matrices by melt processing. This was a first in the field, nanocellulose being so far mostly compounded by solvent casting. The resulting materials presented remarkable properties, especially the strain hardening (increase of the rigidity) at high deformation: this is a basic characteristic for which is a characteristic required by your own tyre if you want it to adhere in the tightest hairpins.

3



1- Comparison of gluten-based materials properties according to the type of industrial lignin used.

2 - (a) macroscopic aerogel SEM micrographs of (b) NFC aerogel (c) and (d) modified with TiCl4 and further carbonized.

3- TEM micrographs of elastomer filled with (a) silica (b) CNC and (c) NFC. Stress-strain curves of elastomer filled with (a) CNC and (b) NFC.

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Transverse Flexibility of Cellulose Crystals

Cellulose nano-crystals are often compared to perfect metallic whiskers: their longitudinal modulus estimated at 150-200 GPa is indeed comparable to that of steel. However, despite numerous experimental evidences that have revealed their propensity to get transversally deformed and delaminated, little is known about their transverse flexibility.

CONTACTS

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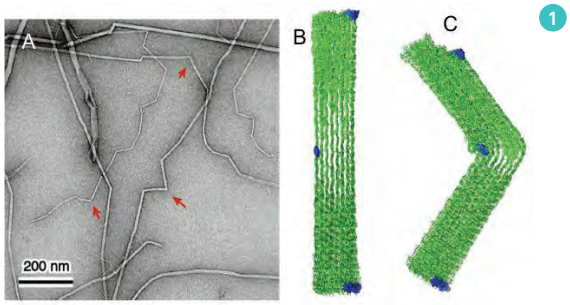
Cellulose nano-crystals (CNCs) observed using transmission electron microscopy often depart from their expected ideal straight shape (Figure 1A). Indeed they often display a continuous curvature together with sharp kinks, which suggest that their transverse moduli are much smaller than the longitudinal one. Experiments aimed at measuring the lateral mechanical property of CNCS face huge difficulties due to (i) a lack of control of the bending direction of the load and to (ii) the imprecision in the measurements of their lateral dimensions. This problem may well be resolved by atomistic simulation.

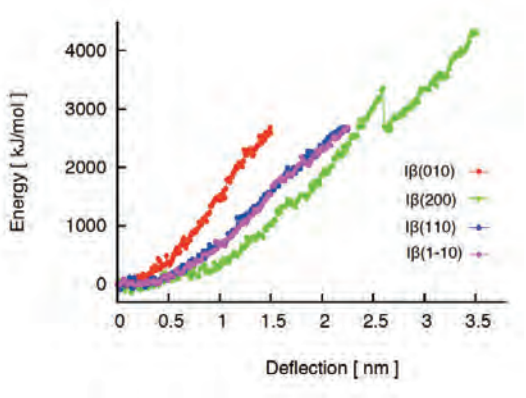


1

1- *A : Typical TEM micrograph of sonicated CNCs. B : Initial model of Iβ. C : Iβ model kinked by 4.5 nm deflection.*

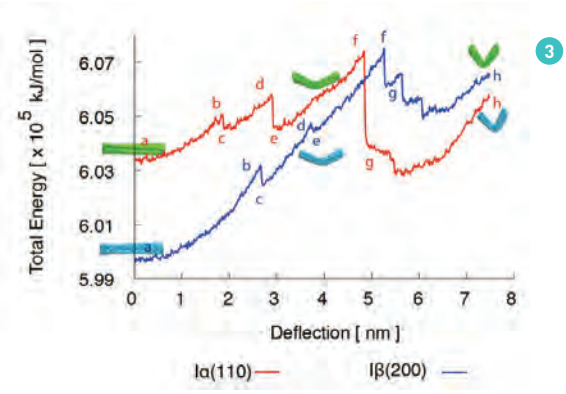
To do this, we have generated molecular models of CNCs (3.5 nm x 3.5 nm x 20 nm), starting from the experimental coordinates of the Iα and Iβ allomorphs. An original simulation protocol applied to the four surfaces of each crystal phase was developed in order to mimic a typical AFM 3-points bending test. Figure 1 gives a snapshot of a Iβ CNC in its initial state (Figure 1B) and after a deflection of 4.5 nm perpendicular to its (110) surface (Figure 1C), leading to a bending angle of 60°. Note that the resulting kinked model looks similar to the defects details revealed in sonicated CNCs (Figure 1A).

Figure 2 reports the energy variation of a cellulose Iβ crystal as a function of deflection for various loading directions. It shows that bending the crystal perpendicular to its hydrophobic (200) surface is easy: in such a deformation, the hydrogen-bonded cellulose sheets can slip past each others and the structural adaptation to deformation has a weak energy cost. On the opposite, the crystal is extremely rigid in the direction perpendicular to the (010) rough surface, as it requires a significant amount of energy for bending.



2

2- *Energy-deflection curves for Iβ CNC for various loading directions.*



3

3- *Energy-deflection curves of Iβ (blue) and Iα (red) kinked by their hydrophobic surfaces.*

When the constraints forcing the bending of the CNCs are released, the nano-crystals recover their straight shape if the initial bending angle had been lower than 60°, which corresponds to the elastic to plastic limit. Above 60°, the crystal is permanently deformed.

The energy-deflection curves when the Iα and Iβ crystals are bended by their hydrophobic surfaces show occasional drops (Figure 3). These energy accidents are caused by relative longitudinal displacements of the hydrogen-bonded cellulose sheets by about 0.5nm, causing a local allomorphic conversion. This conversion is permanent for the Iα phase as the energies of the bended forms, which contain a certain amount of Iβ phase, are lower than the energy of the straight form, made exclusively of Iα allomorph. In contrast, the conversion Iβ → Iα is only temporary for the Iβ phase, since the energy of the mixture of the two allomorphs is systematically higher than that of the neat Iβ.

Many intrinsic properties of the cellulose crystal have yet to be characterized. In this context Cermav is the place of choice.

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The Fascinating Shapes of Polysaccharide Crystals

Polysaccharide crystals offer a rich variety of shapes and structures. Differences are found between native crystals, whose features are controlled *in vivo* by the biosynthesis machinery, and recrystallized material prepared *in vitro* from dilute solutions. In both cases, the size and morphology of the crystals are generally well adapted to imaging techniques such as scanning and transmission electron microscopies (SEM and TEM, respectively), both of which have always been integral to the fundamental study of polysaccharide structures at Cermav.

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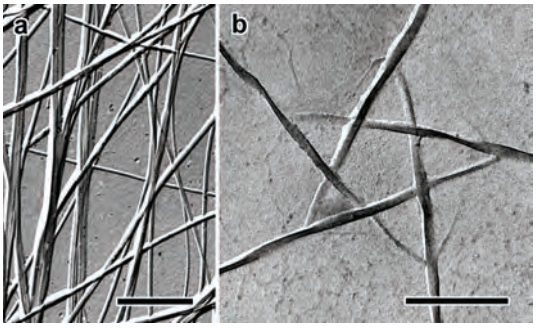
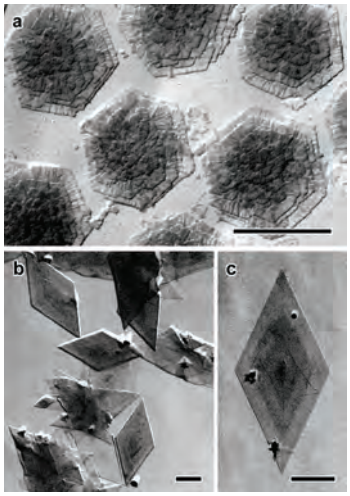
Crystalline polysaccharides such as cellulose and chitin generally occur *in vivo* in a microfibrillar form, acting as structural elements in algal and higher plant cell walls, in the tunic of some sea animals and in the cuticle of some insects and crustaceans. Moreover, some bacteria and microalgae excrete or eject crystalline filaments that entangle and allow the development of colonies. Consisting of parallel molecules, these slender nanocrystals are 2 to 50 nm wide and several micrometers long (Fig. 1).

1



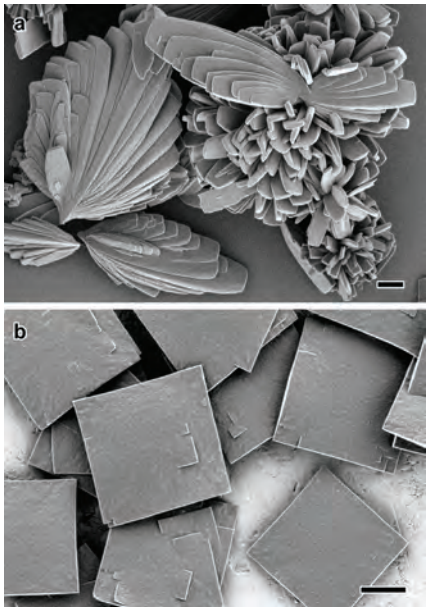
When crystallized *in vitro* from dilute solutions, most linear polysaccharides form lamellar crystals similar to those obtained with synthetic polymers. Their lateral size can reach a few micrometers but their lamellar thickness never exceeds one to a few tens of nanometers. The chain axis is generally perpendicular to the crystal base plane. The strikingly well-defined geometric shape of these single crystals is generally the expression of the unit cell symmetry: hexagonal for xylan (Fig. 2a), lozenge for orthorhombic cellulose triacetate (Fig. 2b), among numerous examples [1].

2



Significantly larger single crystals have been recently grown using short to moderately long chains of linear amylose synthesized *in vitro* by enzymes. In particular, when crystallized in the presence of acetone vapors and 1-naphthol, respectively, amylose forms spectacular flower-like aggregates of acicular crystals (Fig. 3a) [2] and square inclusion complexes (Fig. 3b) [3]. These two examples are quite unique in the field of polymers as the molecular structure of these unusually large single crystals was directly solved using synchrotron X-ray microdiffraction data collected at ESRF [4].

3



At Cermav, TEM has been in operation since 1969 with three successive microscopes under the supervision of H. Chanzy and R. Vuong. The latest one, the Philips CM200 Cryo, installed in November 1993, was selected to deal with the constraints of radiation sensitive polysaccharides (low illumination, low temperature) and perform electron crystallography. SEM has been complementary, the first microscope being installed in November 1991 and operated for many years by D. Dupeyre. Its successor since November 2014, the Quanta-FEG 250 from FEI, is a significant step forward, allowing to observe the crystals with a higher resolution, at low voltage, under variable pressure and in a hydrated environment (ESEM). Since January 2015, both microscopes and related sample preparation equipment are constituting the PMIEL facility, part of the larger NanoBio chemistry platform of Institut de Chimie Moléculaire de Grenoble [5].

1- a) Cellulose microfibrils from Valonia;
b) 5-fold organization of ejectile chitin filaments from Phaeocystis. *TEM images of specimens shadowed with W/Ta. Bars: 500 nm.*

2- Lamellar single crystals prepared by recrystallizing xylan (a) and cellulose triacetate (b,c). TEM images of specimens shadowed with W/Ta. Bars: 1 µm.

3- Large single crystals prepared by recrystallizing amylose in the presence of acetone (a) and 1-naphthol (b). SEM images. Bars: 2 µm.

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Design of Hyaluronic Acid Hydrogels for Tailored Control of Three-Dimensional Neurite Outgrowth

CONTACTS

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Anna Szarpak-Jankowska

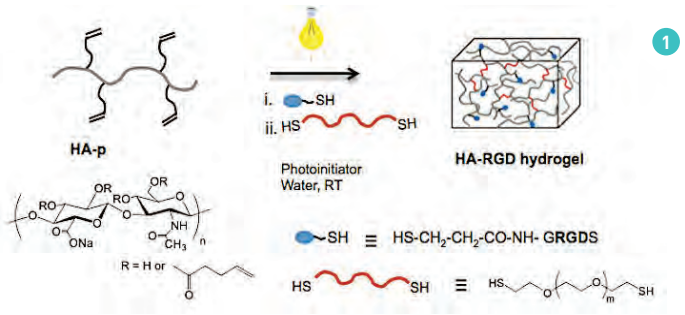
Hydrogels, due to their unique biocompatibility, structural similarity to the natural extracellular matrices, and tunable physical and biochemical characteristics, have been the material of choice for many applications in regenerative medicine. In particular, these water-swollen polymeric networks have emerged as a promising treatment for brain injury. The benefit provided by these scaffolds may partly be attributable to their mechanical properties that can be tailored to mimic native brain tissue. This offers a means to modulate and control neural stem cell behavior. Hyaluronic acid (HA) is a glycosaminoglycan naturally present in the brain extracellular matrix. Although HA-based hydrogels have been previously used for neural tissue engineering, there is still a need to develop HA-based scaffolds with customized functional properties that can provide greater control over cell fate.

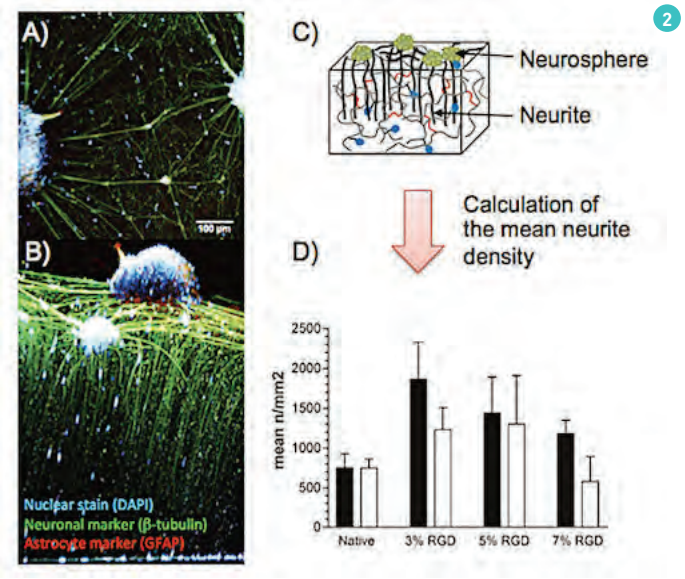
Our aim was thus to optimize adhesion and proliferation of exogenous neural progenitor cells (NPCs) and, to promote neurite outgrowth within HA hydrogels during neuronal differentiation, by modifying the elastic modulus or cell-adhesive RGD (Arg-Gly-Asp) ligand density. To this end, we developed HA-based hydrogels that offer independent control over adhesion site density and mechanical rigidity using thiol-ene photochemistry [1] (Fig. 1).

As neural stem cells preferentially differentiate into neurons when cultured on soft materials, we adjusted here the conditions of the HA gel synthesis to obtain substrates with low stiffness (elastic modulus, G' < 1 kPa). When neural progenitor cells were seeded on the HA gels (G' = 400 or 800 Pa), these scaffolds appeared to promote neuronal differentiation and neurite outgrowth. Interestingly, using two-photon microscopy, we demonstrated for the first time that neurites extended not only on the gel surface but also into the HA gels (Fig. 2).

We also showed that increasing the density of the adhesive peptide anchors did not result in a plateau of mean neurites density but rather in a nearly normal Gaussian distribution going from native (no RGD) HA matrices via gels of HA-RGD with degree of substitution (average number of substituents per repeating disaccharide unit, DS) of 3%, 5% to 7% with the maximum around 3%. Here, we additionally analyzed the impact of substrate stiffness. Whatever the substrate elastic modulus (400 or 800 Pa), the neurites density was maximum for the gels prepared from HA-RGD with a DS of 3%. However, the mean neurites density was higher in the softest gels (G' = 400 Pa).

Given the ability of our HA hydrogels to efficiently promote 3-D neurite outgrowth, these materials may be useful in developing therapies to facilitate neuronal regeneration.





1- Synthesis of HA-based hydrogels with independently tunable crosslinking and RGD ligand density using thiol-ene photochemistry.

2- Analysis of the impact of hydrogel RGD density and elastic modulus on neural progenitor cells by two-photon microscopy: Top (A) and side (B) views of two photon images of a hydrogel of HA-RGD (DS= 5 %, G' = 400 Pa) seeded with NPCs after 17 days of culture. The green β3 tubulin staining indicates the neurites. (C) Calculation of the mean neurite density in the gels at D17. (D) Impact of the GRGDS peptide on the mean density of neurites in 400 and 800 Pa HA gels at D17 after plating.

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Light and Thermoresponsive Hyaluronic Acid-based Nanogels for Photocontrolled Release of Hydrophobic Drugs

Many drug delivery systems have been developed with the aim to overcome several technical issues related to drug administration, such as poor bioavailability, lack of target selectivity and toxicity. To improve the biodistribution of drugs, nanoparticles have been designed for optimal size and surface characteristics to increase their circulation time in the bloodstream, as well as to perform active targeting. In this regard, stimuli-responsive polymeric nanoparticles are attractive platforms for drug delivery due to their ability to change their physical and/or chemical properties in response to an external stimulus, such as light, magnetic field, pH or temperature. Thermoresponsive polymers are widely studied due to their ability to undergo a reversible thermally-induced phase transition without the need of additional reagents. More recently, light-responsive materials have been explored for 'on-demand' drug release by remote activation, with spatial and temporal control.

CONTACTS
Rachel Auzély-Velty
Talitha Fernandes Stefanello

Our group demonstrated that grafting thermoresponsive ethylene-glycol based copolymers onto hyaluronic acid (HA) allowed temperature-triggered assembly of HA into nanogels (1,2). These gel nanoparticles possess many interesting features for drug delivery, like: facile formation, tunable size, easy loading of hydrophobic molecules, possible recognition by the CD44 receptor that is overexpressed on the surface of many tumor cells, contributing for an efficient cellular uptake of the nanogels. Interestingly, the incorporation of light-cleavable units (coumarin ester derivatives) also makes these copolymers light-responsive. The coumarin derivatives act as photoremovable protecting groups inducing a shift in the lower critical solution temperature (LCST) of the copolymer due to the conversion of coumarin esters into carboxylate groups upon light irradiation (near-infrared or UV exposure).

By coupling a copolymer based on diethylene glycol methacrylate (DEGMA) and coumarin methacrylate (CMA) on HA (Fig. 1), we successfully obtained light and thermoresponsive nanogels (diameter ≈ 250 nm) of which the LCST is shifted from 27 °C (below human body temperature) to 38.5 °C (above human body temperature) after UV exposure (Fig. 2). *In vitro* studies demonstrated that our nanogels are biocompatible with mammalian VERO cells and that they can be successfully uptaken by HeLa cells, a human cervical cancer cell line (Fig. 3).

These results encourage further biological studies to gain better insight into the behavior of these light and thermoresponsive nanogels *in vitro* and *in vivo*. These will advance understanding of the molecular parameters that must be tuned to obtain a clinically applicable drug delivery system.

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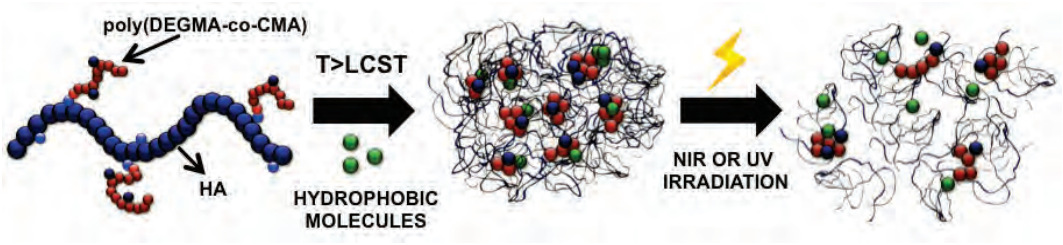

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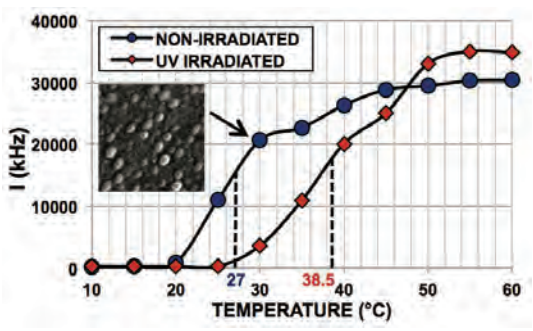

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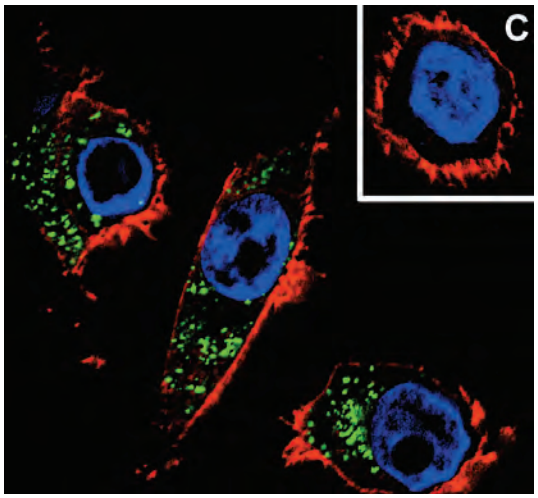

1- Light and thermoresponsive HA-poly(DEGMA-co-CMA) nanogels: formation by temperature increase and disruption by light exposure (shift of LCST).

2- Light-sensitivity of nanogels of HA-poly(DEGMA-co-CMA): the LCST shift from 27 °C to 38.5 °C after UV exposure is determined from the variation of the light scattering intensity as a function of the temperature. Scanning electron microscopy of non-irradiated nanogels (37°C).

3- Confocal microscopy images of HeLa cells incubated for 16 h with HA-poly(DEGMA-co-CMA) nanogels loaded with di-styryl benzene derivative (DSB). Cell nuclei = Hoechst; cellular membrane = Alexa Fluor 667 Phalloidin; DSB = green fluorescence. C = control cell (not treated)

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Engineering LbL Capsules Based on Cyclodextrin Modified Polysaccharides to Control Hydrophobic Drug Delivery

Administration of hydrophobic drugs that constitute a large part of currently available drugs is a great challenge in the field of biomedicine. The encapsulation of hydrophobic substances allowing to prevent their degradation, to control their release, to maximize the efficiency and reduce side-effects is an important topic for scientists and pharmaceutical companies.

CONTACTS

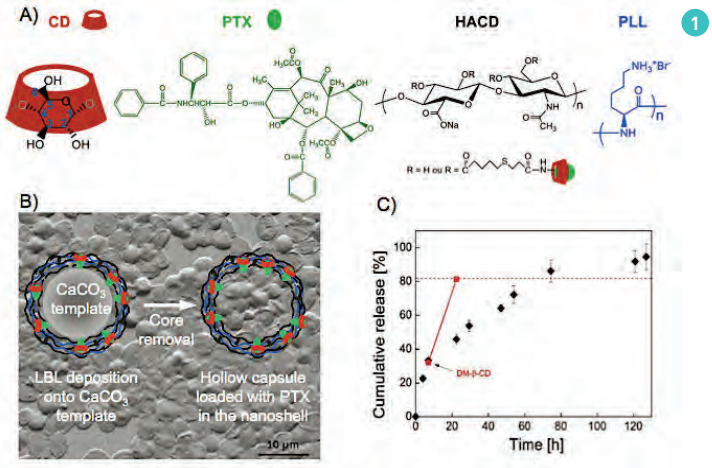
Anna Szarpak Jankowska

Rachel Auzely Velty

Several approaches are used to incorporate hydrophobic molecules in polymeric vehicles such as liposomes, amphiphilic block copolymer assemblies, dendrimers or hyperbranched polymers. However, they are often unstable structures and meet problem of aggregation. Introduced in the 90's, the layer-by-layer (LbL) assembly of polyelectrolytes has emerged as an interesting approach to produce hollow micro-/nanocapsules with tailored architectures and properties. These capsules are fabricated by the sequential deposition of polymer layers onto a sacrificial template followed by dissolution of the core material. Although many studies focused on synthetic polyelectrolytes for designing functional capsules, biocompatible and biodegradable polysaccharide capsules attract a special attention for application in biotechnology and drug delivery.

We built LbL capsules by alternating deposition of hyaluronic acid (HA), a negatively charged polysaccharide, and a positively charged partner such as poly(allylamine hydrochloride) (PAH), poly(L-lysine) (PLL), or chitosan (CHI), on a sacrificial $CaCO_3$ template, followed by its dissolution in mild conditions.[1,2]. We thus established the feasibility of obtaining HA-based microcapsules with an aqueous cavity surrounded by a hydrophilic multilayer shell. However, to entrap hydrophobic drugs in the hydrophilic multilayer film, we proposed to graft β-cyclodextrin (CD) on HA polymer chains and to incorporate the poor water-soluble anticancer drug paclitaxel (PTX) in CD cavities via host-guest interactions. (Fig. 1A) The presence of CDs enables not only the selective entrapment and transport of the drug, but its grafting on HA polysaccharide improves the solubility of PTX and increases the loading capacity.[3] HA-CD complexed with PTX was LbL assembled with PLL, a biocompatible and biodegradable polypeptide (Fig. 1 B). After dissolution of the $CaCO_3$ core, the PTX loaded CD-HA/PLL capsules remain stable in physiological conditions and the hydrophobic anti-cancer drug can be slowly released. Interestingly, the release can also be triggered by addition of dimethylated β-CDs (DM-β-CD) as competitive host molecule in the external medium (Fig. 1C).

Finally, to evaluate the potential of our HA-based capsules for breast cancer treatment, the PTX loaded capsules were incubated with MDA-MB-231 cells (Fig. 2A). It was found that the cells are bound specifically to the capsules through the CD44 receptor that is overexpressed on their surface. At the day 3, the number of cells was ~8-fold lower than the cells cultivated in the absence of capsules or in the presence of unloaded capsules. We have also found that our capsules strongly limit the proliferation and metabolic activity of cancer cells (Fig. 2B). The mechanism of action of PTX loaded capsules is based on the combined action of several phenomena: specific interactions with cancer cells via the CD44 receptor, passive transfer of PTX from CD cavities to the hydrophobic lipid membrane of the cells and local degradation of the capsules by hyaluronidase enzyme secreted by these cells.



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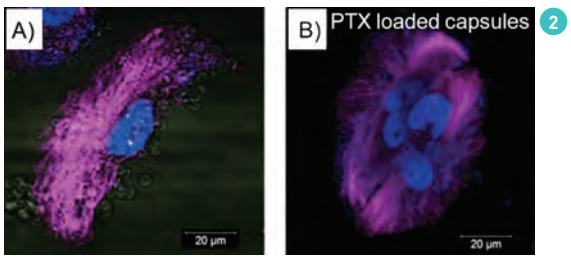

2

1- A) Structure of β-CD, paclitaxel and of the biopolymers (HA-CD and PLL) forming the nanoshell. B) Schematic representation of the fabrication of HA-CD/PLL capsules with the SEM image of the dried hollow capsules. C) In vitro release profile of paclitaxel from the [(HACD-PTX)/PLL]$_4$/HA capsules triggered by addition of DM-β-CD.

2- Microscopic observations of microcapsules/MDA-MB 231 cells interactions after 24h of culture. A) Interaction of HA-containing capsules with MDA-MB-231 cells (nuclei stained in blue, β-tubulin in pink). B) Fragmentation of nuclei and bundles of mictotubules in presence of PTX loaded capsules.

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Self-Assembly of Carbohydrate-Based Block Copolymers: Sub_10nm Thin films

The self-assembly paradigm in science has matured over the past two-decades to a point of sophistication that one can begin to exploit its numerous potentialities in nanofabrication. Indeed, among the bottom-up strategies, self-assembly is an incredibly powerful concept in macromolecular engineering that offers an invaluable tool for the preparation of 2D and 3D discrete nanostructures, ranging from materials science to molecular biology, which are often not accessible by any other fabrication process.

CONTACTS
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Sami Halila

The design of smaller, faster, more efficient devices is a major scientific and technological challenge, driven in part by a constant need for smaller features and higher resolution patterns. To this end, self-assembling soft materials continue to play an important role in societal and economical goals for smaller and hierarchically nanostructured devices. Carbohydrate Block Copolymer Systems leading to thin films belong to this class of materials where self-assembly takes place on nanometer length scales. This makes them ideal for emerging nanotechnologies, including applications, for instance, in drug delivery, nano-templating, nano-porous membranes, organic optoelectronics and anti-reflection coatings. To make those systems more attractive for bit-patterned media applications, attempts were made to achieve smaller domains and sizes. These attempts have, however, had only limited success. To overcome these two bottlenecks – substitution of petroleum molecules and resolution limit – our group has recently made an important breakthrough by using carbohydrate-based BCP and has achieved the highest resolution ever reached to date (5 nm features) [1].

Among the recent achievements using those new carbohydrate BCP systems is the high-performance of organic field-effect transistor (OFET) memory devices. Reported here are the nonvolatile electrical characteristics of pentacene-based organic field-effect transistor (OFET) memory devices created from the green electrets of olisaccharide-based block copolymer maltoheptaose-block-polystyrene (MH-*b*-PS), and their supramolecules with 1-aminopyrene (APy). The very hydrophilic and abundant-hydroxyl MH block is employed as a charge-trapping site, while the hydrophobic PS block serves as a matrix as well as a tunneling layer. The orientation of the MH nanodomains could be well controlled in the PS matrix with random spheres, vertical cylinders, and ordered horizontal cylinders via increasing solvent annealing time, leading to different electrical switching characteristics. The electron-trapping ability induced by the horizontal-cylinder MH is stronger than those of the random-sphere and vertical-cylinder structures, attributed to the effective contact area. The electrical memory window of the device is further improved via the supramolecules of hydrogen-bonding of APy to the MH moieties of MH-b-PS for enhancing the hole-trapping ability. The optimized device using the horizontal cylinders of the supramolecule electret exhibits the excellent memory characteristics of a wide memory window (52.7 V), retention time longer than 10(4) s with a high ON/OFF ratio of > 10(5), and stable reversibility over 200 cycles.

This study reveals a new approach to achieve a high-performance flash memory through the morphology control of carbohydrate-based block copolymers and their supramolecules [2 & 3].

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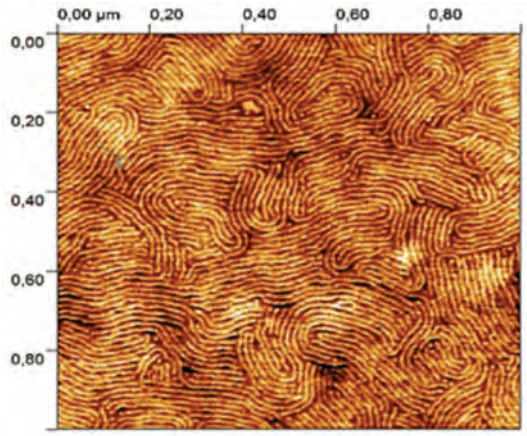

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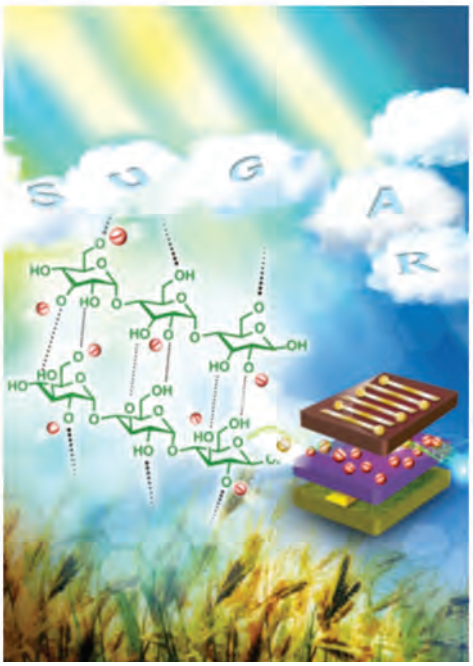

Carbohydrate Block Copolymers:

1- Sub_10nm thin film.

2- Dielectric thin film toward high performance non-volatile transistor memory devices.

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[2] Y.C. Chiu, I. Otsuka, S. Halila, R. Borsali & W.C. Chen, Adv. Funct. Mat., 24, 2014, 4240-4249

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Self-Assembly of Carbohydrate-Based Block Copolymers: Nanoparticles

Carbohydrates are one of the most abundant raw materials that have attracted growing interest due to their "green" aspects, biocompatibility, biodegradability, and bio-recognition properties. It is currently attracting much interest in various sectors and their industrial applications at the nanoscale level will have to expand quickly in response to the transition to a bio-based economy. In this context, the self-assembly of carbohydrate block copolymer systems (BCP) at the nanoscale level via the bottom-up approach, has allowed the conception of original glyconanoparticles.

CONTACTS

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This challenge is strongly motivated by the potentials offered by mimicking nature and by exploiting, at the nanoscale level, the potential of oligo- and polysaccharides towards the developments of novel glyco-nanoparticles: design and control of the shape, their encapsulation with hydrophobic/hydrophilic active molecules, their decoration/coating and ability to interact via specific sugars with proteins.

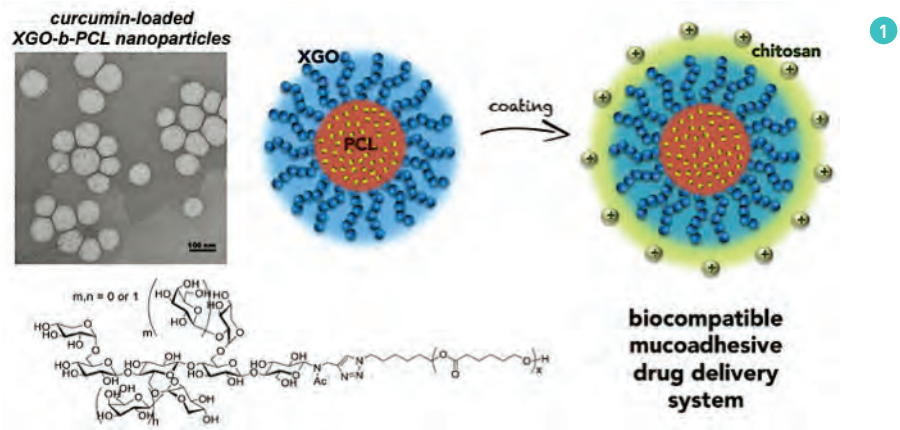
Recently, a variety of poly-/oligosaccharides-based block copolymer systems involving maltoheptaose (MH), xyloglycan (XG), cyclodextrine (CD), have been synthesized using 'click chemistry' and studied their self-assemblies in solution. In aqueous media, poly-/oligosaccharide-containing BCPs form glyconanoparticles (micelles or vesicles) with an outer shell/external corona made of the carbohydrate block and an inner core made of the hydrophobic synthetic blocks.

For instance, the controlled self-assembly of maltoheptaose-block-polystyrene (MH-*b*-PS), into nanoparticle in aqueous media and their capacity of gold nanoparticle encapsulation was demonstrated [1].

Second example deals with xyloglucan based BCP: XGO-*b*-PCL systems loaded with curcumin (anti-inflammatory active molecules). Curcumin was successfully encapsulated within the PCL-based hydrophobic core. The coating of XGO-*b*-PCL nanoparticles with chitosan resulted in an increase of approximately 10 nm in the mean particle radius and positive surface charge.

Surface plasmon resonance (SPR) measurements demonstrated the mucoadhesive properties of chitosan-coated nanoparticles by its exceptional ability to interact with mucin through electrostatic forces. Finally, *in vitro* studies demonstrated that loading curcumin into copolymer nanoparticles showed reduced cytotoxicity when compared to free drug, and higher cytotoxic effects against B16F10 melanoma cells than L929 fibroblast cells [2].

Another example deals with the conception of biocompatible and UV-degradable glyconanoparticles made from maltoheptaose and poly(methyl methacrylate) [3], the micelles obtained from self-assembly of the BCPs from acetone solution into water through nanoprecipitation method showed the ability to encapsulate Nile red and then release it in a polar/non-polar interface. This feature can be considered as a promising concept in the development of news drug delivery systems.



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1- Novel xyloglucan-block-poly(ε-caprolactone) (XGO-b-PCL) nanoparticles coated with the mucoadhesive polysaccharide, chitosan, for delivering of hydrophobic drugs in mucous membranes.

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Synthesis of Neoglycoconjugates for 'Clickosylation' of Polymers

Today, polymer synthesis has attained a control of the architectures at the molecular level allowing a fine-tuning of final properties. At the opposite, even if significant progress have been achieved over the last decades, carbohydrate chemistry still suffers in the control of polyfunctionality and stereochemistry (chirality) of carbohydrate building blocks. From the association of polymer & glyco-science was born the term glycopolymers which are defined as pendant sugar moieties along the polymer backbone or at the end of the chain. Glycopolymers are able to strongly interact with proteins, carbohydrates or others species through multivalent interactions, mimicking natural polysaccharides.

CONTACTS
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Issei Otsuka
Redouane Borsali

Since 10 years, in the 'Physico-Chemistry and Self-assembly of Glycopolymer' Group of Cermav, a new research area has emerged with the preparation and studies of self-assembly of block or graft amphiphilic copolymers. These sugar-based BCPs self-assemble in solution to give nanoparticles made of outer corona of sugars and in thin film giving rise to controlled nanostructured materials.

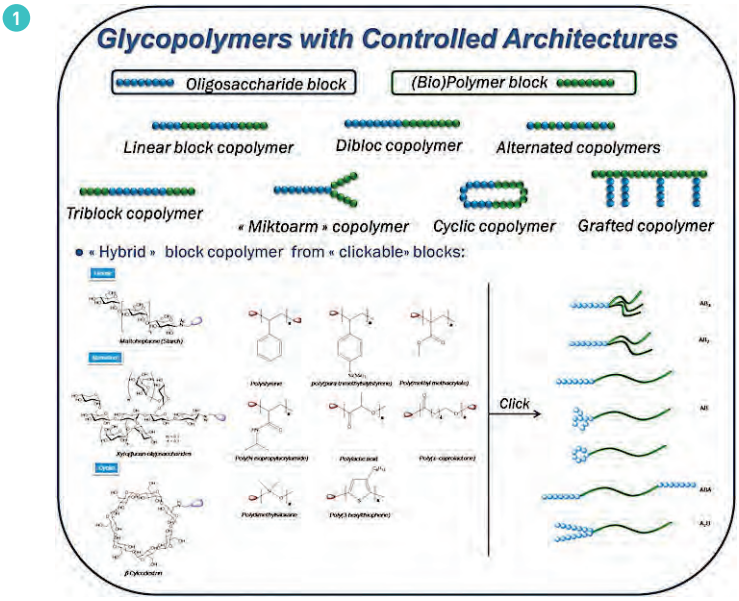
The applications areas are diverse and includes cosmetic, nanomedecine and nanoelectronic. A significant breakthrough was realized with the obtaining of nano-organized thin films made from hybrid glycopolymers with size and domain spacing approaching 10 nm while the state-of-the-art is 20 nm when using petroleum molecules [1]. For both applications, the introduction of biosourced poly-/oligo-saccharides block is highly desirable in regards to their high hydrophilicity, chemical functionalities, biocompatibility, biodegradability and their relevant biological activities.

To achieve a high degree of homogeneous and ordering nanostructures, narrow molecular weight distribution of blocks are mandatory. Well-defined maltoheptaose and cyclodextrin as well as oligosaccharides from xyloglucan, chitosan and chondroitin sulfate were coupled to various synthetic and functional polymers.

Usually, the synthesis of carbohydrate-based copolymers followed a three-step strategy consisting of:

1. preparation of scalable and size-homogeneous oligosaccharides from biosourced polysaccharides obtained by enzymatic or chemical processes;
2. conjugation with "clickable" functions at anomeric position. The regioselective introduction of clickable functions into specific positions of unprotected oligosaccharides is one of the key steps. The traditional reductive amination, modified Kotchekov reaction and Shoda's activation using 2-chloroimidazolinium salt in aqueous solution were used [2];
3. « Click » coupling of pre-formed blocks referred as « grafting onto » method. Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC) was first performed with high yield. Residual metal catalyst in BCPs could limit their future development. Currently, we investigate the metal-free and/or cleavable click reaction such as thiol-maleimide addition [3].

All those systems are generally characterized with the state-of-the-art techniques including NMR, MALDI, GPC..... and light scattering.



1- *Precise synthesis of carbohydrate-based BCPs with controlled architectures*

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Lectins as Targets in Bacterial and Fungal Infections

Microbial infections are the leading cause of mortality worldwide and represent a real public health problem with the increase of immunocompromised patients. With the emergence of resistant strains and the lack of new effective therapeutics, it is now urgent to develop new anti-infectious strategies.

CONTACTS

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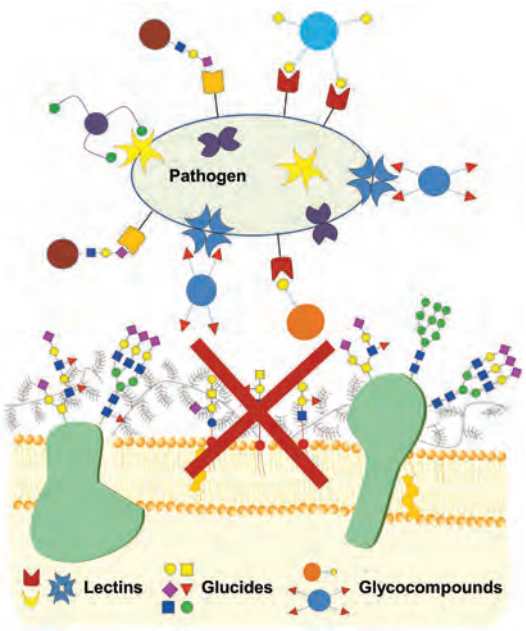
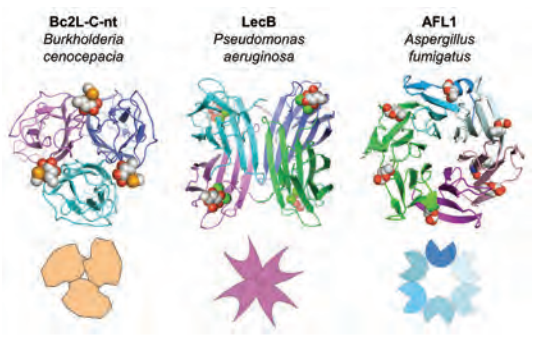
Anne Imberty

Pathogenic microorganisms often use glycoconjugates present on the host epithelia during the first steps of infection. Microbial receptors called lectins binds to the host glycans resulting in specific recognition and adhesion. Lectins play therefore an essential role in host-pathogen interactions and have become the targets for the development of glycocompounds as new anti-infectious agents in antiadhesive therapeutical strategies (Figure 1). It is essential to identify and characterize the lectins involved and their ligands in order to better understand the host recognition mechanisms and we used a structural glycobiology approach from gene to 3D structure.

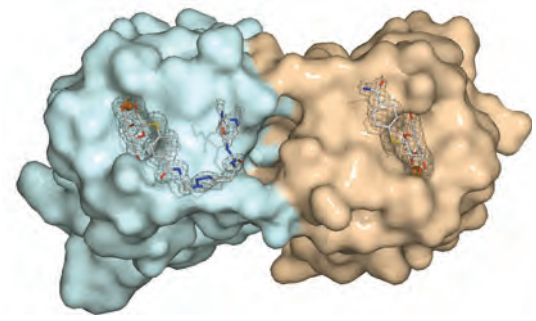


1- *Schematic of microbial anti-adhesive strategy.*



2- *Diversity of fold, arrangement and multivalency of microbial lectins.*

Pseudomonas aeruginosa is a life-treatening pathogen for cystic fibrosis patients and is responsible for 10% of nosocomial infections worldwide. It presents two tetrameric lectins, LecA and LecB, specific for galactose and fucose, respectively that are involved in the pathogenesis of this bacterium. Nanomolar affinity was recently obtained against LecA with a bivalent galactosylated molecule characterized by X-ray crystallography and molecular modelling (Figure 3) [1]. Tetravalent calixarene-based glycoclusters have been shown to reduce biofilm formation, bacterial adhesion to human epithelial cells and lung injuries in vivo in a murine model [2].



3- *Electron density for a divalent glycocompound bound to LecA from* Pseudomonas aeruginosa *at 1.65Å.*

Soluble lectins from several bacterial and fungal opportunistic pathogens mainly responsible for severe broncho-pulmonary infections are under scrutiny. Thanks to high resolution crystal structures with human oligosaccharides, important information were obtained on the lectin multivalency and the lectin binding sites were dissected at the atomic level (Figure 2). In collaboration with glycochemists and physiopathologists, glycocompounds have been designed, synthetized and evaluated. Multivalent molecules are the most promising since they permit to attain higher affinity and specificity than monovalent one.

AFL, a fucose binding lectin has been identified and characterised in a major airborne opportunistic fungal pathogen *Aspergillus fumigatus* whose infections are associated with high mortality rate. AFL has been localised at the surface of conidia, has pro-inflammatory activity and presents six non-identical fucose binding sites [3]. Only di, tetra or hexavalent fucosylated molecules with sufficient linker have shown an increase of about one order of magnitude in affinity. In vitro tests are underway.

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New Lectins for Research and Diagnostics

The large diversity and complexity of glycan structures together with their crucial role in many biological or pathological processes require the use of novel receptors for deciphering their structures and conformations. Lectins are proteins that bind complex oligosaccharides with fine specificity. They are therefore classically used for characterising, imaging or targeting glycoconjugates and, when printed on microarrays, they are very useful tools for profiling glycomes. Lectins can be purified from natural sources, such as plants, bacteria or invertebrates, but the availability and quality of natural-occurring lectins could be an issue for reliable and reproducible data.

CONTACTS
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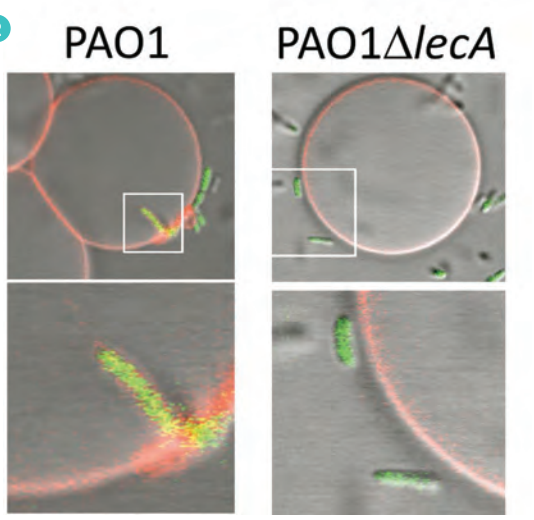
Annabelle Varrot

Development of recombinant lectins gives access to such reliable and reproducible material. Lectins from pathogenic micro-organisms evolved together with human tissue glycosylation and therefore exhibit high specificity for our sugars. The LecA lectin from *Pseudomonas aeruginosa* binds specifically to the oligosaccharide epitope on human glycosphingolipid Gb3. It is produced in recombinant form in *Escherichia coli*, and can be used as a research tool to study the formation of "glycolipid zipper". *In vitro* experiments with Gb3-containing giant unilamellar vesicles revealed that LecA multivalent binding to glycolipids is able to force the formation of invagination in the vesicles [1]. The interaction between bacterial lectins and human glycosphigolipids result in the engulfment of bacteria into ephitelial cells through LecA/Gb3-mediated lipid zippering.

2



1- Lipid zipper induced by Pseudomonas aeruginosa on giant unilamellar vesicles dependent of Gb3 binding by LecA.

Lectins from more exotic origin such as mushrooms or algi can also be produced in bacteria. *Psathyrella velutina* is a wild mushroom that can be collected around Grenoble and which produces a seven bladed beta-propeller lectin with six binding sites able to recognize oligosaccharides with terminal N-acetylglucosamine (GlcNAc). The PVL lectin, produced in recombinant manner, is an excellent marker for cancer cells since their altered glycosylation pathway exposes GlcNAc that is not usually detected on the surface of healthy cells [2].

The next step is the design of artificial lectins, with controlled specificity but also controlled number of carbohydrate binding sites. Such synthetic glycobiology approach already resulted in the design of libraries of neo-lectins presenting a variable number of binding sites [3]. From the accumulated knowledge on protein–carbohydrate interactions, it will be possible to alter the specificity at each site for precise targeting of specific glycol-epitopes. The panels of artificial lectins that could be produced will be invaluable tools for deciphering the glycocode, and targeting cells with altered glycosylation. Such tools can be used for research, for quality control in the production of glycoproteins, but also as new markers for diagnostics and vectorisation.

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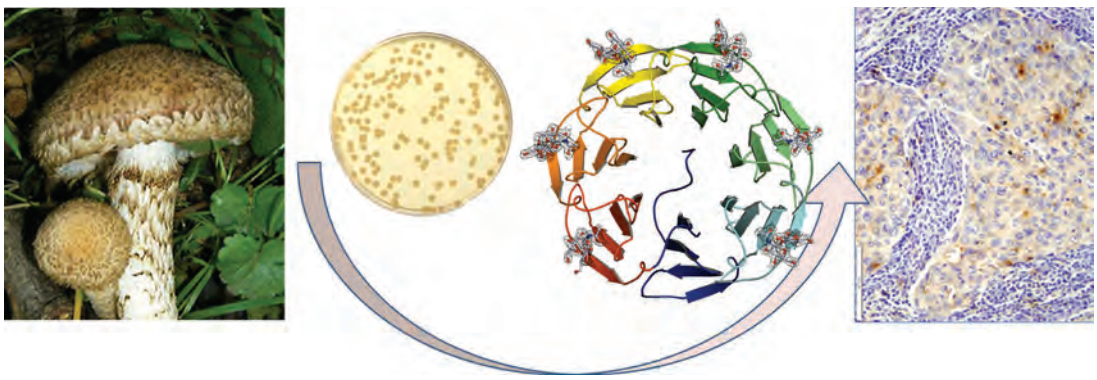

2- A gene from fungi was used to produce PVL in bacteria, resulting in a crystal structure. The recombinant lectin was used for labelling of cancer tissues.

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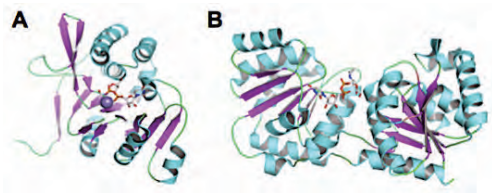
Glycosyltransferases: The Enzymes that Build Sugars

CONTACT

Christelle Breton

Glycosylation is quantitatively the most significant biochemical reaction on earth and the key player is the large glycosyltransferase (GT) family. These enzymes form glycosidic bonds by catalyzing the transfer of saccharides from a donor (usually a nucleotide-sugar) to a wide variety of acceptors. Acceptor substrates consist of carbohydrates, proteins, lipids, and numerous small molecules such as antibiotics, flavonols, ... The diversity of GT reaction products is reflected in their numerous biological roles ranging from structure and storage to signaling. This diversity is also largely exploited in the pharmaceutical industry in the search for new active molecules (i.e. as antibiotics, anti-diabetics and anti-cancer agents). Aberrant cell surface glycosylation is a hallmark of numerous cancer cells and defects in the glycan biosynthetic machinery in humans generally have severe deleterious consequences. Given their prevalent role in both normal and pathological processes, GTs are attractive targets.

Structural information available to date has revealed that the large GT family is characterized by a conserved 3D architecture since these enzymes exhibit only two types of folds (and variants thereof), termed GT-A and GT-B (Figure 1). A better understanding of the structure and catalytic mechanisms of GTs represents an important biological issue. This will be exemplified through the following examples that are studied at Cermav.



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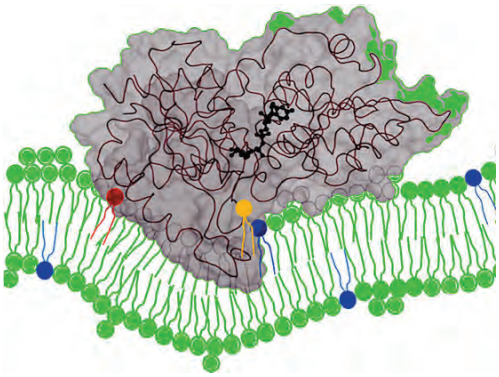

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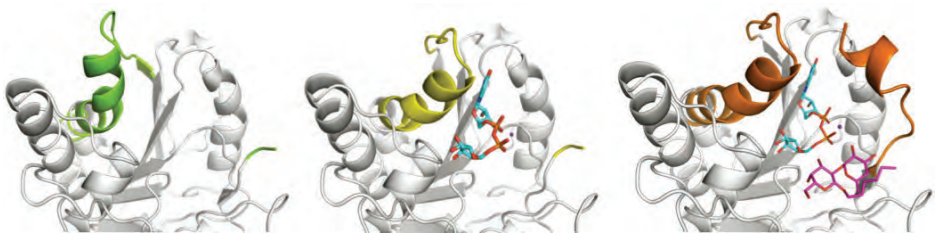
1-Classical GT-A fold (A) and GT-B fold (B) of glycosyltransferases.

2- 3D structure of MGD1 and proposed model for its membrane association.

3- Loop movements upon ordered donor and acceptor binding in a blood group synthase.

Galactolipids are considered as the most profuse lipid class on earth and are essential for assembly and function of photosynthetic membranes. In *Arabidopsis*, MGD1 is the major enzyme providing the bulk of MGDG (monogalactosyl-diacylglycerol) necessary for the massive expansion of thylakoids. MGD1 is a monotopic protein localized to the inner envelope membrane of chloroplasts. The molecular characterization of this enzyme has been recently determined as well its membrane binding properties using model lipid monolayers (Figure 2). This paved the way for a better understanding of its mode of action and gave insights into chloroplast membrane biogenesis. Future structural studies will explore the regulation of MGD1 activity by phosphatidic acid, a known allosteric activator of the enzyme. This represents a central mechanism coupling phospholipid and galactolipid syntheses in plants.

One major challenge in the field of glycobiology remains the dissection of the reaction mechanisms of GTs. The rational design of potent inhibitors has been complicated by the complex reaction mechanism and unusual conformational plasticity of these enzymes, which undergo significant conformational change in the active site during the catalytic cycle (Figure 3). This research area suffers from a lack of insightful 3D-structures of ternary complexes. Our aim is to advance our knowledge in the catalytic mechanisms of GTs using a combination of computational methods and kinetic crystallography, focusing on model enzymes (such as the blood group synthases). Mechanistic information that is expected from these studies will provide insights into the donor-acceptor interplay in the active site, and will help in the search for potent drug/inhibitor candidates for this class of enzymes.



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Enzyme'n Click Assembly of Neoglycoconjugates Towards Biomedical Applications

Carbohydrates are essential components of cells in all living organisms, not only as structural component or as energy source but also as promoter of the cellular communication. Indeed, oligosaccharides and glycoconjugates (glycolipids and glycoproteins) play a major role in the social life of cells and are involved in many physiological and pathological processes (immunity, inflammatory response, cancer, infection by pathogens). Unraveling the structure/function relationship of complex oligosaccharides should have a significant impact on human health. It could contribute to improve the early diagnosis of several pathologies and to develop efficient treatments against pathogen infections or tumor progression among others. However, the low bioavailability of natural glycoconjugates, their large heterogeneity, their difficult isolation and characterization are obstacles to these ambitions.

CONTACTS
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Bernard Priem
Eric Samain

The development of efficient tools for the synthesis of glycoconjugates is thus required and is identified as an international strategic issue. Despite significant advances in the last decade, the chemical synthesis of oligosaccharides remains a difficult work which generally leads to low amount of isolated products. Bio-inspired enzymatic approaches offer much better outcomes. Natures has developed sophisticated synthesis of complex oligosaccharides with a wide range of different enzymes and proteins are now becoming readily available with the rise of genomics, bioinformatics and biotechnology.

A low cost and efficient microbial process for the synthesis of oligosaccharides at gram scale has been developed at Cermav. High cell density culture of metabolically engineered E. coli strains allows the production of a wide range of biologically important glycans (gangliosides, lewis antigens, blood group antigens, human milk oligosaccharides) from an exogenous disaccharide lactose acceptor. Efficient internalization and glycosylation of lactoside precursors allows a straightforward access to conjugatable glycans through click chemistry. Alkene, alkyne, amino-functionalized oligosaccharides are produced and conjugated by CuAAc, thiol-ene, acylation or Diels Alder reaction to proteins, polymerizable groups... The resulting neoglycoconjugates can be used as antigens for cancer immunotherapy, as cell adhesion molecules for tissue engineering, as antivirals or as ligands in carbohydrate arrays. Glycomimetics with improved stability against glycoside hydrolases, or increased immunogenicity or affinity toward lectins have also been efficiently synthesized from exogeneous carbohydrate analogues. Inihibitors of both neuramaminidase and hemagglutinin of influenza viruses have been obtained by biosynthesis from N-modified sialic acids.

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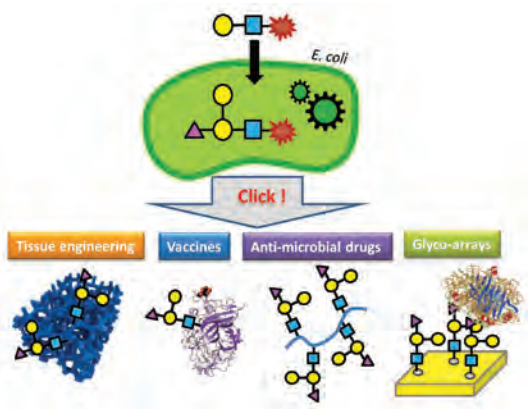

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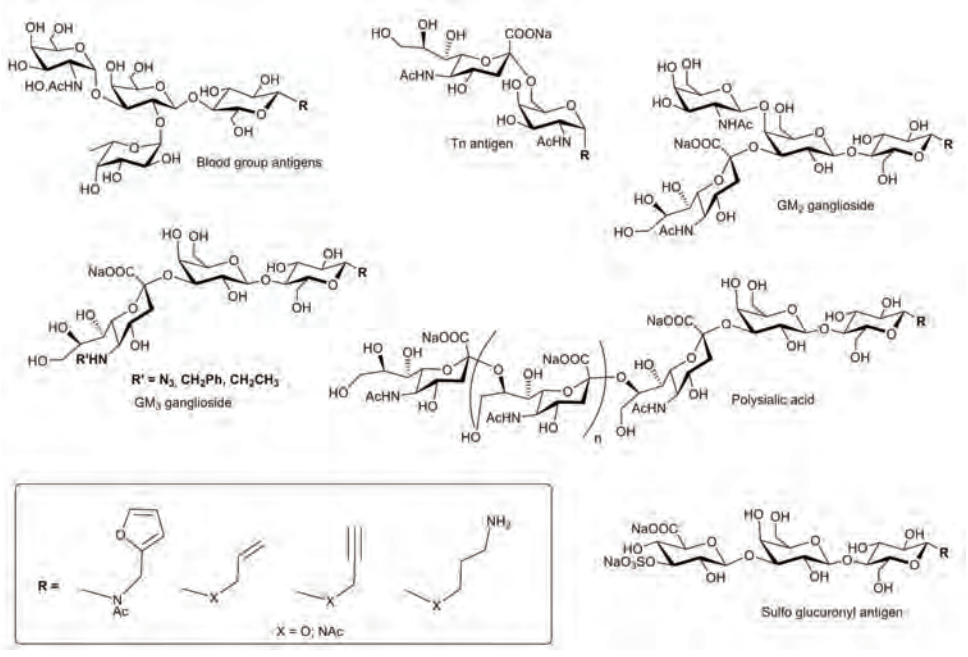

1- Production of neoglycoconjugates by microbial enzymatic synthesis combined with click chemistry.

2- Examples of clickable oligosaccharides produced in recombinant E. coli cells.

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Crazy Polysaccharides: Screening Active Enzymes on Polysaccharides

CONTACT
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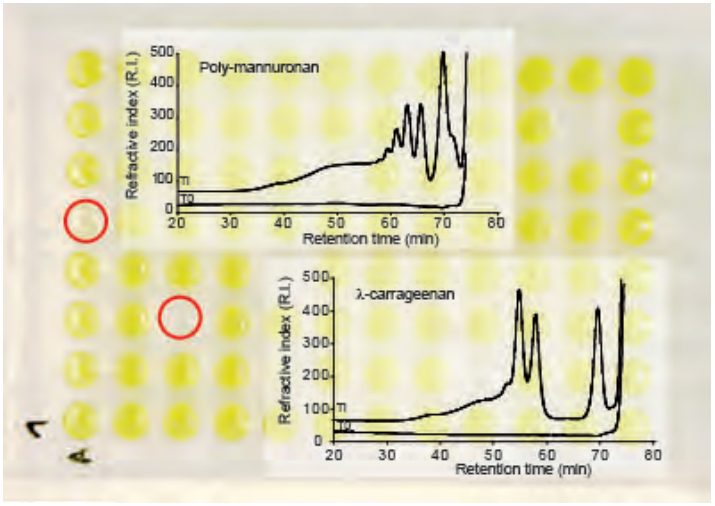
Enzymes can cleave the glycosidic bonds that join the monosaccharide moieties of polysaccharides by hydrolysis (glycoside hydrolases, GH), by β-elimination (polysaccharide lyases, PL) and, as found recently, by oxidation (lytic polysaccharides mono-oxygenases, LPMO). The structural diversity of polysaccharides requires a large number of enzymes for their biodegradation. For the hydrolytic mechanism alone, more than 160 E.C. numbers have already been described, corresponding roughly to the number of substrates that they cleave (IUM-MB enzyme nomenclature at www.chem.qmul.ac.uk/iubmb/). Independently, the classification of these enzymes in families based on amino acid sequence similarity has led to the description of 132 families (CAZy classification at www.cazy.org), most of which grouping together enzymes of differing specificity.

For long time the discoveries of novel polysaccharide degrading enzymes resulted of the identification of enzymes activities in complex microorganism extracts followed by several purification steps. This strategy needs, at starting point, the availability of the polysaccharide substrate prior to seek for the corresponding degrading enzymes. The "substrate-driven" exploration of new enzymes allowed evidencing most of the enzymes activity which peptide sequences are in databank.

Despite the exponential deluge of sequence data resulting from modern sequencing technologies (i.e. genomic era), the pace of discovery of new GH, PL or LPMO enzymes remains constant and comparatively modest. The new sequence data essentially increases the number of enzymes classified in existing families, and does not contribute to the number of biochemically-characterized enzymes or of enzymes with a known 3-D structure. Therefore, the gap between the number of biochemically characterized and uncharacterized enzymes increase exponentially. However, "sequence-driven" strategies consisting in biochemical characterization of proteins having divergent sequences with characterized enzymes or studies of proteins located in polysaccharides utilizing loci (PUL) allowed to ascribe function to new polysaccharides degrading enzymes.

In this context, we have developed a methodology allowing to screen complex microorganisms extracts [1] or heterologous overexpressed proteins selected based on bioinformatics analyses against a wide collection of polysaccharides. The screening is conducted at microtiter plate scale and enzyme activities are revealed by detecting the reducing end produced during cleavage of the glycosidic bonds (colorimetric method) or by the observation of newly formed oligosaccharides by mass spectrometry or chromatography.

The screening of complex bacterial extract allowed evidencing new enzymes (e.g. ulvan lyase of Nonlabens ulvanivorans) or enzymes with new mode of action (e.g. methyl-porhyranase of *Pseudoalteromonas atlantica* T6c [2]). Bioinformatic analyses of genomes combined with functional screening of overexpressed protein lead, for example, to the discovery of the polysaccharide lyase PL24 family [3].



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Biotechnological Syntheses of Lipochitin-Oligosaccharides as Plant Growth Promoters

Some soil micro-organisms are able to associate with plant roots to form symbioses which play very important ecological and agronomic roles. Rhizobial bacteria enter a symbiotic association with leguminous plants, resulting in differentiated bacteria enclosed in intracellular compartments called symbiosomes within nodules on the root. The nodules and associated symbiosomes are structured for efficient nitrogen fixation. Arbuscular mycorrhizal symbiosis, which results from an association with fungi of the Glomerales order, allows plants to improve their water and mineral nutrition. Both symbioses involve signals molecules that are synthesised by the microbial partner.

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These Nod and Myc factors are lipochitin-oligosaccharides (LCOs) which consist of an oligomeric backbone of β-1,4-linked *N*-acetyl-D-glucosaminyl residues (GlcNAc), *N*-acylated with a fatty acid chain at the non-reducing terminal residue. Their structures differ in the number of GlcNAc residues present in the backbone, the nature of the fatty acyl group, and in the substitution of the reducing and non-reducing terminal residues (e.g. *O*-acetyl, *O*-fucosyl, *O*-carbamoyl or *O*-sulfate group). In the context of growth of global population which will inevitably increase pressure on the environment, reduction of the use of fertilizers and pesticides, via their replacement by bio-inspired growth promoters like LCOs might prove an efficient alternative. The availability of LCOs is a critical issue, since chemical synthesis or purification from rhizobia cultures have only enabled access to minute quantities of such compounds so far. The use of high-cell-density culture of metabolically engineered *E. coli* has allowed gram-scale production of precursors of Nod and Myc factors. Tetra-*N*-acetyl-chitinpentaose and tri-*N*-acetyl chitintetraose have been isolated from *E. coli* expressing nodC and nodB genes encoding for chitin oligosaccharide synthase and chitin oligosaccharide *N*-deacetylase, respectively. A chemical acylation of the only free amino group with fatty acids afforded a range of natural LCOs.

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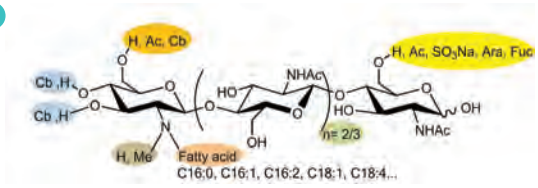

Sulfated LCOs were also synthesized by overexpressing nodH, the gene encoding for the 6-*O*-sulfotransferase. More recently a complementary approach based on *in vitro* enzymatic remodelling of chitinoligosaccharides has also been developped. Chitinoligomers (COs), which result from depolymerization of chitin, the second most abundant polysaccharide after cellulose but also a major byproduct of the crab industry, are industrially produced. *In vitro* production of natural LCOs as well as analogues has been carried out at preparative scale with a recombinant chitin deacetylase from *Vibrio cholerae* able to regioselectively withdraw an acetate from the non-reducing penultimate GlcNAc unit of COs. This activity provides a free amino group which can be further *N*-acylated with a fatty-acid to give analogues of LCOs. Alternatively the non-reducing GlcNAc unit was removed by β-*N*-acetylglucosaminidase treatment, followed by N-acylation to give natural LCOs.

1- *Molecular structures of lipochitin-oligosaccharides.*

2- *Chemo-enzymatic routes towards synthetic lipochitin-oligosaccharides.*

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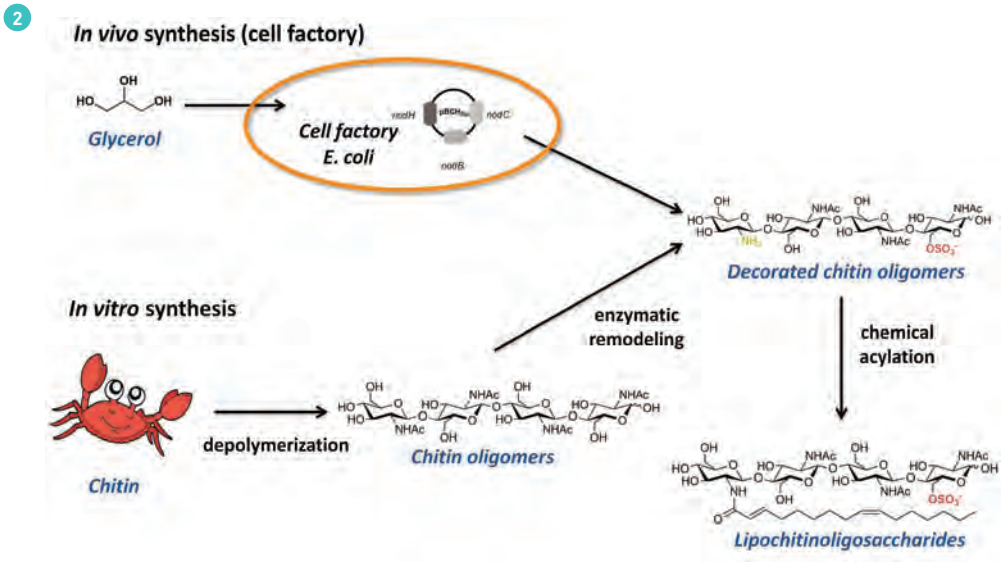

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Structural Insights into Xyloglucan Biosynthesis

Expression, purification and structure of glycosyltransferases responsible for xyloglucan biosynthesis.

CONTACTS

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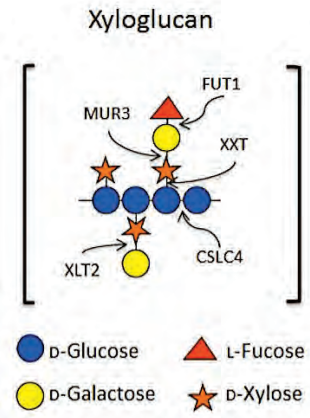
Christelle Breton

The synthesis of plant cell wall matrix polysaccharides occurs through the concerted action of hundreds of glycosyltransferases. These enzymes catalyze the transfer of a sugar residue from an activated nucleotide-sugar onto a specific acceptor. Because cell wall matrix polysaccharides exhibit an important structural complexity, their biosynthesis must be adequately controlled within the plant cell. Xyloglucan (XG) is the most common and abundant hemicellulose found in the primary cell wall of vascular plants, where it is commonly described to play a role in coating and tethering the cellulose microfibrils, and many enzymatic and non-enzymatic proteins are involved in finely tuned rearrangements of its structure or interaction with cellulose microfibrils, thus permitting cell wall dynamic and eventually plant growth [1].



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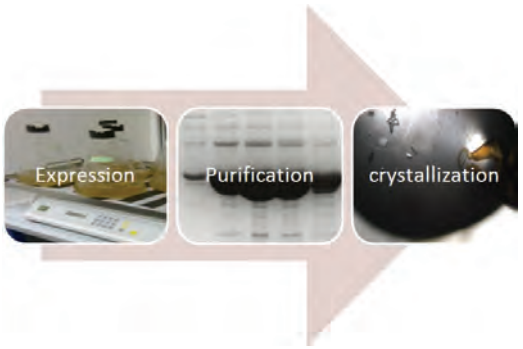
Xyloglucan and other complex polysaccharides of the plant cell wall are also envisioned for human uses, as a sustainable bio-resource comprising high potential molecules for varied applications. Plant polysaccharides are, for example, cornerstone additives in food industry and cellulose can potentially be converted to glucose then ethanol for biofuel production. These potent applications raised an urgent need for a better understanding of plant glycosyltransferases (GTs) characterization, from gene identification to protein expression and purification and the structural study of their enzymatic mechanisms [2].



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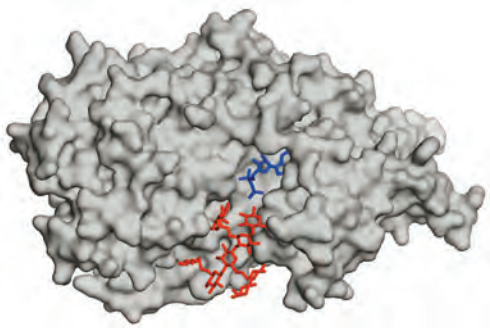
At Cermav, we developed a fruitful strategy for plant GTs expression using eukaryotic host systems (Insect cells, Pichia pastoris...), affinity purification, protein quality control assessment and eventually protein crystallization. In 2015, this effort was rewarded by the structural characterization of the xyloglucan α-1,2-fucosyltransferase in complex with a xyloglucan-derived acceptor named XLLG and a structural analog of the substrate donor (GDP), at a 2.5Å resolution. To the extent of our knowledge this is the first time that a plant cell wall biosynthetic enzyme has been successfully characterized at the structural level, offering the opportunity to decipher at the molecular level the catalytic mechanism for xyloglugan polysaccharide fucosylation.



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1- Xyloglucan structure and name of enzymes involved in its biosynthesis.

2- Flow-chart for GTs crystallization studies.

3- Structure of AtFUT1 protein (in grey) with GDP (blue) and xyloglucan oligosaccharide (red).

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Production of Glycosaminoglycans by Recombinant Bacteria

Glycosaminoglycans (GAGs) are anionic polysaccharides composed of repeating disaccharide units of uronic acid and N-acetylhexosamine. Animal glycosaminoglycans except hyaluronic acid are normally sulfated. GAGs are found in extracellular matrices and present a broad variety of structures depending of the tissue and the physiological state of the cells.

CONTACT
Bernard Priem

GAGs possess very important biological properties, an example being the GAG-issued 'heparin', broadly used a an anticoagulant medicine. There is an increasing interest in finding biological activities of GAGs for human therapy. This purpose obviously relies on the availability of GAGs, which are traditionally extracted from animal tissues such as bones, chicken cockscombs, shark fins, among others. However, the natural complexity of GAGs makes it impossible to obtain well-defined polymers suitable for medical applications.

GAGs are also encountered in the capsula of some bacteria such as *Escherichia coli*, *Streptococcus* and *Pasteurella multocida*. Bacterial GAGs are rather simple because they are not sulphated (Fig. 1). Several highly-producing selected strains are already used to produce hyaluronic acid, chondroitin and heparosan at industrial scale.

Deciphering biological activities of GAGs and enlarging their utilisations in human medicine require structurally characterized compounds. This can be achieved by controled chemical or chemo-enzymatic synthesis. Unsulphated bacterial chondroitin and heparosan can serve as precursors of chemical or enzymatic sulphation.

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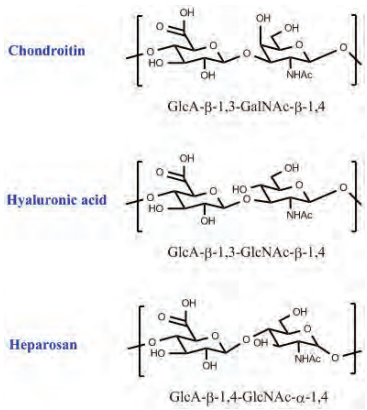

We have recently adressed the design of recombinant bacteria producing well defined GAGs. Contrary to natural strains, the metabolic pathways of recombinant microorganisms can be more elaborated leading to new valuable products. Hence, the way to go is to express selected enzymes involved in the synthesis of animal GAGs to produce the desired defined animal-like GAGs in confined bio-reactors. For now, we have been able to settle a recombinant *E. coli* strain co-expressing recombinant heparosan-synthases as well as an heparosan-degrading enzyme, thus producing heparosan oligosaccharides in gram scale (Fig. 2) [1].

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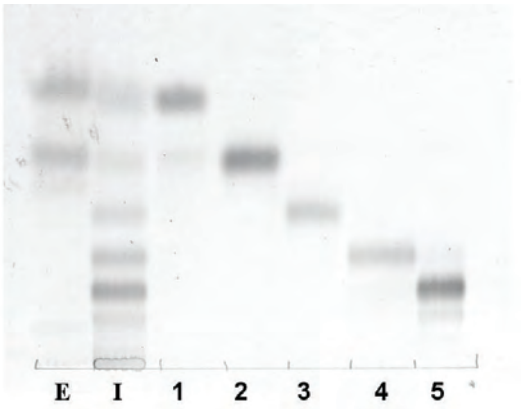

Another achievement is the design of new strains synthesizing GAGs possessing "clickable" or conjugatable groups (alkyne, alkene, amine, diene...) groups at their reducing extremity, providing a cheap source of building blocks for coupling chemistry (Fig. 3) [2].

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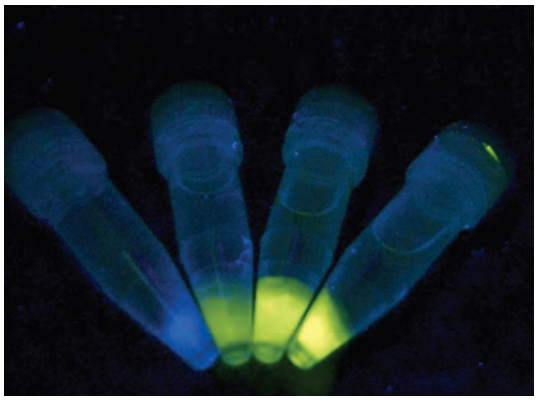

1- Disaccharide units of bacterial glycosaminoglycans.

2- Thin layer chromatography profiles of culture extracts of recombinant E. coli *strain producing heparosan oligosaccharides. From left to right: E, extracellular fraction; I, intracellular fraction; purified heparosan oligomers, the number being the repeating disaccharide unit number.*

3- Ethanol pellets of fluorescein heparosan prepared by click-chemistry. From left to right: normal heparosan; increasing ratios of FITC/heparosan coupling conditions.

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Databases for Glycosciences

CONTACTS

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Structural glycoscience is a progressing field of research where the diverse structural and functional role of carbohydrates (in the form of oligo, polysaccharides and glycoconjugates) are investigated and established throughout a wide diversity of experimental and theoretical methods. Glyco3D is a portal of several inter-linked databases covering the 3-D features of mono-, di-,oligo-, poly-saccharides, glycosyltransferases, lectins, monoclonal antibodies, and glycosaminoglycans-binding proteins. A common nomenclature has been adopted for the structural encoding of the carbohydrates: every molecule is described by 4 different types of representations to cope with the different usages in chemistry and biology.

BiOligo contains the 3D structural information of more than 250 entries of bioactive oligosaccharides which have been subjected to conformational sampling to determine their conformational preferences: several low energy conformations are available for each entry. Collection of annotated NMR data of about 180 oligosaccharides is also provided.

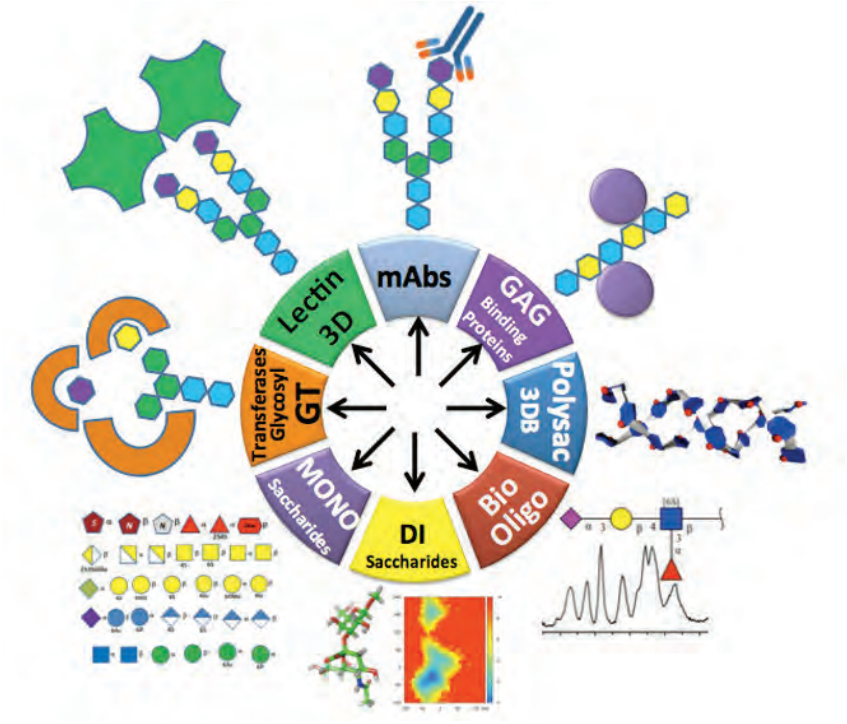
PolySac3DB contains the structural information of about 150 polysaccharide entries that have been established using various structure determination techniques. Attention was given to the recording of the available diffraction patterns as the original experimental data from which the structures were established (120 diffractograms have been collected).

Lectin DB. Lectins are oligomeric proteins which as per present knowledge act as macromolecular tools to decipher sugar-encoded messages. More than 1500 lectin 3D structures among which 60% have been determined in complex with a carbohydrate ligand are available in the database.

GTs-DB. Glycosyltransferases (GTs) constitute a ubiquitous group of enzymes that catalyze the synthesis of glycosidic linkages by the transfer of a sugar residue from a donor to an acceptor. At present, more than 400 GT crystal structures are available that have been grouped into 38 families.

GAG-binding proteins DB and MAbs-DB gathered the 3D data from protein-oligosaccharide complexes which have been solved from X-ray crystallography, respectively. There is a relatively low number of structures available at the present time, but unvaluable insights are provided into the binding mode of monoclonal antibodies to carbohydrates, and to the interaction involving physiologically important molecules as heparin, heparan sulfate, chondroitin sulfate, dermatan sulfate, hyaluronic acid and keratan sulfate.

Each individual database stands by itself. A search engine is available that scans the full content of all the databases for queries related to sequential information of the carbohydrates. The interconnection of these databases provides an opportunity to characterize the 3D features that a given oligosaccharide molecule can take in different environments. The databases, which have been manually curated, were developed with non-proprietary software. They are available at http://glyco3d.cermav.cnrs.fr.



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S. Pérez et al., Methods in Molecular Biology, 2015, 1273

The Quality Management System

The ISO 9001 quality management standard, a well-recognized standard in the international community, was chosen in 2004 to improve and manage the organization of the laboratory. Thus, Cermav set up a quality management system answering all the requirements of the standard: process approach (see figure 1), procedures, internal audits...

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The organization system, called 'Source' (for Système d'Organisation de l'Unité de Recherche CErmav), was elaborated in order to centralize and structure the data of the unit and provide management and information tools to all the scientific, technical and administrative staffs. The new ergonomic intranet (figure 2) was designed into five parts, developed so as to initiate and involve the staff in the Quality Management System: organizational structure, procedures, processes, resources, and... improvement. The available documents are precisely realized by the permanent staff and the updating is ensured through an interactive interface. Source was selected in 2010 by the CNRS in order to provide a documentary management tool for the implementation of a quality management kit for the research units.

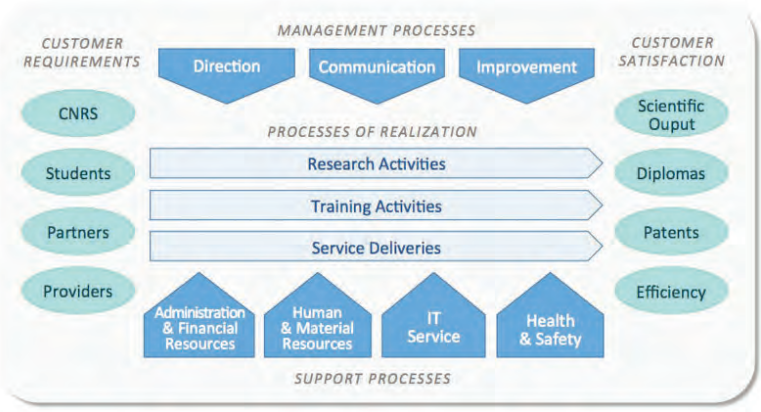
According to the principle of continuous improvement, inherent to any quality system, Source is constantly evolving. Now, our efforts aim two axes : the knowledge capitalization and the traceability of our research activities.

Knowledge Capitalization consists in perpetuating the scientific information of the laboratory according to three axes. Firstly, the laboratory notebooks, which use is generalized, are being followed up since 2013 in a database allowing multicriteria research. Secondly, the protection of the scientific data needs the development of databases which contain all the information resulting from experiments or analyzes (mass and NMR spectroscopy, amidotheque....). Finally, the regular digital archive of the scientific data, is realized according to a standard operating modus operendi.

Traceability of research activities. The second objective is to extend the quality to the research activities with an organization, in project mode, nowadays largely used in the H2020 or ANR projects. At Cermav, PhD students must follow a roadmap indicating schedules, a set of tasks and other objectives. The traceability of research work is ensured by a project map which contains various information on the research project (financing, planning...) and allows to make a link with the laboratory notebook and the associated digital archive.

The association of the intranet and the quality approach has fully reached the expected objectives by helping to improve the overall functioning of the laboratory, allowing the transmission of knowledge and providing good visibility for our partners.

1



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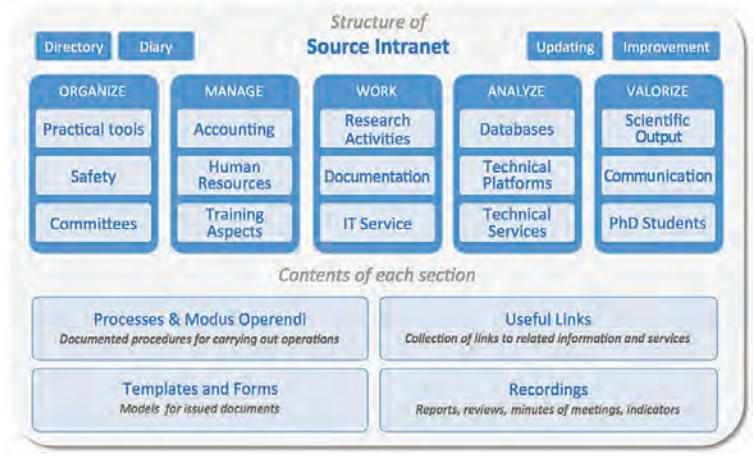
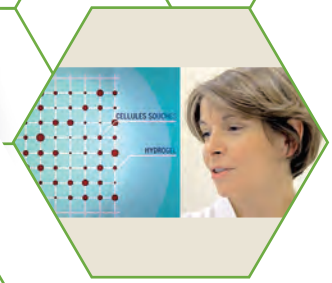


FURTHER READING
A. Rivet et al., La démarche qualité d'une plate-forme technologique, 2013, 978-2-9546225-0-7
H. Valeins et al., Démarche qualité en recherche publique, Techniques de l'Ingénieur, 2011, 7

Hall of Fame

Dr. Yoshiharu Nishiyama receiving the prestigious Markus Wallenberg Prize 2015 from the hands of Carl XVI. King of Sweden (© photo Ulf Palm).

Dr. Nishiyama also received the Hayashi Jisuke Award in 2012 and the Japan Wood Research Society Prize in 2007.



The Scientific Innovation Prize of the BNP Paribas Fondation was awarded in 2013 to Prof. Rachel Auzély Velty.

CNRS MEDALS

Silver Medal of the Chemical Institute to Dr. Anne Imberty (2013). Dr. Henri Chanzy (1981)

Bronze Medal. Dr. Laurent Heux (2003). Dr. Bernard Henrissat (1988). Dr. François René Taravel (1978)

Crystal to Dr. Eric Samain (2005)

Dr. Anne Imberty received the Charles Dhéré Prize of the Académie des Sciences in 2011 and the Roy Whistler Carbohydrate Award in 2004.

In 2009, the Cermav was awarded the Trophée INPI de l'innovation en Rhône-Alpes.

SCHOLARLY SOCIETY AWARDS

The Prize of the Groupe Français des Glucides des Sociétés Françaises de Chimie et Biochimie to Dr. Sébastien Fort (2008), Prof. Rachel Auzély (2002), Prof. Sylvain Cottaz (1998)

The Prize of the Groupe Français des Glucides to Dr. Bernard Henrissat (1986)

The Prize of the Groupe Français des Polymères to Dr. Henri Chanzy (1975)

Dr. Henri Chanzy receiving the Anselme Payen Award of the American Chemical Society in 1991. Dr. Chanzy also received the Lifework Alceru Prize in 2006.

Prof. Fernand Barnoud (1926-2013) received the Foulon Prize of the Académie des Sciences in 1980.

Contributors

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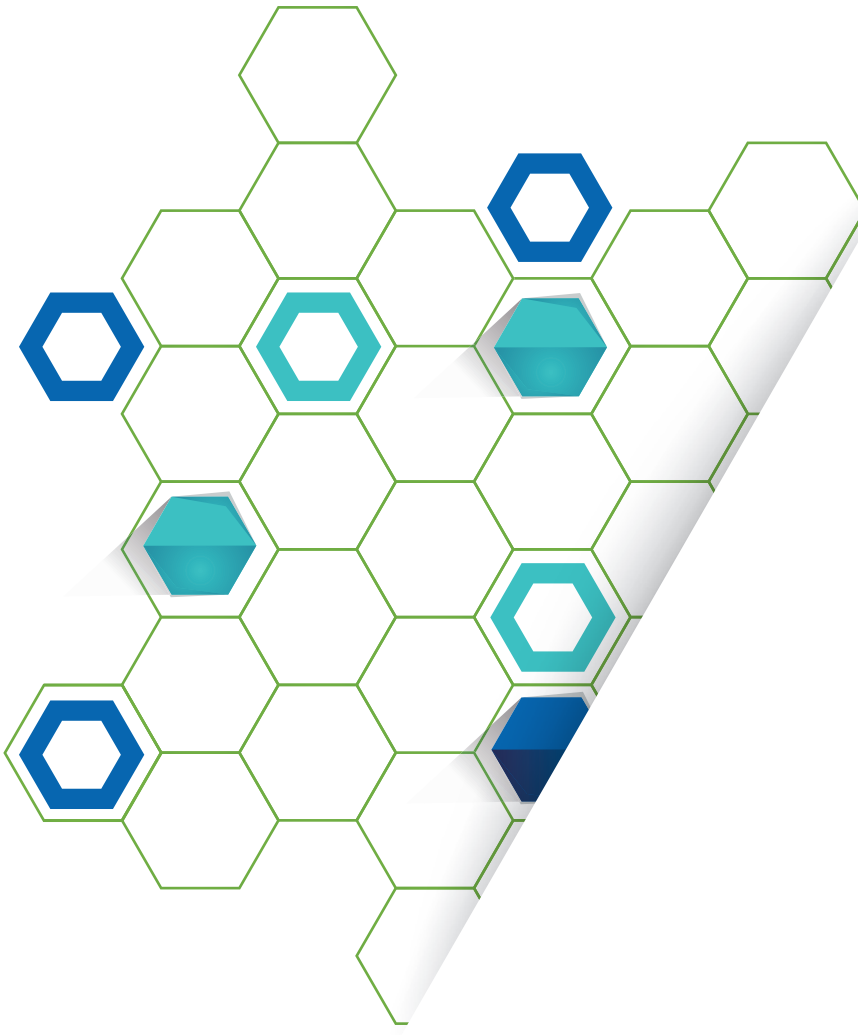

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