INRA
SCIENCE & IMPACT

UR 1268

UR I268 bia Biopolymères Interactions Assemblages

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Key figures
71 scientists and engineers
48 technicians and administrative staff
39 doctoral and post-doctoral students
1 Platform (BIBS: Biopolymers Structural Biology)
1 UMT Nova²cidre
1 SFR IBSM
5 technical facilities

Research Unit

Biopolymers Interactions Assemblies

General presentation

In a context of demographic and food transitions, environmental and energy constraints, and awareness of the impact of these factors on public health, the actors of agriculture, agri-food and agro-forestry of the 21st century must face a major challenge of **sustainability of production and food chains**. Taking this issue into account will lead to fundamental changes in terms of production, processing, transport, distribution, consumption, valorization, … of agricultural raw materials.

To support these breakthrough *scenarii* and promote sustainable agriculture and food in its bioeconomic, social, environmental, health and cultural dimensions, academic research must focus its efforts on this major challenge.

In this context, the **Biopolymers Interactions Assemblies Unit (BIA)**, a component of the CEPIA department (Characterization and Elaboration of Agricultural Products), focuses its research project towards the **sustainable transformation of agricultural resources and plant biomass**. The main challenge is to integrate the entire chain of transformation of these resources from their **construction** to their **deconstruction** during their final use.

Research Topics

The overall scientific objective of the BIA unit is to better understand the **construction and deconstruction phases of assemblies of biopolymers** (protein, polysaccharides) and biomolecules (lipids, phenolic compounds) in **plant organs** (seeds fleshy fruits)**, or**, through their transformation, in **formulated foods and bio-based materials** (liquid foams, cellular solids, emulsions, gels, films, particles...), to improve their quality and functionality in a context of sustainability.

To achieve this objective, **3 scientific priorities** have been identified:

1. to control the quality of crop production to consolidate the different uses in a context of sustainable agriculture
2. to improve and develop healthy and sustainable formulated foods for targeted functionalities
3. to design bio-sourced composite materials in a perspective of biomass valorization

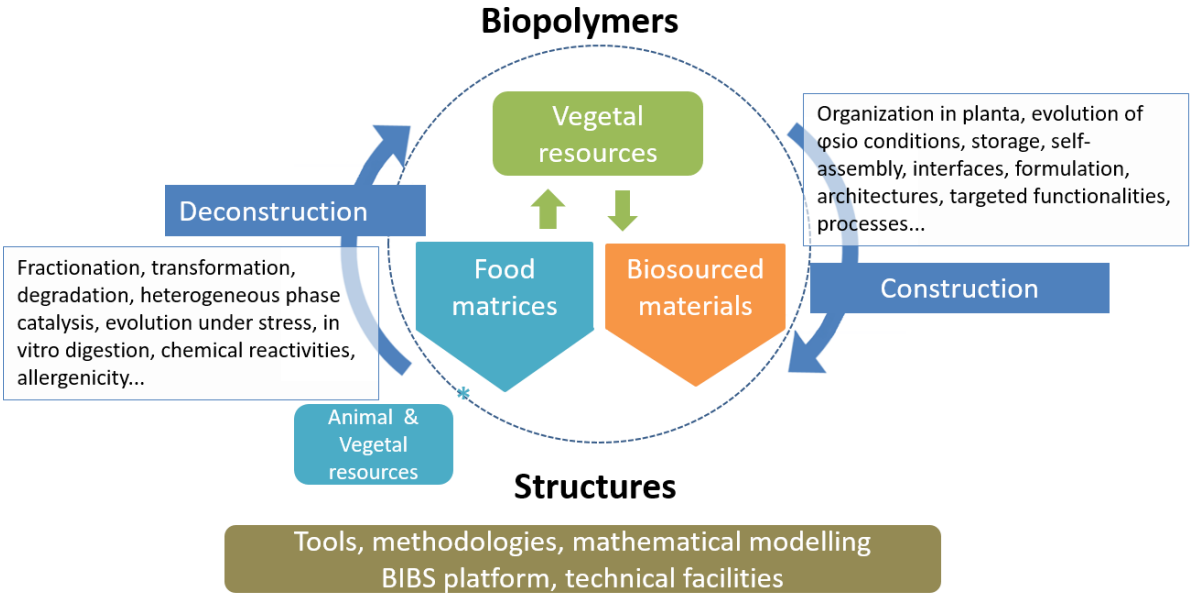
This research is conducted within the Unit's 7 thematic research teams:

- Plant cell wall and parietal polysaccharides (PVPP)
- Lipoprotein and polysaccharide-protein structures (ELIPS)
- Polyphenols, reactivity, process (PRP)
- Interfaces and dispersed systems (ISD)
- Allergy to food proteins (ALL)
- Materials, creation and behavior (MC2)
- Nanostructured assemblies (NANO)

which benefit from the methodological and instrumental support of the Structural Biology Biopolymers Platform (BIBS).

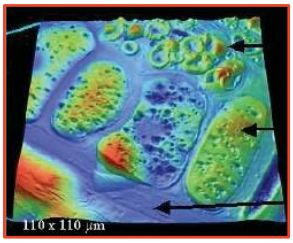
In addition, in 2012, we established a Federal Research Structure: Biopolymer Engineering for Matrices and Materials Structuration (SFR IBSM n°4202) with the UMR Engineering of Processes, Environment, Agri-food (GEPEA- Nantes University, Oniris, IMT, CNRS) in order to propose a more integrated research offer from the molecule to the final product.

Concept



Instrumental platform and technical facilities

Platform Biopolymers Structural Biology (BIBS)



The BIBS platform offers a set of analytical methods to characterize the fine structure, organization, interactions and location of biopolymers of plant or animal origin in biological systems (plants, organs, cells...), food and bio-sourced materials. This structural characterization covers scales from nanometer to millimetre. It makes it possible to describe very precisely the architecture of systems in relation to their biological, functional or technological properties.

The platform's tools also allow to evaluate the variability of structure and composition on large series of samples. The BIBS platform includes four analytical domains: mass spectrometry, NMR, microscopy, and phenotyping / chemotyping. It is IBISA certified, part of the BioGenOuest GIS and ISO9001 certified. It is recognised as Strategic Collective Infrastructure by INRA. Contact: H. Rogniaux, www.bibs.inra.fr

Five facilities complete the device of the BIA Unit

- Protein purification – V. Solé
- Antibody production – O. Tranquet
- Recombinant proteins – V. Solé
- Experimental bakery – H. Chiron
- Design and manufacture of accessories for scientific instrumentation (CRAIS) – P. Papineau

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Partnership

In addition to the thematic collaborations inherent in the different objectives of the research teams, the BIA Unit has undertaken certain strategic collaborations.

UMT Nova²cidre : through a five-year collaborative program, the UMT Nova2cidre Joint Technology Unit, located on the INRA Le Rheu site (35), formalizes a research and development partnership between the PRP (Polyphenols, Reactivities & Processes) team of the BIA Unit and the Institut Français des Productions Cidricoles (IFPC, Technical Centre). Currently, this program meets two main objectives: (i) adapt products to consumers based on taste, visual and olfactory perceptions; (ii) adapt processes to environmental requirements and participate in the emergence of more ecological raw material production systems, while guaranteeing product quality.

SFR 4202 IBSM : the BIA Unit has created in 2012 a Federal Structure of Research with the GEPEA joint research unit (Engineering of Processes, Environment, Agri-food) under the supervision of Nantes University, Oniris, IMT, CNRS (100 permanent staff, including 68 research professors, 3 CNRS researchers). The 2 units formed the new IBSM Federal Research Structure: Biopolymer Engineering for Matrices and Materials Structuration, led by INRA. The challenges of IBSM are to create an attractive and visible national centre in the agri-food sector by exploiting the complementarities of disciplinary skills in the field of structural studies and functionalities, multi-scale approaches, process engineering and the valorization of agri-resources. The federation, through its multidisciplinary approaches, also makes it possible to offer a wider range of training to Master students and doctoral candidates.

Research Teams

The **Plant cell wall and parietal polysaccharides** team aims to develop knowledge on the plant wall, its implementation, evolutions and physicochemical and mechanical properties in relation to the processing capacity and quality of products derived from plant matter. The main plants of interest are fruits, cereal grains, grass stems and fibre plants.

The **Lipoprotein and polysaccharide-protein structures** team is specifically interested in the structure and assembly of fruit cuticular bases (including cereal caryopses) and the formation of the amyloid-protein matrix of cereal grains.

The objective of the **Polyphenols, reactivity, process** team is to better understand the reactivity and fate of phenolic compounds during apple juice and/or cider transformation processes. The aim is to characterize the new polyphenolic structures and study their functional properties (solubility, macromolecular interactions, colour, etc.) in order to better control the consequences of this reactivity on organoleptic and nutritional qualities.

The objective of the **Interfaces and dispersed systems** team is to understand the role of assemblies, at different scales, in the structuring-stability-destructuring mechanisms of dispersed systems. The structuring of formulated foods, via interactions between lipids and other components of the environment (proteins, polysaccharides), modulates their physical and chemical stability, as well as their evolution during their simulated digestion *in vitro* (deconstruction), and its control makes it possible to propose ways of optimizing formulations guaranteeing their nutritional, health and sensory qualities.

The objectives of the **Allergy to food proteins** team are to elucidate the allergenicity of foods (1) by understanding the allergen and its structure (native or modified) within the food matrix, (2) by elucidating the biological mechanisms involved (passage of the intestinal mucosa, sensitization or initiation of the allergic reaction). Using several cellular and animal models, in vitro, ex vivo and in vivo, we decompose allergen/organism interactions at different scales (molecular, cellular, tissue) and study the environmental factors that modulate it.

In the context of products from major cultures, particularly starchy ones, the objectives of the **Materials creation and behavior** team, based on increasing structural scales, are to determine (1) the molecular and supramolecular organization of native starches and starchy materials, (2) the links between the molecular mobility of biopolymers and the behavior, in particular mechanical, of composite solids, (3) the mechanisms governing the creation of starch-based cellular solids, in particular the growth of bubbles in viscoelastic media, and their mechanical behavior.

The work of the **Nanostructured assemblies** team is positioned in the field of biosourced materials for (i) the substitution of petroleum-based materials with materials from renewable resources, (ii) the reduction of the impact of human activity on the environment by using both renewable and low environmental impact resources, and (iii) the design of innovative materials exploiting specific properties of polymers of biological origin.

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Highlights 2017-2018

Unclouding the mechanisms behind haze episodes in cider-chain beverages – *S. Guyot*
This thesis work carried out as part of a Casdar project of the UMT Actia Nova²cidre aimed to understand the mechanism of appearance of physicochemical haze that occur in three cider-chain beverages (cider, apple juice and pommeau) initially limpid. The composition of the haze particles was accurately analyzed (polyphenols, polysaccharides, proteins, metals and minerals) and the mechanisms of interactions responsible for the appearance of the haze were studied in model solutions.

Towards controlled lipid content in cereal starches – *D. Marion & H. Rogniaux*
Starch-lipid complexes, along with storage proteins, make excellent markers of corn endosperm (the seed's storage tissue) vitreousness. Here we studied the source of these lipids, and particularly lysophosphatidylcholine, during corn kernel endosperm development. By coupling MALDI imaging with the spatio-temporal expression of genes involved in lipid metabolism in the developing seed, we were able to forge a scenario connecting lipid trafficking in the endosperm, storage protein biosynthesis, and lipid metabolism in the starch. These findings set the stage for potential new strategies on the farming practice front and on the varietal selection front (nitrogen nutrition, resistance to environmental stressors), for controlling the lipid content of cereal-crop starches.

African insects with huge potential for nutritious human diet – *C. Genot*
Entomophagy- eating insects- is endorsed by the FAO as a way to address global food security challenges. Our research has demonstrated excellent nutritive potential for human diet in three insects eaten in Cameroon: *Rhynchophorus phoenicis* larvae, wether wild or farmed, and caterpillars of *Imbrasia truncata* and *I. epimethea*. *Rhynchophorus* larvae are rich in lipids and thus make a potentially good energy supplement. Less lipid-rich but with alpha-linolenic acid as the main fatty acid, *Imbrasia* caterpillars are a rich source of high-quality proteins, containing all the essential amino acids. These caterpillars thus make a good option to address protein-energy undernutrition and readjust ω3 fatty acid intakes. The storability of the flours was also assessed.

Wheat grain growth is strangled by bonds between cell-wall polymers – *A-L. Chateignier-Boutin, L. Saulnier & F. Guillon*
Polymers in the cell walls of cereal grains are assigned important biological, nutritional and technological roles. By studying the composition and wall structure of the developing wheat grain, unexpected results were observed for external tissues 1) the presence of lignins at early growth stages 2) changes in cell-wall properties likely due to covalent crosslinking between cell-wall polymers. Our findings suggest that major structural cell-wall changes occur late into development in the outer pericarp and that these changes could be part of a system that arrests grain growth and thus determines grain size.

Synchrotron Soleil sheds real-time light on the enzymatic deconstruction of lignocellulosic biomass – *M-F. Devaux & B. Chabbert*
The enzymatic conversion of lignocellulosic biomass is an attractive route to bio-based substitutes for fossil-fuel compounds as it can mobilize highly-selective enzymes in "soft chemistry" processes. However, if not pretreated, the lignocellulosic cell walls often prove recalcitrant to enzymatic digestion. An ambitious strategy coupling microscopy imaging with the SOLEIL Synchrotron's deep-UV and infrared sources made it possible to track the action of cellulases on lignocellulosic biomass in real-time. These innovative approaches applied to commercial cellulases and grass stems make it possible not only to monitor the dynamics of wall deconstruction at the microscopic scale but also to visualize contrasting levels of recalcitrance according to cell types in relation to the nature of enzymatic cocktails.

A 3-D picture of hemicelluloses in wheat endosperm – *H. Rogniaux, M. Fanuel, F. Guillon & L. Saulnier*
Wheat grain quality is notoriously variable, which carries significant economic implications for the cereals industry. This makes it an important challenge to gain deeper understanding and control of the determinants and drivers of these variations which affect both the functional and nutritional properties of the grain. The focus of research has turned to the cell-wall components. Mass spectrometry imaging was combined with specific cell-wall degrading enzymes to investigate how these polymers are structured and their spatial distribution at whole-grain scale. Our results shed new light on how the tissue-level partitioning of cell-wall polymers is linked to their structure. The enzymatic tools employed here can readily be adapted to carry this methodological development over to other plant systems and other polymers in order to study, for instance, the degradability of grass stems used in biorefinery processes.

Food allergy aggravates asthma in a mice model of atopic march – *G. Bouchaud*
Allergies are constantly increasing in the most developed countries. In addition, there is a clinical trend characteristic of allergic diseases over the lifespan, from skin and food allergies to respiratory allergies such as asthma. The purpose of this work was to understand the mechanisms involved in this evolution. We have shown that the induction of food allergy to wheat gluten aggravates the symptoms of subsequent mite asthma induced in mice. Moreover, this effect seems to be mediated by specific receptors (CCR9) that allow the migration of immune cells.

Zein: a model material for 3D-printing polymer melts – *L. Chaunier*
3D printing is an additive manufacturing technique that builds 3-dimensional objects based on a computer-file template. One of the most mainstream 3D printing processes is fused deposition modelling (FDM), which works by depositing a thermoplastic polymer filament layer-by-layer. To extend the range of FDM-process applications out to biomedical and pharmaceutical manufacturing, new thermoplastic materials need to be purpose-developed. All-natural biodegradable biopolymers, some biocompatible and potentially even edible, could make good candidate materials, but only with a purpose-tailored formulation. Our research has shown that zein, the seed storage protein found in maize, is suitable for 3D printing by deposition in the molten state.

Droplet-based millifluidics repurposed for plant protein assembly – *D. Renard & A. Boire*
A regular dietary supply of plant proteins is widely recommended. However, their use as an ingredient is limited by the fact that they have an inherently low water solubility and a heavily aggregated fraction. To better understand and control this aggregation, we developed a medium-throughput screening tool working by droplet-based millifluidics. This experimental setup made up of cheap and

commercial off-the-shelf tubing and connecting, considerably reduces the size of the devices, the required quantity of raw materials, but also the reaction and analysis times. Using millifluidics made it possible to use ten times less material and run experiments five times faster than with a conventional-scale tubing system approach, while at the same time being less expensive than microfluidics and requiring less expertise to deploy.

Field-flow fractionation coupled with static light scattering, a powerful technique for characterizing mixed milk protein aggregates – A. Riaublanc & V. Solé
During heat treatment, the soluble milk proteins get denatured and then aggregate together or at the surface of casein micelles to form mixed aggregates. These supramolecular aggregates possess potentially useful functional properties for replacing certain food additives, but these properties are sensitive to supramolecular structure and the presence of soluble proteins. Working up from a separation system based on asymmetrical flow field-flow fractionation coupled with multi-angle laser light-scattering and refractive index detectors (A4F-MALLS-DRI), we developed appropriate methods for separating different populations within heated solutions of milk proteins. This technique, when applied on a complex mixture, can separate protein aggregates over a very broad size range (5 nm–1µm), determine the size and apparent molar mass of the component structures, and quantify each population. Although developed for milk proteins, this novel technique should be extended to plant proteins, which are often aggregated, and lead to a better understanding of their functional properties.

Testing scientific theories using conceptual models: enzymatic degradation as a case-in-point example – K. Kansou
The literature review, which fundamental to natural science, is getting more and more difficult for domain specialists due to the inexorable inflation of scientific papers. To facilitate the capture and computation of scientific domain knowledge, we proposed a method for assessing and integrating published scientific models. The method has been applied to understanding the mechanism limiting the enzymatic hydrolysis of cellulose, which is pivotal to research in a number of domains, including the production of biofuels. We applied our model to test assess two published kinetic models which are thought to explain the rate-limited hydrolysis of cellulose. We showed that neither of the two models can fully explain all the target behaviors, whereas a third model integrating the first two is able to explain all the target behaviors.

Quantifying the histological profile of maize ear internodes to unravel plant response to hydric stress– D. Legland
Carefully controlled crop irrigation is crucial for sustainable agriculture. In this respect, the development of drought-adapted plant varieties is desirable. Moreover, plant degradability and plant agronomic properties (such as drought-resistance) are both crucial factors that depend on plant-growth development and variations in plant cell-wall composition. Therefore, it is the diversity of these responses that can serve to identify genotypes combining stable agronomic performances with good degradability under varying environmental conditions. We custom-developed an automated image processing and analysis procedure for quantifying the histological tissue-type pattern of maize stem sections using high-res colour-stained input images digitalized with whole-slide scanners.

SpecOMS: software for exploring the protein universe – *D. Tessier & H. Rogniaux*
The SpecOMS software developed in collaboration between the BIA unit and the LS2N laboratory demonstrates that the fragment ion accuracy reachable with the latest generations of mass spectrometers paves the way to a new generation of spectra algorithms. By exploiting a reorganization of the spectral data at the all-sample level in an appropriately-geared data structure and with efficient data access modes, SpecOMS can handle pairwise comparisons of the tens of thousands of experimental spectra from tandem mass analysis against hundreds of thousands of spectra, such as the spectra corresponding to the human proteome. SpecOMS is currently the world's fastest mass spectral analysis software and the least-memory intensive, taking just minutes on a standard desktop PC, which makes it easy to use on any and all mass spectrometry platforms. The method provides a profile of the modifications brought by the proteins in a sample, and can even reveal modifications that are simply impossible to get with other approaches available.

A chimeric antibody for allergenicity assessment of functionalized glutens – *O. Tranquet & S. Denery*
Since the 1990s, glutens have been functionalized by acid hydrolysis for use as ingredients in foods or cosmetics. However, these modified glutens turned out to be neo-allergenic, as the deamidated proteins induce the production of specific IgE-type antibodies that trigger an allergenic reaction, and the first reports of allergies were described in Europe and Japan just a few years after commercial release of products containing deamidated gluten. We have produced a chimeric antibody specific to these deamidated glutens that can serve as a tool to characterize their allergenic potential.

Innovative processes for oil encapsulation – *D. Renard*
Encapsulation of active compounds is used to shield often-fragile compounds like oils from manufacturing/storage process-induced degradation and decay. We have produced microcapsules whose size is such that they minimize these process interferences with the texture or appearance of the final product. We developed a process that consists of forming droplets by dispersing the emulsion in a stirred alginate bath, and that works with either oil-in-water or water-in-oil emulsions. Alginate crosslinking to form the membrane then produced microcapsules at sizes in the range of 370 to 500 µm. The dispersion protocol was then re-engineered for a millifluidics process in order to control microcapsule size. We thus managed to produce monodisperse capsules size-bracketed to within diameters of 140 µm to 1.4 mm.

Carbohydrate-degrading enzymes to restore gut health in broilers – E. Bonnin & L. Saulnier
Gut health problems cause big economic losses in poultry farming. Carbohydrate-degrading multi-enzyme preparations (MEPs) have shown an improvement in animal performance related to the partial depolymerization of parietal polysaccharides from wheat grain. The presence of MEP increases the amount of short-chain arabinoxylans (SC-AX) without producing oligosaccharides. These fractions were incorporated into a wheat-based diet to feed broilers for 2 weeks after hatching, and their effects on broiler performances, gut health, short-chain fatty acid production and gut microbiota composition were studied.