Industrial biotechnology at BASF

Where chemistry and biology meet

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Résumé **Les biotechnologies industrielles chez BASF : quand la chimie rencontre la biologie**
Les biotechnologies industrielles, ou biotechnologies blanches, sont des technologies clés dans le développement de l'industrie chimique du XXIe siècle. BASF exploite depuis longtemps déjà les éléments de la nature afin de développer pour ses clients des solutions innovantes qui préservent les ressources. Quelques exemples de ces procédés biotechnologiques ainsi que des applications potentielles sont mis en avant dans cet article.

Mots-clés **Biotechnologie industrielle, biocatalyse, fermentation, intermédiaires optiquement actifs, enzymes, ingénierie métabolique et enzymatique.**

Abstract Industrial or white biotechnology is a key technology to augment the competencies of a modern chemical company in the 21st century. BASF has a long tradition of exploiting nature's synthetic tool box to develop innovative and resource-conserving solutions for its customers. Some examples of these biotechnological processes are highlighted in this article as well as potential future applications.

Keywords **Industrial biotechnology, biocatalysis, fermentation, optically active intermediates, enzymes as products, metabolic and enzyme engineering.**

White biotechnology, also named industrial biotechnology, uses living microorganisms or enzymes to produce a variety of chemical and biochemical products. It is a key technology at the chemical company BASF, complementing its wide-ranging chemical expertise. BASF investigates methods and processes for efficient and resource-conserving manufacture of chemical products in areas that include nutrition, agriculture, fine chemicals, and biopolymers. White biotechnology gives access to products which cannot be produced competitively by conventional chemical methods and reactions.

White biotechnology not only offers the opportunity to develop completely new products, but also contributes towards sustainable production as it allows a number of products to be manufactured with less consumption of energy and resources. Thanks to white biotechnology, both new and existing products might be manufactured using renewable resources such as sugars and vegetable oils. BASF has more than three decades of experience in this field with products such as vitamins, enzymes and chiral intermediates.

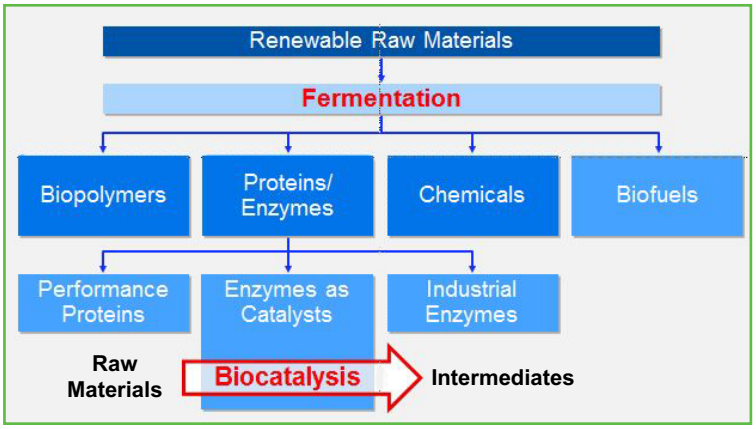

Figure 1 - White biotechnology comprises both whole cell conversions which are usually based on renewable raw materials as *e.g.* sugar as feedstock as well as biocatalysis. Here enzymes are used to catalyse single step chemical conversions.

Within white biotechnology, a distinction is made between fermentative and enzymatic processes. In fermentation, the native metabolic functions of living microorganisms are utilized. The organisms are "fed" with a simple raw material such as sugar, and after a number of catalytic conversions, the final product is obtained. On the other hand, in biocatalytic processes, usually a single enzyme is used to catalyse a one-step chemical conversion. The enzymes employed here often originate from microorganisms while the reactants can be synthetic or natural compounds (*figure 1*).

White biotechnology *per se* has no inherent advantage; it has to be considered as one technology for chemical synthesis among several others. Consequently, the key performance parameters for a biotechnological process are identical to those of conventional chemistry: low manufacturing costs in combination with adequate product properties. In the past, these requirements predestined biocatalysts for the production of optically active fine chemicals as well natural products. Biocatalysis often outperforms classical chemistry due to its notorious selectivity. Generally the reactions conditions are much more benign than in classical chemistry, resulting in real savings with respect to energy and investment in equipment. This shows that white biotechnology can complement and even surpass classical chemistry in many cases, which do not need to be limited to fine chemicals. In essence BASF opts for a biotechnological solution wherever economic leverage becomes apparent.

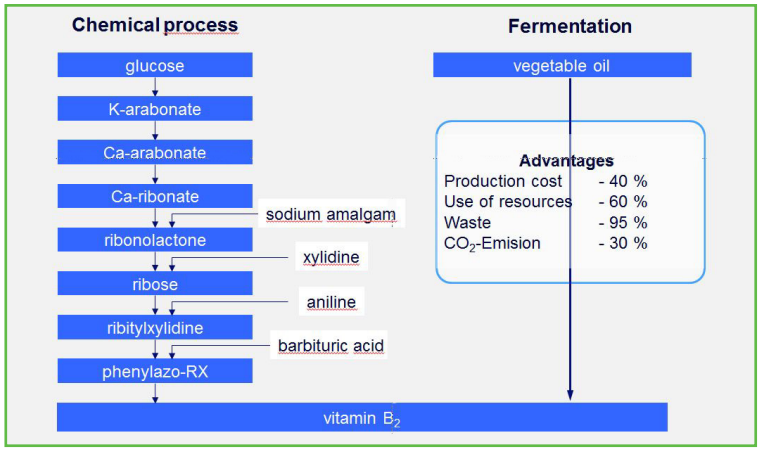

Figure 2 - Some significant advantages of white biotechnology become apparent when comparing the classical synthesis for the production of riboflavin (vitamin B2) to the fermentation process: less unit operations based on non hazardous chemicals give access to the same product in excellent quality.

Another more recent example is the microbial production of succinic acid based on renewable resources. Succinic acid is a very promising building block for bio-based polymers and the demand of succinic acid is anticipated to grow strongly in the next years. Together with its R&D collaborators, BASF has isolated a novel microorganism capable of synthesising succinic acid from renewable resources. Strain improvement and process development resulted in an industrial process which was scaled up to supply BASF's in-house products. Now a production capacity of 25,000 tons per year is anticipated for 2013 to make large volumes of succinic acid available for external customers. Additionally a world-scale plant with a capacity of 50,000 tons per year is planned. The newly developed process combines high efficiency with the use of renewable substrates and the fixation of the greenhouse gas CO_2 during the production. This results in a positive eco-footprint and makes bio-based succinic acid an economically and ecologically attractive alternative to petrochemical substitutes.

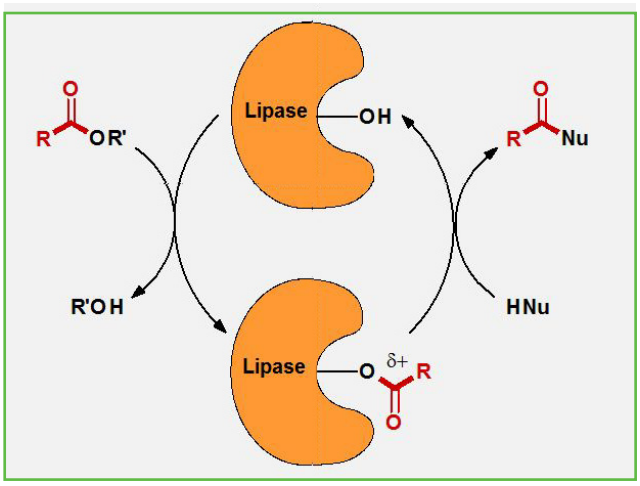

Figure 3 - Lipase acts as an acylation catalyst.
Depending on the presence of water, it either hydrolyses an ester bond or transfers the acyl moiety to another available nucleophile as *e.g.* an amine or alcohol.

BASF activities in white biotechnology

Fermentation processes

Microbes have been streamlined in such a fashion that they produce naturally occurring metabolites as *e.g.* an amino acid or vitamin in several thousand fold excess compared to their wild-type ancestors. This was illustrated quite nicely by BASF's riboflavin (vitamin B2) process (*figure* 2). This vitamin ensures health and performance in animal nutrition. BASF pioneered the biocatalytic production of vitamin B2 more than twenty years ago by substituting a chemical synthesis with biotechnology. Since then BASF produces vitamin B2 with the fungus *Ashbya gossypii.* Due to less unit operations, substitution of hazardous chemicals and successful R&D efforts, the biotechnological production of vitamin B2 is significantly favoured over the chemical synthesis.

Biocatalysis

Not only entire metabolic pathways but also single enzymes may be exploited successfully for industrial applications.

A prime example for enzyme catalysis is certainly the BASF process for the resolution of racemic amines. Mechanistically lipases act as acyltransfer catalysts (*figure 3*): the enzyme cleaves an ester bond forming an intermediate enzyme-acyl-complex while liberating the alcohol moiety. The enzyme-acyl-complex then reacts with a nucleophile which is ultimately acylated. *In vivo*, the enzyme would transfer the acyl group to water as a strong nucleophile forming the carboxylic acid. Since lipase is highly stable in the presence of organic solvents as *e.g.* amines or alcohols, these compounds may serve as nucleophile as well and will thus be acylated by the lipase.
A chiral molecule, lipase preferentially interacts with one enantiomer of a racemic mixture of nucleophiles, resulting in enormously different reaction velocities for each antipode. Most surprisingly, the high enantioselectivity of the lipases used by BASF is combined with a stupendous substrate spectrum. This gives access to a vast variety of different optically active amines and alcohols (*figure 4*).

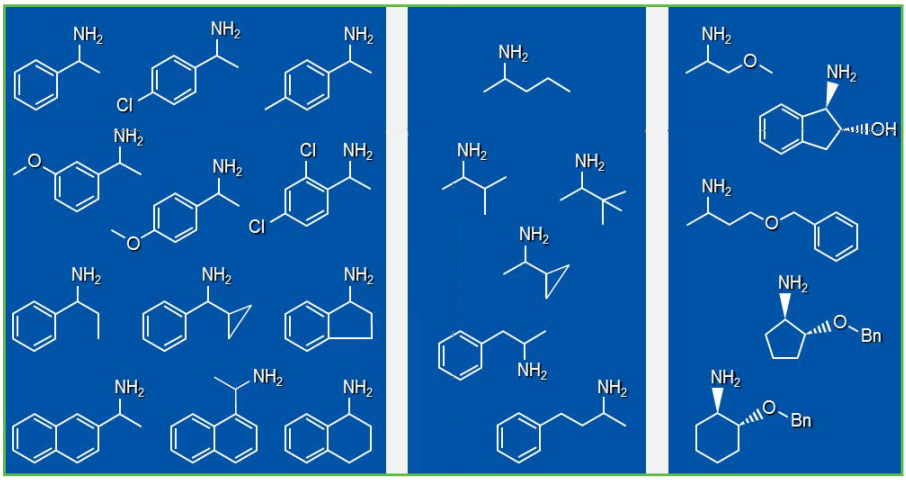

Figure 4 - Lipase as biocatalyst gives access to a very broad range of optically active amines and alcohols.
Although promiscuous in the substrate specificity, the enzymes generally show a very high enantioselectivity.

Specialty Monomers

Acrylates	Methacrylates	Amine Methacrylates
• Behenyl Acrylate 1822 • Behenyl Acrylate 1822 F • iso-Butyl Acrylate • tert-Butyl Acrylate • n-Hexyl Acrylate • iso-Decyl Acrylate • Stearyl Acrylate 1618 • Stearyl Acrylate 18 • Lauryl Acrylate 12 • Lauryl Acrylate 1214 • Ethyldiglycol Acrylate • Hydroxypropylcarbamat Acr. • 2-Hydroxyethyl Acrylate • Hydroxyethylcaprolactone Acr. • 2-Hydroxypropyl Acrylate • 4-Hydroxybutyl Acrylate	• Allyl Methacrylate • Behenyl Methacrylate 1822 • tert-Butyl Methacrylate • tert-Butyl Methacrylate low acid • Cyclohexyl Methacrylate • iso-Decyl Methacrylate • Lauryl Methacrylate 1214 • Stearyl Methacrylate 1618 • Stearyl Methacrylate 1618 F • iso-Tridecyl Methacrylate • Ureido Methacrylate	• N,N-Dimethylaminoethyl Methacrylate • N,N-Diethylaminoethyl Methacrylate • tert-Butylaminoethyl Methacrylate **Others** • N-Vinylformamide

Figure 5 - Overview of acrylates commercialized by BASF.

As this and other examples from the ChiPros® portfolio show, enzyme catalysts are currently used predominantly for the production of optically active compounds. There are however also examples of non-chiral products prepared by enzyme catalysis due to a superior performance compared to a chemical route. When it comes to technical examples which have been realized recently, the new process for "specialty acrylates" implemented by BASF may illustrate the expansion of biocatalysis from "classical" chiral applications to specialty chemicals which – at the first sight – may appear to be less sophisticated than optically active intermediates.

BASF produces a vast variety of specialty (meth)acrylate esters which are employed in a range of polymer products such as coatings, flocculants or adhesives (*figure 5*).

Those specialty acrylates are bulk products usually produced *via* classical acid-catalyzed esterification. Competing with such processes appears to be challenging, as commercially successful biocatalytic reactions usually tackle enantiopure high-price chemicals such as the ChiPros® mentioned above. So, why to use enzymes in acrylation reactions? Once again, enzymes play out their typical strengths. Their ability to work at low temperatures and mild conditions reduces the amount of side products, which are in the case of acrylates mainly Michael-adducts, elimination or condensation products. The risk of uncontrolled polymerization is also drastically reduced with mild reaction conditions, thus allowing access to new classes of acrylate monomers which are not stable under classical chemical reaction conditions. Lower energy consumption in the process also helps to reduce the overall process costs. Additionally, enzymes are able to tolerate functional groups such as carbamates, which is an essential advantage exploited for the synthesis of hydroxyl propyl carbamate acrylate (HPCA). HPCA is a main component of a carbamate clearcoat, a protective coating usually used in automotive coating with high resistance towards mechanical, chemical, UV or weather exposure. As this monomer is not accessible by classical chemical synthesis, introduction of the carbamate functionality was traditionally realized in an unspecific transesterification step with methyl carbamate after polymerization of hydroxyethyl methacrylate. In order to obtain a broader formulation flexibility of the polymers, a chemo-enzymatic process was developed which gives access to the monomer HPCA. This process is now fully integrated in the BASF-Verbund (see *info box*).

Starting from propylene carbonate, aminolysis leads to a mixture of 1- and 2-hydroxypropylcarbamate. Transesterification with ethyl acrylate, catalysed by lipase, gives a mixture of 1- and 2-HPCA (*figure 6*).

However, analysis of the acrylation reaction kinetics revealed that substrate conversion is slowing down at ~ 50% conversion. Detailed investigation of the catalyst has shown that the typical advantage of the enzyme, its high substrate specificity, turned out to be disadvantageous in this context. Molecular docking experiments were employed to identify sites for mutagenesis of the lipase with the aim to reduce its substrate specificity. This was realized by increasing the size of the substrate binding pocket (*figure 7* p. 40). Several mutants have been prepared and analysed leading to the identification of two mutants with reduced substrate specificity without loss of activity compared to the initial wild type enzyme. Hence, substrate conversion was substantially improved compared to the wild type within the same reaction time [1].

BASF Verbund

Our Verbund is one of BASF's assets when it comes to efficient use of resources. Production plants at large sites are closely interlinked, creating efficient value chains that extend from basic chemicals right through to high-value-added products such as coatings and crop protection agents.
In addition, the by-products of one plant can be used as the starting materials of another. The system saves resources and energy, minimizes emissions, cuts logistics costs and utilizes infrastructural synergies. Our global production Verbund is the foundation for BASF's competitiveness in all regions.
With its six Verbund sites and some 385 production sites, BASF supports customers and partners in almost every country in the world. The Verbund is all about intelligent interlinking of production plants, energy flows, logistics and infrastructure. Chemical processes consume less energy, produce higher product yields and conserve resources.

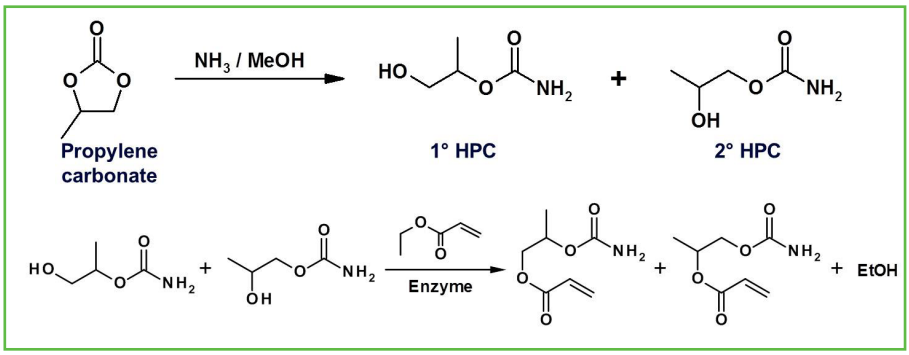

Figure 6 - Reaction scheme of the chemo-enzymatic approach to HPCA.
Aminolysis of propylene carbonate gives a mixture of 1- and 2-HPC (top). Subsequent, enzyme catalysed acrylation yields the HPCA products (bottom).

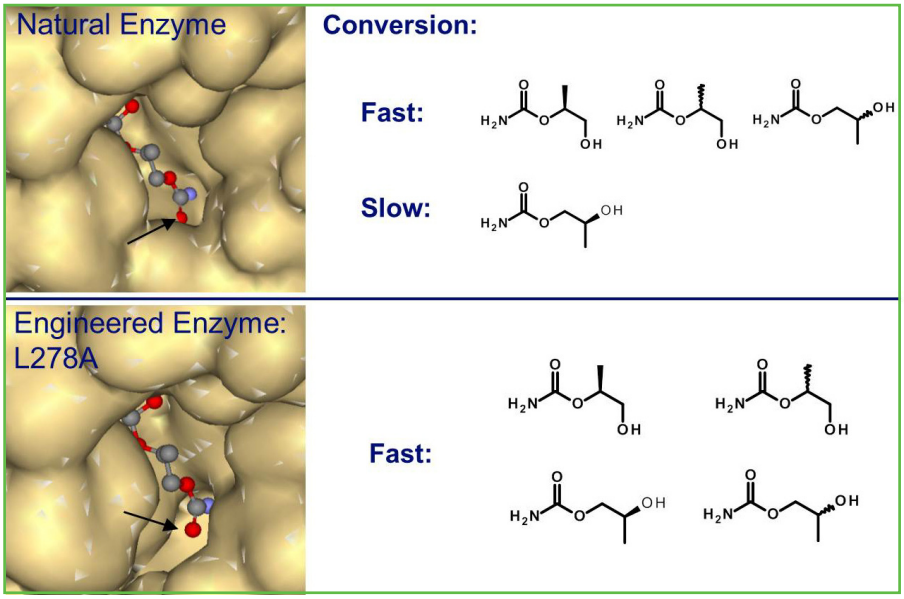

Figure 7 - Model of wild type lipase (top) and mutated lipase (bottom) with 2-HPC in the active site and trends for the respective conversion rates for all four substrate isomers.

The process was successfully scaled up and pilot scale quantities could be synthesized using the chemo-enzymatic approach. Commercial production started in 2010 using a technical set-up which is fully integrated into raw materials streams from acrylic ester plant. This set-up allows for maximum flexibility for the synthesis of additional products, so that the process is used as a platform technology for the manufacture of various other acrylate esters, *e.g.* those shown in *figure 5*.

Further attractive targets are glucoside acrylates, which are not accessible by classical chemical approaches as mixtures of isomers are formed, whereas an enzymatic approach allows selective acrylation at C6. Butanediol monovinyl ether acrylate (BuVEA) cannot be accessed by chemical approaches due to its low onset temperature for polymerisation and the high polymerization enthalpy of >1000 J/g.

Glycerol carbonate acrylate, an interesting crosslinking monomer decomposes under chemical esterification reactions, rendering the enzymatic approach ideal for production of this monomer. All above mentioned examples have in common that post-polymerization cross-linking is possible, *e.g. via* hydroxyl groups (glucoside acrylates), ring-opening with diamines (carbonates) or separate polymerization using a vinyl-function (BuVEA), which makes them versatile building blocks for polymer production.

Enzymes as products

Besides catalysts for the production of chemicals enzymes are important products on their own. For more than two decades, phytase is used as feed additive in animal nutrition. Here, BASF was the first to introduce phytase into the market in the early 1990's. Used in diets for poultry and pigs, phytase dramatically minimises the amount phosphate excreted by the animal. The phosphorus load of manure is reduced by approx. 30%, leading to a significant decrease of the environmental burden in animal husbandry. In addition to the beneficial environmental impact, phytase also helps our customers to be more successful economically: Natuphos® cleaves phytate – the plant bound phosphorous storage form – that is indigestible for poultry and pigs, and thereby minimizes the need of supplementation of inorganic phosphorous. In addition it releases other phytate-bound nutrients such as calcium, zinc, copper and magnesium but also amino acids. Finally it also allows animals to metabolize more energy out of the same amount of feed. Feed enzymes like phytase are produced by fermenting the filamentous fungus *Aspergillus*. Today Natuphos® is one of the most important feed additives and is used all over the world.

Another example from this area are glucanase and xylanase which are distributed by BASF under the brand name Naturgrain® TS. In feed Naturgrain® TS helps to increase digestibility and nutritional value by acting on feed components that contain non-starch polysaccharides that would be otherwise indigestible to poultry and pigs. Both enzyme products are characterised by a substantial thermo stability. This is a crucial feature as modern animal feed needs to be treated at fairly high temperatures prior to feeding. Heat treatment serves to sanitize the forage. This would usually destroy non-thermo stable enzymes by protein denaturation. BASF was successful however to develop thermostable xylanase as well as glucanases which are now produced using *Aspergillus* strains (*figure 8*). Natugrain® TS was introduced to European market in 2008; worldwide approval procedures are on the way.

Figure 8 - *Aspergillus niger* is capable to produce massive amounts of useful enzymes.
Phytase, glucanase and xylanase are examples from BASF's current enzyme portfolio. This figure shows fungal mycelium, an agglomeration of cell filaments with diameter of approx. 2-5 µm.

Outlook

White biotechnology can help industrial chemistry by providing novel, selective catalysts and processes operating under ambient, energy conserving conditions. The synthesis of complex natural compounds based on renewable resources is possible.

In the future, modern tools of molecular biology, bioinformatics and analytics will continue to revolutionize industrial biotechnology. Screening for novel biocatalysts expands from classical screening of biodiversity to prospecting microbial ecosystems on the genome level. Sequence data are fed into rapidly growing databases, which then serve as platform for *in silico* screening.

Enzymes are optimised by mimicking evolution in the test tube; a simple trial-and-error approach can – in combination with powerful high throughput assays – lead to quickly evolved enzymes with significantly improved properties. In the future, we will see single enzymes designed from scratch to meet the needs of industrial chemists.

Wild type organisms have fine-tuned networks of different interdependent metabolic pathways which are perfectly adapted to the needs of the natural environment. Obviously, the needs of an industrial process are quite different. The more is known about the genetic and metabolic set-up of a given production organism, the more leverage exists to optimise this strain by rational approaches. Metabolic engineering has refined to such an extent that there are for the first time opportunities to dramatically alter the metabolism of a production strain both quantitatively as well as qualitatively in swift fashion. Tailor-made “designer strains” capable of producing non-natural chemicals are no longer fiction, but reality. Even though this field of research is still in its infancy, first encouraging examples have been published recently.

When scientific progress translates into broad technical applicability and economic benefit, we will see further increase in the importance of white biotechnology in the chemical industry. In this sense, BASF will continue its successful path of implementing this exciting and promising technology.

[1] Liu D., Trodler P., Eiben S., Koschorreck K., Müller M., Pleiss J., Maurer S.C., Branneby C., Schmid R.D., Hauer B., Rational design of *Pseudozyma antarctica* lipase B yielding a general esterification catalyst, *ChemBioChem*, **2010**, *11*, p. 789.

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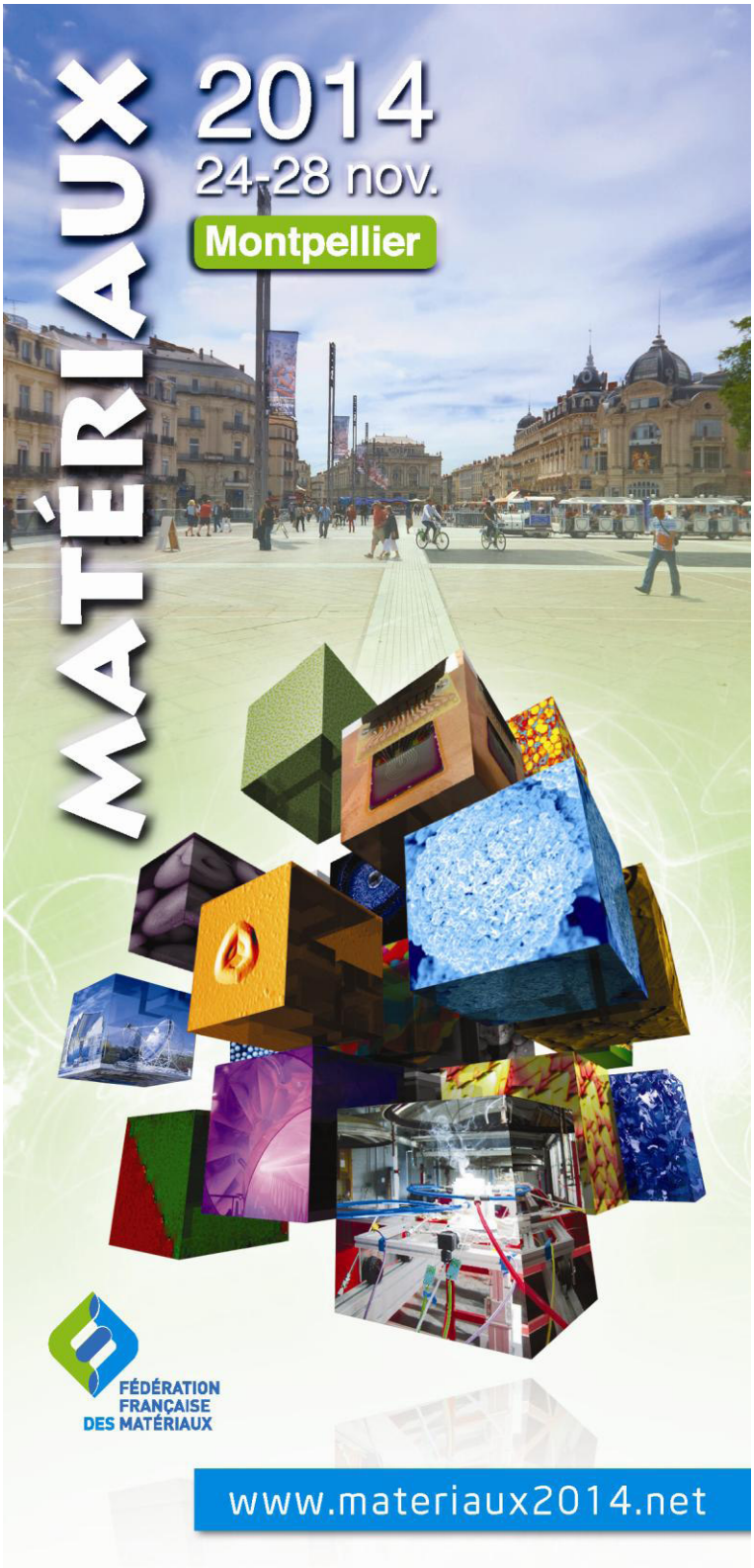

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