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Hot from the press!

Innovation Centre Peptide and Protein Technologies
HES-SO Sion: Pioneer in an Emerging Market

Abstract: Peptides are small proteins typically containing a chain of up to 100 amino acids. In our bodies, they are produced every day in vast quantities and perform highly specific biological activities. As there is an increased interest in peptides for pharmaceutical applications, the HES-SO Valais-Wallis created a research group to focus on Peptide and Protein Technologies (P^2T).

Keywords: Peptide and Protein technologies

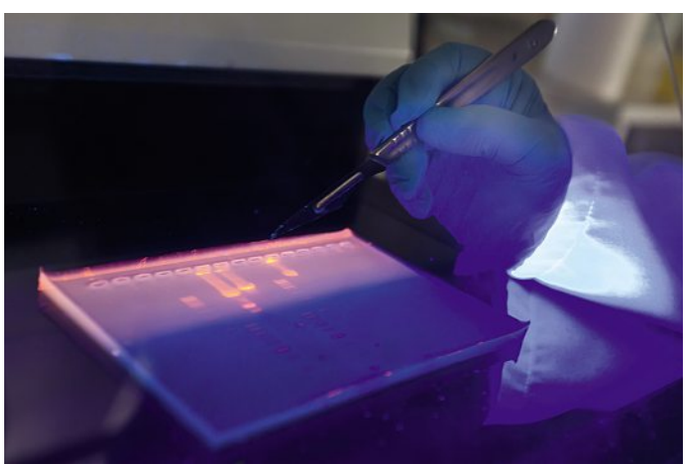
There is huge potential in peptide-based therapeutics as they are closely involved in different processes of the nervous and the cardiovascular system as well as the gastrointestinal tract. As certain peptides can pass through cell membranes, they may be used as carriers for targeted drug delivery. Bioactive peptides also open up an important new market segment for medical diagnostics, nutraceuticals, anti-microbials and cosmetics. However, for a successful breakthrough in peptide technology we need industrial volume production, and must therefore improve manufacturing methods to increase productivity, manage cost pressure and reduce the time to market without compromising on quality.

Everything under One Roof

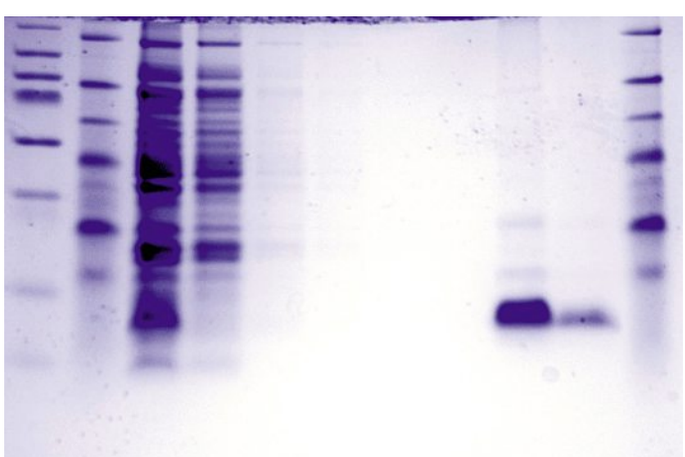
The Institute Life Technologies of HES-SO Valais-Wallis has created the 'Peptide and Protein Technologies' Innovation Centre to discover and optimize peptides and proteins for therapeutic, diagnostic and food applications. With the P^2T research team, industry and research institutes can complete their value chain, including the development, characterization and production of peptides and proteins. The focus is on developing peptides and manufacturing batches of up to 300 litres. "Recent technological advances in delivery and formulation tools have resuscitated the field of peptide therapeutics, resulting in approx. 60 approved peptide drugs and an annual predicted market growth rate of approximately 10%", comments Dr. ***Sergio Schmid***, head of the institute. "Today, most peptides are produced by chemical synthesis. But recombinant peptide production will become more important in the near future and play a key role in the competition landscape of peptide therapeutics companies. In particular, this is the case for long and complex peptides containing natural amino acids." Although development of a biotechnological process for recombinant production may be time-consuming, larger quantities of peptide can be produced and the environmental impact of the generated waste is lower compared to chemical synthesis. "However, recombinant peptide expression must overcome several obstacles in order to be cost-effective and competitive with chemical synthesis", emphasizes Sergio Schmid.

Recombinant Synthesis as an Alternative

For instance, there is the possibility of recombinant peptide production in microbial expression platforms, in particular the expression hosts *Escherichia coli* and yeast and their respective vectors. Recombinant production is an alternative to chemical synthesis, especially for longer peptides/short proteins and peptides containing post translational modifications such as multiple disulphide bonds. The major advantage of secretory production of recombinant peptides is the ease of downstream processing which leads to lower production costs. This is due to a reduction in contamination with cellular compounds, a limited number of naturally secreted extracellular proteins and less degradation by cellular proteases.



Agarose electrophoresis of DNA.



SDS of the purification steps of a recombinant peptide.

In a project supported by the Commission for Technology and Innovation, the P^2T researchers considered – together with their colleagues from the Debiopharm group and the EPFL – different expression systems for manufacturing and purifying snake venom-derived three-finger toxins containing four disulphide bonds. "To express the peptides in the cytoplasm of *Escherichia*

coli, where the reductive environment normally prevents the formation of disulfide bridges, we compared several strains with mutated reductases and expressing inducible auxiliary proteins to correct S-S bridge formation", explains Dr. ***Sarah Wegmueller***, Senior Researcher Molecular Biology. "In another approach, the peptides are expressed in the oxidative environment of the ER (endoplasmic reticulum) of the methylotrophic yeast *Pichia pastoris* and then secreted into the medium." The favourable aspects of this yeast make it increasingly popular for the recombinant production of secreted peptides.

An Insidious Disease Attacking Babies

One of the most common infections around the world is caused by the Respiratory Syncytial Virus (RSV), which attacks the upper respiratory tract and the lungs. By the age of 2, most children have been infected with the virus. While the infection is usually asymptomatic, the emergence of the disease can require hospitalization of the infants in an intensive care unit, and in some instances it can lead to death. For the time being, there is only one treatment available, a prophylactic antibody. This preventive therapy is recommended for infants that are high-risk because of premature birth or other medical problems such as congenital heart disease.

In a joint project with the French National Institute of Agricultural Research (INRA) and the School of Engineering and Architecture of Fribourg (EIA-FR), Dr. ***Origène Nyanguile***, HES-SO, took a closer look at various viruses belonging to the same family as RSV. Peptides derived from the phosphoprotein P can inhibit the replication of the virus into the host cell by disrupting the binding to N^0, the RNA unbound form of the nucleoprotein N. However, nobody has spent any time on developing such antivirals for RSV because there are no structural data available for the N^0/P complex. "Recently our collaborators at INRA identified the sequence of P that folds into an α-helix upon binding to N^0", explains the Harvard University-educated professor. "With my group, we investigated whether helix stabilization can increase the inhibitory activity of RSV-derived P peptides and lead to cell permeable molecules."

SDS analysis of recombinant peptides.

Large-scale microbial fermentation in a bioreactor.

Manual solid-phase peptide synthesis.

HPLC purification of synthetic peptides.

The researchers identified novel molecules that interfere with the binding of N^0 to P, in particular HEVS 77 and HEVS 78. "These peptides are derived from the phosphor-protein P and inhibit viral replication in Hep2 cells with EC_{50} values of 60 and 15 µM respectively", comments Origène Nyanguile. "While HEVS 77 shows no cytotoxicity, HEVS 78 is cytotoxic at 100 µM." The results show that peptide stapling can significantly enhance the α-helical content of the peptides. Through further chemical modifications the scientists identified the lead peptide HEVS 124 that displays better inhibitory potency in a cell-based assay. Intranasal administration of HEVS 124 in BALB/c mice inoculated with a Luciferase-encoding RSV showed that the peptide is capable of dramatically reducing the replication of the virus *in vivo*.

However, although HEVS 124 appears to show promising data, the molecules have only modest activity in cellular settings, and further work has to be undertaken to improve the potency of this lead.

PHAs, the Promising Linear Polyesters

As part of the collaborative work being undertaken within the Life Technology Institute at the HES-SO in Sion (Valais), the laboratory of peptide expert Dr. ***Marc Mathieu***, researchers are investigating chemically modified PHAs, polyhydroxyalkanoate biopolymers. These are bacteria-synthesized, intracellularly accumulated polyesters of selectively unsaturated hydroxy-alkanoic acids. They belong to a rapidly growing class of biocompatible polyesters which can thus undergo chemoselective conjugation with peptides at specific binding sites (M. Bassas-Galià, A. Gonzalez, F. Micaux, V. Gaillard, U. Plantini. S. Schintke. M. Zinn. M. Mathieu, FH-HES: 'Chemical Modification of Polyhydroxyalkanoates (PHAs) for the Preparation of Hybrid Biomaterials', *CHIMIA* **2015**, *69*, 627). "In our laboratories we performed preliminary assays for the assembly of a new prototype of such hybrid biomaterial by binding amino acid units to PHA. Our aim is to generate – using peptides – an innovative biomaterial for applications in the medical and therapeutic areas", says chemist Marc Mathieu, Head of the Peptide & Peptidomimetic Platform.

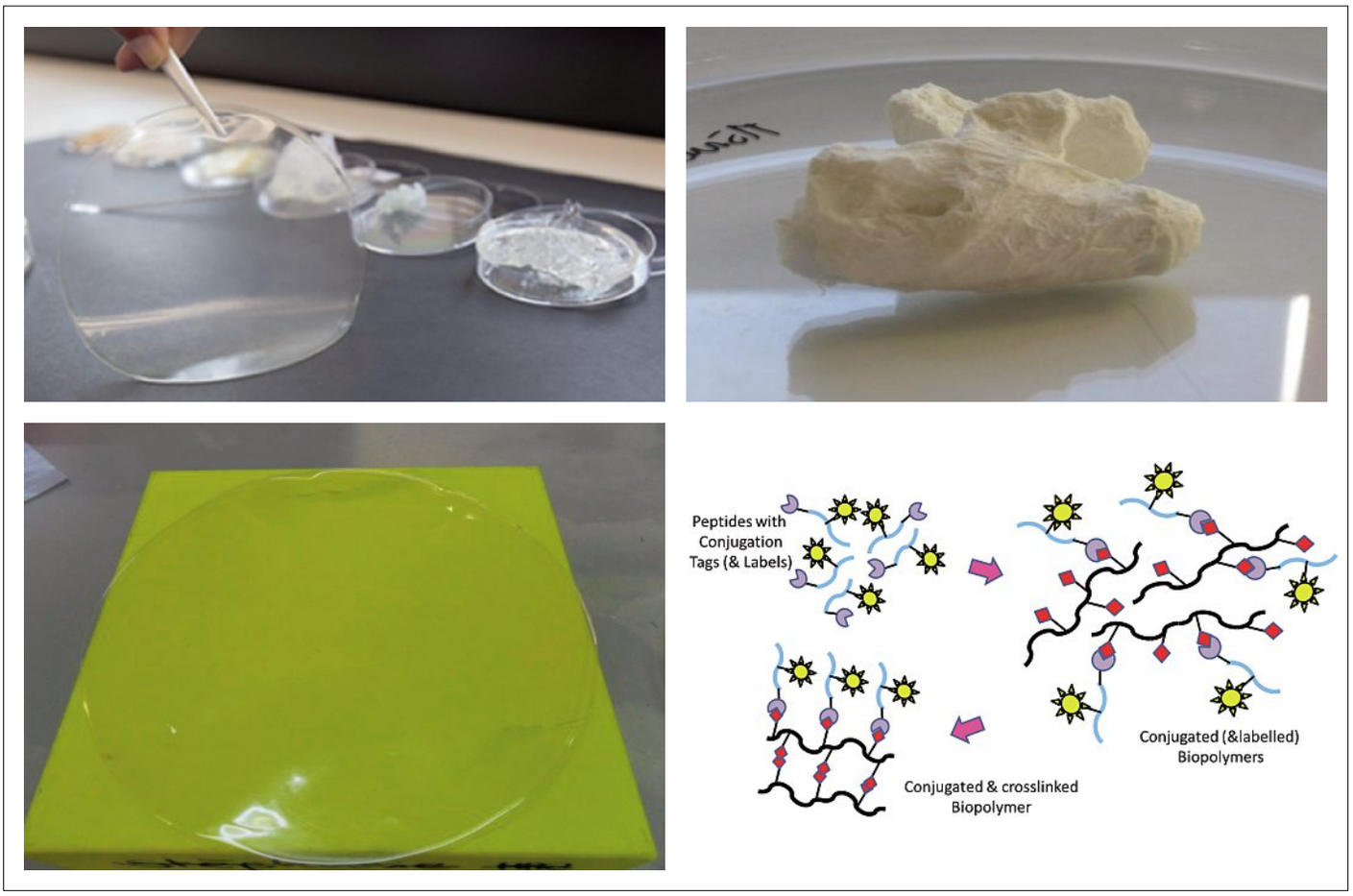
UPLC analysis of synthetic peptides.

UPLC analytical system.

Today, peptides are becoming more widely used in pharmaceutics and cosmetics such as protein therapeutics, for drug delivery, gene delivery and tissue engineering. Peptides provide – together with biocompatible biopolymers – a perfect material for skin contact applications. "Their advantage is that they can be tailored for desired adhesive properties by modifying selected peptide sequences", comments Marc Mathieu. "This property is highly promising for an improved skin-contact material. So far our efforts were directed towards the conjugation of short peptidic sequences, such to enable optimization of downstream processing (DSP) procedures and optimal characterization by NMR and FT-IR."

Complex Research, but Great Economic Benefit

"For the time being, there are about 60 approved peptide drugs on the market", explains Dr. Sergio Schmid, Head of the Institute. "Seven of them reached global sales over USD 500 million and five of these had sales of over USD 1 billion each in 2011. The increasing number of peptides entering clinical trials reflects the ongoing interest of pharmaceutical companies in this molecule class as drugs." The scientist observes a trend towards longer peptides and applications where peptides are needed in large quantities. There is also a strong demand for environmentally friendly production methods. "That means we are facing new



Researchers at the HES-SO investigate chemically modified PHAs, polyhydroxyalkanoate biopolymers. Photos by Prof. Dr. M. Zinn.

challenges", explains Sergio Schmid, who has already spent many years working on the development of microbial expression platforms for the production of recombinant peptides and proteins. From this perspective, recombinant peptide manufacturing offers many advantages and has a promising future for the production of long peptides composed of natural amino acids for industrial volume production.

But he is aware of the fact that the path to recombinant technology is a rocky one: "While the molecular toolset for the expression of peptides is steadily increasing, and various expression systems have been successfully developed and applied, it is still hit-or-miss as to which peptide will perform better with which strategy, as even a single amino acid change in a peptide can lead to altered properties during production and purification." This means that the efficacy of an expression system is highly peptide-dependent and the most suitable expression systems have to be identified and optimized for each individual peptide. Currently, it is not possible to predict the best host-vector combinations – this must be determined empirically. "The availability of large strain collections combined with a wide variety of vectors, promoters, and gene regulatory systems requires innovative medium- and high-throughput screening approaches to choose the optimal system", comments Sergio Schmid. "Medium and high throughput screening methods such as these will accelerate process development and give a competitive edge to the biotechnological production of peptides." But the peptide research scientists at the HES-SO are well prepared for the challenges to come and ready to benefit from the opportunities arising from them.

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