doi:10.2533/chimia.2021.539 Chimia 75 (2021) 539–542 © M. Mathieu, O. Nyanguile

Peptide Technologies at Energypolis

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Abstract: The new Energypolis campus brings together the skills of EPFL Valais-Wallis, HES-SO Valais-Wallis, and the Ark Foundation's services. Together these partners respond to today's major concerns in the domains of energy, health, and the environment cutting-edge technology. The spirit of this new campus is to foster innovation in these disciplines and emulate the creation of start-up companies. The HES-SO hosts the School of Engineering (HEI) at this campus, which includes the following degree programmes: Life Technologies, Systems Engineering and Energy and Environmental Engineering, as well as their corresponding applied research institutes. Peptide technologies belong to the many activities that are carrying out in the Institute of Life Technologies. The present review summarizes the peptide technologies that are currently under development, that is, the regioselective labeling of therapeutic antibodies for cancer imaging, the development of peptide antivirals and antimicrobials for the treatment of infectious diseases, targeting of drugs conjugated to peptidic scaffolds as well as engineering of biomaterials.

Keywords: Biomaterials · Cyclic peptides · N^0-P complex · Peptide–drug conjugates · Peptidomimetics · Regioselective labeling · Stapled peptides · Therapeutic antibodies

1. Peptides Technologies at Energypolis (Fig. 1)

1.1 *Development of a Regioselective Labeling Method for Clinical Imaging of Therapeutic Antibodies*

In a collaboration between Debiopharm Research & Manufacturing, the CHUV, and the HES-SO Valais-Wallis, a novel methodology was developed to regioselectively label therapeutic antibodies with an imaging cargo. The project was initially supported by Innosuisse,[1] and is now fully funded by Debiopharm Research & Manufacturing S.A. A peptide binding specifically to the antibody Fc domain[2] was engineered with a spacer, a reactive center and an imaging cargo to yield a peptide reactive conjugate. Upon mixing of the antibody with the peptide reactive conjugate, the imaging cargo is attached regioselectively to the antibody Fc domain. Because the Fc domain is conserved in IgG immunoglobulins and because the conjugation reaction occurs regioselectively at this site, labeling does not compromise binding of the therapeutic antibody to its cognate antigen. The method was initially intended to serve as a non-invasive diagnostic tool to help physicians identify patients that express the target antigen and thus benefit from an antibody-based treatment. The technology can also be used to identify micrometastases. Briefly, the therapeutic antibody is labeled directly at the clinic with a radioisotope such as ^{68}Ga, ^{111}In and ^{89}Zr, followed by a microdose injection of the labeled antibody into the patient. Trafficking of the antibody and tumor accumulation is then visualized by Positron Emission Tomography (PET) or Single Photon Emission Computed Tomography (SPECT) directly at the clinic.[3] As a proof of concept, we labeled the anti-HER2 monoclonal antibody Trastuzumab[4] with ^{111}In and demonstrated that the conjugation did not affect binding to HER2$^+$ SK-OV-3 tumor cells. Following the injection of ^{111}In-Trastuzumab in mice bearing a SK-OV-3 tumor, the increase of tumor uptake was observed with time, being the highest at day 6[5] (Fig. 2).

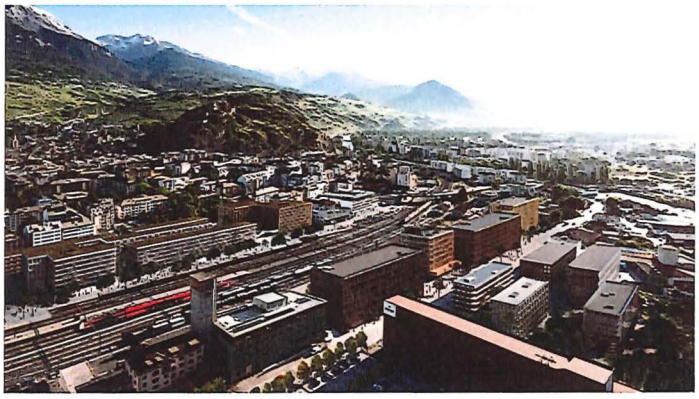

Fig. 1. Campus Energypolis© Evéquoz Ferreira Architectes SIA.

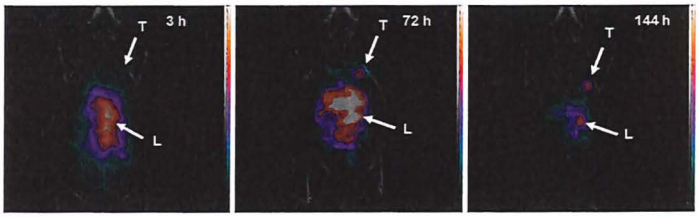

Fig. 2. SPECT acquisitions at 3, 72 and 144 hours post injection of 18 MBq ^{111}In-labeled trastuzumab in a mouse bearing a SK-OV-3 tumor (HER2 positive). Uptake of ^{111}In-TzmAb was observed in tumor (T) and liver (L).

Our data demonstrate that the use of therapeutic antibodies for human imaging purposes should be feasible in the clinic. Furthermore, the technology can be expanded to broader applications such as the preclinical evaluation of multiple pharmacodynamic and pharmacokinetic parameters of antibody candidates, the synthesis of antibody drug conjugates (ADCs),[6] and more generally to the regioselective labeling of any biological target.

2. Targeting Conserved Viral Fusion and Replication Mechanisms with Peptide Antivirals

At the HES-SO Valais-Wallis, we are interested to develop peptide antivirals for infectious diseases.[7] We are targeting two

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highly conserved protein–protein interactions involved in the mechanisms of viral cell entry and replication.

2.1 *Stapled Peptide Inhibitors of Viral Fusion Proteins*

Viral fusion proteins are present at the surface of many unrelated virus families. Their role is to fuse the viral membrane with the host cell membrane in order to deliver genomic viral material into host cell. The viral fusion proteins are divided into three classes based on their structures. The class I fusion proteins include many well-known pathogenic viruses such as HIV, Influenza, Ebola, Rabies, Respiratory syncytial virus (RSV) and SARS-CoV-2, the class II include the Flaviviridae family such as hepatitis C virus and dengue, and the class III include the rhabdoviruses, herpesviruses and baculoviruses.[8] Despite the high diversity of these enveloped viruses, they all use a universal mechanism, whereby a fusion peptide anchors the fusion protein into the host cell membrane, followed by a dramatic conformational change of the fusion protein into a cluster of trimer of hairpins leading to the post-fusion state.[8] This mechanism pulls the viral and host cell membranes simultaneously until they fuse and open a pore into the host cell membrane, thereby allowing the delivery of viral genomic material. Targeting the large interface of the prehairpin intermediate with a peptide antiviral is therefore a universal means to inhibit all class of enveloped viruses. Through screening peptides derived from the heptad repeat-2 (HR2) of the RSV F fusion protein (collaboration with the CMU at UNIGE and INRAE in Jouy en Josas), we identified short double-stapled peptides inhibiting RSV entry in HeP-2 cells with IC_{50} values of 500 nM[9] (Fig. 3A). The potency of these peptides has recently been improved 10-fold, to reach IC_{50} values as low as 50 nM in a HeP-2 cell viral inhibition assay (unpublished data). Stapled peptides are alpha-helical peptides stabilized with an all-hydrocarbon crosslink (the staple) at the face of the helix that does not interact with the target. This macrocyclic ring has been shown to improve binding affinity, proteolytic resistance and cellular permeability of the stapled peptide.[10]

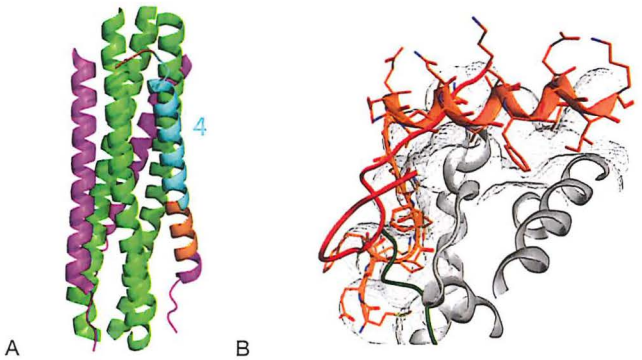

Fig. 3. A) Schematic representation of the RSV F six-helix bundle in the post-fusion conformation. The stapled peptide inhibitors were derived from peptide **4** highlighted in cyan (figure reproduced from Gaillard *et al.*[9]). B) Model of the RSV N^0-P binding site. The surface of N^0 is presented in grey. The ribbon structure of peptide P(1-28) is shown in orange. This model was generated with Molecular Operating Environment (MOE) using the PDB access codes 2WJ8 and 5FVD (figure reproduced from Galloux *et al.*[12]).

2.2 *Stapled Peptide Inhibitors of Viral Replication*

Non-segmented negative strand RNA viruses (NNSV), including the respiratory viruses RSV, human metapneumovirus, Parainfluenza 5, and the hemorrhagic viruses Ebola, Marburg, Nipah, encapsidate the neosynthesized antigenome and genome with a nucleoprotein N to form ribonucleocapsids (RNCs). This RNC protects RNA from RNAse degradation and is used as a template for viral replication. The neosynthesized N is prone to oligomerize and to interact non-specifically with RNAs. To prevent this, neosynthesized N is kept in an assembly competent formed known as N^0 through binding to the chaperone phosphoprotein P, until delivered to nascent synthetized viral RNA. Although similar, the P binding domain on N^0 is specific for each virus. For RSV, the phosphoprotein N-terminus (P 1-40) binds to a large cavity of N^0, impairing N oligomerization. More specifically, NMR and biochemical studies have suggested that P (13-25) of RSV folds into an alpha helix upon binding to N^0[11] (Fig. 3B). Through screening peptides derived from this domain, we identified stapled peptides that act as replication inhibitors. We demonstrated that these peptides inhibit RSV replication *in vitro* and *in vivo* in BALB/c mice, through preventing the formation of the N^0-P complex.[12]

3. Peptidomimetics Applied to Antimicrobials

Small linear peptides have proven their limitations as therapeutic agents due to their low stability towards proteolysis, consequently reducing their feasibility and profitability for the pharmaceutical industry.[13]

More recently, the peptide laboratory at the HES-SO Valais-Wallis has been active in the development of approaches with efficient and economic peptide methodology where synthesis can contribute to revitalize peptide-based drugs in the current pharmaceutical market.[14] Diverse chemical modification protocols that have evolved to diminish drawbacks such as solubility and half-life are tackled in this project and have very promising pharmacological potential and like natural peptides, are able to interact with natural proteins and meet important criteria for good solubility and permeability.[15] These include cyclization, *N*-methylation, incorporation of non-natural amino acids and other structural constraints. Stability by incorporation of D-amino acids improves stability up to 50% in human serum and can be entirely tailored. Stability after incubation with lysosome is increased in the range from hours to weeks depending on length of D-amino acid retro-inverso stretches.[16]

3.1 *Novel Tools against Microbes: Head-to-sidechain Peptidomimetics*

The global spread of microbiological diseases has raised the need for the development of novel, effective, and safe drugs. New antibacterial, respectively antiviral agents with innovative mechanisms of action, exempt of pre-existing cross-resistances seem very necessary.[17] In our laboratories at Energypolis cyclic peptides constitute a most promising platform for drug development due to their biocompatibility, chemical diversity, and similarity to proteins. Our Head-to-sidechain peptidomimetics focus on an approach of novel cyclodepsipeptide scaffolds (Fig. 4) for the treatment of biofilms.[18] Our consortium behind this project (2016-ongoing) combines expertise from academia with extensive industrial experience in organic chemistry, peptide synthesis, microbiology as well as material science, crucial in the development of novel peptide-based antimicrobial formulations for applications in medical fields.[19]

In this project our focus is to investigate highly innovative antimicrobial peptidomimetics by combining the active entities of selected active ingredients.

3.2 *Vectorized Peptides Anti-cancer Drugs*

A targeted therapeutic approach in the treatment of cancer has become one of the top priorities for industry.[20] Small chemical molecules and biologics have demonstrated a certain number of therapeutic successes, but suffer from limitations such as toxicities, resistance, immunogenicity or biodistribution. Our know-how in linker and chemoselective conjugation of selected drugs to targeting peptides helped us to contribute at the edge of inno-

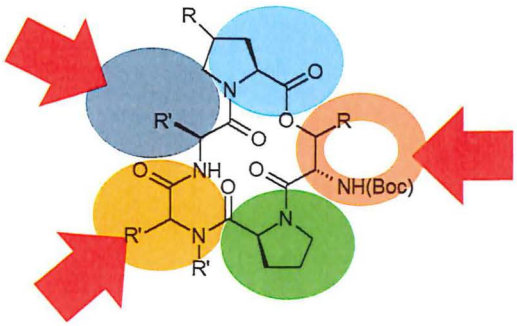

Fig. 4. Pentameric cyclodepsipeptide with color-coded residues and arrows indicating sites where modifications are investigated.

vation in the field of peptide drug conjugates (PDCs). Ongoing collaborative work at the pre-clinical stage relates in the targeting of over-expressed surface polymers on tumor cells. Our technology platform investigated a modular approach that allowed the development of customized PDC scaffolds for various types of cancers.[21]

3.3 *Hybrid Biomaterials*

Nowadays, 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.[22] Their advantage is that they can be tailored for desired adhesive properties by modifying selected peptide sequences. Our HES-SO project (2013–2015),[23] developed a novel class of hybrid biomaterials – conductive hybrid peptide-biopolymers which have high application potential as novel flexible electrodes for medical signal diagnostics, and/or therapy.

Based on a novel combination of state-of-the-art technologies from chemistry, biochemistry and nano & microtechnology thin film deposition and patterning technique, our new prototype electrode materials were optimized for their electrical conductivity, mechanical flexibility, and dermal adhesion properties (Fig. 5). 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. Such hybrid biomaterials belong to the rapidly growing class of biocompatible polymers, which are of great interest for medical and therapeutic applications. In the course of this project, the biosynthesis of a new PHA homopolymer and the chemical modification, an epoxidation reaction, are investigated.[24]

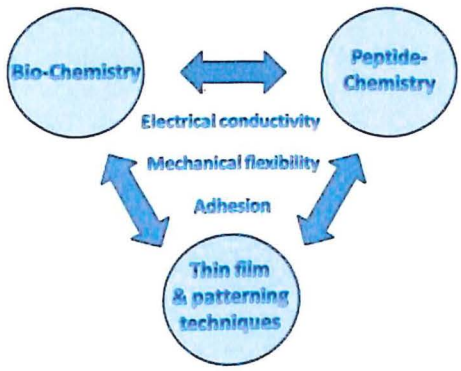

Fig. 5. Triangular HES-SO consortium with competency poles illustrating the broad competencies required for implementing such interdisciplinary projects.

To improve biocompatibility for the application of PHA polymers as biomaterials, molecules of biological interest such as cell-recognizing peptides (*e.g.* RGD, KQAGDV, LDV) can be used to functionalize polymer surfaces by means of covalent linkages[25] and thus improve significantly cellular adhesion onto biopolymers such as PHA. In our laboratories we performed to this stage initial 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, to improve the sensitive skin-interface of electrodes as in our model.

With respect to peptide surface presentation and cell growth control, MAPs (Multiple Antigenic Peptides) or *peptide dendrimer* strategy offers an alternative to peptide–protein conjugates by presenting multiple copies (typically 2, 4, 8) of the peptide synthesized on a small multivalent lysine core (crosslinked lysine residues).[26,27] This approach, when applied to our particular application for the preparation of biocompatible biopolymers for improved interactions between interfaces such as in our skin-electrode model, has the advantage of increasing the number of copies locally presented through a peptidic core and thus minimizes the entropic cost of interaction with a biological matrix such as skin. Our model, in a fully controlled manner, is thus of interest when related to cell-growth promoting sequences such as RGD and peptide sequences presented above.[28]

Received: April 15, 2021

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