Press release

New technology CF LINK for protein bioconjugation and structural proteomics from collaboration of a Brno biotech startup and Czech Academy of Sciences

Brno, Czechia, 3rd December 2019 - *The cooperation of two Prague research institutes – Institute of Organic Chemistry and Biochemistry and Institute of Microbiology of the Czech Academy of Sciences and the Brno based start-up CF Plus Chemicals, a spin-off of ETH Zurich, has brought a new technology called CF LINK for site-selective bioconjugation of proteins and also their structural characterisation. The recently published, patent pending technology, allows to selectively prepare protein conjugates via their tryptophan residues and perform posttranslational modification of aromatic amino acids. Furthermore, it can also be used as a tool for mapping of protein surfaces and studies of protein-protein interactions.*

The company CF Plus Chemicals, an ETH Zurich spin off founded in 2014, is based on almost ten years of cooperation of the group of Dr. Petr Beier at the Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences (IOCB) and Dr. Václav Matoušek, a Ph.D alumnus of Prof. Dr. Antonio Togni at ETH Zurich.

Reagents based on cyclic hypervalent iodine-perfluoroalkyl compounds, nowadays also termed as the Togni reagents, have become widely used and popular tools in organic synthesis, especially in medicinal chemistry for preparation of new fluorinated drug candidates, in-line with the growing demand for fluorinated scaffolds in drug design. While in the early 1990s fluorinated molecules accounted for 5% of the total number of approved drugs, now it is 15% and roughly 30% for newly approved therapeutics.

This family of chemical compounds was subsequently expanded in 2013 in collaboration of ETH Zurich and Dr. Petr Beier from IOCB to a new generation of patented Togni reagents that carry a more complex RCF_2CF_2 group instead of a simple CF_3 group.[1] The new family of these chemicals shares not only the rich reactivity of the first generation of Togni reagents, but also brings an extra dimension due to the virtually unlimited structural variability of the β-substituted tetrafluoroethyl group which they are able to transfer to a variety of substrates relevant to medical chemistry of small molecules.

In 2017, the application potential of the second generation of Togni reagents was extended to proteins.[2] Their high affinity towards the thiol group allows to perform selective bioconjugation through cysteines to form stable conjugates which, unlike maleimide conjugates, are not subject to slow deconjugation and thiol exchange.

The present invention[3,4] builds on the previous knowledge of the radical nature of Togni reagent-mediated reactions. In the SME Instrument Horizon 2020 project, supported by the City of Brno and in cooperation with IOCB and Dr. Petr Novák from the Institute of Microbiology of the Czech Academy of Sciences (IMIC), it was showcased that Togni reagents, when mixed with sodium ascorbate, a cheap, non-toxic and biocompatible reducing agent, immediately generate ß-substituted tetrafluoroethyl

radicals that selectively attach to sterically accessible tryptophan residues of the protein under transition metal-free conditions.

Once azidofluoroalkyl groups are attached to the protein, various functional groups, such as fluorescent dyes, radionuclides or ADC-toxins for targeted oncotherapy can be subsequently linked via click reaction to afford the corresponding protein conjugates. The disclosed tryptophan-selective bioconjugation method does not disrupt protein disulfide bridges and offers an alternative solution where conventional cysteine conjugation is not possible, for example due to an undesirable disulfide scrambling.

This bioconjugation method can also be extended to other aromatic amino acids and hence to proteins lacking tryptophan. Thus, it was possible to successfully modify human recombinant insulin and attach up to 7 modifications to its aromatic amino acids, demonstrating the potential for post-translational modification of proteins.

The extremely rapid nature of this reaction that targets solvent-accessible aromatic amino acids makes it a convenient tool for mapping protein surfaces and studying protein-protein interactions. Using the example of human carbon anhydrase, it has been demonstrated that the results of surface mapping are in excellent agreement with its published native structure.

The second generation of Togni-CF_2CF_2R reagents is protected by a worldwide patent owned by ETH Zurich and IOCB and are exclusively licensed by CF Plus Chemicals. The new CF LINK ascorbate technology, a subject of another patent application, further strengthens the intellectual property protection and forms a technology package which is currently offered to licensing to business partners for bioconjugation of diagnostically and therapeutically relevant proteins/antibodies, preparation the corresponding immunoconjugates with fluorescence dyes, radionuclides or ADC-toxins, post-translational protein modification, structural proteomics and study of protein-protein interactions.

Prof. Dr. Martin Fusek, CEO of IOCB Tech: *„The basis of successful commercial outcomes from basic research results is excellent scientific work. This is an important result that is not only useful as a tool for basic research, but also as a means for development of new protein-based drugs. The uniqueness, which should rather be the rule, is that the project was created by cooperation of two academic and one commercial subject. I am very glad that we could have been part of the process, albeit small."*

Dr. Petr Beier, Head of research group at IOCB: "*I am glad that we have been able to develop a successful cross-disciplinary collaboration of organic synthesis and biochemistry. It turns out that the specific properties of fluorinated compounds can be utilized not only traditionally in the medical chemistry of small molecules, but as recently showcased also for bioconjugation of proteins and studies of their structure. I believe that in the future we will be able to identify other attractive uses of Togni reagents in biochemistry.“*

Dr. Petr Novák, Head of research group at IMIC: *„Thanks to Togni reagents we have been able to introduce a fluorinated probe into the protein structure in an aqueous environment in a matter of few*

seconds. We are now able to use this technology to selectively tag proteins for clinical diagnostics or use to it to identify the interaction interface of proteins with their ligands.

Dr. Václav Matoušek, CEO of CF Plus Chemicals: "*I am excited to see that the reactivity of Togni reagents could be extended to aromatic amino acids and aromates in general, thus opening a plethora of potential applications, especially in protein science and protein-based therapeutics. We are now actively looking for established industrial partners who could apply our technology to solve their challenges.*"

About CF Plus Chemicals: CF Plus Chemicals (www.cfplus.cz) is a Brno, Czechia-based ETH Zurich spin-off founded in 2014 focusing on life science applications of fluoroorganic chemistry both in the field of small molecules in medical chemistry and in the field of large molecules, especially of proteins.

About IOCB: The Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences / IOCB Prague (www.uochb.cz) is a leading internationally recognized scientific institution whose primary mission is the pursuit of basic research in chemical biology and medicinal chemistry, organic and materials chemistry, chemistry of natural substances, biochemistry and molecular biology, physical chemistry, theoretical chemistry, and analytical chemistry. An integral part of the IOCB Prague's mission is the implementation of the results of basic research in practice. Emphasis on interdisciplinary research gives rise to a wide range of applications in medicine, pharmacy, and other fields.

About IMIC: Institute of Microbiology of the Czech Academy of Sciences (www.mbucas.cz) represents the largest scientific body in the Czech Republic focused on physiology, cell and molecular biology of various microorganisms. Main research areas include genetics, biochemistry, physiology and ecology of bacteria, yeasts and filamentous fungi, microscopic algae and immunology. IMIC has also a unique position in multidisciplinary research mainly due to scientific excellence in already existing infrastructure modules. Namely, the next generation sequencing, mass spectrometry, nuclear magnetic resonance, electron microscopy, and germ-free animal rearing facility are developing in respect to basic research questions and to their prospective practical exploitation in medicine and industry.

References:

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