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Chemie, Life Sciences & Biotechnologie

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ASSOCIATION SUISSE DES CHIMISTES DIPLÔMÉS HES

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VOM MASTER IN DIE KREBSFORSCHUNG

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Das Wort des Präsidenten

Liebe SVC-Mitglieder
Liebe SVC-Sympathisanten

Ich freue mich, Ihnen das zweite À JOUR des Jahres 2021 zu präsentieren. Und dies auch wegen der noch immer anhaltenden, speziellen Situation einer Pandemie. Denn fällt es Ihnen auch auf? Unser À JOUR wird immer dünner. Wegen den Massnahmen gegen die Ausbreitung des vermeintlich neuen Coronavirus SARS-CoV-02 fielen viele Veranstaltungen von uns ins Wasser, über die wir natürlich berichten wollten.

Geplante Weiterbildungskurse mit und bei unseren Partnerorganisationen fanden nicht statt, und interessante Forschungsarbeiten in Chemie, Biotechnologie oder Life Sciences an den Fachhochschulen konnten nicht veröffentlicht werden. Auch die beliebte Vergabe der SVC-Preise für Bachelor- und Masterabsolventinnen und -absolventen fand ohne unsere physische Präsenz statt.

Der notwendige Austausch mit kantonalen und eidgenössischen Parlamentarierinnen und Parlamentariern sowie Behördenmitgliedern, denen die Entwicklung des naturwissenschaftlichen Bildungsangebotes an den Schweizer Fachhochschulen genau wie uns am Herzen liegt, war ebenfalls nicht möglich. Es fehlt an allen Ecken der persönliche Kontakt zur Weiterentwicklung des Netzwerkes unseres so wertvollen Berufsverbandes.

Aus diesem Grund müssen wir alle daran arbeiten, dem SVC über das À JOUR seine ursprüngliche Strahlkraft zurückzugeben. Ich rufe hiermit alle Leserinnen und Leser auf, sich für die nächsten Ausgaben um viele freiwillige naturwissenschaftliche Autorinnen oder Autoren zu bemühen oder gleich selber einen spannenden Artikel zu schreiben. Und zwar kostenlos.

Die Zeichen der Zeit stehen gut. Erinnern Sie sich noch an mein Vorwort im ersten À JOUR des Jahres 2020? Die gesamte Welt stand damals im Bann der Pandemie. Durch die damals postulierte Neuartigkeit des Coronavirus SARS-CoV-02 und dessen deshalb nicht aufhaltbare, exponentielle Ausbreitung haben Bundesrat und kantonale Behörden den Notstand ausgerufen und die Bevölkerung gebeten, nur noch dann aus dem Haus zu gehen, wenn es dringend notwendig sei.

Mittlerweile hat sich die Lage meiner Meinung nach entspannt. Allen Menschen in der Schweiz wurde ein Impfangebot gemacht. Die Spitalbetten waren bis heute nie über 80% belegt, obwohl diese in den letzten 18 Monaten um bis zu 40% abgebaut worden sind. Nun mangelt es an medizinischen Fachkräften. Dies ist eine Herausforderung für die Gesundheitspolitik.

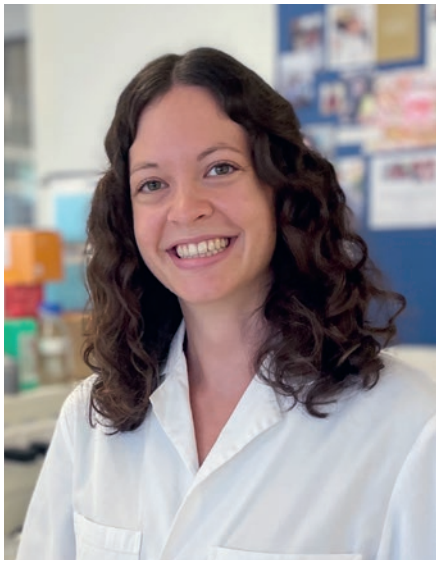
Zwei für mich wesentliche Institutionen haben je eine wichtige Information publiziert. Im Februar 2021 hat die WHO die Infektionssterblichkeit von über 3.5% auf unter 0.2% korrigiert. Und die Berliner Charité publizierte Ende August 2021 eine Studie, dass es auch bei SARS-CoV-02 eine stabile Kreuzimmunität gäbe, basierend auf der zellulären Immunabwehr. Die bereits vorhandenen T-Gedächtniszellen seien auf andere Erkältungsviren zurückzuführen. Damit wird auch klar, warum über 80% der Infizierten nicht einmal Symptome entwickelt haben. Diese wissenschaftlichen Publikationen stimmen mich zuversichtlich.

Aus den obigen Gründen hoffe ich, dass wir Ende Oktober 2021 unsere Generalversammlung inklusive unserer 75-Jahr-Jubiläumsfeier gemeinsam und persönlich auf der Burg Lenzburg feiern können.

Im Namen des Vorstandes
Marc Oliver Bürgi
Präsident

Bildquelle: Marc Bürgi

Vom Master in «Pharmaceutical biotechnology» zur Wissenschaftlichen Mitarbeiterin in der Krebsforschung



Bettina Kritzer, 04.02.1997 aus Urdorf ZH

Autorin: Bettina Kritzer

Bereits während meiner Lehre zur Pharmaassistentin entdeckte ich (Bettina Kritzer, Abbildung 1) mein Interesse für Biologie und Biochemie, weshalb ich eine Weiterbildung in diese Richtung ins Auge fasste. Ich entschied mich für das Studium der Biotechnologie an der ZHAW in Wädenswil. Dort faszinierte mich besonders die Arbeit mit Zellen im Labor, wobei ich mein erlerntes Wissen in der Praxis anwenden und vertiefen konnte. Die Arbeit im Labor wurde zu meiner Leidenschaft und so entwickelte ich den Wunsch, nach dem Studium in die Forschung einzusteigen. Aus diesem Grund absolvierte ich nach dem Bachelorstudium noch den Master in «pharmaceutical biotechnology». Dank meiner Masterarbeit hatte ich die Möglichkeit, mich in das Gebiet der Zelldifferenzierung von induzierten pluripotenten Stammzellen einzulesen und praktische Erfahrungen zu sammeln. Ich differenzierte die Stammzellen zu verschiedenen neuronalen Zellen und etablierte eine *in-vitro*-Modellierung der Blut-Hirn-Schranke. Die Masterarbeit mit dem Titel «Developing an *in vitro* model system of the blood-brain barrier using iPSCs» durfte ich im Labor für Zellphysiologie und Zell-Engineering von Prof. Dr. Jack Rohrer an der ZHAW durchführen.

Die Entwicklung eines repräsentativen *in-vitro*-Modells der Blut-Hirn-Schranke ist mitunter ein aktueller Teil für die Medikamenten- und Therapieforschung. In den letzten Jahren wurden wichtige Erkenntnisse und Erfolge in der Forschung für Medikamente gegen bisher nichtbehandelbare Krankheiten wie Parkinson, Alzheimer oder Krebs erzielt. Die Umsetzung von neuen Medikamenten in der Klinik gestaltet sich jedoch schwierig, da die Blut-Hirn-Schranke (BHS) den Blutkreislauf vom Gehirn trennt und dieses somit vor giftigen Stoffen schützt. Es ist beinahe unmöglich, eine wirksame Dosis eines Medikamentes über die BHS ins Gehirn zu transportieren. Aus diesem Grund ist es für die Entwicklung neuer Medikamente essenziell, über ein *in-vitro*-Modell der BHS zu verfügen, welches so ähnlich wie möglich die menschliche BHS simuliert. Somit können die entwickelten Medikamente auf ihre Penetrationsrate durch die Blut-Hirn-Schranke untersucht und optimiert werden.

Meine Masterarbeit beschäftigt sich mit der Entwicklung eines *in-vitro*-Modellsystems der Blut-Hirn-Schranke. Die BHS besteht grundlegend aus drei verschiedenen Zelltypen: den mikrovaskulären Endothelzellen, welche in direktem Kontakt mit dem Blut stehen, Perizyten und Astrozyten, welche die Endothelzellen auf Seiten des Gehirns unterstützen (Abbildung 2). Die verwendeten Zellen

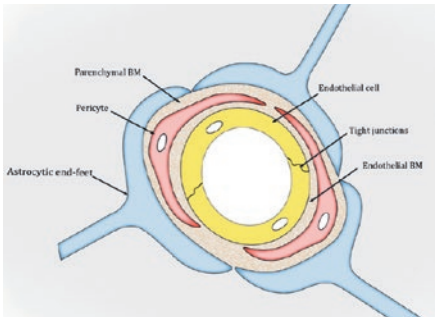


Abbildung 2: Schematische Darstellung des Aufbaus der Blut-Hirn-Schranke mit ihren Zellen: den Endothelzellen, den Perizyten und den astrozytischen Endfüssen (Xu et al., 2019).

für das Modell wurden aus humanen induzierten pluripotenten Stammzellen, auch als iPSCs bekannt, differenziert (Abbildung 4). Für das Modellsystem wurden die iPSCs zu Mikrovaskulären Endothelzellen, Perizyten und Astrozyten differenziert. Dabei wurden die generierten Zellen bezüglich ihrer Morphologie und der Expression von relevanten, biologischen Markern untersucht. In Abbildung 3 ist die Expression von spezifischen Bio-

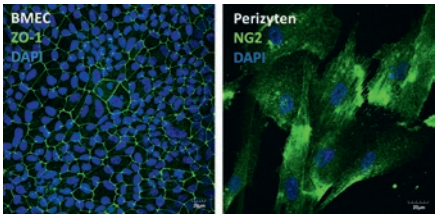


Abbildung 3: Links: BMECs mit angefärbtem Protein ZO-1 (grün), welches in der Zellmembran lokalisiert ist und dem Zellkern (blau). Rechts: Perizyten mit dem Protein NG2 (grün) in der Zellmembran und dem Zellkern (blau). Massstab: 20 um

markern für BMECs und Perizyten sichtbar, welche mithilfe eines konfokalen Mikroskops ausgewertet wurden.

Die erfolgreich differenzierten Zellen wurden auf ihre Funktionalität und somit Fähigkeit, eine funktionierende Blut-Hirn-Schranke zu bilden, geprüft. Die Integrität der gebildeten Barriere wurde mithilfe des transendothelialen, elektrischen Widerstands (TEER) sowie ihrer Permeabilität und Transporter-Funktionalität evaluiert.
Das Ergebnis dieser Masterarbeit ist ein *in-vitro*-Modellsystem der BHS, welches physiologisch vergleichbare TEER-Werte erreicht und bis zu 30 Tage stabil bleibt.

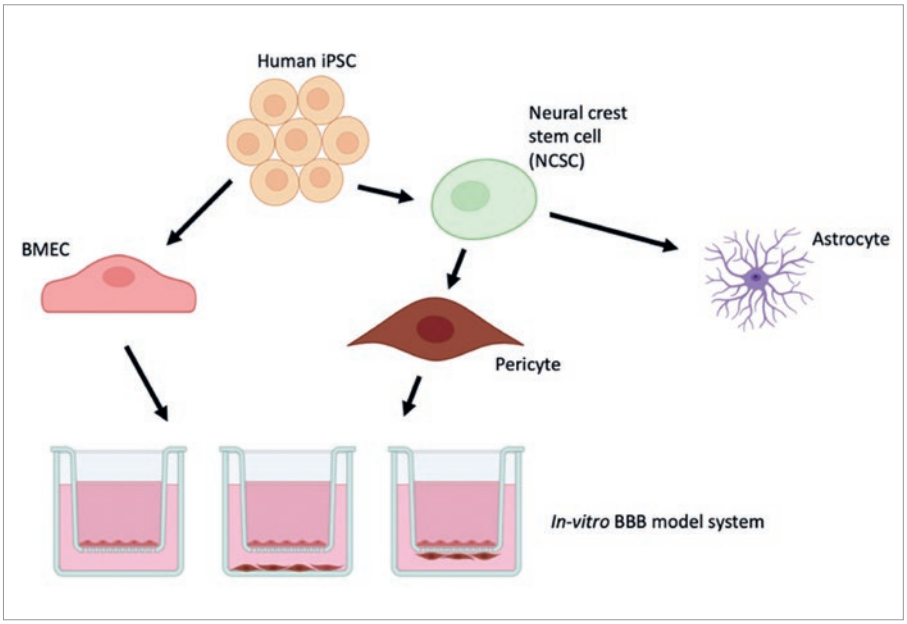
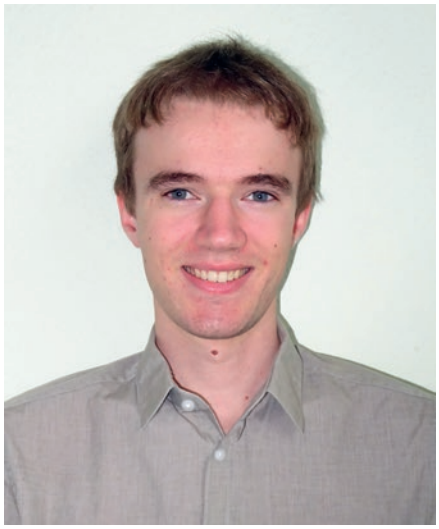


Abbildung 4: Schematische Übersicht der Methode der Masterarbeit mit der Differenzierung der Zellen sowie der Erstellung des in-vitro-Modells.

Dank meiner Masterarbeit und den dadurch erlernten Fähigkeiten ist es mir gelungen, eine Anstellung in der akademischen Forschung anzutreten. Seit Abschluss des Studiums bin ich Teil der Forschungsgruppe von Prof. Dr. Javad Nazarian am Kinderspital Zürich und forsche an pädiatrischen Hirntumoren. DIPG (diffuses intrinsisches Ponsgliom) ist ein selten auftretender und inoperabler Tumor mit minimaler Überlebenschance. Als Research Associate widme ich nun meine Zeit der Forschung nach neuen Behandlungsmöglichkeiten für Kinder mit DIPG, um zukünftigen Patienten eine bessere Überlebenschance zu bieten.

Bildquellen: Bettina Kritzer

Process Development of Bis(2-oxazoline)-based Poly(ester amide)s



Fabien Neuenschwander, 10.11.1997, Grand-Lancy GE

At the end of my compulsory schooling, I started an apprenticeship as chemistry lab technician with integrated technical vocational baccalaureate at Rolex in the R&D laboratory in Geneva. After its completion, I followed my studies with a BSc in chemistry at the School of Engineering and Architecture of Fribourg (HEIA-FR). After it, I decided to continue with a MSc with a major in chemical development and production at the University of Applied Sciences and Arts Western Switzerland (HES-SO), during which I had the opportunity to carried out this Master thesis under the supervision of Prof. Roger Marti.

Author: Fabien Neuenschwander

Abstract
The aim of this Master thesis was the process development of poly(ester amide) (PEA) from 2,2'-bis(2-oxazoline) in a kneader reactor. In this study, a wide range of poly(ester amide)s produced by polyaddition of 2,2'-bis(2-oxazoline) with different dicarboxylic acids were synthesised either in molten state and in solution in N,N-dimethylformamide (DMF). The impact on polymer thermal properties of chain length, aromaticity and heteroatoms present in the dicarboxylic acids was studied as

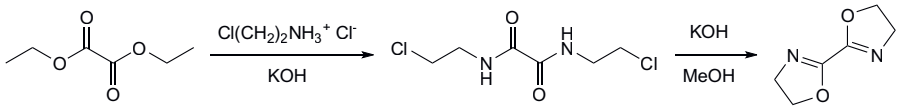
these dicarboxylic acids have a big impact on polymer thermal properties. Copolymers enable access to a wide range of melting points (mp) by varying the molar ratio between the two components. After thermal safety process analysis, the two-step synthesis of 2,2'-bis(2-oxazoline) from diethyl oxalate and 2-chloroethylamine hydrochloride was scaled up from laboratory scale to kg-scale. The overall yield decreased from 63% to 48% due to formation of impurities. The scale-up of the PEA based on sebacic acid in kneader reactor was also carried out giving a PEA with a weight average molecular weight (Mw) of 17 kDa and melting points of 146/172 °C.

Introduction

PEAs are very interesting and promising polymers, because they take advantage of the best properties of both polyesters and polyamides. Indeed, they have good degradability characteristics, specific to the polyesters, and simultaneously typical good thermomechanical qualities of polyamides. Since amide moieties provide the crystallisation properties due to the formation of hydrogen bonds and that ester groups provide the rubber characteristics to the polymer, the properties can be adapted as desired by varying the ester/amide ratio. For example, by increasing the amide ratio in the polymer, the modulus and yield stress grow. [1, 2] These two main characteristics are very interesting for various applications, as in medicine for its use as biocompatible and bioresorbable implants, but it could also be used as disposable bags, agricultural films, or drug carriers. [3, 4]

Result and discussion

After process safety analysis, the scale-up of the synthesis of 2,2'-bis(2-oxazoline) from diethyl oxalate and 2-chloroethylamine hydrochloride (cf. scheme 1) was successfully done from laboratory scale to kg-scale despite a decrease of the overall yield from 63% to 48%. As summarised the yield remained constant at 78% during the synthesis of N,N'-bis(2-cholorethyl) oxamide (1st step). However, for the second step, the yield dropped from 80% to 62% due to the formation of the hydrolysis product, N,N'-bis(2-hydroxyethyl)oxamide, and a red-orange compound. The causes of the side product formation were not identified.



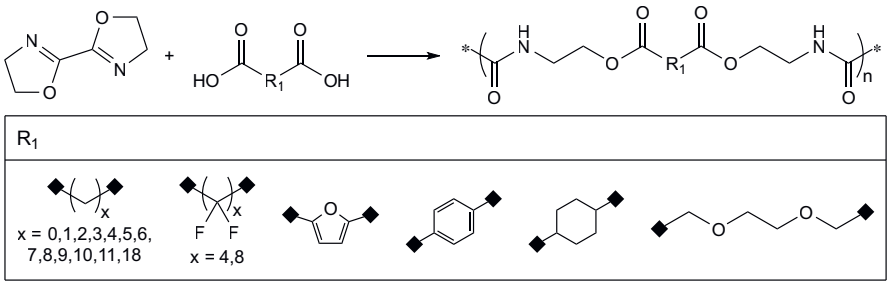
Scheme 1: Two-step synthesis of 2,2'-bis(2-oxazoline).

	Laboratory scale		Kg-scale
Synthesis / Reactor volume	**100 mL**	**750 mL**	**15 L**
N,N'-Bis(2-cholorethyl)oxamide	79% (16 g)	78% (0.15 kg)	78% (3.0 kg)
2,2'-Bis(2-oxazoline)	80% (7 g)	81% (72 g)	62% (1.1 kg)
Overall yield	**63%**	**63%**	**48%**

Table 1: Summary of yield (with isolated masses) as a function of scales and syntheses.

PEAs were obtained by polyaddition of 2,2'-bis(2-oxazoline) with various dicarboxylic acids (cf. scheme 2) both in solution in DMF and in bulk. Polymerisation in solution is a good way to test new dicarboxylic acids at small scale (around 0.5 g) and to obtain information on polymer melting points, even if the Mw is only around 3 kDa compared to up to 36 kDa in bulk conditions. Consequently, melting points of polymers synthesised in solution are lower (about 5 °C of difference) in comparison with those manufactured in bulk (cf. figure 1).

On DSC thermograms, almost all polymers show two melting points separated by a cold crystallisation between 130°C and 175°C, due to the recrystallization of oxalamide moieties. As summarised in table 2, the impact of various factors related to the dicarboxylic acids on polymer thermal properties was studied. The increase of the number of carbon atoms in the dicarboxylic acid implies a rise in the first melting point and a decrease in the second until they join as a point, as observed in bulk conditions with eicosanedioic acid at 157 °C. However, no change is observed on glass transition temperature (Tg). For smaller dicarboxylic acids with a number of carbon atoms comprised



Scheme 2: Reaction scheme of all PEAs synthesised by polyaddition of 2,2'-bis(2-oxazoline) and dicarboxylic acids.

between four and eight, bulk synthesis was not conclusive in molten state, since Weissenberg effect occurs, and polymerisation cannot be achieved. For oxalic, malonic and fluorinated acids, no polymer was recovered in both solution and bulk, because they are decomposed due to process temperatures. For dicarboxylic acids containing a ring (aromatic or not), such as terephthalic acid, 2,5-furandicarboxylic acid or 1,4-cyclohexanedioic acid, phenomena similar to those observed for small dicarboxylic acids occur, but in addition they show melting points very close to the degradation temperature.

Copolymers produced with an equimolar proportion of two straight-chain saturated dicarboxylic acids have melting points lower than PEA manufactured with only one of their dicarboxylic acids, with a difference of up to 40 °C. This decrease can be adjusted by varying the ratio between the two dicarboxylic acids. Indeed, by increasing the molar proportion of one dicarboxylic acid, the melting point will get closer to the one of its respective PEA. This enables access to a wide range of melting points without using additives. Polymers and copolymers made with straight-chain saturated dicarboxylic acids with a number of carbon atoms upper or equal to eight are thermally stable, and their thermal stability increase with the number of carbon atoms.

All polymers were characterised by infrared spectroscopy and did not show any signals corresponding to a crosslinking, but these thermoplastics could be easily transformed in thermosets by using a large excess of 2,2′-bis(2-oxazoline) and triphenylphosphite as catalyst, as reported by Wilsens et al. [5] The scale-up of PEA in the kneader reactor was carried out with sebacic acid as it is a biobased dicarboxylic acid. During the scale-up, no decline of thermal properties was observed. Melting points remained constant around 150 °C and 172 °C.

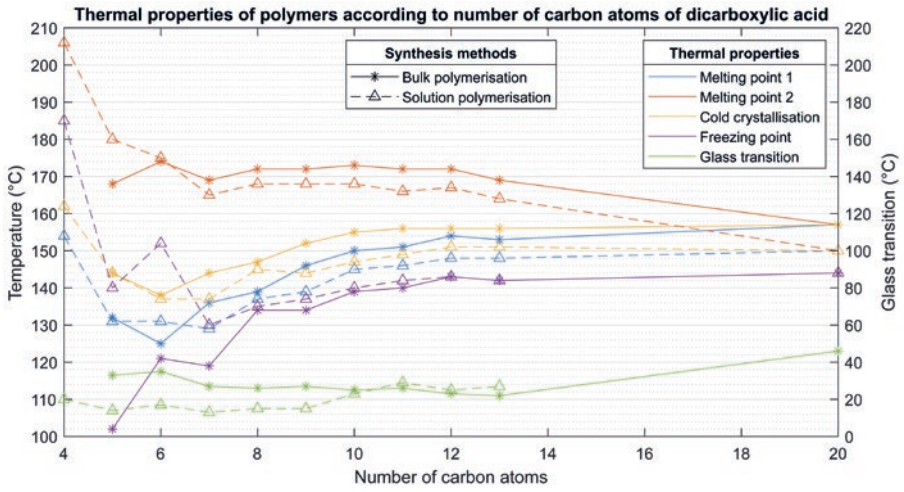


Figure 1 Thermal properties of PEAs according to the number of carbon atoms of the straight-chain dicarboxylic acid and the synthesis method.

Conclusion

A promising family of PEAs with tuneable melting points was synthesised in this work by polyaddition of 2,2′-bis(2-oxazoline) and dicarboxylic acids and successfully scaled-up in the kneader reactor. A better understanding of impurities formation during the second stage of 2,2′-bis(2-oxazoline) synthesis is required to improve the overall yield and to reach at least an overall yield of 63% obtained at lab scale. Additional tests should also be done to screen the impact on polymer thermal properties using bis(2-oxazoline) derivatives with various spacers between the two 2-oxazoline rings. These trials are currently done at the HEIA-FR and the results will be soon published.

Impact of	**Observations**
Chain length [a]	• ↗ n(C): → T_g, ↗ 1st mp, ↘ 2nd mp, ↗ thermal stability. • 4 ≤ n(C) ≤ 6: Weissenberg effect observed in bulk conditions. • n(C) ≤ 3: Dicarboxylic acid decomposed due to process conditions.
Ring / aromaticity [b]	• Weissenberg effect observed in bulk conditions. • Mp > 250°C, but close to degradation point.
Heteroatoms	• 3,6-dioxaoctanedioic acid: no melting point, but liquid above T_g and flexible at room temperature (20°C). • Fluorinated dicarboxylic acids decomposed due to process conditions.
Copolymers [a,c]	↘ mp up to 40°C compared to mp of PEAs produced with one dicarboxylic acid. By adapting the molar ratio, mp can be adjusted.

Table 2: Summary of the impact of various factors on polymer thermal properties. a For straight-chain saturated dicarboxylic acids with a number of carbon atoms between 4 and 20. b For 2,5-furandicarboxylic acid (FDCA), terephthalic acid and 1,4-cyclohexanedioic acid. c Made with sebacic acid as second dicarboxylic acid.

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Source: Fabien Neuenschwander

Engineering of Epimerases towards Pharmaceutical Applications

Sandro Giger completed his apprenticeship as a chemical laboratory technician at Novartis and then studied chemistry at the Zurich University of Applied Sciences (ZHAW). During his military service after graduation, he realized that he wanted to further deepen his knowledge. Back at the ZHAW, he was able to complete his master's degree under the supervision of Prof. Dr. Rebecca Buller, head of the biocatalysis group at the ZHAW. Currently, Sandro Giger is a PhD student at the ZHAW in collaboration with the Vienna University of Technology. His research focuses on engineering enzymes for synthetic applications.

Author: Sandro Giger

The World Health Organization (WHO) states that the current clinical pipeline of antibacterial agents is insufficient to tackle the challenge of increasing emergence and spread of antimicrobial resistance [1]. Many pharmaceutical companies have abandoned research on new antibiotics leading to a shortage of new drugs available [2]. Making matters worse, antibiotics are excessively used as human therapeutics and for livestock farming, thus facilitating and accelerating the development of bacterial resistances against existing drugs.

Antimicrobial peptides (AMPs) are found in all five kingdoms of live. They offer an alternative to classical antibiotic substances by selectively acting upon the negatively charged bacterial membranes, while the uncharged cell membranes of plants and animals remain unaffected [3]. So far, only a few AMPs such as vancomycin or polymyxin B are used in human therapy. AMP's oral bioavailability is typically limited due to low stability and poor penetration of the intestinal mucosa. Furthermore, regulatory agencies struggle with guidelines defining the minimum purity for peptide therapeutics [4]. D-amino acid containing peptides, however, offer a promising approach to overcome some of the drawbacks of AMPs [5]. In various studies, which compared D-amino acid containing AMPs with their all L-counterparts, D-amino acid containing AMPs have shown higher protease stability while maintaining or increasing their antimicrobial activity without exhibiting toxicity against mammalian cells [6, 7].

Radical S-adenosylmethionine (rSAM) enzymes create a radical species by cleaving S-adenosylmethionine (SAM) reductively, which enables them to catalyze a wide range of different reactions, such as epimerisations [8]. The first rSAM enzyme capable of selectively inverting the stereoconfiguration of amino acids in peptides, called PoyD, has been described in 2012 [9]. Later, OspD, AvpD and PlpD [10], as well as YydG [11] have been added to the rSAM epimerase family. The proposed reaction mechanism of rSAM epimerases works as follows: SAM is cleaved reductively by a [4Fe – 4S] iron-sulfur cluster resulting into a radical 5'-deoxyadeonsine species, which then abstracts the C_α-hy-

drogen atom from an amino acid in the peptide substrate. A solvent derived thiol hydrogen atom, provided from a cysteine residue, quenches the radical species stereoselectively resulting in the formation of a D-amino acid [11]. In my thesis, the rSAM epimerase YydG was expressed as a Strep-tag fusion protein in E. coli. In vivo YydG epimerizes one valine and one isoleucine residue in YydF [11]. The activity of YydG was verified by in vitro reactions with a truncated version of its native substrate, called YydFcore. To allow for analysis by LC-MS, the epimerized peptide was digested with trypsin resulting in three short peptides.

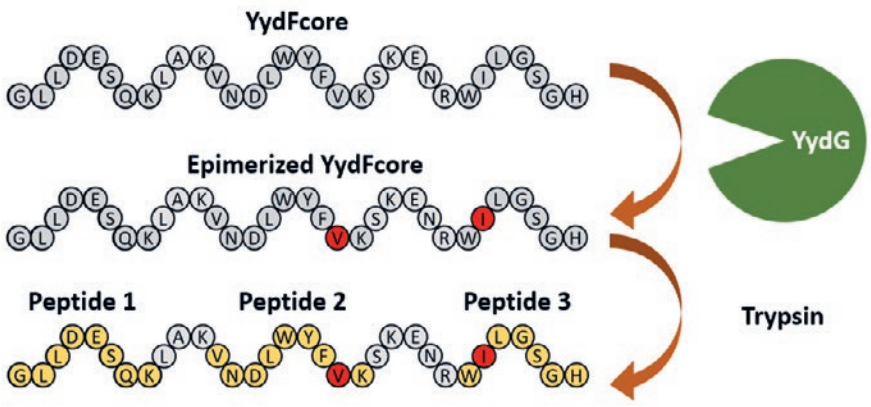

Figure 1: Schematic representation of the epimerization of peptide YydFcore with the epimerase YydG and subsequent digestion with trypsin for LC-MS analysis. The epimerized residues are colored in red, the three resulting peptides after trypsin digest are colored in yellow.

YydG was then tested upon additional substrates, including antimicrobial peptides. Notably, the enzyme was able to epimerize several non-native peptides and their modified analogues. Analysis of the epimerization pattern gave first insights into the mechanism at the basis of the enzymatic epimerization reaction, i.e., which amino acid sequence in the peptide might define the epimerization site.

Since the conformational change of a single or two amino acids in a peptide often only has a low impact on the analyzable characteristics of the molecule, analysis of epimerase products is delicate. To overcome this problem, the in vitro reactions with YydG were performed in buffer that was prepared with deuterated water. As the hydrogen atom which is donated by the enzyme to form the D-configured amino acid is ultimately derived from solvent, these experiments allowed for the incorporation of a deuterium atom into the peptide. Utilizing MALDI-TOF-MS, the isotopic shift of a deuterium incorporation can be observed in this way confirming the epimerization reaction. To pinpoint the exact epimerization site, epimerized peptides were subjected to LC-qTOF analysis, which allowed to determine the epimerization site by investigation of b- and y-ions of the peptides.Overall, only selected peptides were epimerized by YydG possibly indicating that certain

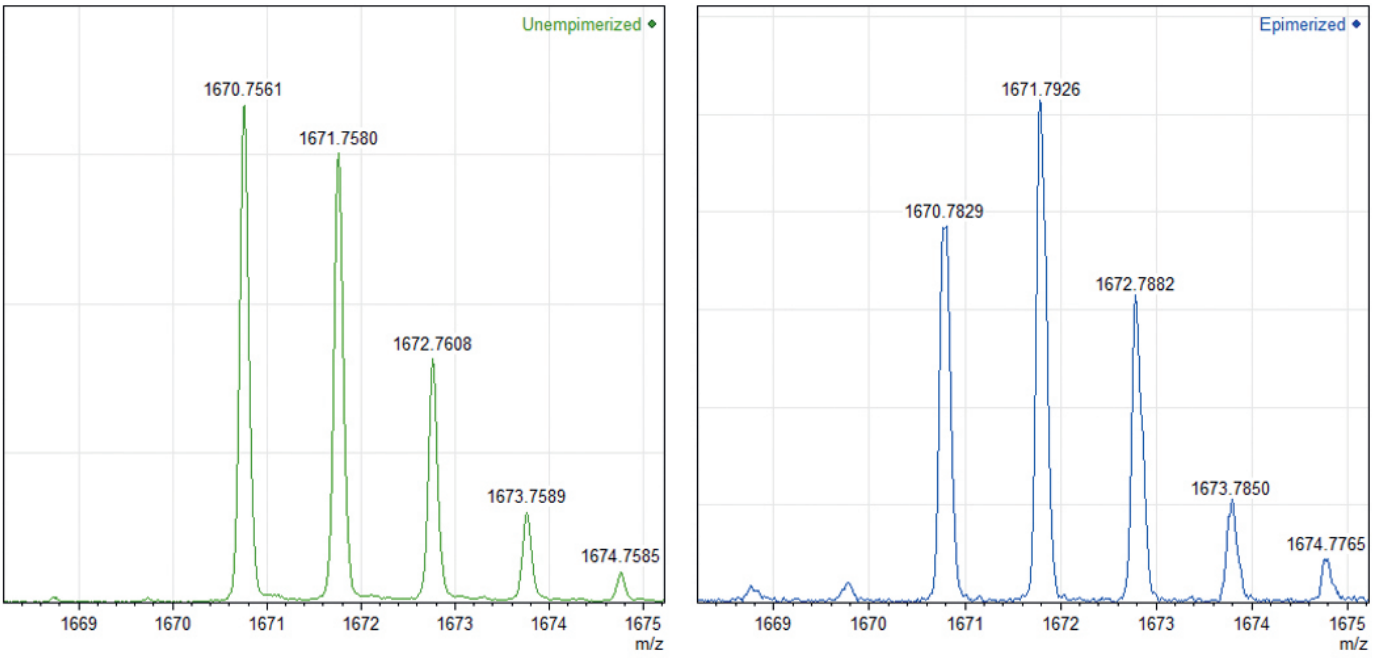

Figure 2: MALDI-TOF-MS spectra of two peptides. Left: Non-epimerized reference peptide. Right: Peptide epimerized in deuterated buffer, resulting in an observable mass shift.

peptide sequence motifs are crucial for epimerization by YydG. Using this knowledge, further studies will aim to investigate if additional non-native peptides can be epimerized by YydG.

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Source: Sandro Giger

Biocatalytic Potential of alpha-Ketoglutarate Dependent Dioxygenases

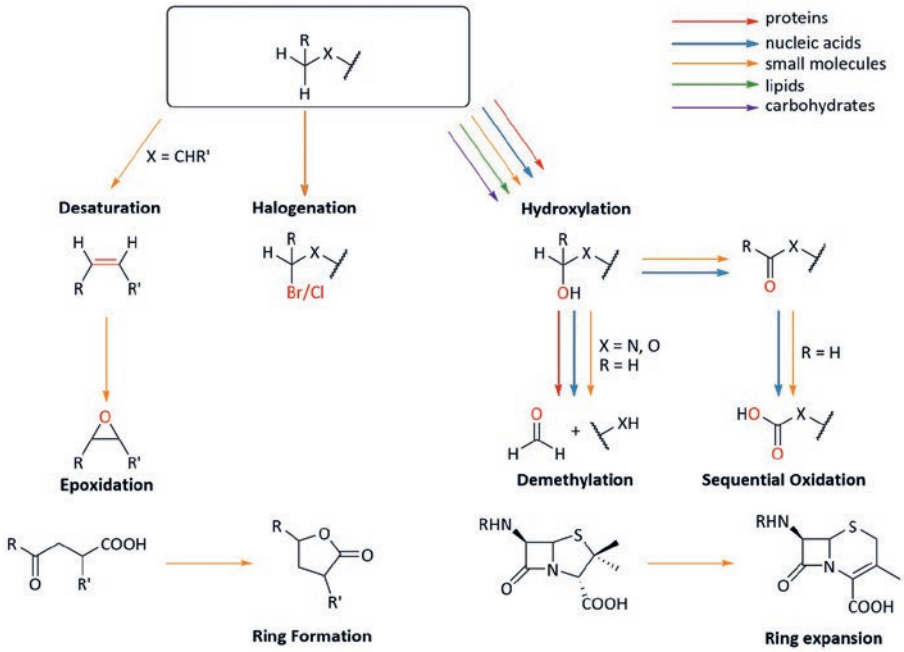
Jan Taubitz started his career with an apprenticeship as a chemical laboratory technician with integrated baccalaureate at the Novartis Pharma AG in Basel. After completion of his apprenticeship, he commenced a bachelor's degree in Chemistry at the Institute for Chemistry and Biotechnology of the Zurich University of Applied Science. Finishing his bachelor's degree with a thesis at the Competence Centre of Biocatalysis (CCBIO) of Prof. Dr. Rebecca Buller, he decided to continue his studies with a Master of Science in Chemistry. Under the supervision of Prof. Dr. Rebecca Buller and Dr. Fabian Meyer, Jan Taubitz completed his degree with a thesis about the biocatalytic potential of α-ketoglutarate dependent dioxygenases (αKGDs). Currently Jan Taubitz carries out an internship in biocatalysis at Givaudan SA in Kemptthal.

Author: Jan Taubitz

The enzyme family of α-ketoglutarate dependent dioxygenases (αKGDs) was first described in the late 1960s. Today, over 80 human αKGDs have been described and even more exist in plants and animals. αKGDs constitute the largest en-

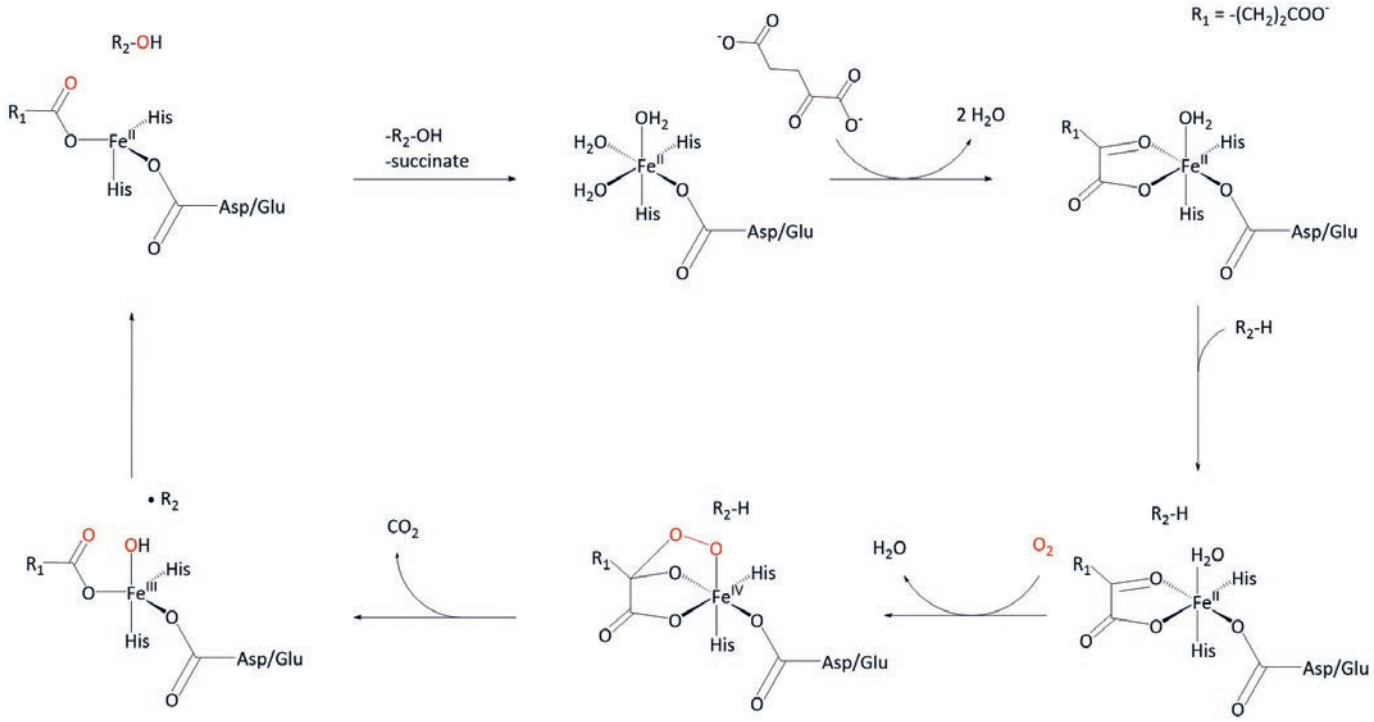
zyme family known to catalyse oxidation reactions without containing a Haem-Group [1, 2]. Notably, the reaction scope of αKGDs is versatile and includes reactions such as hydroxylations, epoxidations, halogenations and many more [3].

As the stereo- and regioselective functionalisation of unactivated C-H bonds by conventional chemical reactions remains challenging, enzymatic approaches have attracted increasing interest. The diverse reaction- and substrate scope of αKGDs makes them ideally suited candidates as biocatalysts for modifications of sp3 C-atoms in molecules. Currently, biocatalytic reactions with αKGDs catalysts are usually monitored by analysis of the substrates and products, for example by LC- or GC-MS. The diverse substrate- and reaction scope requires that the analysis method must be tailored to each enzyme specifically, a process which is time-and resource consuming. However, despite the broad substrate scope and the plethora of reactions catalysed by αKGDs, the general reaction mechanism in αKGDs is conserved and is coupled to the oxidative decarboxylation of the co-substrate α-ketoglutarate to form succinic acid in all cases [3].

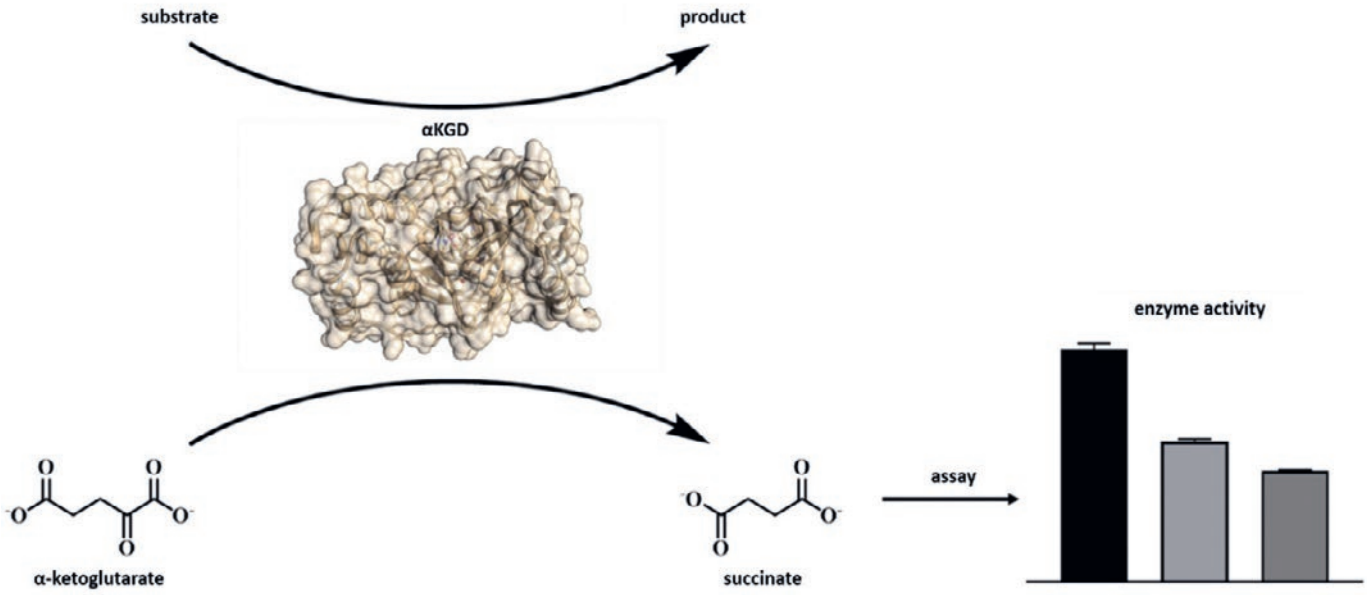


REACTION SCOPE: Overview of the reaction scope of αKGDs. The colour of the arrows indicates the substrates for the respective reactions. Figure adapted from Islam et al [3].

The co-substrate α-ketoglutarate is transformed to succinic acid in equimolar amounts to the main reaction product and the reaction can therefore be used to monitor the enzyme activity. In this way,



MECHANISM: Consensus mechanism of αKG-dependent hydroxylases. Mechanism and figure adapted from Islam et al. [3], Peters et al. [4] and Voss et al. [5].

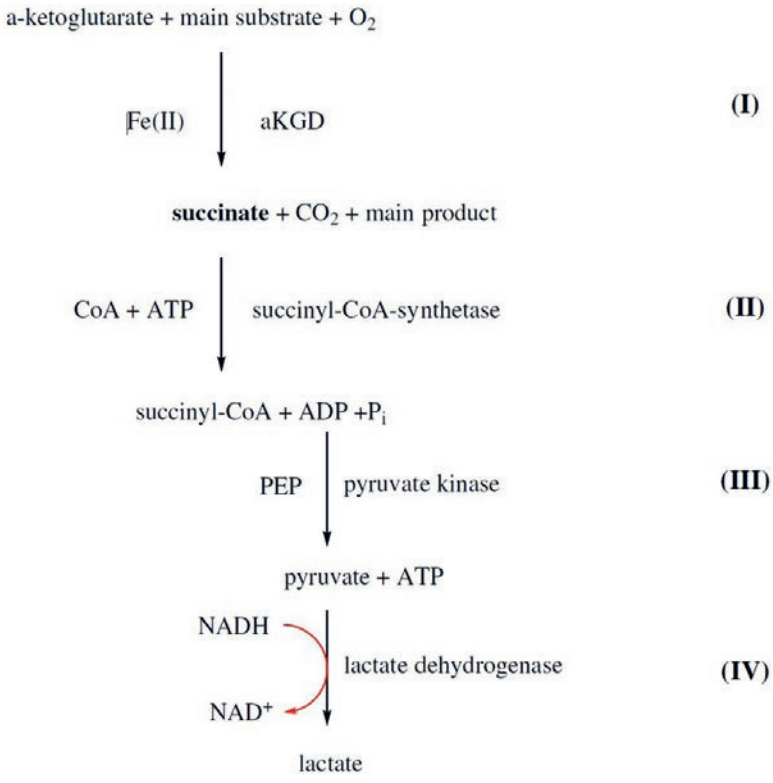


PRINCIPLE: Schematic representation of a universal screen for αKGDs. Crystal structure from Koketsu et al [6].

the activity measurement of various αKGDs can be carried out independently of their main reaction. In this thesis, the concept of two different approaches for the measurement of the side reactions was investigated and their use for the activity determination of a set of representative αKGDs was validated.

The first approach was designed to directly detect succinate formation by liquid chromatography coupled to mass spectrometry (LC-MS), while the second method was based on an enzymatic reaction cascade which allowed to detect succinate formation photometrically.

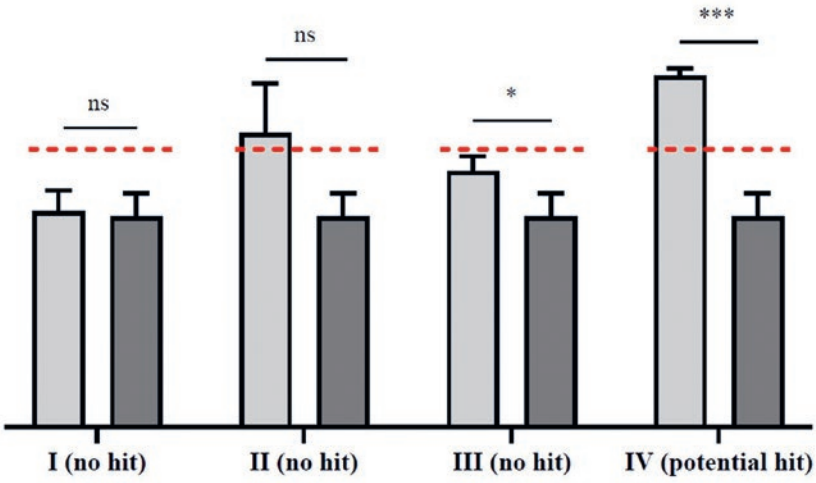
A library of 36 diverse αKGDs was modified by adding a N-terminal his-tag for enzyme purification. Soluble expression for 29 of 36 αKGDs was confirmed by SDS-PAGE. Based on the expression and substrate availability, four enzymes were selected and cultivated in batches. Initial validation of the LC-MS method and enzymatic assay was done by analysis of the biotransformation with the native substrates. For the identi-



ENZYME: Enzymatic assay to determine the activity of αKGDs. The biocatalytic transformation of the main substrate by αKGDs leads intrinsically to the formation of succinate (I). Succinate can be converted to succinyl-CoA by the enzyme succinyl-CoA-synthetase. Thereby, ATP is transformed to ADP (II). In a next step, pyruvate kinase transfers a phosphate group from phosphoenolpyruvate (PEP) to the formed ADP, under the release of an equimolar amount of pyruvate (III). In the last step, the formed pyruvate is reduced by lactate dehydrogenase. Thereby, NADH gets oxidized to NAD+ (IV). The decrease of NADH (indicated with a red arrow) can be measured at 340 nm. Scheme adapted from Luo et al. [7].

fication of potentially active reactions, different statistical tests were conducted. Both methods were able to detect significant formation of succinic acid and thus confirming enzyme activity of the enzymes towards their native substrates. Furthermore, both methods revealed unknown enzyme-substrate pairs as potentially active.

It is anticipated that with the conceptional validation of two methods capable of measuring αKGD activity independently from the enzyme's main reaction, the screening of diverse αKGDs libraries can be accelerated. By optimization of the screening parameters, such as the LC-MS method and conditions of the enzymatic assay, the economics of the screen could be further improved. Combining the here-established high-throughput screening with in silico approaches (virtual screening) is expected to further advance the search for industrially valuable C-H activation catalysts.



STATISTICS: Illustration of the analysis parameters to determine the significance of the difference between succinic acid concentration in biocatalysis (light grey) and uncoupled reactions (dark grey). The significance was determined by one-way ANOVA (enzymatic assay) or t-test (LC-MS), and is indicated above the bars. Additionally, a threshold was added, representing the limit of detection (LOD) and is indicated as a red line. In case I, neither of the parameters is full-filled, the biocatalytic reaction would be considered as not active. Case II shows a scenario, in which the threshold is surpassed, but the non-significance of the biocatalysis result compared to the negative control leads to a classification as "no hit". In case III, the succinic acid formation in the biocatalysis is significantly different from the uncoupled reaction. However, the LOD is not exceeded, which would also lead to a negative classification. Case IV shows a biocatalysis reaction, which would be considered as a hit. Both parameters are satisfied.

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Source: Jan Taubitz

Le mot du Président

Chers membres de la SVC,
Chers sympathisants de la SVC,

J'ai le plaisir de vous présenter le deuxième À JOUR de l'année 2021. Et cela malgré la situation particulière persistante de pandémie. L'avezvous remarqué aussi ? Notre À JOUR s'amincit de plus en plus. En raison des mesures contre la propagation du soidisant nouveau coronavirus SARS-CoV-02, beaucoup de nos événements sont tombés à l'eau, dont nous avons naturellement voulu rendre compte.

Les cours de formation continue prévus avec nos organisations partenaires n'ont pas eu lieu et des travaux de recherche intéressants en chimie, biotechnologie ou sciences de la vie dans les hautes écoles spécialisées n'ont pas pu être publiés. La remise populaire des prix SVC pour les bacheliers et masters s'est également déroulée sans notre présence physique.

L'échange nécessaire avec les parlementaires cantonaux et fédéraux ainsi qu'avec les membres des autorités qui, comme nous, se soucient du développement de l'enseignement des sciences naturelles dans les hautes écoles spécialisées suisses, n'a pas non plus été possible. Partout il y a un manque de contact personnel pour le développement ultérieur du réseau de notre si précieuse association professionnelle.

Pour cette raison, nous devons tous travailler pour redonner à la SVC son éclat d'origine à travers l'À JOUR. J'appelle toutes les lectrices et tous les lecteurs à encourager les nombreux auteurs scientifiques volontaires ou pourquoi pas vousmême à écrire un article passionnant. Et cela bénévolement.

Les signes des temps sont bons. Vous souvenezvous de mon avantpropos du premier À JOUR de 2020? Le monde entier était alors sous le charme de la pandémie. En raison de la nouveauté du coronavirus SARS-CoV-02, et en partant du postulat que sa propagation serait imparable et exponentielle, le Conseil fédéral et les autorités cantonales ont déclaré l'état d'urgence et demandé à la population de ne quitter la maison qu'en cas d'urgence ou d'impérieuse nécessité.

Entretemps, à mon avis, la situation s'est apaisée. Une offre de vaccination a été faite à toutes les personnes en Suisse. À ce jour, les lits d'hôpitaux n'ont jamais été occupés à plus de 80 %, bien qu'ils aient été démantelés jusqu'à 40 % au cours des 18 derniers mois. Maintenant, il y a une pénurie de professionnels de la santé. C'est un défi pour la politique de santé.

Deux institutions à mes yeux dignes de foi, ont chacune publié des informations importantes. En février 2021, l'OMS a corrigé la mortalité par infection de plus de 3,5 % à moins de 0,2 %. Et la Berlin Charité a publié fin août 2021 une étude selon laquelle il existait également une immunité croisée stable avec le SARS-CoV-02, basée sur la défense immunitaire cellulaire. Les cellules T mémoire déjà existantes seraient dues à d'autres virus du rhume. Cela montre également pourquoi plus de 80% des personnes infectées n'auraient même pas développé de symptômes. Ces publications scientifiques me donnent confiance.

Pour les raisons cidessus, j'espère qu'à la fin du mois d'octobre 2021, nous pourrons célébrer notre Assemblée générale, y compris notre célébration du 75e anniversaire, ensemble et en personne au château de Lenzburg.

Au nom du comité
Marc Oliver Bürgi
Président

Source des illustration: Marc Bürgi

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