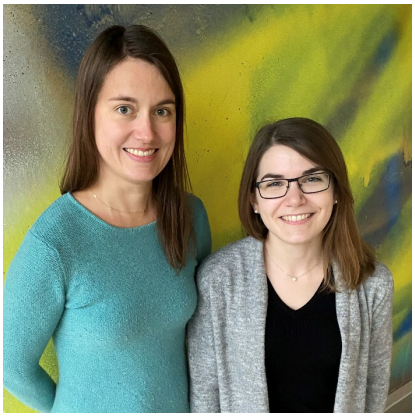
UNIVERSITÄT BIELEFELD

CeBiTec Center for Biotechnology

CeBiTec – Quarterly

Winter 2023/2024

Successful kick-off of new „Early Career Female Scientist Seminar“ series



© M. Sagasser

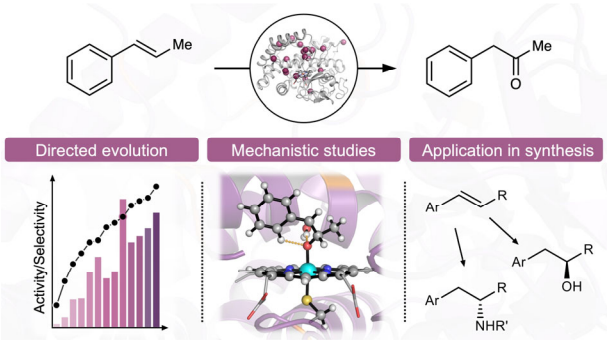
The new „Early Career Female Scientists series“ successfully started in October 2023. The founding members Dr. Nadja Alina Henke (Genetics of Prokaryotes) and Dr. Hanna Schilbert (Genetics and Genomics of Plants) invited Dr. Annina Kemmer (TU Berlin) and Prof. Dr. Susanne Neugart (Georg-August-University Göttingen) respectively to give insights into their research in the CeBiTec Lunch colloquium. Dr. Annina Kemmer talked about scale-down approaches in bioprocess development. Prof. Dr. Neugart presented the impact of UV radiation on the biosynthesis of plant secondary metabolites and its potential use in food applications. The committee is now looking for motivated PhD students as well as PostDocs who want to shape the future of this seminar series to anchor this as part of the CeBiTec symposia.

(N. Henke & H. Schilbert)

Hammer group publishes biocatalytic route to ketones via enzymatic oxidation of alkenes in Nature Catalysis

The development of new catalytic processes to access desired molecules more sustainably is an

important challenge in current synthetic chemistry. Biocatalysis offers one approach to address this challenge. In this context, natural enzymes are used to accelerate and control chemical reactions. Today, enzymes are applied industrially to produce a variety of products. However, the biocatalytic approach is largely limited to enzyme classes that are found in nature. The research group of Stephan Hammer (Faculty of Chemistry) has now succeeded in developing a new enzyme function that converts easily accessible alkenes into important ketones by a catalytic aerobic oxidation process.



Graphical abstract of the published study. © S. Hammer

Ketones are key intermediates in synthesis and are common in many important products. The direct regioselective synthesis of ketones from readily available internal alkenes could shorten synthetic routes and solve a long-standing challenge in catalysis. This sought-after chemical transformation is theoretically conceivable but only partially accessible in practice. The new enzyme function was generated by transferring the process of natural evolution to the laboratory scale through iterative mutagenesis and screening. The evolved enzyme catalyses the direct oxidation of internal aryl alkenes to ketones with several thousand turnovers and benefits from 15 mutations, most of them distal to the active site. Mechanistic studies performed with Dr. Marc Garcia-Borràs (Girona University) revealed how the 15 mutations collaborate to generate a highly reactive carbocation species and control the accessible conformations of this intermediate. The developed enzyme utilizes a metal-oxo compound as the catalytically active species for ketone synthesis and enables various challenging functionalization reactions of aryl alkenes. These include the catalytic, enantioselective oxidation of internal alkenes to ketones as well as the formal asymmetric hydrofunctionalization of internal alkenes in combination with other biocatalysts.

In the long term, such new enzyme functions can not only help to shorten the synthesis of important molecules (e.g. drugs) but can also expand metabolism in living organisms.

This collaborative work was carried out by doctoral researcher Sebastian Gergel (Bielefeld University) and Jordi Soler (Girona University) as co-first authors, and by Alina Klein, Kai Schülke (both Bielefeld University) and Bernhard Hauer (University of Stuttgart) under the direction of Marc Garcia Borras (Girona University) and Stephan Hammer (Bielefeld University). This research was recently published in Nature Catalysis:

Gergel, S., Soler, J., Klein, A. *et al.* Engineered cytochrome P450 for direct arylalkene-to-ketone oxidation via highly reactive carbocation intermediates. *Nature Catalysis*, **2023**, https://doi.org/10.1038/s41929-023-00979-4

(S. Hammer)

Schönhuth group develops “hybrid-hybrid” correction of errors in long reads

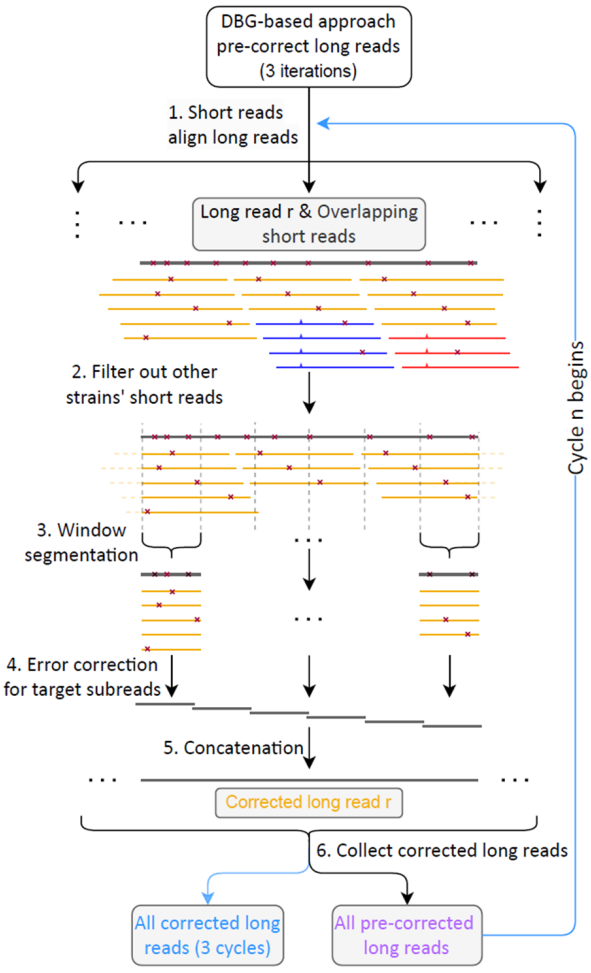
Sequencing reads from inexpensive and affordable third-generation technologies still contain substantial amounts of errors. The high error content puts their length, i.e. their landmark advantage into context in various applications. An obvious, but little sustainable solution is to dispose of the earlier and proceed with more recent third-generation sequencing platforms, where the error rates of the latter type are similar to those of near-error-free short-read sequencing platforms.

The solution just sketched, however, is not only unsustainable, because it means dumping the earlier machines in favour of more recent technology. In fact, it is also very expensive because of the costs of sequencing runs on later TGS platforms. Because the advantages of later TGS platforms are due to considerably more sophisticated sequencing protocols that claim both experimental and computational resources, expenses can be expected to run at 3-5 times the amounts to be expected for earlier, more conventional TGS platforms.

Therefore, solutions that address the exploitation of existing technology are not only truly sustainable but also incur considerably lower costs. In addition, when combining conventional TGS with short-read sequencing of the common type (such as provided by Illumina, for example), the resulting reads are not only cheaper to generate, but also considerably longer, and contain even fewer errors than the reads reflecting the latest advances in sequencing technology.

We have developed HERO, as a method that removes errors from long, still very noisy reads via the integration of little erroneous, short reads. Since almost every sequencing laboratory worldwide can generate both conventional long reads and classic short reads, HERO also offers services for laboratories that operate at rather limited budgets, so crucially depend on sequencing solutions that are sustainable in terms of laboratory equipment.

As alluded, sequencing reads processed with HERO for error removal are near-error-free, with error rates outperforming those of platforms of the latest generation. Of course, near-complete removal of errors is the primary goal of an error correction approach.



HERO: Error correction workflow © A. Schönhut

HERO is “hybrid-hybrid” in nature, which has been key to the success of overcoming certain limitations of conventional hybrid approaches, characterized by drawing from more than one type of sequencing reads. HERO being “hybrid-hybrid” addresses that HERO is not only hybrid in terms of data usage but is also hybrid in terms of methodical paradigms: unlike all earlier error correction approaches, HERO employs both overlap graphs and de Bruijn graphs as data structures to arrange the sequencing reads and systematically exploit their information content, which is a novelty.

Using HERO is generic in terms of applications. In fact, since HERO requires only very small read data volumes by its design, which adds to its benefits in terms of economic features, HERO can also treat low coverage samples of all kinds, for example.

X. Kang, J. Xu, X. Luo, A. Schönhuth (2023) Hybrid-hybrid correction of errors in long reads with HERO. *Genome Biology*, 24: 257; https://doi.org/10.1186/s13059-023-03112-7

(A. Schönhuth)

New Workshop ‚Photosynthesis in Biotechnology‘ in the *teutolab*-biotechnology

Since last summer, *teutolab*-biotechnology has been developing a new workshop called ‚Photosynthesis in Biotechnology‘. In this school year, about 40 courses will be carried out with students of upper secondary levels. The relevance of photosynthesis and metabolic processes has strongly increased as the curriculum includes these topics now in school leaving examinations.

For a deeper understanding of photosynthesis, *teutolab*-biotechnology designed four experiments: Most importantly, the students analyze the growth of algae under the influence of abiotic factors. They compare the development of the optical density in a standard growth medium, under acidic and alkaline conditions and with the addition of glucose. Starting with this experiment in the morning and providing light and ventilation, *Chlorella vulgaris* shows measurable reproduction (or decrease) during the workshop.

Preparing the samples for the growth experiment with *Chlorella vulgaris* © K. Röllke

The students figure out that glucose enhances algae growth and that an appropriate pH value is relevant. The *teutolab*-biotechnology would like to thank the Algae Biotechnology and Bioenergy group (especially Dr. Thomas Baier) for providing *Chlorella vulgaris* and information on cultivation. The *teutolab*-biotechnology would also like to thank the Multiscale Bioengineering group (especially Dr. Domink Cholewa) for supporting us with *Euglena gracilis* for our first approach.

After mixing the algae cultures and measuring for the first time, the students examine both *Chlorella vulgaris* and *Elodea elegans* for comparison with a multicellular plant under the microscope. This gives them an insight into the shape and number of chloroplasts. In the afternoon, the students analyze the photosynthetic activity of both organisms by measuring the production of oxygen under different light conditions (darkness, white, green and red light).

Students measuring the optical density of *Chlorella vulgaris*
© K. Röllke

They learn to explain the results using the absorption spectrum of the different pigments. The photosynthetically active molecules are made visible by chromatographic examination. The students apply the extract of the pigments from samples of *Elodea elegans* and *Chlorella vulgaris* on silica gel plates and use butyl methyl ether with acetone as solvent.

Finally, the students present and discuss the results of all experiments. To illustrate the relevance of photosynthesis in biotechnology, the tutors give insights into the research topics in this field at the CeBiTec as well as practical applications like cultivating them in algae farms.

(K. Röllke, M. Panhorst & N. Grotjohann)

CeBiTec research group 'Organic and Bioorganic Chemistry' Develops Protein Bromination Platform

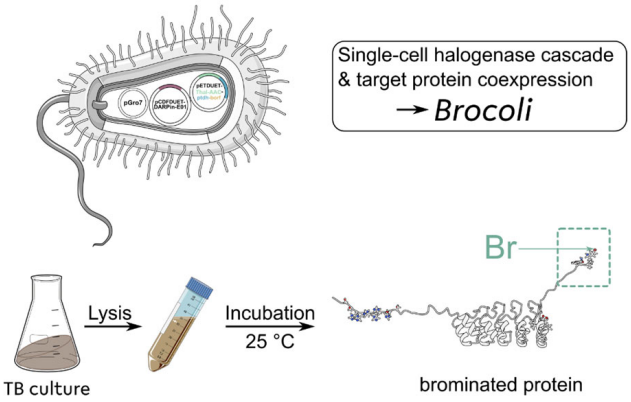
In their article "Enzymatic Peptide and Protein Bromination: The BromoTrp Tag", published in Angewandte Chemie, recent PhD graduate Dr. Nicolai Montua along with co-authors Paula Thye, Pia Hartwig, Matthias Kühle, and Prof. Dr. Norbert Sewald describe a novel method for post-translational protein modification.

Existing approaches for enzymatic protein functionalization are often held back by hydro-lysis or equilibrium formation. In contrast, the perspective of combining biocatalytic halogenation with palladium-catalyzed cross-coupling reactions holds immense promise without the downsides of established methods. While Flavin-dependent Trp-halogenase Thal has some potential for peptide bromination (Schnepel et al., 2023, ChemBioChem 24, e202200569), it showed decidedly poor acceptance of substrates with a C-terminal tryptophan residue, making protein bromination an unlikely proposition.

Therefore, an enzyme engineering campaign was devised to evolve Trp-6 halogenase Thal towards improved acceptance of peptide substrates with a *C*-terminal Trp residue. By following a substrate walking approach, libraries of increasingly longer peptides were synthesized and assayed while iterative mutagenesis was performed on Thal, finally arriving at the tetrapeptide YNIW and the Thal triple mutant variant E461A-N54A-Y454C. Upon examining this Thal variant for halide selectivity, a ca. 6-fold decrease in chlorination activity was noticed.

This preference for bromide was assumed to selectively enable bromination even in the presence of chloride ions. Consequently, protein bromination was demonstrated using three minimally tagged model proteins with sizes ranging from 17 – 31 kDa.

To this end, the identified peptide tag YNIW was *C*-terminally appended and the target proteins conveniently coexpressed with the full halogenase cascade comprising three enzyme functionalities as previously described (Montua et al., 2023, ChemBioChem 24, e202300478) resulting in post-translational protein bromination with high conversion (67 – 93%) after only a single cultivation and purification step. This **bro**minating *E. **coli*** system was named ***Brocoli.***



Using the *E. coli* co-expression system Brocoli, preparative protein bromination is possible in a single step. The icons (bacterium and centrifuge tube) were modified from Servier Medical Art (CC BY 3.0). © N. Montua

Finally, Suzuki-Miyaura cross-coupling was demonstrated by using Pd-catalysts at 37 °C to facilitate protein arylation, hinting at the rich potential of this strategy as a platform for bioorthogonal protein functionalization and conjugation.

(N. Montua & N. Sewald)

Impressum

Centrum für Biotechnologie
Universität Bielefeld
Dr. Lutz Wobbe
Universitätsstr. 27
33615 Bielefeld
Germany

Concept & Idea:
Dr. Stefan Weidner
info@cebitec.uni-bielefeld.de