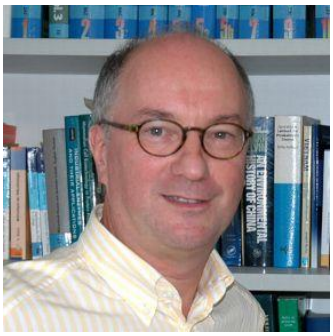
SFB 960-/BZR – Kolloquium

Dienstag, 7. Februar 2023 13.00 Uhr
Neubau Biologie H 53



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BEYOND NATURE - ENGINEERING CYCLASE ENZYMES FOR CHALLENGING APPLICATIONS

Biotechnology promised to be a major driver to the challenging problems we face in the 21st century. Under the term of industrial biotechnology significant contributions are expected for the quest of new system solutions in chemistry. The field of enzyme engineering developed to a mature technology that impacts various areas such as industrial biocatalysis, biomedicine and synthetic biology. Traditionally, new enzyme activities have been discovered by screening microorganisms and mutant libraries. However, it became obvious that these methods have their limitation to identify the much-needed novel enzyme functions. However, we have to expand our portfolio of biocatalyst towards new, non – physiological reactions to boost the impact of biotechnology. It is a restriction to biocatalysis that for many important reactions catalyzed corresponding enzymes are missing.

This presentation will highlight hidden as well as uninvestigated enzymatic activities and illustrate strategies to unmask them. In my talk I will discuss what opportunities are associated with an enzyme like squalene hopene cyclase (SHC). SHC is a class II cyclase and driven by a Brønsted acid catalysis mechanism. The wildtype enzyme shows a few activities beyond the physiological reaction of cyclization squalene to the hopene and hopanol. Interestingly it turned out that the active site of the SHC enzyme is highly evolvable. To initiate our studies, we used a reaction mechanism-guided approach to generate a platform for exploiting the SHCs protonation machinery. Different functional groups can be activated and enable the synthesis of various cyclohexanoids and so uncouple the enzyme from its polycyclization chemistry. Tailoring the enzymes beyond the active site and including the entrance tunnel enabled the development of a most efficient biocatalyst for the cyclisation of homofarnesol to ambroxide. The overall catalytic performance was increased by a factor of almost 400 ensuring the stereocontrol of the reaction. We then expanded our strategy by introducing anchoring sites for very dynamic substrates. Based on designed hydrogen bonding it became possible to induce directed cyclizations and predestinate termination in otherwise barely accessible intermediates. We could demonstrate the synthesis of apocarotenoids in a single step with high selectivities (>99.5% ee) and yields (up to 89%). In a third example the precise carbocation control over an unactivated monoterpenes by biocatalytic Brønsted acid catalysis will be presented. SHC showed initial activity for the promiscuous isomerization of (+)-β-pinene producing a wide mixture of different olefinic and hydroxylated monoterpenoids. Based on iterative saturation mutagenesis we tuned the carbocation control of SHC for borneol production resulting in a variant reaching 90.2% borneol selectivity (99.6% ee). Recently we reported a new concept to increase the overall activity of our system. SHC spheroplasts exhibited up to 100-fold higher activity than their whole-cell counterparts. With this perspective in the right economic context, we can anticipate the relevance of biotechnology to chemistry to accelerate even further.

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