DISCOVERY AND ENGINEERING SYSTEMS FOR MULTI-ENZYME CATALYSIS

Claudia Schmidt-Dannert, University of Minnesota, Twin Cities, USA
schmi232@umn.edu

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Reprogramming and rewiring biological systems by introducing new functionalities offers great promise for the design of cells for the production of new chemicals. In this lecture I will discuss and show examples of our efforts on engineering metabolic pathways and other complex properties into microbial cells and for *in vitro* biomanufacturing. In this presentation I will discuss our efforts in i) discovery and characterization of biosynthetic enzymes for the production of natural products and ii) design and engineering of self-assembling protein systems for *in vivo* and *in vitro* multi-enzyme biocatalysis.

In a first example, I will describe our efforts on characterizing and accessing the natural product portfolio of Basidiomycete fungi with goal of enabling the discovery of new enzyme activities and bioactive compounds. using genomics driven approaches together with heterologous expression and biochemical characterization of enzymes and biosynthetic pathways. Basidiomycota and Ascomycota represent the major phyla of the fungal kingdom. Only a fraction of this diversity has been described and an even smaller fraction of the known species has been characterized in more detail and/or exploited by humans. Ascomycota have so far received the greatest attention while Basidiomycota remain greatly understudied despite their importance for carbon recycling, ecosystem functioning and medicinal properties. This group of fungi holds great promise for the discovery of novel biosynthetic pathways and biocatalysts, especially enzymes for redox catalysis [1]. Homology-based analysis of fungal genomes suggests that the secondary metabolome of Basidiomycota differs significantly from those of other prolific microbial natural products producers [2]. Leveraging genomic information, we have identified and characterized a large number of different sesquiterpene synthases from Basidiomycota. Many of the sesquiterpene synthases are located in associated biosynthetic gene clusters which we have begun to characterize. More recently, we have sequenced additional Basidiomycota genomes that give us access to new types of sesquiterpene scaffolds and clustered biosynthetic enzymes.

In a second example, I will discuss our efforts on engineering enzyme co-localization systems for metabolic engineering and biocatalysis. Cells operate a multitude of enzyme cascade reactions simultaneously with high efficiency, while at the same time controlling metabolic fluxes, preventing the build-up of toxic intermediates and directing metabolites to the correct enzymatic pathways [3]. Key to the optimal function of cellular metabolic networks is the spatial organization and temporal control of these cascades. The same design principles for spatial organization of metabolic enzymes may be adapted to engineer more efficient metabolic pathways and create robust and highly efficient cell-free orthogonal biocatalytic cascade reactions that operate concurrently in one pot. We have engineered protein nano-compartments into *E. coli* and have shown that multiple heterologous cargo proteins can be targeted into these compartments [4, 5]. More recently, we have designed robust self-assembling protein scaffolds that enable co-localization of multiple cargo proteins and enzymes on these protein architectures. Recombinant scaffold building block and cargo proteins can be readily produced for the formation of self-assembled protein systems.

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