In situ Immobilized Biocatalysts – Production and Characterization of Catalytically Active Enzyme Inclusion Bodies

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Project Summary

A more detailed investigation of intracellular protein deposition processes in the recent past has led to a paradigm shift in the classical perception of inclusion bodies (IBs) as inactive agglomerates of misfolded polypeptides. As a consequence, **catalytically active IBs** are currently coming to the focus of attention as a **cost-effective, one-step strategy for enzyme in situ immobilization**, thereby facilitating the rational development of biocatalytic production chains.

Unfortunately, there is still little knowledge available on the **bioprocess designs and parameter settings** to best possibly accumulate catalytically active IBs, and on how to **modify their relevant key attributes in a controlled fashion**. This is also due to the fact that the **analytical methods to characterize IBs**, which may be regarded as 'endogenous biological nanoparticles', have not yet been explored in detail. Importantly, suitable analysis methods should not only yield a maximum of information on the quality-critical key attributes of IBs, but should also be compatible with highthroughput testing in routine manufacture if biocatalytic IB processes shall enter market segments with GMP demands.

In this project, two research groups with focus on bioprocess development and nanomaterial analytics will join forces to (i) generate essential **practical knowledge on the formation kinetics of catalytically active IBs** in microbial host systems, (ii) establish **relationships between process conditions and IB properties** to allow for target-specific process optimization and real-time control, and (iii) develop **best practice guidelines for monitoring enzyme IB manufacturing** in industrial biocatalysis applications. In combination with the constantly evolving technologies for genetic enzyme engineering and synthetic biology, this will further pave the way towards well-controlled enzyme self-assembly and the production of multifunctional, in situ immobilized biocatalysts via coexpression and deposition of multiple enzymes.

The results obtained in this project will help advancing the emerging field of direct IB utilization to a platform technology with a broad range of applications, and provide innovative solutions to Swiss biocatalysis industry and R&D initiatives on carrier-free enzyme immobilizates.