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On demand activity regulation of enzymes in one-pot multi-step catalysed processes using online analytics, automated feeding and light induced enzyme inactivation

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PURPOSE OF THE ABSTRACT

There is an urgent need for the development of greener syntheses procedures if we want to maintain an environment worth living in and keep a high standard in material comfort (or reach a higher one in developing countries). The establishment of more biocatalytic steps in chemical syntheses is one possible solution, as enzymes and whole cells offer sustainable advantages, such as biodegradability, intoxicity, high selectivity, and many more. As a myriad of enzymatic reactions exist for almost any product, their potential is immense. Great scientific achievements and new techniques have enabled the design of economically and ecologically feasible one-step and multi-step enzyme catalysed reactions. However, with these new opportunities, also new challenges arise.

The more enzyme steps are combined, the higher the risk of undesired cross-reactivity. Spatial or temporal separation can solve this problem. In the 'LightCas' project, we are investigating the possibilities of avoiding cross-reactivity in one-pot systems by separating the reaction steps over time. Using sequential enzyme addition and on-demand light-induced enzyme inactivation [1] each step can be turned on and off individually. To achieve selective inactivation of the enzymes, a genetically encoded photosensitizer is coupled to the enzymes. Exposure to (blue) light produces reactive oxygen species. The most suitable photosensitizers produce predominantly singlet oxygen. Due to the short lifetime of these reactive oxygen species, only the bound enzyme is inactivated, but not other enzymes in the same reaction vessel. In combination with suitable online analytics (benchtop NMR) [2], we have exemplarily operated a three-stage light-controlled single-pot enzyme reactor for the production of tetrahydroisoquinolines [3] from cheap (renewable) substrates. The spatially resolved activity control makes it possible to obtain the desired product in high purity even in a one-pot system. Furthermore, we have recently achieved our ultimate goal of a technically self-controlled process in which an automated and FAIR data principles based data collection process is implemented. Time-resolved online concentration determination of substrates, intermediates and products allowed each enzymatic step to be automatically started and stopped upon completion without the need for manual intervention. As long as NMR-signal separation is achieved, this method can in principle be used for the optimisation and control of other one-pot reaction systems.

FIGURES

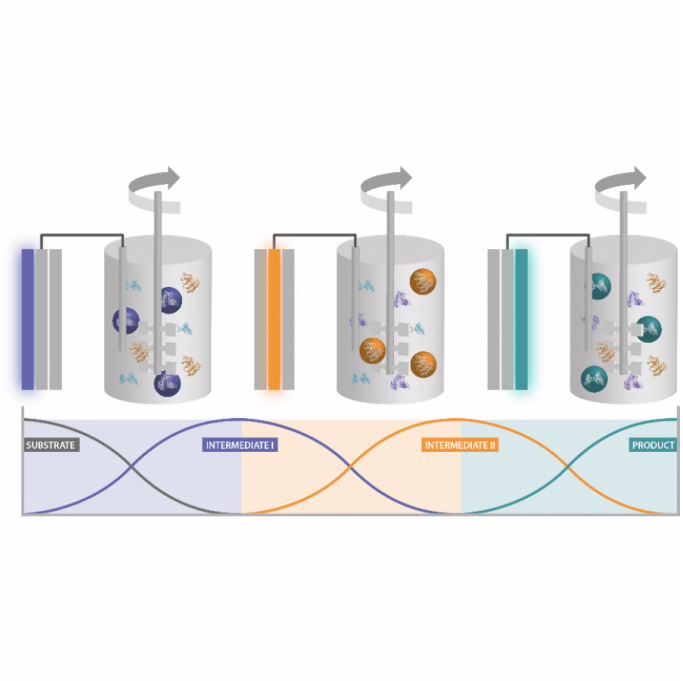

FIGURE 1

Figure 1. Activity controlled one-pot multi-step reactor. With benchtop NMR online analytics, the reactant concentrations can be determined. This makes it possible to start subsequent steps, e.g. by enzyme supply or substrate feeding, and to inactivate the activity of a specific enzyme after completion of a step

FIGURE 2

KEYWORDS

activity regulation | multi-enzyme catalysed processes | automation | cross-reactivity prevention

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