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Physical sciences/Chemistry/Chemical synthesis/Flow chemistry
Biological sciences/Biochemistry/Enzymes/Immobilized enzymes

Subject: Flow Chemistry

Title: Let's stick together for continuous flow biocatalysis

Standfirst: Fusion systems have been designed linking enzymes to cofactors and immobilization modules through appropriate synthetic spacers. These modular biocatalysts (assembling catalysis, cofactor provision/regeneration, and assisted immobilization) are suited for heterogeneous biocatalysis systems and can be efficiently used in continuous flow reactors.

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Biocatalysts can be sustainably employed in continuous reactors for maximizing their usability and minimizing the cost of their production. Flow reactors with immobilized enzymes may be suited for process intensification, thus potentially allowing for high productivity using relatively small apparatus[1]. Despite the advantages of flow-based biocatalysis (*e.g.,* high selectivity, better parameter control, reduced reaction times, improved yields, in-line work up)[2], technical drawbacks are often encountered using cofactor dependent enzymes. Though cofactors may be simply added to reaction mixture with no major problems in diffusion towards the catalytic site,[3] chemical modification for anchoring cofactors to proteins seems a more logical choice. Self-sufficient assembly of different enzymes/cofactors on supports suited for flow chemistry may lead to efficient heterogeneous catalysts, as reported for a coupled alcohol/formate dehydrogenase system and subsequently for PLP dependent transaminases[4,5]. If the reversible immobilization makes the cofactor free to shuttle between the enzyme active sites without leaving the resin pore microenvironment, the non-specific interactions result in a modest enzymatic activity and turnover number under flow mode. Lopez-Gallego et al. suggested the definition of heterogeneous systems biocatalysis for defining the arrangement of heterogeneous biocatalysts mimicking cellular metabolic pathways[4]. Other methodologies fulfil DNA scaffolds for glucose and malate dehydrogenase to obtain an improved catalytic efficiency due to enzyme/cofactor proximity. So far, these systems have never been applied to continuous operation[6].

Now, writing in *Nature Catalysis*, Colin Scott and co-workers report a solution to the above-mentioned problem by using a new concept of fusion protein[7]. Fusion enzymes have been previously designed for improved catalytic activities, affordable production, and coupling of enzymatic reactions for cascade processes[8]. The heterogeneous system presented now by the researchers, is composed of a fusion protein containing a catalytic module that drives the desired

synthesis reaction, a cofactor recycling unit for the *in situ* regeneration of cofactors, a covalently linked cofactor and an immobilization module for site specific, covalent conjugation to an activated support. The first two domains are separated by a short aminoacidic spacer covalently bound to a synthetic arm exposing the cofactor.

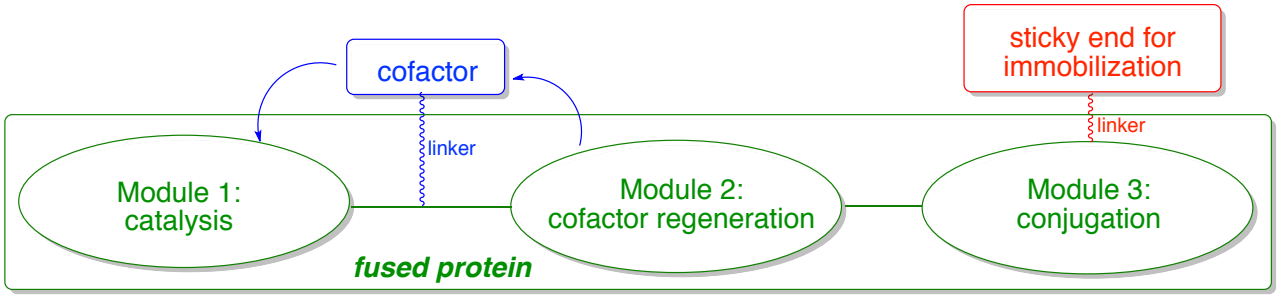

Figure 1: ***Fusion system for cofactor-dependent continuous flow biocatalysis****. The fusion system combines catalysis with cofactor regeneration and easy immobilization.*

As a proof of concept, the researchers prepared three catalytic machineries and operated them in sequence and in flow mode to enable cascade reactions for the preparation of (2*R*,3*R*,4*R*)-2-(hydroxymethyl)piperidine-3,4-diol (an intermediate for the synthesis of the antidiabetic drug D-fagomine). Specifically, the first enzymatic reaction is the phosphorylation of glycerol, followed by the oxidation of glycerol-3-phosphate into dihydroxyacetone phosphate, finally condensed with *N*-Cbz-aminopropanal. The three systems were immobilized and used in a series of flow reactors with high productivity. Moreover, the use of flow mode mitigating the product inhibition allowed for high overall process yield. At the end, the reactions stopped because of the inactivation of the enzymes rather than the loss of cofactor. The successful fabrication of self-sufficient heterogeneous biocatalysts integrating enzymes and cofactors, has been applied here for the synthesis of D-fagomine, but this modular design can be expanded to different enzymatic reactions, keeping the conjugation module fixed and changing the catalytic/cofactor regeneration modules.

The engineered enzymes reported by the researchers appear to be a notable breakthrough due to their applicability in flow biocatalysis. This general framework is rational and likewise uncomplicated to set up for different enzymatic reactions. End-to-end or insertional fusions can be routinely obtained by molecular biology, whereas more detailed protocols for covalent modification of fusion proteins will pave the way to the preparation of fine-tuned modular biocatalysts. This allows the integration of catalytically self-sufficient single molecules into continuous reactors for heterogeneous cofactor-dependent biocatalysis. Caution should be exercised when choosing the auxiliary proteins (especially the one involved in cofactor regeneration), since conditions of optimal activity (temperature, pH, ionic strength etc.) and operational stability of each of the fused enzymes are independent of each other.

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