NANOMACHINE BIOCATALYSTS: TOOLS FOR CELL-FREE ARTIFICIAL METABOLIC NETWORKS

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Assembling cell-free, cascading multi-enzyme enzyme reactions into artificial metabolic networks for the conversion of low value renewable feedstocks into high value products represents a fourth wave of biocatalysis for renewable green chemistry and synthetic biology applications [1]. However, major limitations to both applications include the cost of producing multiple purified enzymes and of providing a continuous supply of diffusible cofactors or cosubstrates [2]. We have applied synthetic biology principles to produce fusion proteins between synthetic enzymes and their cofactor-recycling partner enzymes, with concomitant *in situ* recycling of a modified tethered cofactor, with an added conjugation protein element to allow immobilization of the nanomachines to a surface. This has enabled the construction of nanomachine flow reactors which can be combined in an interchangeable, "plug-and-play" manner to construct complex synthetic networks or Nanofactories. Synthesis of the anti-diabetic drug, D-fagomine, reductive amination to produce various chiral or conjugated amines (Fig. 1) and deracemization of alcohols have been used to exemplify the principles, and we have demonstrated tethered cofactor recycling of ATP, $NAD(H)^+$ and $NADP(H)^+$, as well as ligand-directed immobilization of a variety of enzymes to illustrate the use of these nanomachine biocatalysts as tools for the *de novo* construction of *in vitro* metabolic networks for synthetic biology. Our research is currently exploring the use of frugal innovation principles to integrate key capabilities in reactor design with on-line analytics for real-time reaction monitoring, and, subsequently, dynamic control over the platform's fluidics via feedback loops. We aim to demonstrate the utility of such systems for cell-free metabolic engineering to enable fine chemical synthesis, with additional applications possible in bioremediation and environmental sensing.

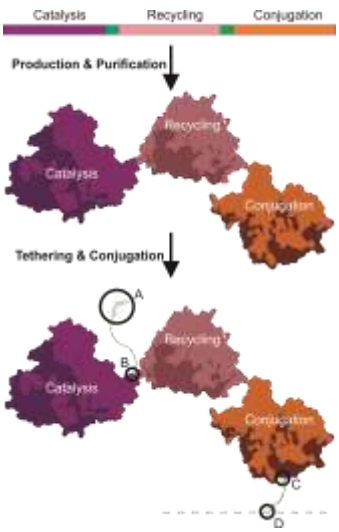

Figure 1 – Nanomachines: engineered enzymes that retain and regenerate their cofactors enable cell-free metabolic engineering.

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[2] Fessner W-D. 2015. New Biotechnology 32:658-664; Zhao et al., 2003. Curr Opin Biotechnol 14: 421-426.