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Synthetic scaffolds for pathway enhancement

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Controlling local concentrations of reactants, intermediates, and enzymes in synthetic pathways is critical for achieving satisfactory productivity of any desired products. An emerging approach to exert control over local concentrations is the use of synthetic biomolecular scaffolds to co-localize key molecules of synthetic pathways. These scaffolds bring the key molecules into close proximity by recruiting pathway enzymes via ligand binding and/or physically sequestrating enzymes and metabolites into isolated compartments. Novel scaffolds made of proteins, nucleic acids, and micro-compartments with increasingly complex architecture have recently been explored and applied to a variety of pathways, with varying degrees of success. Despite these strides, precise assembly of synthetic scaffolds remains a difficult task, particularly *in vivo*, where interactions both intended and unexpected can lead to unpredictable results. Additionally, because heterologous enzymes often have lowered activities in their new hosts, an ideal scaffold should provide a flexible platform that can adapt to kinetic imbalances in different contexts. In this review, we discuss some of the notable advances in the creation of these synthetic scaffolds and highlight the current challenges in their application.

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Introduction

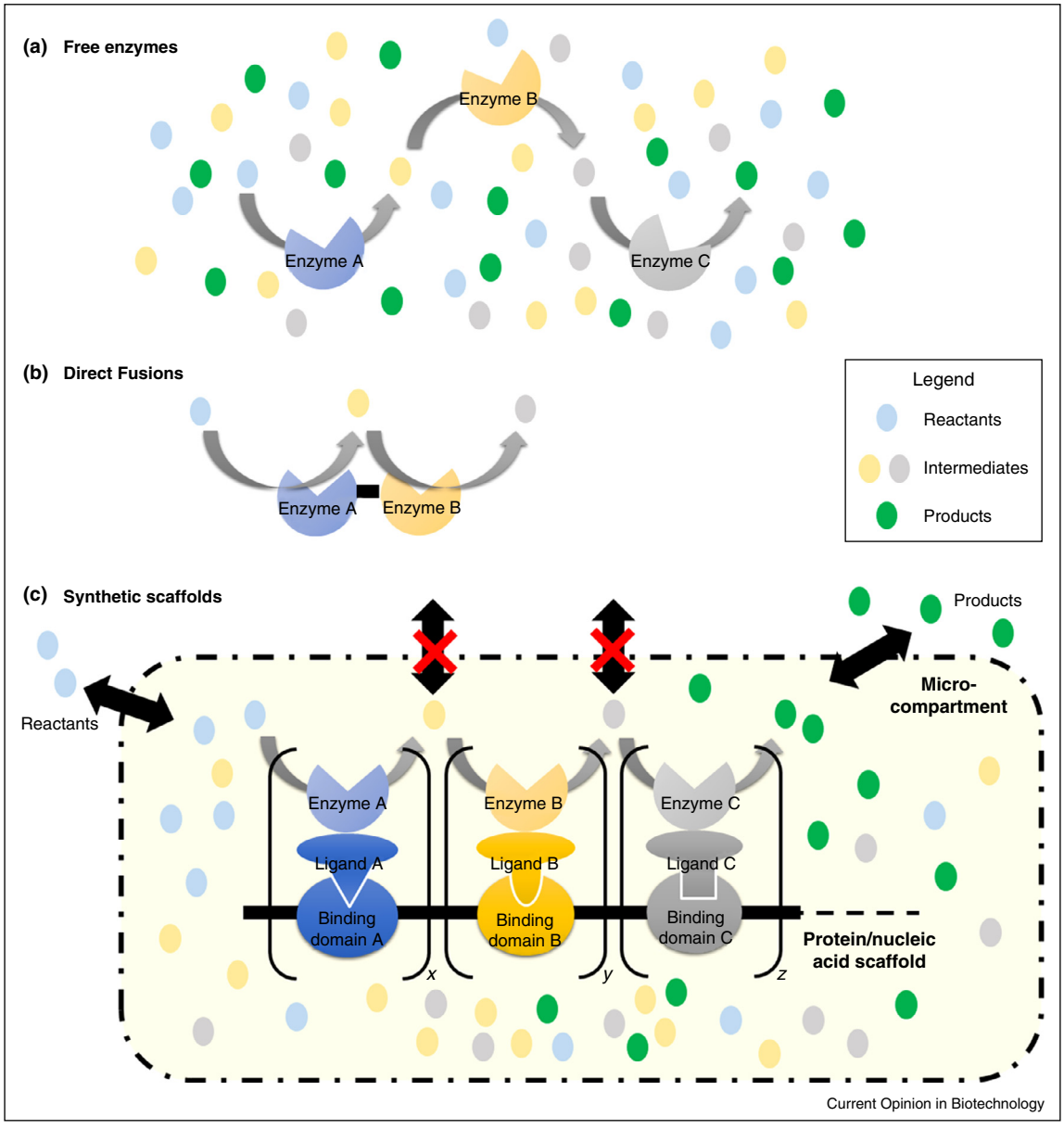
Local concentrations of reactants, intermediates, and enzymes dictate the rates and yields of all biochemical reactions. Controlling local concentrations in synthetic pathways is paramount for achieving satisfactory productivity of the desired products. Unlike natural pathways, exogenous synthetic pathways have not been adapted over eons to generate desirable products; overproduction of desirable products is rarely advantageous or necessary for survival in the host's native environments [1]. This divergence is further exacerbated as synthetic pathways become increasingly complex, introducing problems such as rapid diffusion and degradation of key intermediates, undesirable crosstalk with other cellular pathways, and accumulation of toxic metabolites that ultimately lower product yields [2]. To counter these issues, synthetic biologists have spatially organized pathway enzymes into supramolecular complexes in attempts to elevate local concentrations of enzymes and metabolites that improve reaction flux and minimize cross-reactions. To date, these efforts largely mimic natural metabolic control mechanisms that bring key components into close proximity [3,4].

Analogous to naturally occurring complexes, artificial assemblies of pathway enzymes co-localize multiple molecules to enhance substrate channeling and enzyme clustering effects (see [5–7] for previous reviews). The first attempts to mimic natural complexes are simple fusions of protein domains that catalyze successive reactions. While this strategy has led to increased productivity in multiple circumstances [8,9], it cannot be easily extended beyond two-reaction pathways. The strategy also precludes straightforward changes in enzyme stoichiometry, as catalytic domains are expressed in a fixed ratio on every fusion protein. Instead, synthetic biologists have taken a more modular approach using biomolecular scaffolds to co-localize target molecules [6]. Unlike simple fusions, catalytic domains are not covalently linked, but are co-localized through different interaction domains or physical sequestration. By decoupling binding domains and catalytic domains, generic scaffolds with multiple interaction sites can, in theory, be easily adapted to any number of synthetic pathways by simple substitution of binding and catalytic domains (Figure 1).

The potential advantages offered by this flexibility have driven the development of a wide variety of biomolecular scaffolds in recent years. Many of these scaffolds have been made from ''parts'' taken from interaction domains found in nature, especially protein-based scaffolds. Because the primary requirement to construct these scaffolds is proximity and interactions between the pathway enzymes, nucleic acids (DNA and RNA) [10,11] and micro-compartments [12] have proven to be extremely useful as well.

Despite rapid advances in our ability to design biomolecular scaffolds *a priori*, particularly with nucleic acid-based

Figure 1



Spatial organizations of pathway enzymes. **(a)** Pathway enzymes are ''freely soluble'' and their substrates also freely diffuse in the solution. There is no spatial control over local concentrations and, thus, reaction rates are determined by bulk solution conditions and could be reduced by factors listed in text. **(b)** Direct fusions of enzymes provide so-called ''substrate channeling'' effect and limit exposure of intermediates to potential losses. However, fusions do not allow straightforward changes to enzyme stoichiometry. **(c)** Pathway enzymes are organized onto synthetic scaffolds. Both enzymes and substrates are co-localized by non-covalent binding and/or physical sequestration. Local concentrations of key molecules are elevated to potentially improve reaction fluxes and balance kinetic parameters, in part by changing the number of enzymes (*x*, *y*, *z*) on each scaffold assembly.

scaffolds, precise assembly of synthetic pathways *in vivo* remains extremely challenging, primarily due to incomplete understanding of ligand-scaffold folding, unknown cross-interactions in the crowded intracellular environment, and complications resulted from adapting exogenous enzymes to host organisms. As we will discuss in this review, these challenges present both problems as well as opportunities that can be exploited using different scaffolding strategies.

Protein based scaffolds

In nature, organisms ranging from mammals to bacteria utilize enzyme complexes to optimize pathway performance [5]. The most striking examples of improved