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Biomolecular scaffolds for enhanced signaling and catalytic efficiency

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Proteins inherently are not designed to be standalone entities. Whether it is a multi-step biochemical reaction or a signaling event that triggers several other cascading events, proteins are naturally designed to function cohesively. Several natural systems have been developed through evolution to co-localize the functional proteins of the same pathway in order to ensure efficient communication of signals or intermediates. This review focuses on some selected examples of where synthetic scaffolds inspired by nature have been used to enhance the overall biological pathway performance. Applications encompass both *in vivo* and *in vitro* systems that address two key biological events in cell signaling and biosynthesis will be discussed.

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Introduction

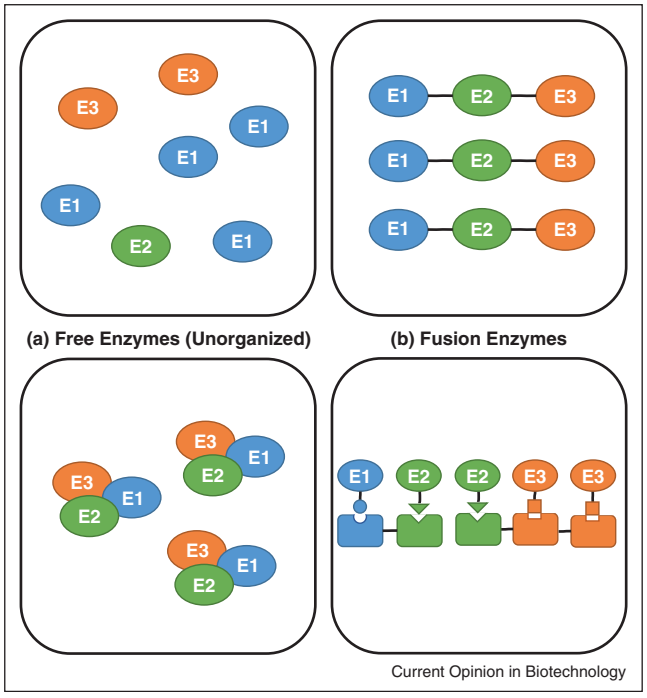
Many enzymes employed in nature are not meant to be standalone entities (Figure 1a) but rather cogs within a complex network of protein partners. The success of many cellular processes depends on the quick communication among these partners within the same enzyme network in order to elicit more complex responses [1–3]. This nanoscale organization has been shown to increase the local concentrations of enzymes and their substrates, to improve intermediate channeling between consecutive enzymes, and to avoid competition with other metabolites present in the cell [4]. In many organisms, these complex enzyme architectures are assembled either by simple enzyme clustering as in the case of metabolons (Figure 1c) [5,6] or by cooperative and spatial organization using a biomolecular scaffold (Figure 1d) [7,8].

From a practical perspective, engineering the required interfacial interactions for efficient enzyme clustering is extremely challenging. In contrast, synthetic scaffolds that permit spatial organization of enzymes have been created by employing orthogonal interaction domains for assembly. The organization of multi-enzyme complexes using a scaffold-based strategy is particularly attractive because of the modular nature of the design, and can be used as a flexible post-translational strategy for controlling the flow of information for complex metabolic and signaling pathways [9,10]. In this review, we will discuss the design principles of different biomolecule scaffolds and their use for enhanced signaling and biosynthesis cascades.

In order to increase the communication between functional domains of protein complexes, a logical plan is to decrease the diffusional path length between enzymes. In literature, this concept has been well documented both through modeling and in practice by fusing enzymes via a non-interacting linker (Figure 1b) [11,12]. A popular approach is to genetically tether enzymes at their N and C termini. For example, Farnesyl diphosphate synthase (FPPS) from yeast and patchoulol synthase (PTS) from *Pogostemon cablin* were fused together using linkers of varying lengths and rigidities, and in particular, the use of a very short three amino acid linker (gly-serine-gly) was found to provide the highest final patchoulol titers with twofold higher yield over free enzymes. Interestingly, placing FPPS on the N-terminal side and PTS on the C-terminal side led to twofold enhancement while the opposite yielded little to no enhancement over free enzymes [12]. This result is a prime example demonstrating the complex nature of enzyme synergy and the need for a highly modular design in order to explore all the possible spatial arrangements.

While the protein fusion approach works well to recruit two enzymes near each other, this strategy becomes less viable for three or more enzymes. Expression of larger fusion enzymes is often challenging since correct folding and retention of activity of all components are not guaranteed. While the effect of the latter can be ameliorated by increasing the linker length, low expression of functional proteins may lower yields despite superior synergy. Furthermore, even minute changes to the fusion enzyme require rechecking the functionality of each fusion partner. This process becomes more cumbersome as the number of fusion partners increases, which is why most fusion proteins reported in literature contain only 2–3 domains.

Figure 1



Different modes of organizing enzyme complexes. **(a)** Free enzymes, **(b)** Fusion enzymes, **(c)** Metabolon (enzyme clusters), **(d)** Scaffolded enzymes.

Synthetic signaling using scaffolds and adaptors

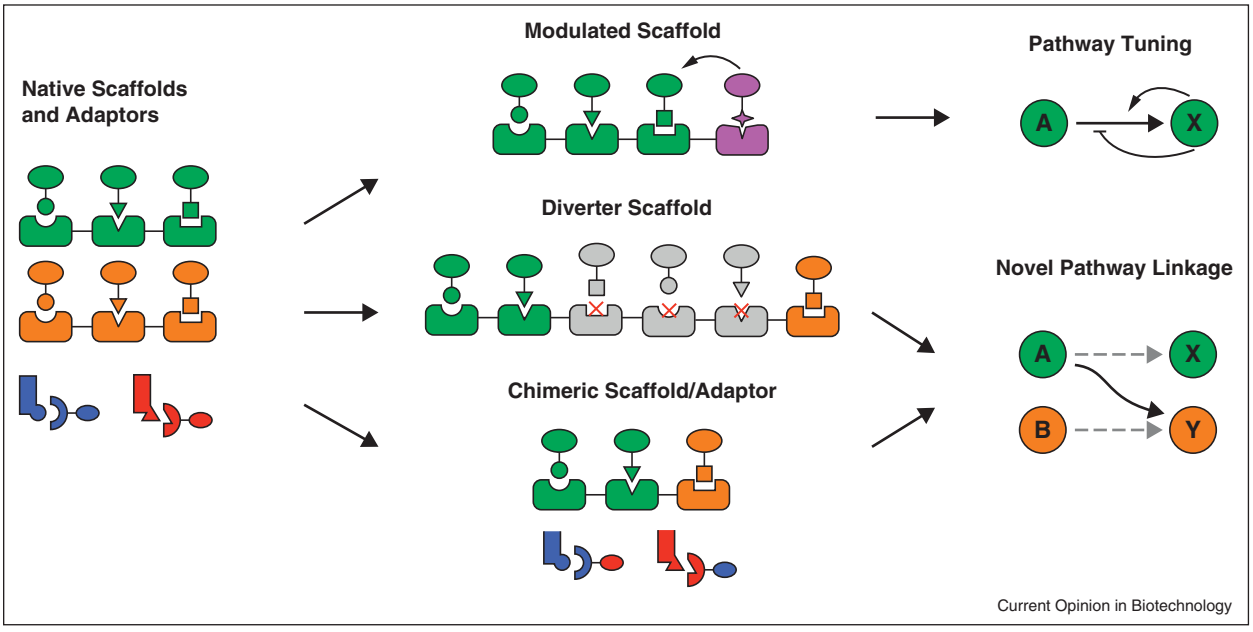
To gain further insight into how to engineer better enzyme complexes, researchers began to look at cell signaling, one of the best synergistic uses of protein complexes in nature, for inspiration. Cells must rapidly respond to a multitude of environmental changes by eliciting precise intracellular signaling cascades, information processing, and specific sets of cellular responses in order to guarantee cell survival and proliferation [13]. Rather than relying on unconnected components for complex signaling, proteins that work sequentially in the signaling cascade are organized together to enable efficient communication [14]. One of the most notable examples is the mitogen-activated protein kinases (MAPKs), a three-kinase regulatory cascade that is central to a highly conserved signaling mode that controls fundamental cellular processes, such as proliferation, cellular survival and differentiation [9]. Cells employ a protein scaffold to bring multiple signaling and effector proteins of the MAPK cascade into close proximity and thereby facilitate efficient signal propagation. Consequently, this protein scaffold acts as a signaling hub, providing intricate spatial and temporal control over MAPK signaling [10].

While many unknowns remain about natural signaling networks, researchers have begun to map out the design principles behind these signaling networks in order to design synthetic signaling pathways to respond to unnatural target inputs and to perform defined tasks as outputs. Inspired by the modularity of natural signaling networks, synthetic signaling networks can be created by rewiring the network with newly engineered scaffolds and kinases (Figure 2). Physically and functionally distinct domains can be added or replaced in the scaffolds and signaling proteins to change their binding partners and localization. By changing scaffold interactions, native signals can be modulated [15] or redirected [16•,17]. A large body of work on yeast MAPK pathways has shown that replacing native tethering interactions with heterologous protein interactions is largely sufficient for scaffold-mediated signaling [16•] and that addition of heterologous interaction domains to the scaffolds or associated signaling molecules is able to tune the output signals [15,16•,18]. Leucine zippers, for instance, can be fused to the yeast mating scaffold protein, Ste5, and native pathway modulators, such as the kinase Ste50 or the MAPK phosphatase Msg5, to create novel interactions between the scaffold and the modulators. These interactions create synthetic feedback loops in which the modulators are artificially recruited to the scaffolds and change the time-response and dose-response behaviors of the pathway [15]. Behaviors such as accelerated or delayed response times compared to wildtype, pulse like behaviors that demonstrated adaptation, and increased switch sensitivity were achieved. Recently, use of bacterial effectors has expanded the available modulators capable of tuning the pathway outputs and changing time-responses in yeast pathways as well as in transformed immune cells [18]. Pathogenic bacteria have evolved effector proteins capable of interacting with MAPK pathways in order to evade immune response. The *Y. pestis* phosphatase, YopH, and the *S. flexneri* MAPK inhibitor, OspF, were utilized to decrease the osmoresponse outputs by threefold. The success of this simple strategy across different platforms proves that we can take advantage of the modularity in signaling networks in the design of synthetic pathways.

Beyond tuning pathway outputs, novel interactions between scaffolds and associated signaling molecules can also be made by changing binding sites on the scaffolds or recombining kinase interactions. In one such example, the yeast mating and osmolarity scaffold protein were fused, and the mating output and high-osmolarity input interaction domains were disrupted to create a synthetic diverter scaffold to reroute alpha-mating inputs into a non-natural osmotic response output [16•]. This strategy could potentially create an entire synthetic signaling pathway using engineered scaffolds with non-natural input–output properties.

Instead of fusing two scaffolds and disrupting certain domains, one can also form a chimeric version of the

Figure 2

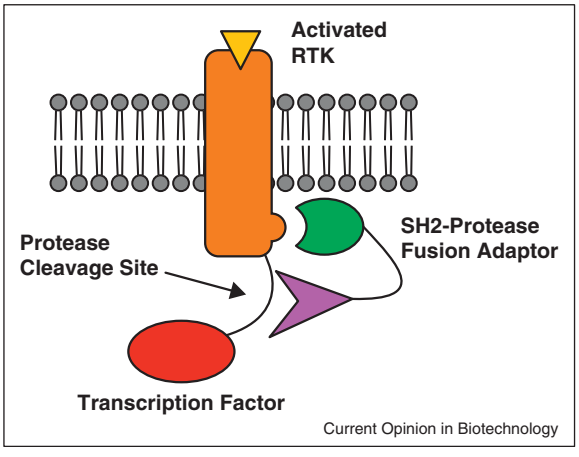


The modular nature of native synthetic signaling pathways can be taken advantage of to engineer synthetic signaling pathways. Modulators can be added to tune output responses of pathways. Diverter and chimeric scaffolds and adaptors can be med to redirect native pathways to form novel pathway linkages. Figure adapted from Wendell *et al*. [54].

original scaffolds to form novel connections between pre-existing signaling molecules. For example, the Grb2 adaptor protein naturally binds activated receptor tyrosine kinases (RTKs) through its Scr homology 2 (SH2) domain and recruits cell proliferation effector domains. By replacing these effector domains with a death effector domain, a synthetic chimeric adaptor redirected mitogenic RTK signals to induce apoptosis in transformed fibroblast cells, tumors, and epithelial cells [17]. Similarly, a chimeric adaptor composed of Abl tyrosine kinase binding domain and a tyrosine phosphatase was made to inhibit the original kinase activity [19]. Besides engineering chimeric adaptor proteins, one can also engineer the kinase itself. Barnea *et al*. fused a transcription factor to a RTK linked by a TEV cleavage site [20]. They also created an adapter protein composed of a SH2 as the kinase binding domain and TEV protease as the effector domain. Upon RTK activation, recruitment of the synthetic adaptor resulted in transcription factor release. The ability to fuse functional domains in different combinations to form synthetic adaptors and kinases is a promising and elegant strategy for designing synthetic signaling pathways (Figure 3).

Another interesting strategy to create synthetic signaling pathways is to use completely artificial scaffolds for docking kinases and effectors fused to heterologous interaction domains. Inspired by scaffold-mediated signaling pathways in yeast, a simple artificial scaffold consisting of a SH3 peptide ligand domain and a leucine zipper domain were introduced into *E. coli*. The corresponding SH3 and leucine zipper domains were fused onto the major components of the native two-component system (TCS), histidine kinases (HKs) and response regulators (RRs), respectively [21]. The heterologous interaction domains

Figure 3



Both kinases and scaffolds can be engineered to create novel signaling outputs. Based upon work by Barnea *et al*. [20], the schematic here shows a transcription factor fused to a RTK with a linker containing a protease cleavage site. RTK activation leads to the recruitment of a synthetic SH2-protease fusion adaptor, which can cleave and release the transcription factor.

Table 1

A summary of the different scaffolds described in this review.

Strategy	Application	Building material	*in vivo/in vitro*	Reference
Fusion enzymes	Synthesis	Protein	*in vivo*	[12]
MAPK scaffold	Signaling	Protein	*in vivo*	[15,16•,18]
Chimeric adapters	Signaling	Protein	*In vivo*	[17,19,20]
Two component systems	Signaling	Protein	*In vivo*	[21]
Cohesin/dockerin	Synthesis	Protein	*in vitro*	[22,23,24••,25,26]
PCNA (ring proteins)	Synthesis	Protein	*in vitro*	[28]
Other (ring proteins)	Synthesis	Protein	*In vitro*	[29,30]
Compartmentalization	Synthesis	Protein	*in vitro*	[31,32,33,34••,35,36]
GBD-PDZ-SH3 scaffold	Synthesis	Protein	*in vivo*	[43]
Single-stranded DNA scaffold	Synthesis	DNA oligonucleotides	*in vitro*	[44,45]
Hexagonal DNA scaffold	Synthesis	DNA oligonucleotides	*in vitro*	[46]
2D DNA origami	Synthesis	DNA oligonucleotides	*in vitro*	[47]
Streptavidin-modified DNA scaffolds	Synthesis	DNA oligonucleotides	*in vitro*	[48]
Halo-tag conjugation of nucleotides to proteins	Synthesis	Modified oligonucleotides	*in vitro*	[49]
Zinc finger proteins/dsDNA scaffolds	Synthesis	dsDNA	*Both*	[50,51•,52]
RNA scaffolding with aptamers	Synthesis	RNA oligonucleotides	*in vitro*	[52]

were able to bind onto the artificial scaffold and allow the scaffold-bound HK/RR pair to interact and actuate a 2.5-fold to 4-fold increase in activation compared to the response in the absence of the scaffold. The addition of the recombinant SH3 domain also changed the specificity of the HK by directing its kinase activity toward the leucine zipper-fused response regulator that co-localized on the same artificial scaffold. On the basis of this work, we can envision using artificial scaffolds and interaction domains to connect pre-existing signaling modules in novel ways to generate customized input–output combinations as part of synthetic signaling (Figure 2).

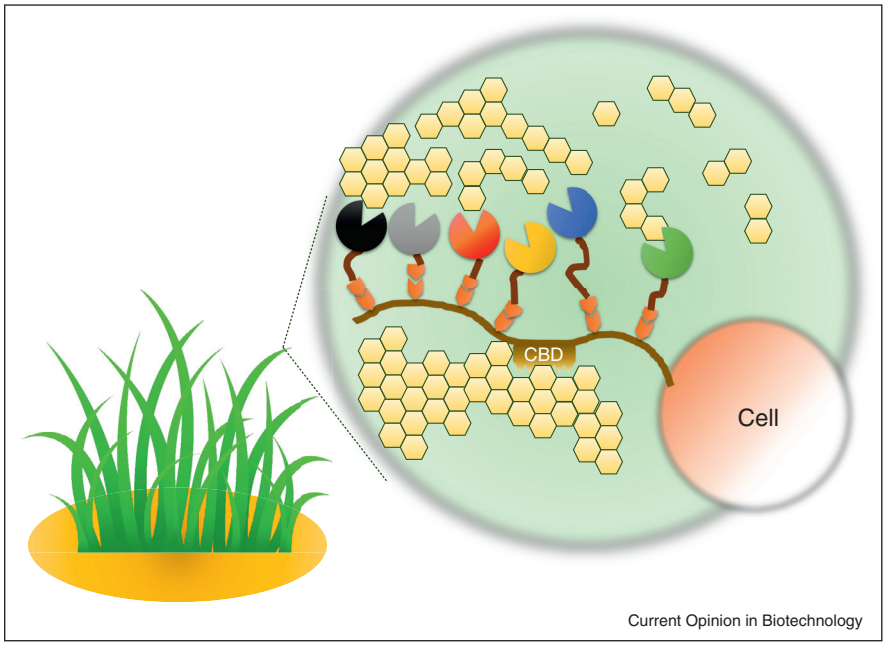
One key challenge in the further development of synthetic signaling pathways using this scaffold-based strategy is to develop more generic modules that can be adapted for any input–output combination. While several orthogonal, well-characterized interaction domains have been catalogued, they may not be able to be transferred from one system to another with ease because of folding and aggregation issues. The potential for cross-talk between engineered scaffolds with native signaling molecules may also become problematic. Hopefully, the use of robust synthetic parts can minimize these risks and allow synthetic pathways to be made with high specificity. Achieving these objectives opens the door to powerful applications, such as cell-based therapeutics against cancer or autoimmune diseases and bioproduction cells which can adapt as fermentation conditions change (Table 1).

Enhancing multi-enzyme biosynthesis using scaffolds

Another prime example of multi-enzyme cascades is the hallmark of anaerobic cellulolytic microorganisms, called cellulosomes (Figure 4). Cellulosomes are cell-bound multi-enzyme complexes responsible for the synergistic deconstruction of both cellulose and hemicellulose, two of the most abundant carbon-rich polymers in the world [8]. This self-assembled system brings multiple enzymes in close proximity to the substrate, and provides a structure that ensures a high local concentration and the correct ratio and orders of the enzymes, thereby maximizing synergy. Consequently, it has a much higher catalytic efficiency than soluble enzymes present in a non-organized fashion.

Inspired by this natural design, a variety of synthetic and modular scaffolds have been created to enhance catalytic efficiency of enzyme complexes. Cohesin–dockerin pairs, orthogonal to each other and any other protein binding domains present, were used to position cellulases in cellulosomes, with reported binding affinities in the sub-nanomolar range. Synthetic scaffolds containing three orthogonal idomains were displayed on the surface of yeast cells to allow the assembly of three different dockerin-tagged cellulases onto the scaffolds [22]. By combining cellulose hydrolysis and ethanol production, the displayed mini-cellulosome was able to yield almost three times the amount of ethanol compared to a mixture of free enzymes [23,24••]. The modular nature of the design suggests that this scaffold may be useful to enhance other sequential biochemical reactions beyond cellulose hydrolysis. This was demonstrated by assembling three dehydrogenases responsible for sequential conversion of methanol to carbon dioxide. The correct ordering of the dehydrogenases resulted in more than fivefold increase in methanol conversion over that for unassembled enzymes [25]. Similarly, three enzymes that were responsible for converting glycer-aldehyde-3-phosphate to fructose-6-phosphate showed increased reaction rates using a similarly assembled structure [26]. It should be noted that the current examples are limited to using only three enzymes due to the degradation problems encountered with producing larger scaffolds. Alternative approaches such as cell-free synthesis may be the key in alleviating this bottleneck [27].

Figure 4



Natural and synthetic cellulosomes displayed on cell surfaces are capable of enhanced hydrolysis of cellulose and hemicellulose to sugar monomers through the complexation of several cellulases and hemicellulases. These enzymes fused with dockerin domains bind specifically to corresponding cohesins in a network of binding sites on scaffolding proteins.

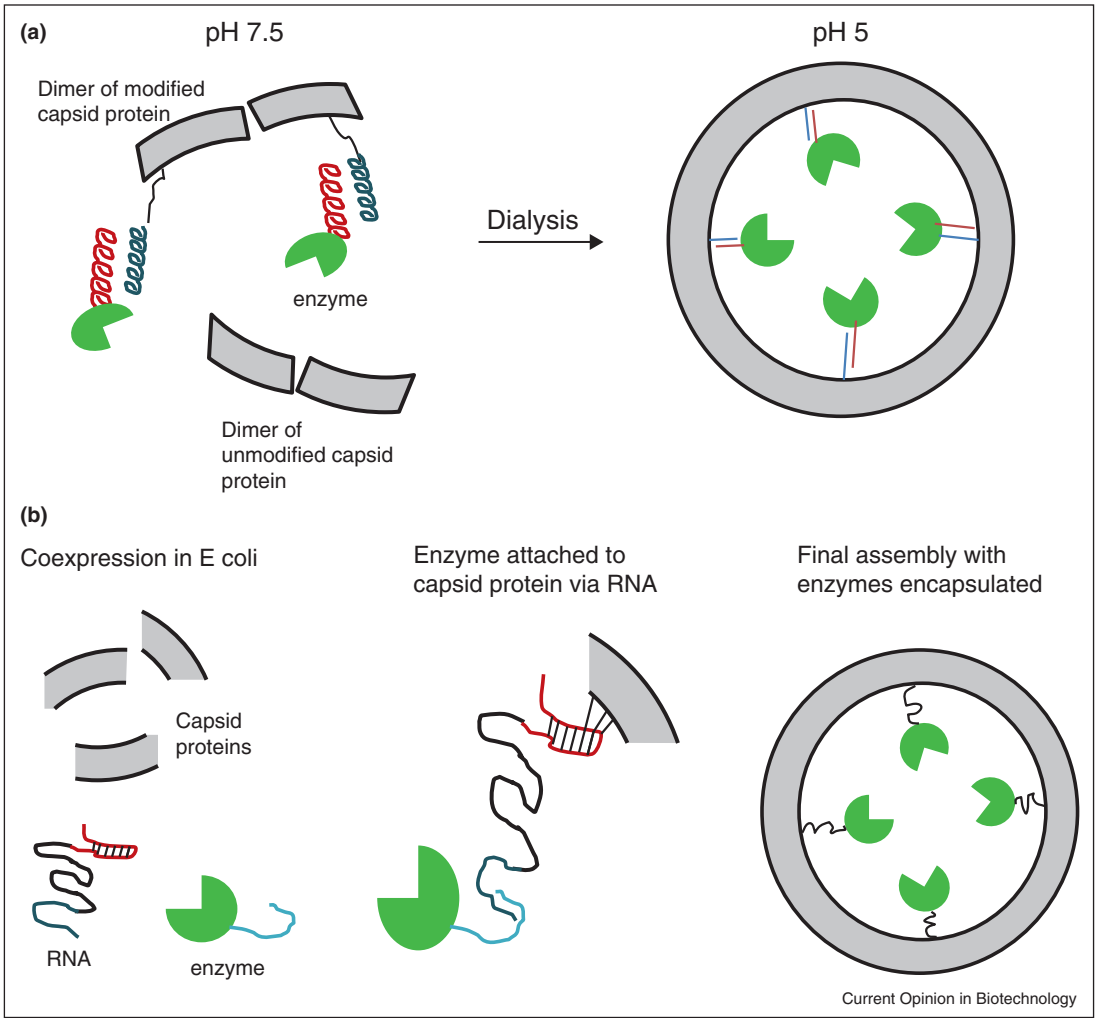
Ring-shaped proteins have also been used as an alternative geometry in organizing enzymes. Proliferating cell nuclear antigen (PCNA) is a heterotrimeric ring-protein that functions as a DNA sliding clamp in *Sulfolobus solfataricus*. Cytochrome P450, putidaredoxin, and putidaredoxin reductases were each fused to the C-termini of the different monomers, yielding a system capable of twofold more efficient transfer of electrons between enzymes [28]. The ability to assembly protein nanostructures with different geometries such as square [29] and hexagonal structures [30] may allow us to explore the benefits of these new architectures for other sequential biochemical reactions.

Compartmentalization is another strategy that nature has evolved to enhance catalytic efficiency. It increases the local concentration of substrates for higher reaction rates and keeps toxic intermediates from entering the cytosol which can lead to cell death. Some of the well-studied microcompartments are the carboxysome, the propanediol utilization microcompartment, and the ethanolamine utilization microcompartment. The carboxysome is a microcompartment of about 100 nm in diameter. It enhances the rate of carbon fixation by threefold by increasing the local concentration of CO_2, which overcomes the slow turnover rate of ribulose-1,5-bisphosphate carboxylase/oxygenase [31]. Efforts have been made to encapsulate other heterologous enzymes within the microcompartments. It has been shown that a wide range of proteins can be encapsulated within the microcompartments by fusing the appropriate targeting peptide sequence while maintaining functionality [32]. This basic design framework can be easily extended to encapsulate multiple enzymes for more complex reaction pathways.

While more understanding is required on how to engineer the shell proteins of microcompartments, capsid proteins from viruses have been studied as a scaffold for organizing enzymes. Capsid proteins can self-assemble into uniform nanometer-scale three-dimensional structures. The capsid of Cowpea Chlorotic Mottle virus (CCMV) is an icosahedral structure of 28 nm. It dissociates into dimers at pH 7.5 and assembles at pH 5 (Figure 5a). Capsid proteins were fused with a positively charged K-coil peptide sequence while enzymes were fused with a complementary negatively charged E-coil peptide sequence, enabling the enzymes to bind to the capsid proteins via the interaction between the charged peptides. The capsid structure was reformed by mixing enzymes with modified and unmodified capsid proteins at pH 7.5 and dialyzed to pH 5 at which point the enzymes were specifically encapsulated by the reassembled capsid [33].

In bacteriophage P22, 100–330 copies of a scaffolding protein are incorporated into the interior of the viral capsid through non-covalent association with the 420 copies of the viral coat proteins. This mechanism has

Figure 5



(a) Schematic representation of encapsulating enzymes in CCMV capsid via interaction between oppositely charged peptides. **(b)** Schematic representation of encapsulating enzymes in Qβ capsid using RNA hairpin structure to link the enzymes to capsid proteins.

been exploited to encapsulate up to 300 copies protein cargos into the P22 capsids by inserting the target protein into the permissive site of the scaffolding proteins [34••], which direct their assembly into the interior of the P22 capsids. Because of the large number of proteins that can be encapsulated, this strategy is highly attractive to create enzyme-based nanoreactors. This feasibility has already been demonstrated using a monomeric alcohol dehydrogenase AdhD from *Pyrococcus furiosus* [35]. Because the morphology of the P22 capsid can be manipulated by gentle heating at 65 °C, resulting in an irreversible volume expansion and the formation of large 10 nm pores, this may be explored to control activity due to differences in enzyme crowding and confinement as well as substrate diffusion. Because of the ability to encapsulate a large number of enzymes, this approach may provide the same benefits as that of the natural microcompartment systems when multiple enzymes in a cascade reaction are encapsulated in the P22 capsids.

RNA can also be used to direct enzyme encapsulation. Bacteriophage Qβ has an icosahedral structure of 25 nm and packages its RNA genome by the interaction between an RNA hairpin structure and certain interior residues of the capsid proteins (Figure 5b). The RNA hairpin sequence was modified by introducing a domain that recognizes an arginine-rich peptide, which was fused with an enzyme. With the RNA sequence bridging the enzymes and the capsid proteins, enzymes were encapsulated inside the Qβ capsid by co-expression in *E. coli* [36].

Although enzymes have been chemically conjugated onto the surface of viral capsids, only a few studies detailed the

in vivo display of enzymes via either N-terminal or C-terminal fusions. One main limitation toward this effort arises from the steric hindrance and the disruption in capsid assembly. One promising way to bypass this problem is to create hybrid viral capsids assembled by a mixture of wildtype capsid proteins and enzyme-fused capsid proteins. Using this method, the lipase B from *Candida antarctica* was displayed onto the surface of Potato virus X and used as an efficient enantiospecific catalyst for chemical hydrolysis reactions [37]. This idea has also been used to display other peptides onto the surface of Qβ capsids [38]. Unfortunately, the ability to co-display multiple enzymes is still difficult due to limitations in protein folding and assembly. Even though proteins have been grafted onto the capsid surface based on biotin–streptavidin interaction [39] or by conjugation with unnatural amino acid residues [40], these methods still require chemical reactions and lack the ability to order enzymes for a multi-cascade reaction. The widespread usage of viral capsids for enhanced biocatalysis will necessitate the use of more robust biological modifications that afford position-specific organization of enzymes. Although still missing the ability for positional assembly, the formation of enzyme hydrogels based on coiled coil peptide interactions [41] or intein ligation [42] provides two promising frameworks toward this direction.

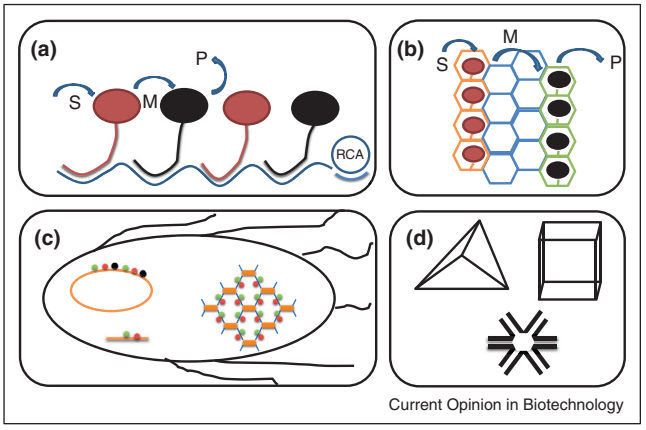
In addition to *in vitro* biocatalysis, synthetic protein scaffolds have also been proven to be useful in increasing *in vivo* product titers. The mevalonate synthesis pathway enzymes were each fused with peptides specific to three different ligand binding proteins, PDZ, SH3, and GBD [43], and organized onto synthetic scaffolds composed of different numbers of binding proteins. The enhancement in mevalonate production was shown to depend on the binding protein ratios and a ratio of GBD, PDZ, and SH3 domains of 1:2:2, which recruits a single copy of AtoB and two copies of both HMGS and HMGR, had approximately a 77-fold increase in mevalonate production over free enzymes. This was especially interesting because no other ratios increased the production by more than 30-fold. Similar synthetic scaffolds were designed to enhance the production of glucaric acid by fivefold using a GBD, PDZ, and SH3 ratio of 1:4:4 [43]. These results suggest that an optimal scaffold design is highly dependent on the target and cannot be easily predicted *a priori*. Rather, a careful balance between the enzyme level, activity, and flux distribution is required to achieve the optimal outcome.

Nucleic acid based scaffolding

Although synthetic protein scaffolds are highly serviceable platforms for multi-enzyme cascade organization, full-length and functional protein scaffolds may be difficult to express as the complexity and size increases. Luckily, the ability to easily predict and manipulate the base-pairing property of nucleic acids along with the ease of synthesis have allowed researchers to create various DNA or RNA-based nanoscaffolds for the organization of multi-enzyme complexes [44]. In one example, glucose oxidase (GOx) and horseradish peroxidase (HRP), chemically modified with DNA oligonucleotides, were spatially organized on single DNA strands generated by rolling circle amplification, which amplified GOx and HRP binding sequences repeatedly using a DNA polymerase (Figure 6a). The long DNA chains (up to 30 nt) enabled multi-enzyme cascades at relatively low enzyme concentrations, at which unassembled enzymes do not exhibit the full cascade activity. This demonstrated the feasibility of using DNA for ordered assembly of enzymes into hybrid composites [45]. However, while effective, single-stranded DNA scaffolds lack rigidity and may lead to unintended folding of the scaffold.

To increase the rigidity of one-dimensional nucleic acid based scaffolds, two-dimensional DNA scaffolds have also been explored. A set of predesigned single-stranded nucleic strands with partially complementary sequences was used to form 'hexagon-like' structures with 10 bp overhanging DNA 'hinges' available for biomolecule tethering. GOx and HRP, chemically functionalized with DNA oligonucleotides, were attached onto two different hexagons by hybridization onto the overhanging hinge areas (Figure 6b). The overall activity of the enzyme cascades can be fine-tuned by controlling their relative position on two-hexagon and four-hexagon structures, with the two-hexagon case showing 1.2-fold higher overall activity than that of the four-hexagon case. This is due to the expected longer diffusional path length of the intermediate using four-hexagons versus two-hexagons

Figure 6



Using nucleic acid scaffolds to organize enzymes. **(a)** Single-stranded DNA as the scaffold. Enzymes are chemically conjugated to oligonucleotides and bind to specific sites on the scaffold. S: Substrate; M: Intermediate; P: product. **(b)** One-dimensional scaffolds created using a hexagon-like structure affords an organelle-like enzyme cascade enhancement **(c)** Nucleic acid based scaffolds can also be used *in vivo* **(d)** DNA can be used to make a variety of geometries.

[46]. Two-dimensional DNA origami structure also looks at the effect of diffusion path lengths of the same two-enzyme system and the results were very similar to the hexagon systems [47]. Unfortunately, this strategy of using single-stranded oligonucleotides when chemically conjugated to random surface residues of enzymes may lead to activity loss, decreasing the feasibility and universality of this strategy [47]. Biotinylated enzymes and streptavidin modified DNA complexes [48] and enzyme-catalyzed crosslinking of proteins onto chemically modified nucleic acids, such as the halo-tag technology [49], were also explored. However, these processes involve some costly modification and purification of DNA to function. Furthermore, any usage of chemical conjugation is highly impractical for *in vivo* applications.

To avoid problems associated with chemically modifying either the DNA or the enzymes, researchers have utilized nucleic acid binding proteins to organize enzymes. For example, target proteins were genetically fused to zinc finger proteins, which have the ability to recognize specific 9–18 base pair sequences of double-stranded DNA. Our group was able to achieve 1.7 fold enhancement of activity over free proteins with endoglucanase and a cellulose binding module fusions to two different zinc finger proteins and immobilized on double-stranded DNA scaffolds as a mini-artificial cellulosome. Similarly, zinc finger protein fused to small laccase enzyme was able to bind onto DNA modified magnetic beads while retaining the enzyme's activity [50].

Zinc finger proteins were also successfully used to enhance synthetic metabolic pathways inside *E. coli*. Using plasmid DNA as the scaffold, a variety of strains capable of self-assembling enzymes of biosynthesis pathways were developed, achieving up to fivefold enhancements of target metabolite titers [51•]. The real strength of this system is the fact that DNA is very easy to produce, allowing researchers to rapidly test several different scaffolds in order to determine the best ratio of enzymes to put on the scaffold (Figure 6c). The three metabolic pathways for resveratrol, 1,2-propanediol and mevalonate production tested in this paper along with L-threonine production enhancement from another paper highlight the universality of this method, which makes DNA scaffolds appropriate for many diverse applications [52]. Similar to single-stranded nucleic acid scaffolds, plasmid DNA can also be floppy and tend to supercoil, and may prevent enzymes from binding to the scaffold.

RNA with dimerization and polymerization domains have also been used to form discrete, one-dimensional and two-dimensional RNA scaffolds with exposed RNA aptamers as binding sites for specific protein partners. Two enzymes, [FeFe]-hydrogenase and ferredoxin, which catalyze the reduction of protons to hydrogen through electron transfer, were tagged with aptamer partners and immobilized on an RNA scaffold showing up to 4-fold, 11-fold, and 48-fold enhancement of H_2 production from one-dimensional, a repeated one-dimensional, and two-dimensional structures respectively. This result is one of the best arguments that higher order geometrical organization of enzymes may lead to unprecedented production titers of cascade reactions [53••].

It is clear that nucleic acids can serve as templates for positional immobilization of enzyme cascades both *in vivo* and *in vitro*. Through rational design, both DNA and RNA have been used to create many one-dimensional, two-dimensional and three-dimensional structures, which are rigid, stable and allow complex arrangements of enzymes [44] (Figure 6d). These structures can potentially further increase the overall activity of enzyme cascades by exploring more favorable arrangements, leading to more favorable substrate channeling.

Conclusion

The benefits gained in enzyme communication through synthetic scaffolding have been significant for both cell signaling and biosynthesis. Even though the majority of the scaffolds employed are fairly simple one-dimensional architectures, the growing interest in synthetic scaffolds will only expand the set of tools that we can utilize to ultimately achieve more complex designs for even more complicated multi-step cascade reactions such as glycolysis.

Acknowledgement

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- • of special interest
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