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Self-assembled nanoreactors based on peptides and proteins

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ABSTRACT

Catalyst function is tightly controlled in biology by means of compartmentalization and positional assembly. Inspired by these innate strategies researchers have developed self-assembled structures that mimic natural control over catalytic activity by employing polymer-, lipid-, DNA-, peptide- and protein-based systems. Here, recent developments in self-assembled peptide- and protein-based nanoreactors will be discussed. This review will cover nanoreactors that are generated by either positional control of catalysts on fibrous supramolecular structures or confinement of catalysts inside protein nanocages. The focus will be on the self-assembly mechanisms that are involved in the formation of these catalytic systems.

1. Introduction

Inspired by the strategies employed by living cells to control multi-step enzymatic processes in a temporal and positional manner, researchers have become interested in positional assembly and compartmentalization of catalysts. Regulation is crucial for proper cellular function, hence cells contain membrane-encapsulated organelles and scaffold proteins to achieve this spatiotemporal control. Importantly, this regulation allows for efficient tunneling of substrates between enzymes in a cascade, prevents toxic effects caused by interference of substrates of incompatible pathways and controls the flux of substrate molecules through metabolic networks via feedback and feed forward mechanisms. Scientists have attempted to mimic biological encapsulation and scaffolding systems by using a plethora of different synthetic and bio-inspired strategies to create nanoreactors [1–4]. For example, compartmentalization of enzymes was achieved in lipid- and polymer-based vesicles, protein and DNA nanocages [5], and repurposed bacterial microcompartments [6–8] and cellular organelles. On the other hand, DNA and protein scaffolds, and self-assembled fibrous structures have been employed to mimic the function of natural scaffold proteins.

The motivations for imitating encapsulation and positional control of catalyst assembly can be found in both fundamental and applied science. Of course, an artificial enzyme or organelle mimic can be used to further our understanding of enzyme, organelle and cellular function. However, a biomimetic nanoreactor can in addition be employed for enhancement of selectivity and efficiency of catalysts with applications in biomanufacturing or green chemistry. Perhaps most intriguingly, a synthetic catalytic system can be utilized in the construction of artificial organelles with the potential to correct defective cells in a therapeutic approach or even expand the functionality of natural cells.

Here, we will review recent developments regarding self-assembled peptide- and protein-based nanoreactors as these materials encompass functionality, flexibility and robustness. Both systems that order catalysts with nanometer precision along a self-assembled structure and systems in which enzymes are encapsulated in confined spaces will be discussed. As the focus of this review is on self-assembled systems, nanoreactors in which catalysts are immobilized or covalently attached to a scaffold [2] are outside the scope of this review. Rather than giving a complete overview of self-assembled peptide- and protein-based nanoreactors that have been developed, we limit ourselves to those systems in which recently most progress has been made and that are most promising for further development, namely organization of catalysts on fibrous structures and confinement of catalysts inside protein nanocages. An overview of the systems that will be described in this review is provided in Table 1. We will discuss the self-assembly mechanisms involved, and the scope and limitations of these nanoreactors. In Section 2 catalytic peptide fibers and peptide- and protein-based hydrogels will be discussed, while the third section elaborates on enzyme encapsulation in protein and viral nanocages. In a concluding

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Table 1
Self-assembled peptide- and protein-based nanoreactors and their catalytic activities.

	System	Catalytic activity
Nanofibers	Lauryl-VVAGHH-NH_2	Suzuki–Miyaura couplings of aryl iodides [9]
	H-HK(H)LLLAAAL(Palmitoyl)-NH_2	Ester hydrolysis [11]
	C_8-GGH-OH	Hydrolysis [12]
	HO-Glu-CO-C_{16}-CO-Glu-OH	Mukaiyama aldol reaction [13]; Diels-Alder reaction [13]
	Fmoc-FFX-OH (X = S, H, D)	Hydrolysis [14]
	Lauryl-VVAGHH-NH_2	Phosphate hydrolysis [15]
	Lauryl-VVAGX (X = D-NH_2, H-NH_2, S-OH)	Ester hydrolysis [16]
	Ac-XLVFFAL-NH_2 (X = K, R, H)	Poly-imine condensation [18]; retro-aldol conversion [18]
	Ac-IHIHIXI-NH_2 (X = Y, Q)	Ester hydrolysis [19,20]
	H-XSGQQKFQFQFEQQ-NH_2 (X = G, H, R)	Ester hydrolysis [21]
	Ac-FGFHFSFDF-NH_2	Amidolytic activity [22]
	H-SMESLSKTHHYRFFKLVFF-OH	Ester hydrolysis [23]
	Ac-FFACD-OH	Reductive activity [24]
Hydrogels	ProVal8	Nitroaldol reaction [26]
	ProValDoc	Direct aldol reaction [27]
	HisVal8	Ester hydrolysis [28]
	SucVal8 + ProValDoc	One-pot deacetalization–aldol tandem reactions [30]; anti-selective Mannich reactions [31]
	H-PFE-C_{12}	Aldol reaction [29]
	CutA-$Tip1_{lig}$ + CutA-Tip1	Electroreduction of oxygen [34]
	PTDH-PCNA-enzyme	Monooxygenation [35]
Protein nanocages	Ferritin	Aerobic oxidation of alcohols in water [44]; templated mineralization of MnO_2 nanoparticles [45]; templated biomineralization of gold nanoparticles [46]; ROS scavenging activity [47]
	Thermosome	Atom-transfer radical polymerization [48,49]; Templated biomineralization of gold nanoparticles [50]
	Lumazine synthetase	Kemp eliminase activity [53]; cyclohexylamine oxidase [53]; catalase peroxidase activity [53]; NADH oxidase activity [53]; ascorbate peroxidase catalyzed polymerization of 3,3-diaminobenzidine [54]; carboxysome-mimetic activity [55]
	Cowpea chlorotic mottle virus	Peroxidase activity [56]; nitroarene reduction [57]; lipase activity [59,61]; two-step conversion of glucose to gluconic acid and D-gluconate-6-phosphate [60]
	Bacteriophage P22	Alcohol dehydrogenase activity [64]; hydrolase activity [65,68]; hydrogenase activity [66]; phosphorylase activity [68]; reductive activity [67]; NADH and hydrogen production [69]
	Bacteriophage MS2	Phosphatase activity [71,72]; two-step production of indigo from L-tryptophan [73]

section we will give an outlook on the potential of these systems for the future.

2. Nanometer-scale positioning of catalysts on self-assembled fibrous structures

Inspired by fibrous structures found in nature, self-assembled peptide and protein fibers and hydrogels have caught the attention of scientists as convenient scaffolding materials for the positional assembly of catalytic species. In these systems, a simple subunit is designed in such a way that it can arrange itself into fibrous bundles with catalytic activity. As a strength of this strategy, the small subunit is fairly easy to produce, while the resulting supramolecular structures can be quite complex and can display novel functionalities. In addition, the supramolecular nature of the fibrous structures results in a dynamic system that can optimize itself via for example molecular imprinting, as opposed to covalently formed fibers. In Sections 2.1 and 2.2 the various peptide-and protein-based catalytic fibers and hydrogels will be discussed more comprehensively.

2.1. Catalytic fibers

Generally, catalytic fibers are peptide-based nanoreactors that are generated by the self-assembly of rationally designed peptide sequences and which receive their catalytic activity from the spatial arrangement of specific amino acids in the sequence. Based on their specific self-assembly mechanisms, peptide-based catalytic fibers can be divided into two classes, namely amphiphilic and amyloid-like peptides, which will be discussed respectively in Sections 2.1.1 and 2.1.2.

2.1.1. Catalytic fibers based on peptide amphiphiles

Catalytic fibers in this class are constituted by a short peptide sequence attached to a hydrophobic moiety (Fig. 1). In most systems, the hydrophobic portion of the amphiphile is composed of an alkyl chain of 8 to 16 carbons in length [9–12] although bolaamphiphilic peptides [13] and peptide amphiphiles with aromatic domains are also known [14]. Despite the catalytic applications of the specific fibers differing substantially, the origin for catalysis generally can be found in the peptide segment. A (combination of) specific amino acid(s) can activate a reaction either directly [11,12,14] or indirectly via coordination to a metal catalyst [9,10,13].

The basis for self-assembly of peptide amphiphiles into catalytic nanofibers can be found in both the peptide sequence and the hydrophobic tail. Guler and Stupp demonstrated that both segments need to be present in order to allow successful self-assembly into nanofibers with a diameter of 7 nm and a length up to several micrometers [11]. Of four similar compounds, only the compound that contained an alkyl chain and a peptide fragment facilitating β-sheet formation, was able to self-assemble into nanofibers. Therefore, β-sheet formation of the peptide domains combined with hydrophobicity-driven aggregation of the alkyl chains is the most probable mechanism of nanofiber self-assembly of these peptide amphiphiles.

More recent studies confirmed these findings and added to our knowledge of peptide amphiphile assembly into catalytic fibers. For example, it was revealed that the alkyl tails are tightly packed in a hydrophobic core that is surrounded by the peptide domain [9,10,12], which allows easy access for interacting metal catalysts [9] or substrates [12]. In addition, novel self-assembly mechanisms were discovered [13,14]. A bolaamphiphile composed of L-glutamate and a 14-carbon alkyl chain was demonstrated to self-assemble into single-walled helical nanotubes that could associate with several metals to yield very efficient catalysts for asymmetric reactions [13]. Alternatively, a peptide amphiphile based on *N*-fluorenylmethoxycarbonyl (Fmoc) diphenylalanine assembled into nanofibers with mixed α-helix and β-sheet structure [14]. Moreover, co-assembly of Fmoc-FFX tripeptide amphiphiles with X representing a Ser, His or Asp residue, resulted in the formation of a complete catalytic triad that displayed esterase activity (Fig. 1b). Optimization of the organization of the catalytic centers via