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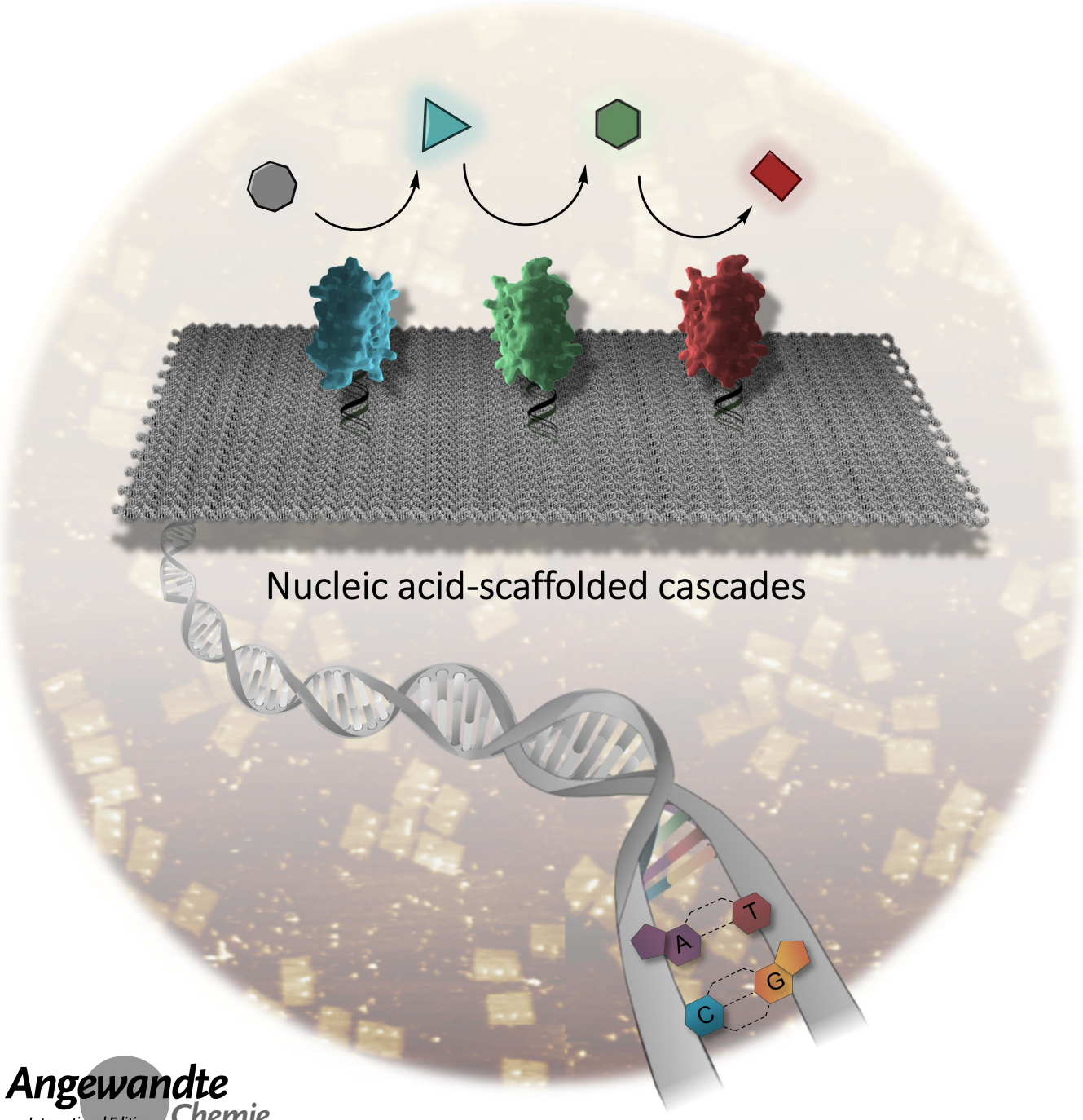
Enzyme Cascades

Nucleic Acid-based Enzyme Cascades—Current Trends and Future Perspectives

*Sandra Kröll and Christof M. Niemeyer**

Dedicated to Professor Manfred T. Reetz on the occasion of his 80th birthday



Abstract: The natural micro- and nanoscale organization of biomacromolecules is a remarkable principle within living cells, allowing for the control of cellular functions by compartmentalization, dimensional diffusion and substrate channeling. In order to explore these biological mechanisms and harness their potential for applications such as sensing and catalysis, molecular scaffolding has emerged as a promising approach. In the case of synthetic enzyme cascades, developments in DNA nanotechnology have produced particularly powerful scaffolds whose addressability can be programmed with nanometer precision. In this minireview, we summarize recent developments in the field of biomimetic multicatalytic cascade reactions organized on DNA nanostructures. We emphasize the impact of the underlying design principles like DNA origami, efficient strategies for enzyme immobilization, as well as the importance of experimental design parameters and theoretical modeling. We show how DNA nanostructures have enabled a better understanding of diffusion and compartmentalization effects at the nanometer length scale, and discuss the challenges and future potential for commercial applications.

1. Motivation and Brief History of DNA Scaffolds for Enzyme Assemblies

In recent decades, structural DNA nanotechnology,[1] has witnessed numerous breakthroughs, especially the development of the scaffolded DNA origami technique,[2] which has now enabled the design and fabrication of complex functional DNA origami nanostructures (DON) with remarkable precision. Such DON readily self-assemble from a circular strand and a variety of short (modifiable) staple strands, enabling for the controlled spatial arrangement of entities such as enzymes into predefined patterns at the nanoscale (Figure 1).[3]

The construction of enzyme-decorated DNA nanoarchitectures holds promise for mimicking multi-enzyme cascades, as precise control of stoichiometry and spatial distance of interacting catalytically active units can be used to unravel molecular mechanisms observed in nature that control reaction, diffusion and transport of substrates between enzymes.[4] Fundamental understanding of the principles of multienzyme complexes is critical for efficient translation to biotechnological applications, such as biosensors or biocatalytic synthesis of valuable molecules. Compartmentalization is the general approach of spatially separating two or more active components of a system to prevent malfunctions from spreading as well as unproductive cross-talk, and it is now considered certain that this spatial confinement of interacting catalysts, substrates, and intermediates also plays a crucial role in biocatalytic processes.[5] In the case of DNA-scaffolded biocatalytic cascades, although impressive progress has been made in the preparation of various complex multienzyme systems, there is still no clear understanding of the underlying mechanisms that explain the frequently observed increase in activity. We will discuss mechanistic aspects below (Chapter 2), but would like to emphasize that experimental investigations require a high degree of methodological reliability, since results can easily be influenced by artifacts.

The history of the development of DNA scaffolds for enzyme assembly dates back to the 1990s, when oligonucleotide-directed self-assembly of proteins was first described to construct supramolecular bioconjugates for applications in biotechnology.[6] This approach was then used in 2002 to arrange nucleic acid-enzyme conjugates using single-stranded DNA (ssDNA) strands as template on a surface to study the coupled reaction of an NAD(P)H:FMN oxidoreductase (NFOR) and a luciferase (Luc) and observe an approximately 2-3-fold enhanced cascade activity (Figure 2A).[7] Methodologically similar work on an cascade composed of glucose oxidase (GOx) and horseradish peroxidase (HRP)[8] showed similar rates of increase, as did later studies on other bienzymatic systems assembled on linear ssDNA.[9] The transition from linear quasi-one-dimensional (1D) to quasi-two-dimensional (2D) oligomeric constructs was achieved by topologically programmed DNA scaffolds that arranged thousands of GOx/HRP pairs in hexagonal patterns and surprisingly exhibited up to 16-fold increased activity rates.[10] For a detailed overview of the increase rates obtained with a variety of DNA-templated enzyme cascades, see Table S1, Supporting Information.

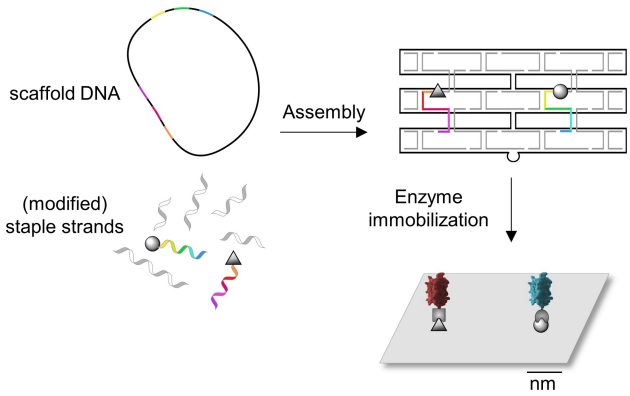


Figure 1. Self-assembly of a circular scaffold strand with modifiable staple strands into DNA origami nanostructures (DON) enables precise arrangement of enzymes.

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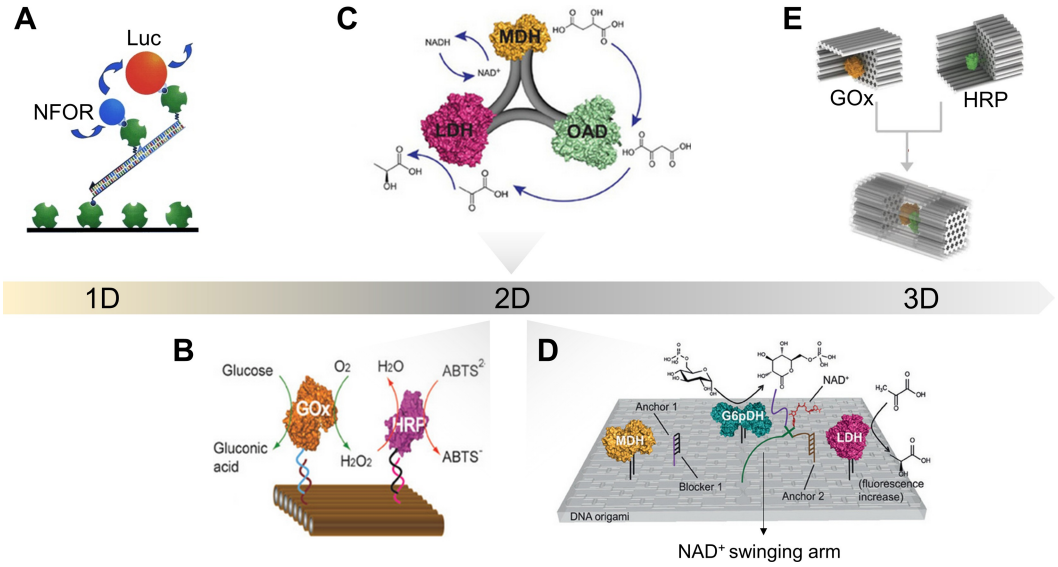

Figure 2. Examples of DNA-scaffolded enzyme cascades. A) Coassembly of Luc and NFOR using linear DNA template, adapted from Ref [7] with permission. Copyright Wiley-VCH, 2002. B) Immobilisation of GOx-HRP cascade in varying nanoscale distances, adapted from Ref [11] with permission. Copyright ACS, 2012. C) Three enzyme cascade of MDH, LDH and OAD on a geometrically optimized DNA nanostructure, adapted from Ref [12] with permission. Copyright Wiley-VCH, 2016. D) Implementation of NAD^+-swinging arm between G6pDH and LDH or MDH on DNA origami, adapted from Ref [13] with permission. Copyright Wiley-VCH, 2016. E) Construction of a 3D nanocage for the immobilization of GOx and HRP, adapted from Ref [14] with permission. Copyright Nature Publishing Group, 2016.

Revolutionary DNA origami technology then provided access to individual 2D DNA enzyme constructs, which were used to systematically investigate the effects of nanoscale spacing on the catalytic properties of the GOx-HRP cascade and to identify distance-dependent dimensionally-constrained substrate diffusion (Figure 2B).[11] The results suggested that nanoscale spacing between enzymes and dimensionally restricted diffusion were responsible for the increased cascade activity. Similar results were also shown using a cascade of sugar-processing enzymes.[15] Likewise, a cascade consisting of the three enzymes malate dehydrogenase (MDH), oxaloacetate dehydrogenase (OAD), and lactate dehydrogenase (LDH) that were immobilized on a tripodal DNA scaffold in different geometric arrangements (Figure 2C), showed significantly higher activity compared to linear constructs, which was attributed to facilitated substrate transfer.[12] In addition to establishing well-defined stoichiometries and optimizing spatial arrangement, DNA origami scaffolds have also been used to target pathways in enzyme cascades. One study demonstrated the directional regulation of a metabolic pathway involving the enzymes glucose-6-phosphate dehydrogenase (G6pDH), LDH, and MDH by attaching a switchable oscillating arm containing the cofactor NAD^+ to the origami surface (Figure 2D), which could be used to physically control the substrate channel between the enzymes.[13] This principle, previously demonstrated for the G6pDH-MDH cascade on a 1D DNA scaffold, could be useful for engineering biological control circuits.[16]

In addition, three-dimensional (3D) DNA scaffolds, such as nanoreactors and nanocages, have been investigated for the production and control of enzyme cascades. For example, the GOx-HRP system was immobilized in a nanocage, providing both an enhanced enzyme activity as

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well as protection from degradation by proteases (Figure 2E).[14] In this study, the observed increase in activity was explained by the formation of a hydrate shell, while protection from degradation was attributed to steric aspects. Also described were reconfigurable 3D origami nanoreactors with a reversible opening and closing mechanism based on DNA strand displacement[17] or pH[18] in which access of the encapsulated enzyme to free substrates can be controlled. Although these systems were not tested in cascade reactions, the results suggest that a closed reactor is permeable to small molecule substrates while effectively keeping large molecules out.

2. Influence of DNA Scaffolds on the Activity of Enzyme Cascades

What causes the altered cascade activity of DNA-templated enzyme assemblies? While the above examples provide an impressive demonstration of what is possible with DNA templates from a design perspective, the literature reports widely varying quantitative increases in activity achieved by nanoscale enzyme arrangement on the scaffold. Although only limited systematic analysis has been performed, largely ($\approx$20-fold) to moderately (1.5-fold) increased activity but also decreased activity has been observed for DNA-templated enzyme cascades[4d,5,19] (Table S1). Since in the literature these amplification rates are often rather vaguely attributed to favored substrate transfer or even direct channeling of intermediates between enzymes on the scaffolds, we would like to briefly summarize the current state. For an extended discussion with specific references, see Supporting Information Discussion SD1. In a first place, one should be clear about the term "substrate channeling", which refers to a process in which intermediates between sequential enzyme reactions are directly transferred from one active site to another without being released into the bulk solution, as realized in nature, e.g., via hydrophobic channels. However, as shown by experimental data and extensive modeling, the spatial proximity of enzymes alone does not contribute to a sustained increase in activity, but can only affect the initial rates at early stages of the reaction.[20] This result is consistent with recent experimental studies[21] and is likely to hold for scaffolds other than DNA structures, e.g., metal–organic frameworks (MOFs) or protein aggregates.[19] However, exceptions to the principle that spatial proximity alone cannot improve mass transfer to enhance the activity of enzyme assemblies are so-called assembly line multienzymes such as polyketide synthases, in which a growing substrate is permanently bound to a swinging arm, resulting in a consistently high local concentration. This swing-arm principle for facilitated substrate transfer has already been applied as a proof-of-concept for DNA-assembled cascades (Figure 2C).

Instead of direct proximity-based channeling, the model consideration of compartmentalization has gained acceptance. Diffusion layers on solid planar surfaces and suspended spheres can act as a physical barrier to diffusive penetration of solute molecules and thus as a microscale compartment. Likewise, microscale compartmentalization can arise from high local concentrations and agglomeration of multienzyme complexes.[22] For particle-immobilized enzyme cascades, it seems that the observed increase in multienzyme activity[23] is most likely due to a high local concentration of enzymes and thus an accumulation of intermediates in the diffusion layer compartment of the particle. For example, the increased productivity of a 10-step sequential reaction of glycolytic enzymes at the surface of nanoparticles was attributed to facilitated substrate transfer through compartmentalization,[23c] and studies of GOx-HRP cascades colocalized on microspheres supported this assumption.[23e]

How and to what extent can a DNA scaffold act as a compartment? Early evidence for a nanoscale diffusion layer of DON constructs[11] is supported by cross-scale effects observed for DON-enzyme cascades immobilized on surfaces of silica materials[24] or polymer microspheres.[21c] In the latter, systematic variations showed that the microscale compartment exerted a strong, but the nanoscale arrangement of enzymes on the DNA scaffold exerted little effect on the productivity of the cascade. Nevertheless, interactions of the DNA scaffold with the enzymes and/or substrates may lead to the formation of nanoscale compartments that form a barrier to diffusive loss of reaction intermediates, resulting in increased productivity of the cascade. This hypothesis is consistent with the fact that substrates in nature often diffuse randomly into compartmentalized reaction spaces that contain many catalytic centers.[20a]

Possible reasons for the formation of a reaction-influencing compartment include the change in local pH due to the high negative surface charge of the DNA scaffolds,[20a,b] the formation of a diffusion-influencing hydrate layer,[11,14,21a,25] and electrostatic modulation or interactions of the active components.[26] While the effects of lowered pH in the DNA scaffold microenvironment and also the formation of a hydrate layer have not yet been verified, electrostatic modulation, as proposed for the activity enhancement of DON-immobilized thrombin,[26c] appears to be a possible explanation, as enzyme activity can be influenced by coupling with DNA in multiple ways (see Supporting Discussion SD2). Of note, a mechanism beyond direct enzyme-DNA interaction concerns the restriction of diffusivity of substrate, intermediate, and product molecules, which are retained by, e.g., electrostatic interactions with the DNA scaffold, so that high local concentrations can affect enzyme activity positively but also negatively (e.g., by product inhibition). Such processes are currently not experimentally tangible, but could be assessable in terms of their quality and quantity through modeling.

Presumably, all of the above mechanisms contribute to the reported increases in activity to varying degrees. Nevertheless, it is questionable whether this can explain the strong differences observed even in the same enzyme cascade (GOx/HRP, see Table S1). To determine true physical effects and exclude experimental artifacts, it is essential for progress in this field that standardized methods are used to acquire and report research data to allow reproducibility

and systematic advancement of these complex systems. It is also important to note for the encouragingly increasing progress in modeling multi-enzyme reactions,[20a,c,22,23e,27] that simulation for quantitative prediction of a specific biocatalytic performance can only be achieved using high-quality experimental data.

3. Advances and Challenges in the Analysis of DNA-based Cascades

In view of the design of sound systematic approaches for the investigation of nucleic acid-scaffolded enzyme cascades, various aspects should be considered in the production and analysis of such systems (Figure 3). Strong variations in activity can be due to differences resulting from the chosen DNA scaffolds and enzymes, the enzyme-DNA coupling strategy, or the purification of the enzyme-DNA complexes. Deviations may also stem from more general aspects, such as insufficient purification, characterization, and quantification of both the free and assembled individual enzymes, technical inaccuracies in the analytics, and reaction conditions that are not clearly defined. Since all aspects lead to a lack of experimental reproducibility, insufficient standardization and inconsistencies in data management are particularly troubling and hamper further development of the field.[4d,5,28] To improve reproducibility and credibility of experimental studies, various efforts to implement guidelines, such as the National Research Data Infrastructure Consortium (NFDI, https://nfdi.de),[29] are recently being applied in biocatalysis research.[30]

3.1. Selection, Bioconjugation and Characterization of Enzymes

Regardless of basic mechanistic research, the potential utility of artificial DNA cascades for application transfer depends largely on the enzyme cascade under investigation. Presumably for reasons of practicality, the vast majority of published studies on DNA-templated cascades deals with robust, stable, preferably commercially available model enzymes that have limited relevance for actual biotechnological applications (e.g., the GOx-HRP system, Table S1). Significantly fewer examples have been carried out with more sensitive enzymes, such as cytochrome P450 BM3,[9,31] ketoreductases,[21c,32] or sugar-processing enzymes,[15,25,33] which are more relevant for biocatalysis.

Bioconjugation of enzymes with DNA can be achieved by a variety of covalent and non-covalent methods, often based on chemoselective and even bioorthogonal techniques, but also include genetically encodable coupling systems. Comprehensive review articles have been compiled on DNA-protein conjugation technology[3–4,28,34] and numerous experimental studies now clearly demonstrate that the method of bioconjugation can have a significant impact on the specific enzyme activity (Supporting Information Discussion SD2). For example, the highly charged polyanionic backbone of DNA as well as the nucleobases provide numerous interaction points with the charged amino acid residues on the protein's surface through electrostatic interaction or hydrogen bonding.[26c,35] Both increased and decreased activity of the individual enzymes was observed after conjugation with DNA, which can generally be explained by altered tertiary structure and/or microenvironmental influences, but cannot be predicted *a priori*. Since the altered activity of a single enzyme can be decisive for the overall performance of a cascade, especially if this enzyme is the rate-determining one,[20c] it is crucial to quantify the influence of DNA coupling on the activity in comparison to the free enzymes with the greatest possible accuracy. Obviously, these studies must take place under comparable temperature and buffer conditions, since, for example, the ionic strength and concentration of EDTA or phosphate ions can affect both the nucleic acid scaffold and the enzyme activity. Absolute determination of enzyme

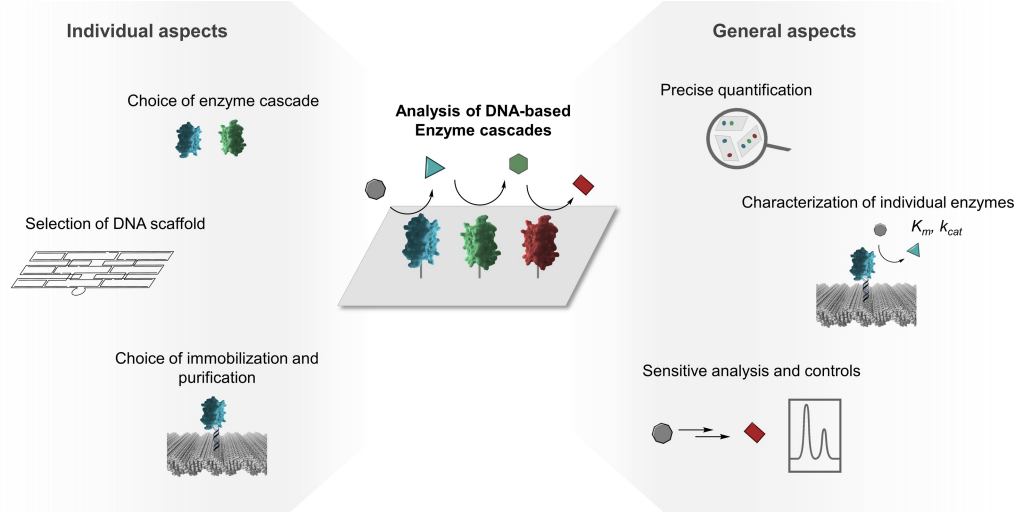

Figure 3. Considerations for the preparation and characterization of DNA-scaffolded enzyme cascades.

activity is essential not only to identify possible differences in cascade activity, but also to use the experimental results for improved modeling of the biocatalytic process and to optimize this cascade activity by stoichiometric adjustments.[36]

3.2. Purification and Characterization of DNA-Enzyme Constructs

Another factor that plays a significant role in the quantitative description of DNA-templated enzyme cascades is the precise quantification of the constructs and the purification from uncoupled enzymes. Usually, the immobilization of enzymes to DNA nanostructures is performed with an excess of the proteins to achieve the highest possible binding efficiency.[3,34a] Since unbound enzymes interfere with activity measurements of the assembled complexes, purification of the constructs is usually required, although in some examples sample contamination with unassembled enzymes was factored out by normalization procedures when determining cascade efficiencies.[11,37]

Numerous methods have been investigated for purification of DNA origami protein constructs and a comparative study showed that magnetic bead purification generally provided the best yield with the highest purity of samples.[38] In general, time-consuming and/or mechanically demanding purification procedures can compromise DNA construct integrity and enzyme activity and may not be suitable for sensitive systems.[19,28] This also applies to methods for concentrating constructs, such as PEG precipitation, which often lead to agglomeration of proteins and thus differences in activity[28] (for detailed consideration, see Supplemental Discussion SD2).

Another integral part of any quantitative study should be the accurate quantification of the coupling of enzymes on the DNA scaffold, including the absolute quantification of the nucleic acid scaffold. Since spectroscopic methods which employ an estimated molar extinction coefficient unfortunately provide an inadequate approximation, the actual concentration of the DNA scaffold should be determined with sensitive methods such as qPCR.[39] Accurate quantification of the DNA scaffold's surface occupancy with enzymes is of great importance because deviating occupancy densities can have a significant impact on the quantification of catalytic efficiency and thus on the assessment of cascade efficiency enhancement. Low occupancy densities, for example, can result in the individual components of a cascade not being co-immobilized on the same scaffold, which can distort the interpretation of the data. To distinguish between fully functionalized and partially functionalized nanostructures, methods such as atomic force microscopy (AFM) or transmission electron microscopy (TEM) are commonly used[15,21a,31–32,40] and can be complemented by additional methods such as western blot analysis.[21c,32c,d]

3.3. Additional Methodological Aspects

The choice of analytical method for characterizing DNA-templated enzyme cascades naturally varies widely in the reported studies. Since much work is done with model systems, chromogenic or fluorogenic substrates may be used, such as $ABTS^{2-}$ or AmplexRed, respectively, for analysis of the HRP/GOx cascade (see e.g. Figure 2B). Since many enzymes require cofactors such as $NAD(P)^+$/NAD(P)H, their conversion can be measured by absorbance or fluorescence spectroscopy. While spectroscopic methods are advantageous because they are simple and can be performed in real time, they quickly provide limitations due to the lower or upper limit of detection. For example, the typically small amounts of available DNA-enzyme constructs often prevent clean analysis of at least early phases of the reaction due to insufficient sensitivity of the method. Likewise, the addition of an excess of the analyte required for kinetic characterization may be limited by the upper detection limit. Cascade activity has also been measured with coupled reporter enzymes,[21a] which provides high sensitivity but can also introduce additional variance due to activity variations or mass transfer limitations. For accurate determination of cascade efficiency and exclusion of measurement artifacts, a highly sensitive and preferably direct measurement of substrate conversion should ideally be performed.[41] This is feasible by HPLC-based methods that have already been used in some studies.[15,25,33] Since enzyme cascades may behave differently at the beginning of the reaction than when reaction equilibrium is reached, HPLC measurements with high-sensitivity mass spectrometric detection can be used to monitor reaction turnover in early phases at small time intervals over a large period of time.[21c]

In addition to analytical methodology, the use of defined, reasonably chosen reaction conditions and the implementation of appropriate negative and positive controls is of great importance. For example, critical controls include reactions in which the DNA scaffold is mixed with the enzymes without being physically coupled to each other to preclude any scaffold effect on the activity of the unassembled enzymes. However, this approach is not a substitute for quantifying the direct effect of physical DNA coupling for the individual enzymes, as discussed above. Similarly, scaffolds carrying only one of the enzymes should be mixed together to ensure that both enzymes must be immobilized on the same scaffold to enhance cascade activity.[20c]

4. Perspectives of DNA Nanotechnology for Biocatalysis

DNA nanotechnology is expected to continue to play an increasingly important role in basic life science research over the next decade, particularly in the study of molecular processes in biological signaling cascades, where it can fully exploit its unique advantage of high-precision controllable stoichiometry and nanoscale arrangement of bioactive ligands. However, the importance of DNA nanostructures for the

development of practically relevant biocatalysis systems is less clear. Both application areas are primarily constrained by production scaling issues,[42] including the generally limited size of individual scaffolds was well as technical difficulties and costs in producing large quantities of staple strands and single-stranded scaffold for origami assembly.

The size of a DNA origami structure is limited by the length of the scaffold strand, so structures can typically reach dimensions of up to 100 nm.[42] For larger structures, the use of longer scaffold strands[43] or assembly of multiple individual DON has been demonstrated,[44] which, however, often results in lower assembly yields. Advances in the cost-effective scalable production of large quantities of staples and single-stranded scaffold include enzyme-mediated *in vitro* amplification of synthetic DNA strands,[45] and, in particular, biotechnological production of staples using enzymatic methods in combination with bacterial production of the starting DNA.[46] Indeed, the use of enzymatic methods for the industrial production of oligonucleotides has advanced significantly in recent years, bringing economically attractive fully automated processes within reach.[47]

The strategy of producing DNA components for origami technology by biological methods has general advantages over classical solid phase synthesis, i.e., very long DNA can be produced with high quality and comparatively low cost. Thus, the efficient production of phage ssDNA as a scaffold for DNA origami was established[48] and in further progress even the bacterial production of complete origami structures was realized, which allowed access to several hundred milligrams at a comparatively low cost.[49] Other developments to simplify and scale origami production include isothermal assembly of origami at room temperature, which, in addition to reducing costs, opens up options for *in situ* functionalization with proteins.[50] In addition, protein-containing DON were constructed as a proof-of-concept in a quasi-one-pot procedure by implementing *in vitro* transcription/translation.[51] While these examples demonstrate the innovation needed to advance the field towards biocatalysis applications, the road to translation into functional enzymes currently appears to be long. However, *in vivo* biological assembly of nucleic acid templated enzyme cascades has been achieved more than a decade ago by using double-stranded plasmid DNA as a scaffold (Figure 4A).[52] By fusing the enzymes with zinc finger (ZF) domains that specifically bind to unique plasmid DNA sequences, the enzymes could be assembled in *E. coli* cells to boost production of various metabolites.

The developments in biologically produced functional DNA nanostructures must also be considered in the context of the development of corresponding RNA nanostructures, which are considered a promising alternative for the production of functional materials.[54] Although structurally more difficult to handle, RNA superstructures have the advantage that they can be directly expressed in the same cell as proteins, so related work on enzyme-functionalized RNA constructs has been known for more than a decade. For example, *in vivo* synthesis of rationally designed RNA nanostructures enabled the intracellular assembly of enzymes for hydrogen production (Figure 4B),[53] and RNA-mediated assembly was used to co-

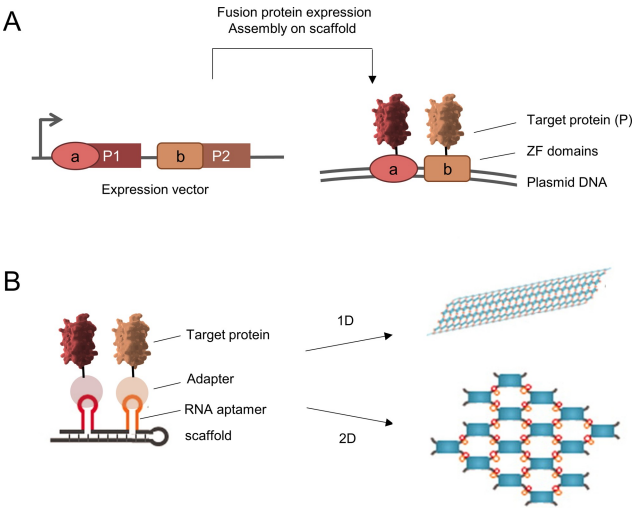

Figure 4. Arranging enzymes on nucleic acid nanostructures *in vivo*. A) Expression of ZF-fusion proteins to assemble target proteins on double-stranded plasmid DNA as a scaffold. ZF: Zinc finger, P: Target Protein. B) RNA aptamer motifs serve as scaffolds for enzymes equipped with binding adaptors. Self-assembled 1D and 2D RNA scaffolds with aptamers (colored circles) enable intracellular assembly of target proteins. Adopted from Ref [53] with permission. Copyright AAAS, 2011.

localize enzymes and increase metabolic production of a two-enzyme pentadecane production pathway.[55]

Since the trends towards scalable production of DNA scaffolds described above clearly point towards biological processes, and since many of the currently available complex chemical methods for protein immobilization are difficult to scale up and control in an industrial process,[19] the development of low-cost, universal immobilization strategies is also likely to come down to genetically encodable coupling tags. Aptamer-based systems (Figure 4B) and SpyTag/SpyCatcher technology[56] appear to be promising for this approach; however, use for commercial applications seems not feasible at this time due to the lack of low-cost production methods for DNA scaffolds (for a consideration of economics, see Supporting Information Discussion SD3). With further elaboration and implementation of the biology-based technologies, the commercial applicability of DON enzyme constructs may be re-evaluated in the coming decade.

Finally, the question should be asked whether our understanding of natural systems is detailed enough to design powerful enzyme cascades on platforms such as DNA origami, or whether such artificially reconstructed systems are informative enough to draw conclusions about biological systems. The influences of the specific environment and the complex interactions of multiple active units in the natural biological system are far from fully understood, and the differences already noted between *in vitro* reconstructed DNA enzyme cascades and natural multienzyme complexes are also substantial. For example, a fundamental problem is that the polyanionic scaffold structure of artificial cascades can have a strong influence on cascade efficiency. An increasing complexity and diversity of artificial systems may

enable reconstructing biological networks and also opening up applications.[4e] In practice, this could be addressed by specifically exploiting cross-scale compartmentalization, as systems with high density of active units, such as on the surface of nanoparticles, show good performance.[23c,57]

5. Summary and Outlook

DNA nanostructures have emerged as powerful and versatile tools for studying mechanistic effects in enzyme cascades due to their ability to arrange proteins with high control at nanoscale distances. However, despite major advances in the fabrication and characterization of these complex systems, currently published data suggest that the overall utility and value of DNA constructs for enzyme cascades is still highly dependent on individual experimental settings.[4d,5,28] From the above discussion, it is clear that the selection of enzymes and DNA scaffolds and, in particular, the sound characterization of all components is crucial, as they all can significantly influence the cascade activity and the reliability of the results.[58] The dependence of the observed increases in activity on the various factors discussed suggests that performance depends on multiple factors whose identity and weighting for the overall process is often unclear. Therefore, as discussed in Chapter 4, robust experimental designs need to be applied to obtain a clearer picture of the actual mechanisms at the nano- and microscale and to translate this into applications.

It is clearly evident that enzyme-decorated origami structures are not suitable for preparative biocatalysis in their current state, mainly because of the high cost, the difficult and expensive fabrication procedures of enzyme-origami constructs, and the numerous uncertainties regarding the influence of DNA immobilization. Nevertheless, the examples described here demonstrate that the development of DNA-scaffolded enzyme cascades to date has led to a high level of widespread interest in such coupled reaction-diffusion networks and has thus contributed substantially to increased activity in modeling such systems. We believe that, in particular, the current approach of focusing mechanistic principles on length-scale cross-cutting considerations points in the right direction to achieve the translation of this fundamental research into routine processes that are also of economic interest.

Supporting Information

The authors have cited additional references within the Supporting Information, Refs. [59–85].

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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Minireviews

Enzyme Cascades

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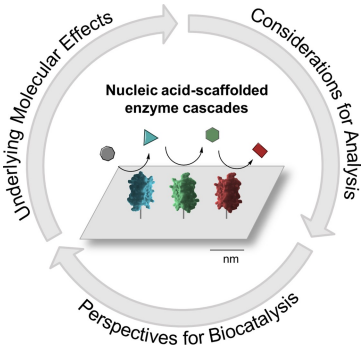
Nucleic Acid-based Enzyme Cascades—Current Trends and Future Perspectives



Nucleic acid nanostructures are capable of precisely arranging proteins at nanoscale distances and have thus proven to be powerful scaffolds for studying mechanistic effects in enzyme cascades. This minireview summarizes the current status of proposed underlying mechanistic effects, derives aspects for further systematic analysis, and discusses future perspectives for biocatalytic applications.