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Unlocked potential of dynamic elements in protein structures: channels and loops

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Enzymes are nature's powerful catalytic proteins to perform reactions with often outstanding activity, selectivity and specificity. Moreover, the access to non-natural functions of biocatalysts can be facilitated by enzyme engineering. While rational approaches are often focused on an enzyme's active site, from random directed evolution we know that further functional hotspots must exist beyond the active site. Addressing flexible structural elements of these biocatalysts like loops and channels in enzyme engineering has the potential to fill this knowledge gap. The structural dynamics of enzyme catalysts are vital to promote their remarkable functions. This influences for example the access, recognition and orientation of substrates. Herein, we review recent examples of loop and channel engineering and classify them according to their use of simulation methodologies, predictions prior to engineering, the engineering methodologies themselves and discoveries found after the engineering. Thereby we highlight current possibilities and make suggestions to further unlock the potential of this yet underexplored strategy.

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Introduction

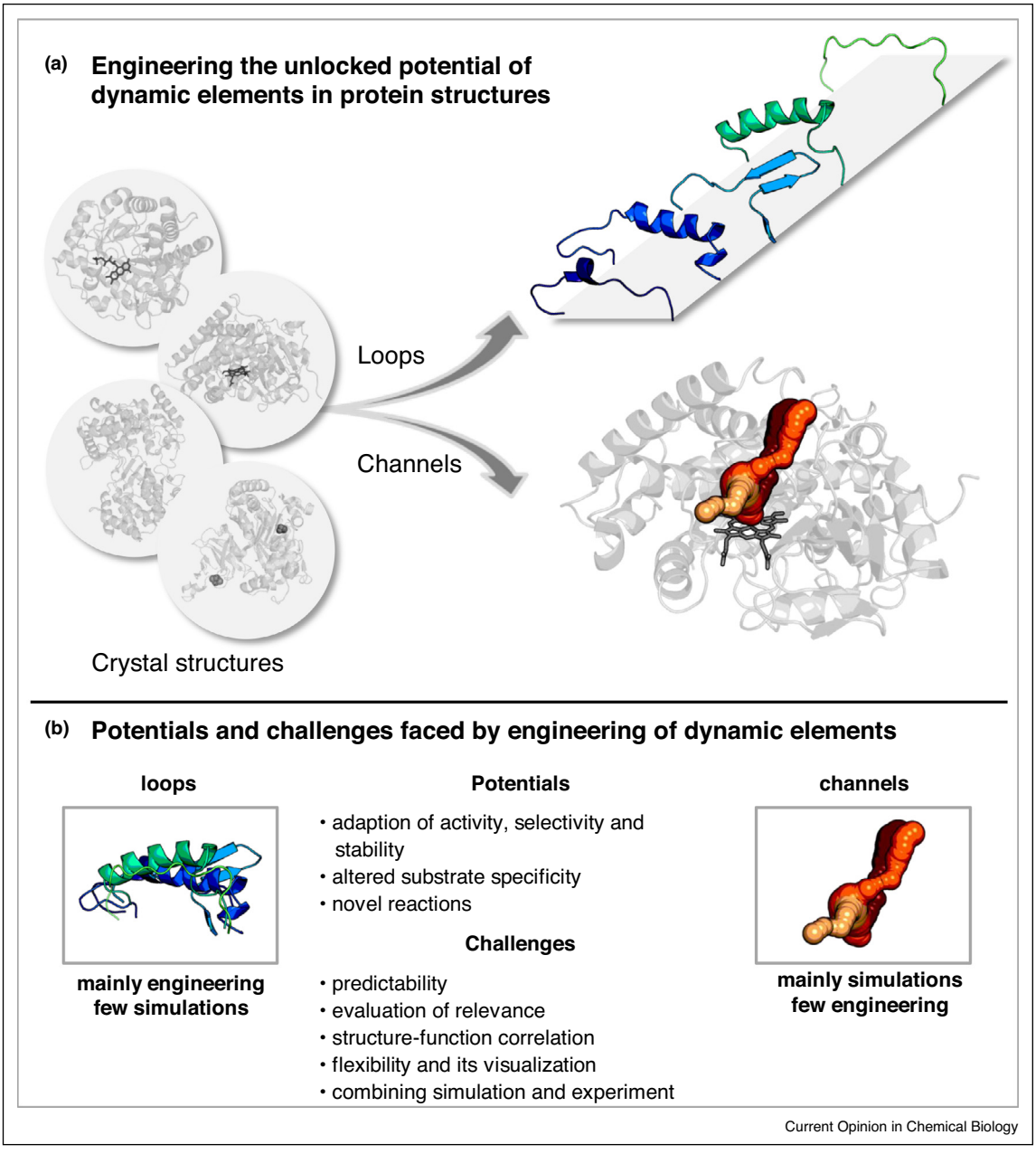
Enzymes have a great potential that goes beyond organic chemistry [1]. To develop stable and synthetic applicable enzymes with defined functions is one of the major challenges in enzyme engineering over the last few decades [2]. Most engineering efforts have been focused on rationally chosen alterations (rational design) [3,4] or strategies introducing random mutations by screening large mutant libraries (directed evolution) [5]. While rational approaches often focus on the catalytic pocket of an enzyme to directly influence transition states, directed evolution studies, however, often introduce variations outside the active site pocket [6]. From these studies we can thus conclude that for the creation of smart enzyme libraries, which drastically accelerate the enzyme identification process, we need to find further functional hotspots beyond the active site.

But what lies beyond the active site? Internal void space such as channels— dynamic paths leading from the active site of a protein to its surface—and flexible loop structures also greatly influence the behavior and properties (e.g. specificity, stability) of enzymes [7,8]. While these structural elements often also form parts of the active site, they are mostly located beyond. Flexible protein structures are widely acknowledged for their essential role and the detailed investigation of these elements is crucial for the development of improved catalysts [9]. Not only ligand recognition and binding, but also transport and enzyme catalysis require rearrangements of these flexible structures [10,11].

Due to their intrinsically less-defined secondary structure, a large portion of an enzyme's dynamics is based on flexible loop regions [12,13]. Within enzyme superfamilies these highly versatile structural elements usually allow a great functional diversity [14]. They often lie at the surface of proteins, surround or are part of the active site and can thus interact with substrates. Moreover, in more than 60 % of annotated proteins, the active site is either located in a surface pocket or buried within the protein [15]. The pathways leading to these active sites, channels, are themselves often highly dynamic structural elements forming complex networks where channels may either merge into one another or branch [16]. The importance of such channels is demonstrated by their appearance in all enzyme classes [17,18]. Amino acids lining these channels may substantially influence the behavior of substrates on their way to a buried active site.

Hence, we propose that a conscious engineering of loops and channels unlocks the potential of these dynamic structural elements and will help finding additional hotspots outside the active site for the creation of smart enzyme libraries filling a gap between current rational mutagenesis and directed evolution approaches (Figure 1). We see great potential in this strategy to adapt catalytic activities and selectivities, to create altered substrate specificities or even to promote the attainment of novel reactions. However, such engineering strategies

Figure 1



Enzymes contain dynamic loops and channels, which are often diverse and non-conserved structural elements. **(a)** Loops and channels can be identified based on crystal structures directly or by further simulation techniques and serve as valuable engineering targets. **(b)** The engineering of these dynamic elements has many promising potentials regarding enzyme catalysis. However, many challenges remain to fully unlock this potential and need to be addressed by a combination of simulation and experimental approaches. Currently, loop engineering mainly focuses on experimental results and few simulations are included. In contrast, channel engineering attracted a lot of attention by simulation research, however, being still a rather unexplored field with regard to experimental exploitation.

are not trivial and face several challenges. Despite our knowledge about the importance of these dynamic structures, their intrinsic flexibility often complicates an exact understanding of their biological function concerning their shape, position and directing energetics [8,13,19]. This also impedes the easy predictability of altered structure-function correlations by crystal structures alone. Therefore, especially the correct visualization of enzyme dynamics is important in order to make correct evaluations on the impact of loop and channel engineering. Facing these challenges, we see the necessity of smartly combining simulation and experimental approaches.

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