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Strategies for designing non-natural enzymes and binders

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The design of tailor-made enzymes is a major goal in biochemical research that can result in wide-range applications and will lead to a better understanding of how proteins fold and function. In this review we highlight recent advances in enzyme and small molecule binder design. A focus is placed on novel strategies for the design of scaffolds, developments in computational methods, and recent applications of these techniques on receptors, sensors, and enzymes. Further, the integration of computational and experimental methodologies is discussed. The outlined examples of designed enzymes and binders for various purposes highlight the importance of this topic and underline the need for tailor-made proteins.

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Introduction

Enzymes catalyze virtually every chemical reaction within a cell. The design of enzymes with high activity and selectivity is a thorough test of our understanding of protein folding, small molecule recognition, and biochemical activity. Above all it can result in widely applicable customized catalysts. This review focuses on recent advances in the design of folds and functions. In particular, we cover the design of new protein scaffolds either *de novo* or by repurposing natural protein fragments, describe computational and experimental methods for sequence optimization, and present recent examples on designed enzymes and ligand binders (Figure 1). For a review covering similar topics before 2013 we refer to Feldmeier and Höcker [1].

Some recent reviews have covered related subject matters. We want to highlight the articles by Goldsmith *et al.* describing strategies and prospects to reach the maximal catalytic activity in different systems [2], MacDonald *et al.* [3] focusing on the use of computational protein design and backbone plasticity, and Romero-Rivera *et al.* [4] presenting in depth explanations on computational tools for biocatalyst design.

Which is the best scaffold for a design?

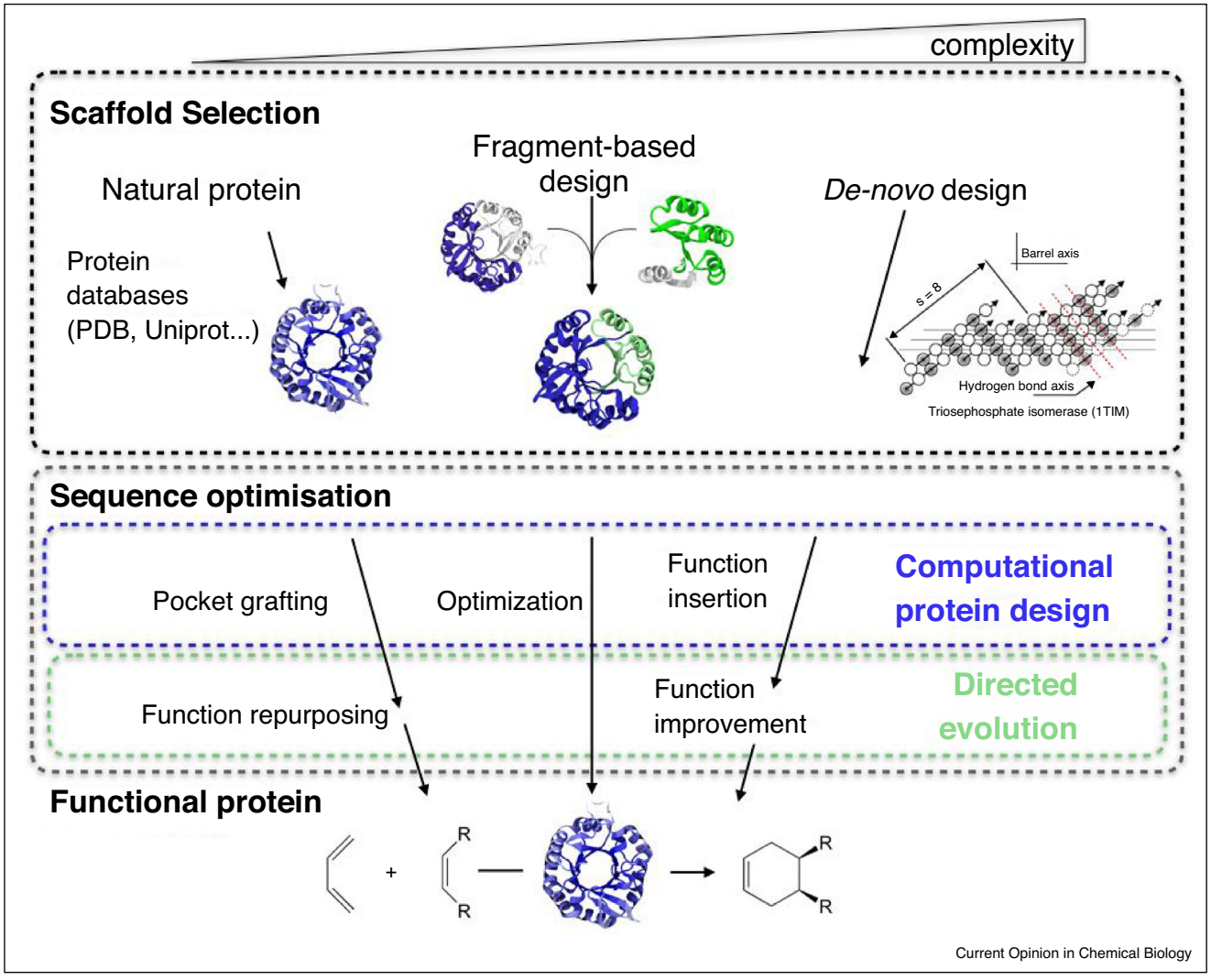
The design process usually starts with the identification of protein topologies that can serve as scaffolds for the introduction of new binding pockets and/or catalytic residues. Several approaches have proven successful to obtain this starting point, ranging from repurposing an existing natural protein [5,6] via the recombination of protein fragments to create new domains, to entirely designing a *de novo* protein with no evolutionary history [7••].

While natural proteins can provide preformed pockets for substrate recognition or offer parts of catalytic machinery that can be repurposed, they can also carry features that are not obvious due to the environment they adapted to in the course of evolution. These features might not be necessary or might even be potentially interfering with the newly designed functionality. Thus, the cleanest way would be a bottom-up approach in which the functional site is designed into a fresh and newly built scaffold that does not carry any evolutionary baggage and in which the key determinants of the fold are understood, for example, proteins that have been generated by *de novo* design. A third approach is to combine the best of both worlds by using parts of natural proteins as building blocks and recombining them to generate new scaffolds. We discuss the latter two approaches below.

De novo scaffold design

One of the most successful protein scaffolds in the evolution of enzymes is the $(\beta\alpha)_8$-barrel (or TIM-barrel) fold. Proteins that adopt this fold tend to be extremely stable and display an interesting division of labor with the active site found on one side of the barrel while stability appears to be defined by the opposite end of the structure. A *de novo* $(\beta\alpha)_8$-barrel design with simplified loops and fourfold symmetry was recently achieved in a joint effort by the Baker, Fernandez-Velasco, and Höcker labs. Out of 22 computational designs, six showed CD spectra corresponding to the expected $(\beta\alpha)_8$-barrel fold. One of these was improved further by computer-guided rational design and its structure confirmed by X-ray crystallography. The newly designed amino acid sequence is clearly distinct

Figure 1



Schematic representation of the enzyme design process. First, a scaffold is selected; either from an existing natural protein or a synthetic one either constructed from natural fragments or designed *de novo*. Then, the sequence is optimized using computational or experimental approaches, or both, depending on the scaffold and desired output.

from all known TIM-barrel sequences and provides an interesting new scaffold for functionalization [7••].

Furthermore, the Baker lab has designed a range of very stable helical bundles consisting of three to five α-helices with a length of 7, 11 and 18 residues, respectively. The designs did not require human intervention after visual inspection nor subsequent rounds of evolution [8]. Similarly, and at the same time, the Woolfson lab used a series of different software packages combined in CCBuilder to design helical barrels consisting of 5–7 α-helices. Seven out of 22 chemically synthesized designs showed the intended oligomerization state of which four were confirmed by X-ray crystallography [9]. One of these designs served as a scaffold for the insertion of a catalytic triad as discussed below [10].

New scaffolds through fragment recombination

While *de novo* design is still developing, nature has long generated a wide variety of scaffolds. It has become accepted that evolution achieved an impressively large protein universe by replicating and recombining a set of small, stable elements. These elements, termed domains, are independent folding units of about 100–200 amino acids and constitute the basic evolutionary unit. Several lines of evidence suggest that the origin of domains themselves occurred through the duplication and recombination of smaller fragments [11,12]. Alva *et al.* identified