Emergent Mechanisms in Photoenzymatic Catalysis

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Biocatalysts for Sustainable Chemical Synthesis

Organic molecules are essential to multiple industries whose products define modern human existence, ranging from materials to flavor and fragrances to pharmaceuticals and agrochemicals. While some of these molecules can be isolated from natural sources, the vast majority need to be synthesized. Consequently, there is a premium placed on sustainable catalytic methods that enable shorter synthetic routes from renewable feedstocks, that offer high levels of chemo-, regio-, and enantio- selectivity, that produce decreased waste and require less energy [1]. Nowhere is this more apparent than in the pharmaceutical and fine chemical sectors, which collectively produce more waste than other chemical industries or the automotive industry [2]. Efforts over the past two decades have been focused on developing catalytic platforms that can be modularly deployed for the diverse range of molecules required by these industries. Hallmark examples include efficient transition metal catalysis [3], base metal catalysis [4], organocatalysis [5], and biocatalysis [6].

Biocatalyst is a rapidly developing field of chemical synthesis [7]. While perhaps the oldest field of catalysis, it was viewed as effective for only a small range of chemical transformations, with specific substrate preferences, and having insufficient stability or activity to be useful as an industrial tool. The advent of directed evolution in 1993, pioneered by Frances Arnold and Pim Stemmer, provided a general strategy for tuning an enzyme function for a particular property (thermal stability, organic solvent tolerance, activity on a specific substrate, or a desired selectivity outcome) [8,9]. Yet, despite these towering advances, biocatalysis was not immediately adopted because other enabling technologies lagged. For directed evolution to be truly useful, technological advances in chromatographic separations, high-throughput experimentation, analytic chemistry, DNA sequencing, and synthesis were required. Only after these

technologies had developed could chemists envision deploying biocatalysis industrially [10].

The watershed moment for applications of biocatalysts was in 2010, when Merck reported a biocatalytic synthesis of Sitagliptin, a type 2 diabetes treatment. Their original award-winning synthesis involved a rhodium-catalyzed asymmetric reduction of an unprotected enamine [11]. However, given the high demand for this molecule, there was a significant financial benefit to developing a more efficient process. Merck's chemists identified an amino transaminase as promising reactivity for streamlining their synthetic efforts and eliminating multiple purification events. However, there were no natural transaminases capable of catalyzing the formal reduction amination on their substrate of interest. However, over 11 rounds of engineering, they were able to develop a catalyst that could be run on a scale that offered increased productivity (53% increase in kg/L/day) and yield (10-13%) while decreasing overall waste production by 19% and eliminating the need for a precious metal catalyst and expensive phosphine ligand. This report was a watershed moment that led to the process chemistry group throughout the industry adopting biocatalysts to improve their manufacturing routes [12].

Despite these incredible advances, there remain significant reactivity limitations to biocatalysts. In general, enzymes are engineered to catalyze the reactivity patterns they exhibit in nature [13]. As nature is inherently conservative in its synthetic strategies and faces different limitations than the ones that chemists experience when producing a drug or fine chemical. Over the past 25 years, researchers have extensively examined how natural or artificial metalloenzymes can be used to catalyze non-natural reactions [14,15]. In the following section, I will describe the strategy that our group has used to expand the functions of proteins using photochemistry [16]. I will discuss our general approach, foundational examples, and future directions and challenges for this area.

Our work in Emergent Mechanisms in Photoenzymatic Catalysis

Photoexcitation is a well-established strategy for unlocking new reactivity in organic and inorganic complexes. While this mode of reactivity is widely used in chemical synthesis, it is rarely employed by enzymes. In fact, there are only three natural photoenzymes known in nature, DNA photolyase, protochlorophyllide reductase, and fatty acid photodecarboxylase [17].

In 2015, our group became interested in whether proteins had latent photochemical functions that were not found in nature. Our initial studies aimed to harness the rich photochemistry of biomimetic molecules by utilizing their biological

counterparts. While our initial studies were focused on the photochemistry of NADPH [18], we found flavoproteins to be more general photoenzymes for organic synthesis. Throughout our studies, we have identified instances of mechanistic emergence where molecules or reactive intermediates behave differently within the protein active site than they do in a solution-based reaction.

We found that flavin-dependent 'ene'-reductases (EREDs) can catalyze various inter- and intramolecular reductive alkene hydroalkylations reactions with excellent enantioselectivity when irradiated with cyan light. In these reactions, radical initiation occurs via excitation of a charge transfer complex involving the flavin hydroquinone and organic substrates. These complexes only formed within the protein active site because of weak electrostatic attraction between the substrates and cofactor [19]. As these complexes require both the alkene and the alkyl halide, they enable selective intermolecular coupling reactions with minimal hydrodehalogenation (Fig 1A).

The protein scaffold is essential in controlling the properties of the CT complex, enabling optimization by directed evolution. Using various mutagenesis strategies, we engineered EREDs to use red and near-infrared light for radical initiation [20]. The protein's ability to tune CT complexes was highlighted when engineering a protein for regioselective indole alkylation [21]. The parent enzyme (GluER-T36A) required irradiation with cyan light to initiate the reaction. Over six rounds of engineering, the selectivity was improved to greater than 9:1. More importantly, the final variant can facilitate the reaction without the need for light. The emergence of this dark reactivity is due to improved mixing of the donor and acceptor wave functions.

Beyond radical initiation, proteins can also change the energy landscape to make challenging steps feasible. In the reductive coupling of alkyl halides with nitroalkenes, we found that EREDs can facilitate mesolytic cleavage of nitroradical anions [22]. In the absence of the protein, this C–N bond cleavage is slow by comparison to electron transfer. We found proteins that can either break the C–N bond or oxidize the nitroradical anion, highlighting the ability of enzymes to precisely control the fate of reactive intermediates (Fig. 1B).

In another example, we established a new mechanism of C–N bond formation that is exclusive to an enzyme active site (Fig 1C). We engineered a flavin-dependent Baeyer-Villiger monooxygenase to catalyze a 5-exo-trig hydroamination of anilines with pendant styrenes to afford pyrrolidines with excellent yield and enantioselectivity [23]. This reaction initiates via the reduction of the alkene, affording a tertiary radical. The protein then templates a through-space interaction

between the aniline and radical to attenuate its oxidation potential, enabling a concerted oxidation/C–N bond forming reaction. Importantly, photoredox catalysts cannot facilitate these transformations because they cannot template the through-space interaction.

Outlook

The mechanisms outlined above highlight the unique reactivity available to common reactive intermediates when localized within the microenvironment of the protein active site. These results only scratch the surface of the types of unique mechanisms that proteins can enable. Contemporary studies by Arnold demonstrate that the protein scaffolds can allow iron porphyrins to catalyze carbene and nitrene transfer reactions that are slow with the cofactor alone [24,25]. As more research groups explore new reaction mechanisms using existing proteins, new and unique mechanisms will be revealed. Moreover, de novo protein design often focuses on known mechanisms for specific transformations. These emergent mechanisms can serve as a design principle for designing proteins with new, novel functions.

For these new photoenzymes to be truly valuable tools for the synthesis of societally valuable molecules, further protein engineering is required to increase their activity and selectivity. As many of these proteins are already used industrially for their native mechanism, they already possess the stability for high concentrations of organic solvents and substrate. Engineering needs to be focused on enhancing the quantum efficiency and molar absorptivity. These advances should be coupled with improved instrumentation and techniques for large-scale photochemical reactions. Alternatively, for reactions involving enzyme-templated charge transfer complexes, engineering can be used to enable these reactions to be run in the dark.

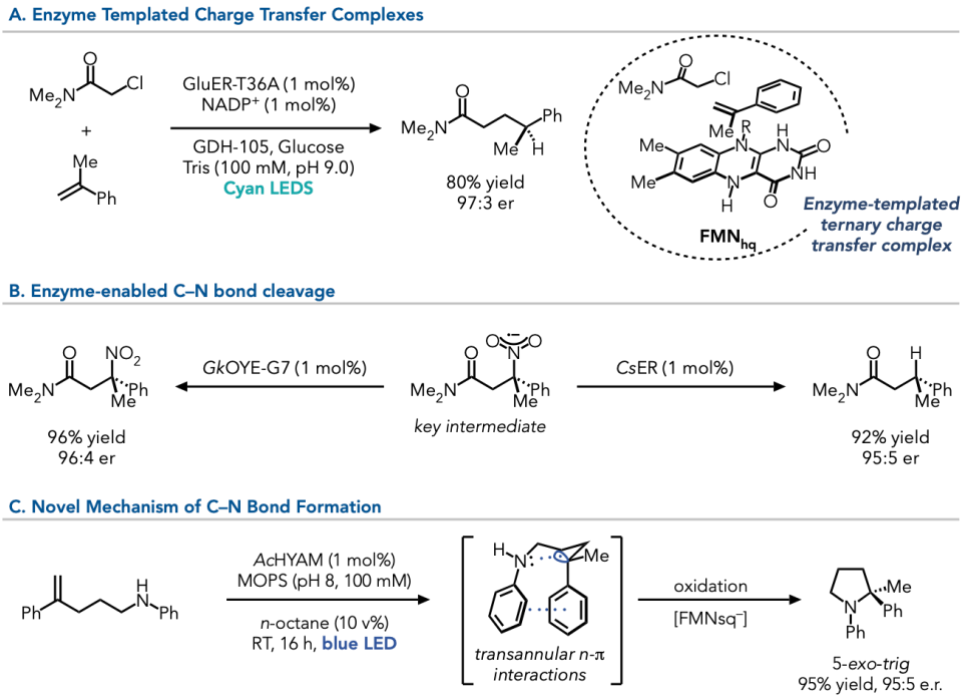

Fig. 1. Emergent Mechanisms in Photoenzymes. A. Enzyme-templated charge transfer complexes enable intermolecular C–C bond formation. B. Active site control over the fate of a nitroradical anion. C. An enzyme-templated interaction between a tertiary radical and amine facilitates oxidative C–N bond formation.

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