Fusion proteins as a versatile biocatalyst for different reactions

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Synthetic dyes have become important to food, pharmaceutical, and textile industries. However, usage of these dyes has environmental and health implications. To circumvent these problems, biological approaches such as the use of whole-cell and enzymatic systems have been investigated. Enzymatic approaches involve the use of proteins such as azoreductases (AzoRo) to reduce synthetic dyes. However, AzoRo require the use of expensive co-substrates such as NAD(P)H and thus, makes the application quite impractical. The use of NAD(P)H recycling systems such as formate dehydrogenases (FDH) has been promising. Therefore, the combination of AzoRo and FDH makes it an efficient duo.

We previously investigated the fusion protein comprised of FDH and AzoRo with histidines as the peptide linker. As the construct improved substrate scope, we further investigated the linkers – using different linkers with varying length and properties. Based on our results, linker properties affect dye reduction with 2x helical linker being the best with 12 out of 20 dyes tested being reduced. We have also observed the production of hydrogen peroxide with and without the addition of FMN up to 2 to 3 mM – making our fusion constructs having oxidase activities. We also coupled our construct with an unspecific peroxygenase (*Cvi*UPO) to fuel hydroxylation and sulfoxidation reactions. *Cvi*UPO with 1 mM H_2O_2 alone cannot produce 1-phenylethanol at all but linker constructs that showed less and slower hydrogen peroxide production exhibited activities and improved enantioselectivity of 1-phenylethanol.

Therefore, our construct shows that we can have a plug-and-play system for different reactions. We can have a myriad of downstream applications like NADH production, dye degradation, H_2O_2 delivery, and cascade reaction with UPO all available from one biocatalyst.

References

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