IN VIVO AND *IN VITRO* FMN PRENYLATION AND (DE)CARBOXYLASE ACTIVATION UNDER AEROBIC CONDITIONS

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Key Words: prenylated FMN, FMN prenyltransferase, UbiX, UbiD, ferulic acid decarboxylase, cofactor regeneration

Prenylated FMN (prFMN) is a newly discovered redox cofactor required for activity of the large family of reversible UbiD (de)carboxylases involved in biotransformation of aromatic, heteroaromatic, and unsaturated aliphatic acids (White et al., 2015). Despite the growing demand for decarboxylases in the pulp/paper industry and in forest biorefineries, the vast majority of UbiD-like decarboxylases remain uncharacterized. Functional characterization of the novel UbiD decarboxylases is hindered by the lack of prFMN generating system. prFMN cofactor is synthesized by the UbiX family of FMN prenyltransferases, which use reduced FMN as substrate under anaerobic conditions and dimethylallyl-monophosphate (DMAP) as the prenyl group donor. Here, we report the *in vivo* and *in vitro* biosynthesis of prFMN and UbiD activation under aerobic conditions. For *in vivo* biosynthesis, we used newly discovered UbiX proteins from *Salmonella typhimurium* and *Klebsiella pneumonia*, which activated ferulic acid UbiD decarboxylase Fdc1 from *Aspergillus niger* under aerobic conditions (0.5-1.5 U/mg). For *in vitro* biosynthesis of prFMN and UbiD activation, we established a one-pot enzyme cascade system that uses prenol, polyphosphate, formate, and riboflavin as starting substrates and (re)generates DMAP, ATP, FMN and NADH. The system contains 6 different enzymes: prenol kinase, polyphosphate kinase, formate dehydrogenase, FMN reductase, riboflavin kinase and FMN prenyltransferase. Under aerobic conditions, this system showed up to 80% conversion of FMN to prFMN and generated active Fdc1 decarboxylase (0.2-1 U/mg). Thus, both systems represent robust approaches for *in vivo* and *in vitro* prFMN biosynthesis and UbiD activation under aerobic conditions. The developed FMN prenylation systems will facilitate the exploration and biochemical characterization of UbiD-like decarboxylases and their applications in biocatalysis.

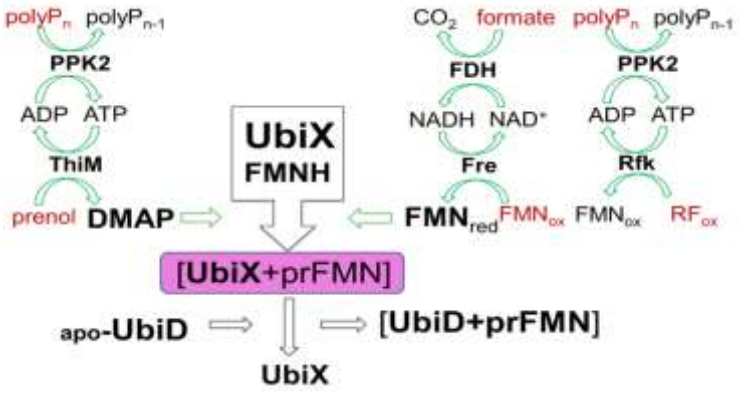

Figure 1 – Schematic diagram showing the in vitro cascade system for prFMN biosynthesis and UbiD decarboxylase. The system includes cyclic and linear (re)generation reactions for ATP, NADH, DMAP and FMN, as well as for enzymatic oxygen removal (FDH).

1. White MD, Payne KA, Fisher K, Marshall SA, Parker D, Rattray NJ, Trivedi DK, Goodacre R, Rigby SE, Scrutton NS, Hay S, Leys D. UbiX is a flavin prenyltransferase required for bacterial ubiquinone biosynthesis. Nature. 2015 Jun 25;522(7557):502-6.