

# NADPH Regeneration via Renewable Hydrogen: Exploiting Oxygen-Resistant [FeFe]-Hydrogenase and BMR Reductase in a Non-Physiological Cascade

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NADPH cofactor plays a pivotal role in bio-catalysis, facilitating the production of high-value compounds like regio- and stereo-selective intermediates utilized in fine chemicals and pharmaceuticals synthesis. Many proposed cofactor regeneration systems encounter challenges such as the accumulation of by-products, acidification, and the need for purifying the desired products for which the cofactor regeneration is ancillary.

Alternatively, employing H<sub>2</sub> enables efficient NADPH recycling without by-products that could disrupt pH balance or complicate product recovery. Additionally, the availability of inexpensive H<sub>2</sub> derived from renewables like solar and wind-powered electrolysis or dark fermentation of waste materials ensures sustainability of the process and aligns with the principles of circular economy.

The system proposed here is the first of its kind, based on a modular combination of non-physiological partners already successfully employed in the “Molecular Lego”<sup>1,2</sup>. We exploited the very robust, highly active and oxygen resilient [FeFe]-hydrogenase CbA5H from *C. beijerinckii*, previously identified in our group<sup>3,4</sup>, combined with a reductase (BMR) from *P. megaterium*. The system showed a good stability as evaluated by DSC and it reached up to 28±2 nmol NADPH regenerated s<sup>-1</sup> mg of hydrogenase<sup>-1</sup> (TOF: 126±9 min<sup>-1</sup>). To demonstrate feasibility in biotechnological applications, a cascade reaction employing [FeFe]hydrogenase, BMR, and a mutated Baeyer-Villiger monooxygenase<sup>5</sup> confirmed efficient indigo production across multiple reaction cycles.

1. Sadeghi and Gilardi, 2013; *BiochAppBiotec* 60(1):102-10

2. Giuriato et al., 2022; *Protein Sci* 31(12):e4501

3. Morra et al., 2016; *Biochemistry*, 55(42), 5897–5900

4. Winkler et al., 2021; *Nat Commun* 12:756.

5. Catucci et al., 2022; *Biocatal Agric Biotechnol* 44, 102458

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