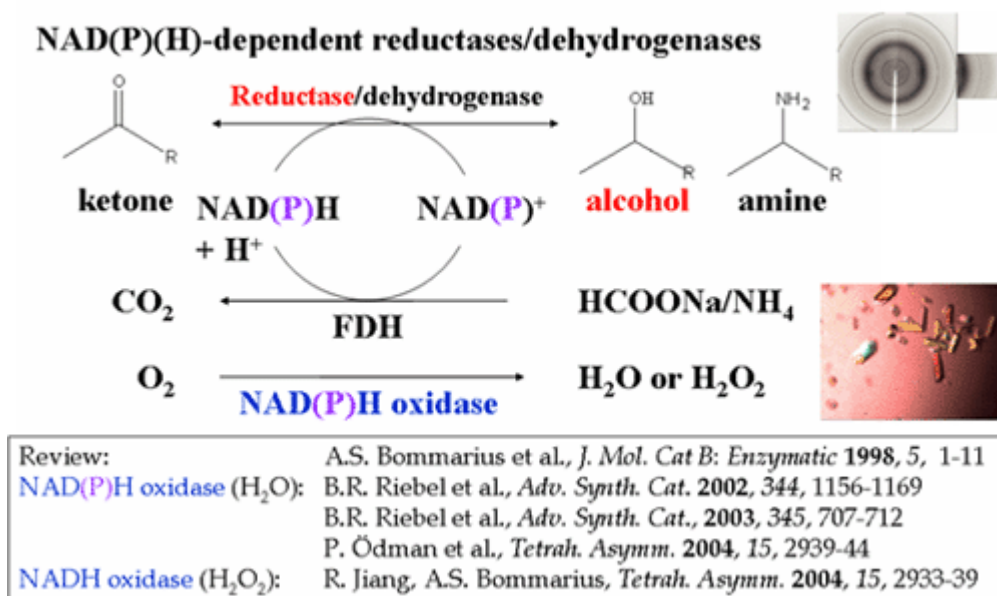


Development of Novel Biocatalysts - Bommarius

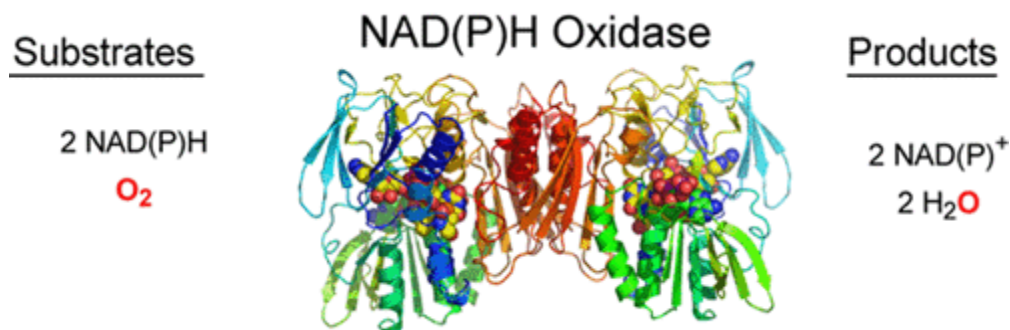
Development and characterization of NAD(P)H oxidase from Lactobacillus sanfranciscensis

A possible solution for the regeneration of NAD⁺ from NADH is the oxidation of NADH with concomitant reduction of oxygen catalyzed by NADH oxidase (E.C. 1.6.-.-). We employ NADH oxidase from *Lactobacillus sanfranciscensis*, which reduces O₂ to innocuous H₂O, and (R)-alcohol dehydrogenase ((R)-ADH) from *Lactobacillus brevis* to perform enantioselective oxidation of racemic phenylethanol to acetophenone and (S)-phenylethanol with regeneration of either NADH or NADPH to their respective oxidized precursors. NADH oxidase from *L. sanfranciscensis* accepts both NADH and NADPH; in contrast, the wildtype (R)-ADH only accepts NADP(+)(H) whereas its G37D mutant strongly prefers NAD(+)(H). Highly pure NADH oxidase (221 U/mg, two-step protocol) was coupled with wildtype-ADH from *L. brevis* on NADP(H) and mutant ADH from *L. brevis* on NAD(H) to achieve 50% conversion of racemic phenylethanol to (S)-phenylethanol and acetophenone. Depending on the relative concentration of alcohol to cofactor, up to more than 100 turnovers were observed. We believe that this is the first demonstration of a regeneration scheme for both NAD⁺ from NADH and NADP⁺ from NADPH with the same enzyme.



Solving the crystal structure of NAD(P)H oxidase

In collaboration with Allen Orville's lab, we recently published the first crystal structure of an NADH oxidase.



Coupled reaction from MSG to alpha-ketoglutarate with regeneration of NADH to NAD⁺ with NADH oxidase

Alpha-ketoglutarate, employed to treat mild chronic renal insufficiency, was obtained through enzymatic oxidation of monosodium glutamate (MSG) catalyzed by L-glutamate dehydrogenase (L-gluDH) coupled with NADH oxidase for the regeneration of NADH back to NAD⁺. The irreversible reduction of molecular oxygen to water by NADH oxidase is demonstrated to drive oxidation of MSG to α -ketoglutarate to completion. L-gluDH was found to be inhibited by all three oxidative deamination products, α -ketoglutarate, NADH, and ammonia. As the pH in the current system was balanced by sodium and NADH was recycled to NAD⁺, inhibition of L-gluDH by α -ketoglutarate is believed to present the biggest challenge to an efficient process. In a batch experiment, we achieved a volumetric productivity of 1 g/(L-d).

