Electronic Supplementary

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I Primer Sequences

Primer A: tttaagaaggagatatacatATGAAACATCACCATCACCATCACGCAGAAATTGGTACG

Primer B: tttattcagcagacgataCGCGGCCGCTTTCGCCGC

Primer C: cagcggcgaaagcggccgcgATGTCTAACCGTTTGGATG

Primer D: gctttgttagcagccggatcCTATTGAGCAGTGTAGCC

Primer E: cagcggcgaaagcggccgcgATGGCTTCGGTACACGGCACCAC

Primer F: gctttgttagcagccggatcTTAAGATCTCTTCACCGGGCTTAC

Primer G: GATCCGGCTGCTAACAAAG

Primer H: CGCGGCCGCTTTCGCCGC

II DNA sequences encoding for the corresponding fusion enzymes

- red sequences encode for the HaloTag
- green sequences encode for the helical linker
- black sequences encode for the corresponding enzyme

II.I *halotag-ppbfd L476Q*

ATGAAACATCACCATCACCATCACGCAGAAATTGGTACGGGATTTCCGTTTGACCCGCATTATGTGGAGGTTCTGG
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TAA

II.II *halotag-lbadh*

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TAG

III. Protein sequences

- red sequences: sequence of the HaloTag
- green sequences: sequence of the helical linker
- black sequences: sequence of the corresponding enzyme

III.I HaloTag-*Pp*BFD L476Q

MKHHHHHHAEIGTGFPFDPHYVEVLGERMHYVDVGPRDGTPVLFLHGNPTSSYVWRNIIPHVAPTHRCIAPDLIGMG
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III.II HaloTag-*Lb*ADH

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IV Effect of acetaldehyde on the production of (*S*,*S*)-PPD

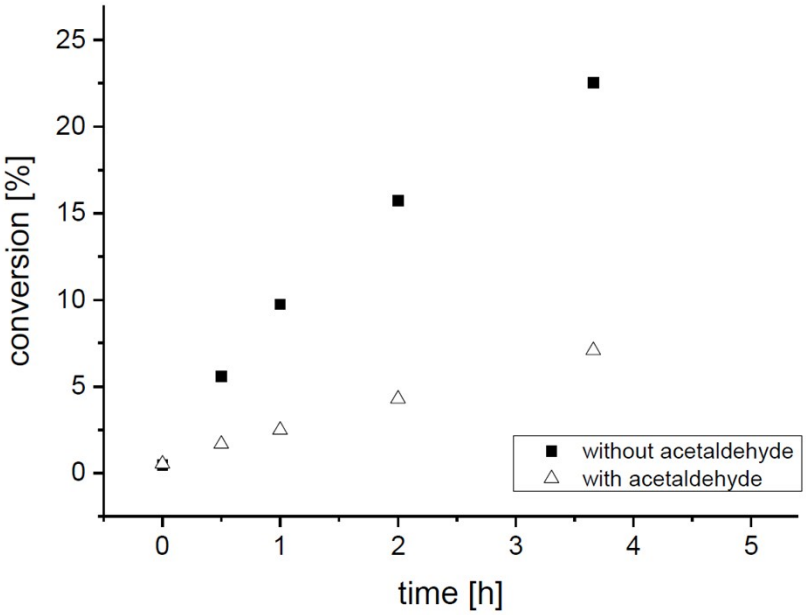

Figure S1: Production of (*S*,*S*)-PPD catalyzed by HaloTag-*Lb*ADH in the presence of acetaldehyde. The reduction of (*S*)-HPP towards (S,S)-PPD was analyzed in batch using immobilized HaloTag-*Lb*ADH. (*S*)-HPP resulting from the carboligation of benzoylformate and acetaldehyde was used either without removal of residual acetaldehyde or after removal by membrane supported stripping. Assay: 20 mM (*S*)-HPP containing either 40 mM or no acetaldehyde, 10 % (v/v) isopropanol, 0.5 mM NAPH, 50 mM TEA, 0.15 mM ThDP, 2.5 mM $MgSO_4$, V = 1 ml, 25 °C, 1200 rpm.

V Photograph of flow setup

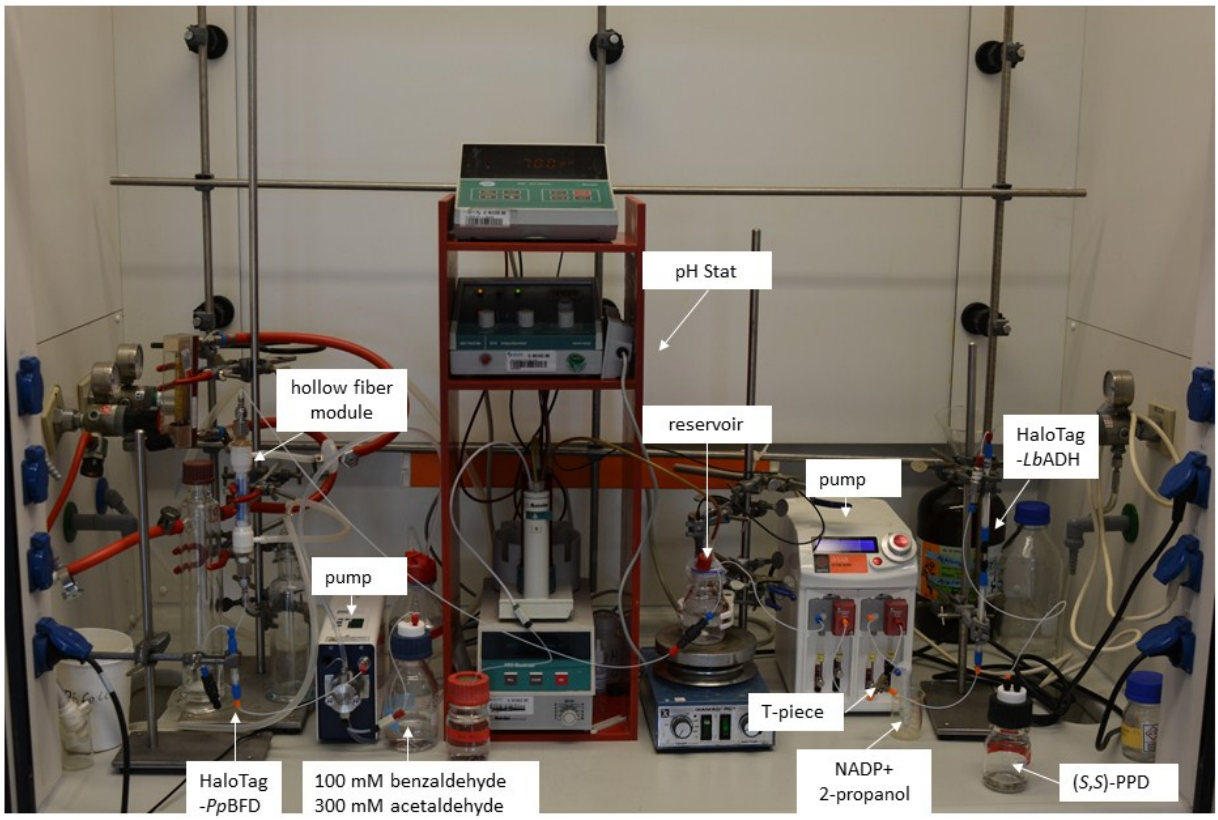

Figure S2: Enzymatic 2-step cascade for the continuous production of (S,S)-PPD. In the first step catalyzed by immobilized HaloTag-PpBFD L476Q, benzoylformate is decarboxylated towards benzaldehyde and carbon dioxide. The resulting benzaldehyde is then further converted into (S)-HPP by a carboligation in the presence of excess acetaldehyde. Afterwards, residual acetaldehyde is removed by membrane supported stripping using a hollow fiber module and the pH is automatically adjusted to pH 7.0 with a pH-Stat. In the second step catalyzed by immobilized HaloTag-LbADH, (S)-HPP is reduced to (S,S)-PPD under consumption of NADPH, which is produced from the cheaper oxidized cofactor NADP+ using 10 vol% 2-propanol as a cosubstrate for cofactor regeneration.