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Supporting Information

Design of Artificial Alcohol Oxidases: Alcohol Dehydrogenase–NADPH Oxidase Fusions for Continuous Oxidations

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cbic_201800421_sm_miscellaneous_information.pdf

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Table S1. List of primers: Primers used in this study are listed in the table below. The underlined part of the sequence is the BsaI recognition site, which is incorporated to allow Golden gate cloning. Gene1 indicates the gene coding for the enzyme that would be at the N-terminus side of the fusion, whereas Gene2 would be C-terminal.

Primer name	**Primer sequence**
Tbadh_gene1_fw	CGGTCTCGCCATATGAAAGGTTTCGCCATGC
Tbadh_gene1_rv	CGGTCTCTGCAGAGCCCGAGGCCAGAATAACAACTGG
adhA_gene2_fw	TAGGTCTCGCTGCAGCTGGTATGACGTCCTCCTCTTCTCC
adhA_gene2_rv	CAATGGTCTCTCAAGTTAGGCGCAGTAGCCGTTGTCG
Lbadh_gene2_fw	TAGGTCTCGCTGCAGCTGGTATGAGCAACCGTCTAGATG
Lbadh_gene2_rv	TGGTCTCTCAAGTTATTGTGCCGTGTAGCC
Nox_gene1_fw	CGGTCTCGCCATATGGCCGGGCAGACGAC
Nox_gene1_rv	CGGTCTCTGCAGAGCCCGAGGTGAGGACGAAACCTTCGTAGCCC
Nox_gene2_fw	TGGTCTCGCTGCAGCTGGTATGGCCGGGCAGACGAC
Nox_gene2_rv	TGGTCTCTCAAGTTAGGTGAGGACGAAACCTTCGTAG
QuikChange	**Primer sequence**
PAMO_C65D_fw	GGGGGCGCGGGACGACATCGAGAG
PAMO_C65D_rv	CTCTCGATGTCGTCCCGCGCCCCC
PAMO_bsai_fw	TGGTGTCTATCGAACAGCAC
PAMO_bsai_rv	ATAGACACCAGCATGTTGCTGAG

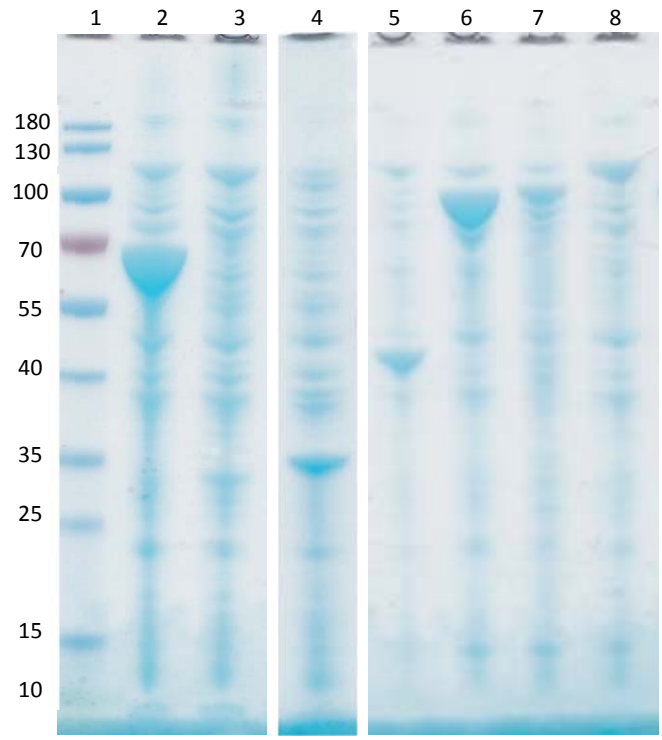


Figure S1. SDS-PAGE. Gel from the cell-free extract from each contstruct: 1. Ladder, 2. NOX, 3. ADHA, 4. LbADH, 5. TbADH, 6. NOX-A, 7. NOX-L, 8. T-NOX

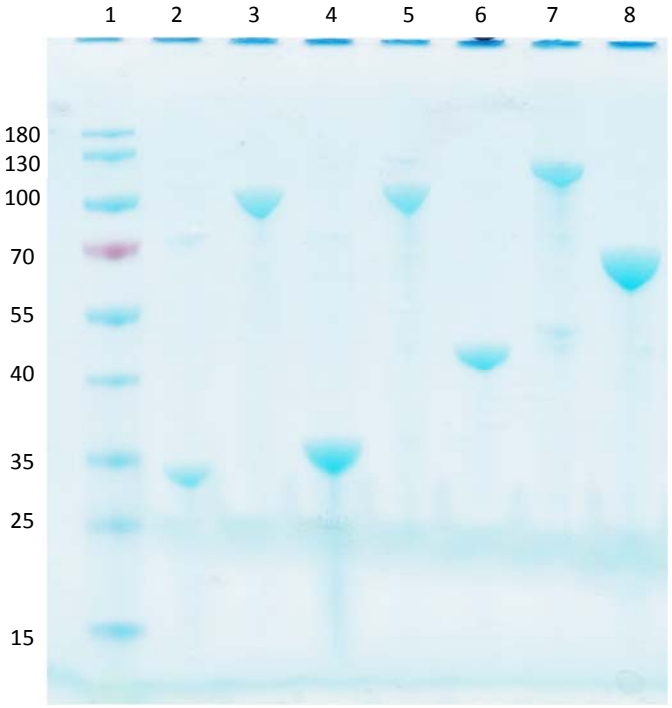
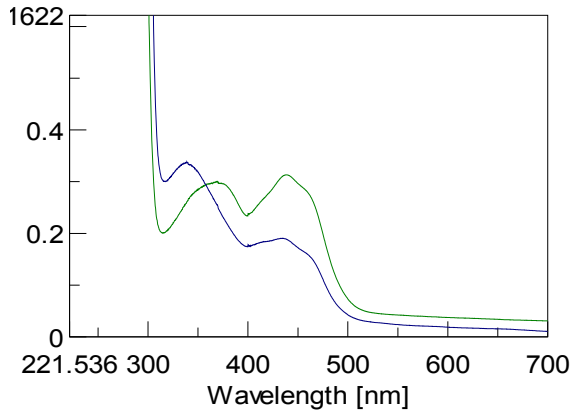
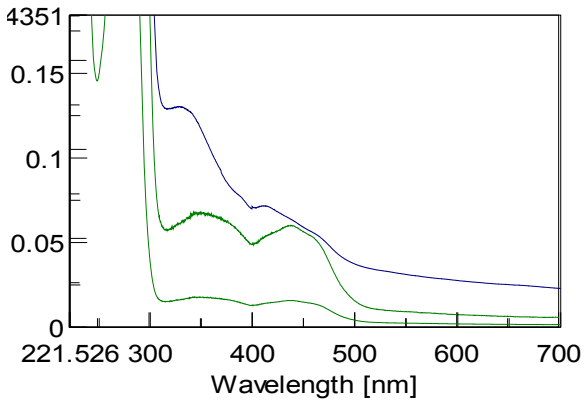


Figure S2. SDS-PAGE purified fractions. Gel of each purified fraction: 1. Ladder, 2. ADHA, 3. NOX-A, 4. LbADH, 5. NOX-L, 6. TbADH, 7. T-NOX, 8. NOX.

a) NOX-A before (blue) and after (green).



b) NOX-L before (blue) and after (green), with high and low concentration.



c) Tb-NOX before (green) and after (blue), with high and low concentration.

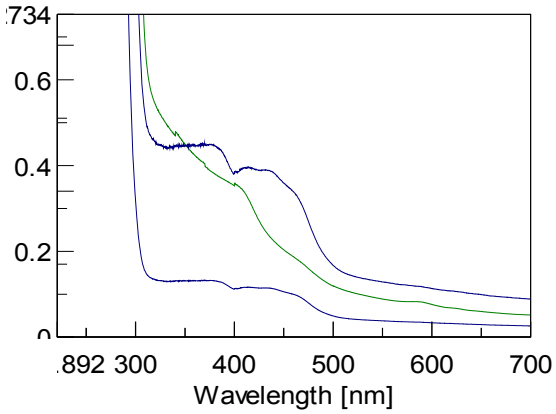


Figure S3. FAD spectra from NOX-ADH fusions. Absorption spectra taken from 200-700 nm, before and after treatment with ferricyanide.

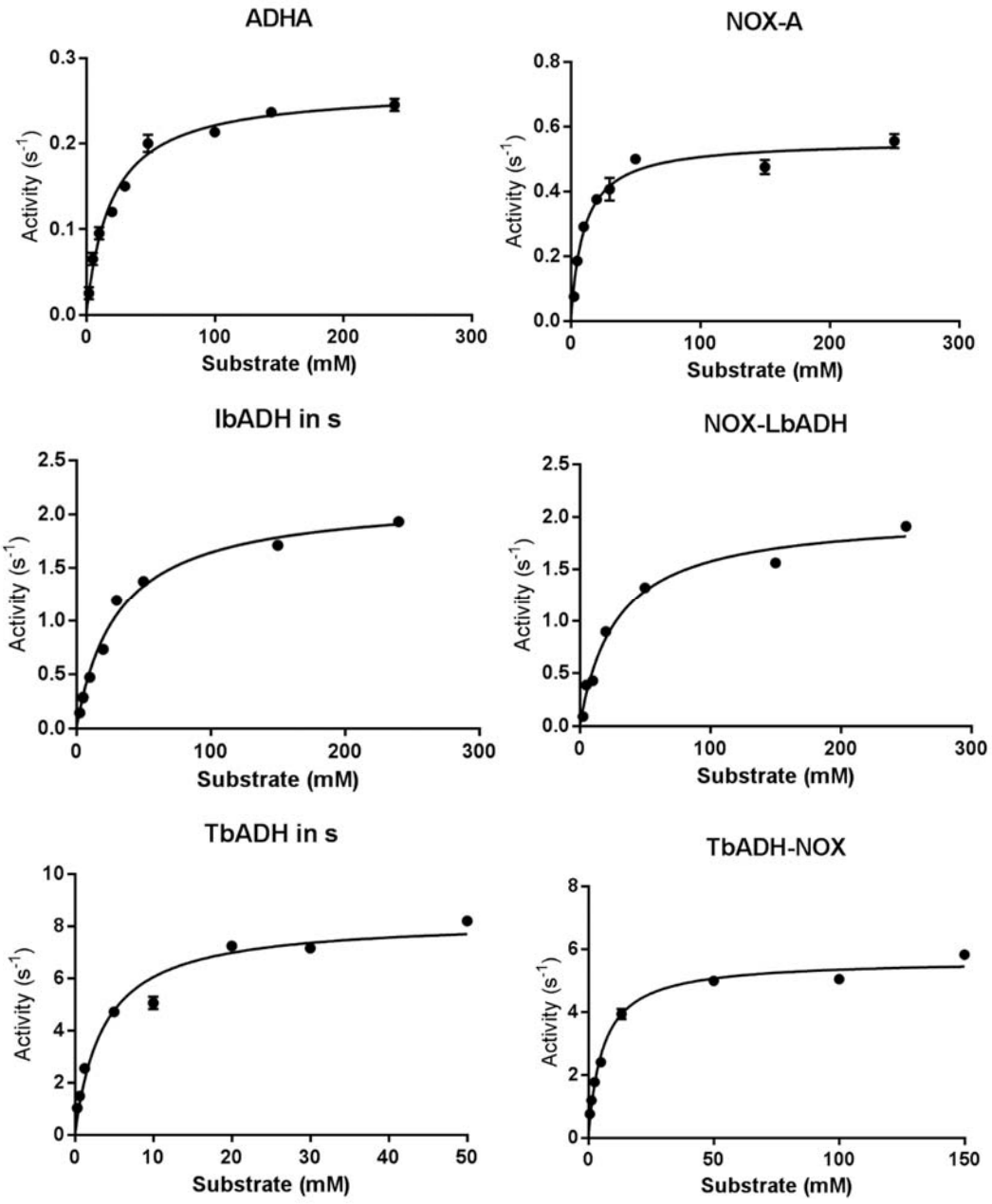


Figure S4. Plots of initial rates (activity, y-axis) against cyclohexanol concentration (x-axis). Reaction conditions: 25 °C, 20 mM KPO_4 pH 7.5, 0.2 mM $NADP^+$, 0.01-1 μM of enzyme. Activity measurements were taken in duplicate or triplicate, with 7-10 different substrate concentrations. The data was plotted in GraphPad Prism 6, and fitted to the Michaelis-Menten equation using non-linear regression with least squares fit. Coefficient of determination ranged from R^2 = 0.96 to 0.98. Kinetic values obtained are presented in Table 2.

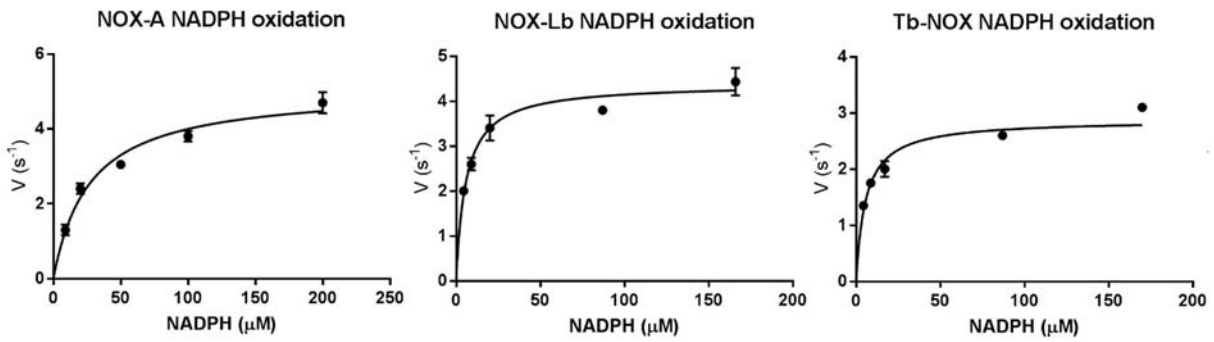
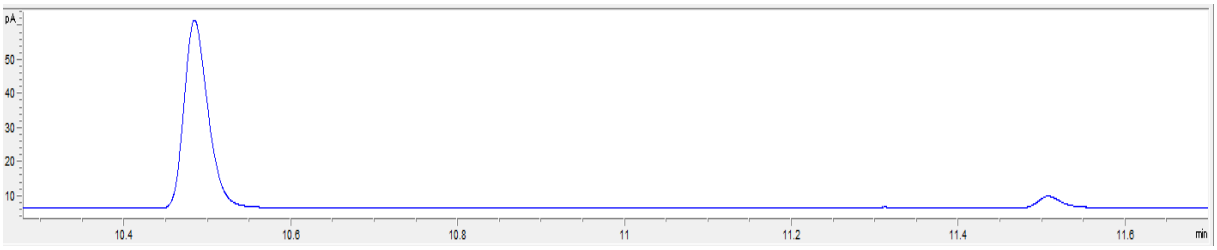
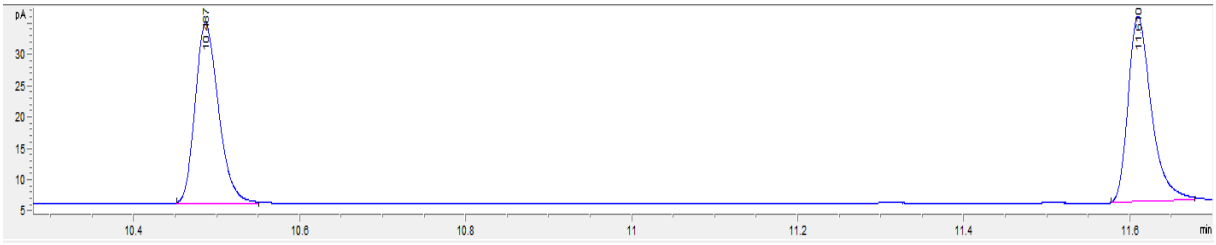


Figure S5. Plots of initial rates (activity, y-axis) against NADPH concentration (x-axis). Reaction conditions: 25 °C, 20 mM KPO_4 pH 7.5, varying concentrations of NADPH, 0.01-1 μM of fusion enzyme. Activity measurements were taken in duplicate or triplicate, with 6 different substrate concentrations. The data was plotted in GraphPad Prism 6, and fitted to the Michaelis-Menten equation using by nonlinear regression with least squares fit. Coefficient of determination: NOX-A R^2 = 0.96, NOX-L R^2 = 0.93, T-NOX = R^2 = 0.92. Kinetic values obtained are presented in Table 2.

a) NOX-A chromatogram (94% conversion)



b) NOX-L chromatogram (50% conversion)



c) Control rac-1-phenylethanol

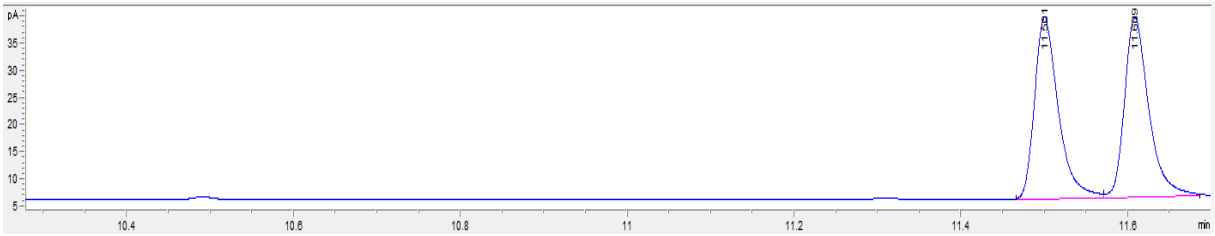
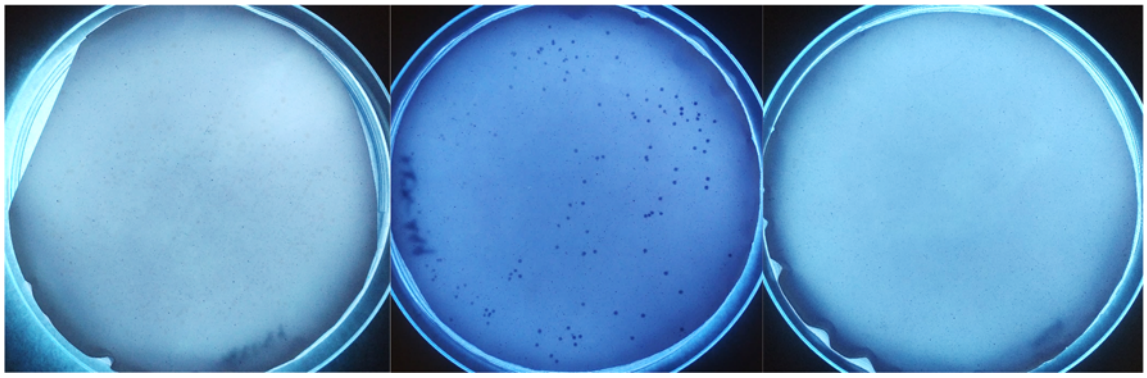


Figure S6 Chiral GC analysis. Results from kinetic resolution. Samples were extracted with ethyl acetate, dried over magnesium sulfate, and analyzed by chiral GC (Hydrodex β-TBDAc column (Aurora Borealis, The Netherlands)). The retention times were: acetophenone (10.5 min), (*R*)-1-phenylethanol (11.5 min), (*S*)-1-phenylethanol (11.6 min).

a)



b)

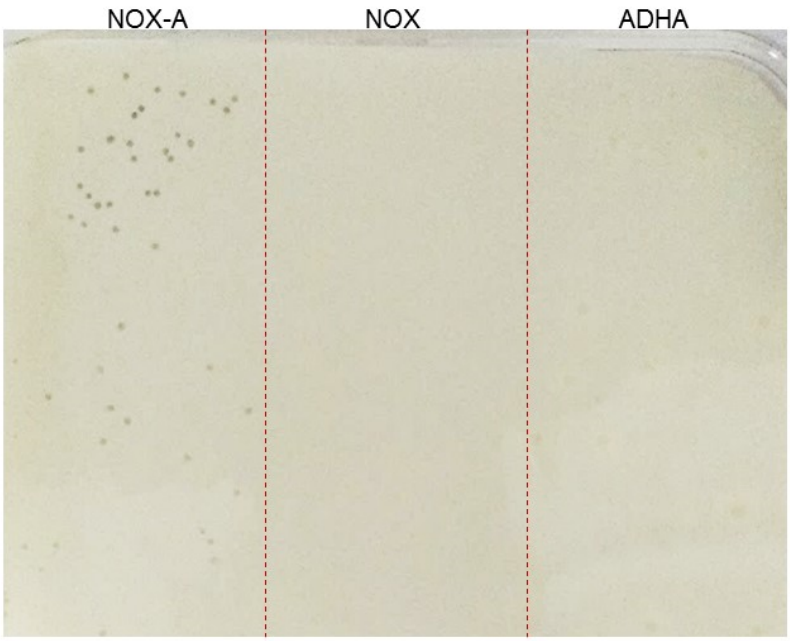


Figure S7: Colony-based oxidase screening. a) Colonies expressing: ADHA (left), NOX-A (middle), NOX (right), then treated with assay mix (see experimental section). b) Replicate, with the three constructs on the same plate, plated in separate lanes (red striped lines).