Supporting Information

A green-by-design system for efficient bio-oxidation of an unnatural hexapyranose into chiral lactone for building statin side-chains

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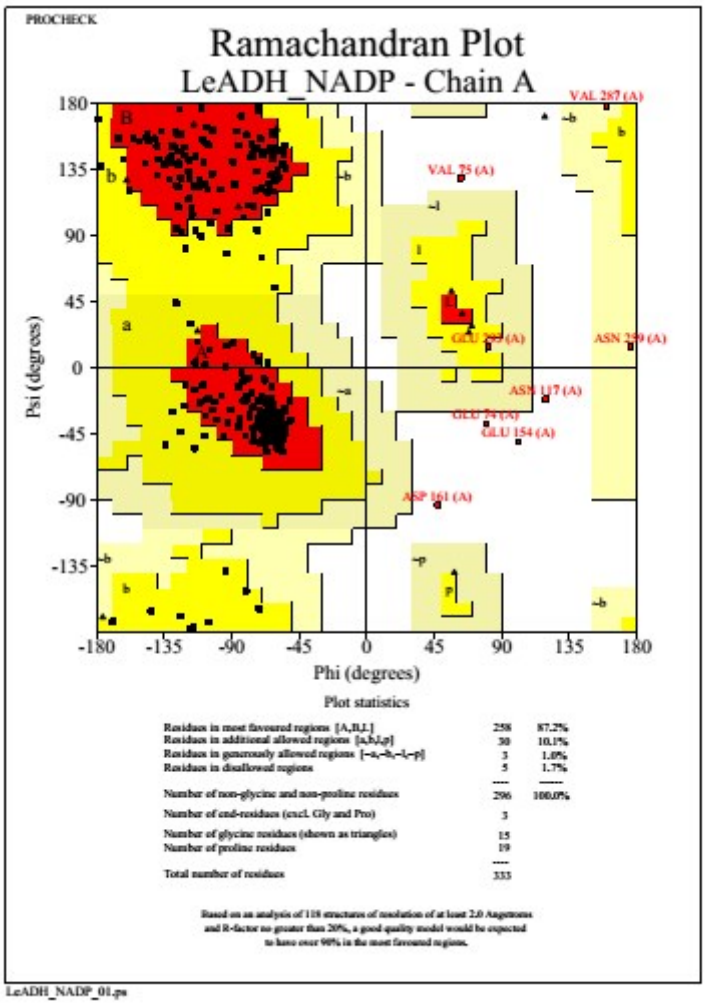

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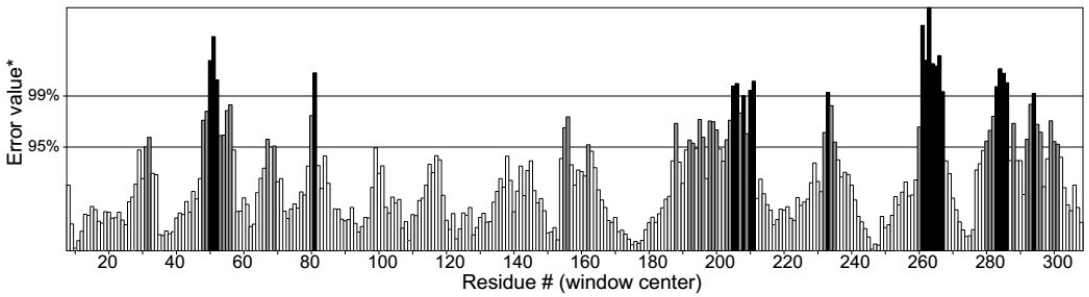

*On the error axis, two lines are drawn to indicate the confidence with which it is possible to reject regions that exceed that error value.

**Expressed as the percentage of the protein for which the calculated error value falls below the 95% rejection limit. Good high resolution structures generally produce values around 95% or higher. For lower resolutions (2.5 to 3A) the average overall quality factor is around 91%.

Figure S1. Ramachandran plot and errat result of *Le*ADH homology structure.

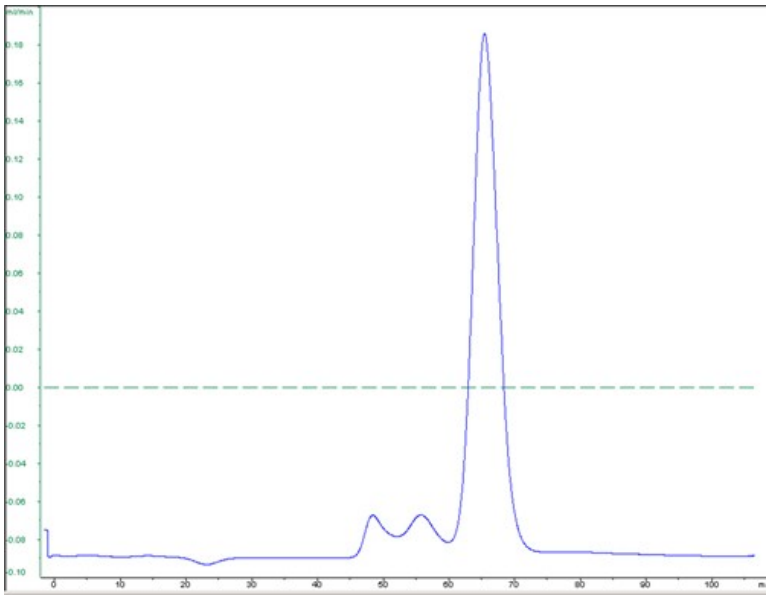

Figure. S2 Spectra of size-exclusion chromatography on superdex 75, elution volume of *Le*ADH is 66.0 mL. Y = -12.955*ln*(X) + 111.8 (Y: elution volume; X: molecular weight), the molecular weight determined to be 33 kDa.

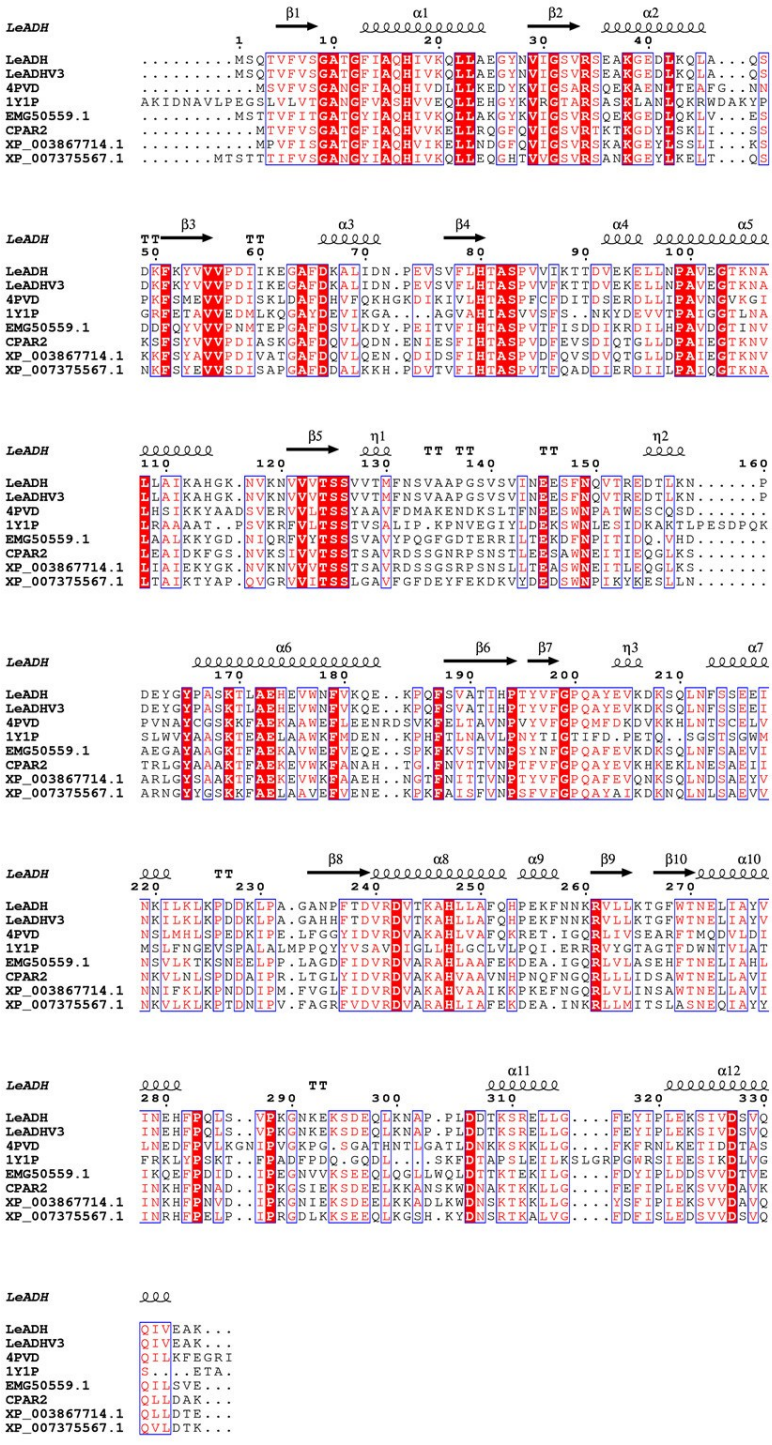

Figure S3. Multiple sequence alignment of *Le*ADH with proteins from different sources. Conserved residues are shaded in red background. The catalytic residues are shown in blue stars. The schematic diagram of secondary structure of *Le*ADH is shown on the top of sequence. The α-helices, 3_{10}-helices and π-helices are displayed as medium, small and large squiggles respectively. β-strands are rendered as arrows, strict β-turns as TT letters and strict α-turns as TTT.

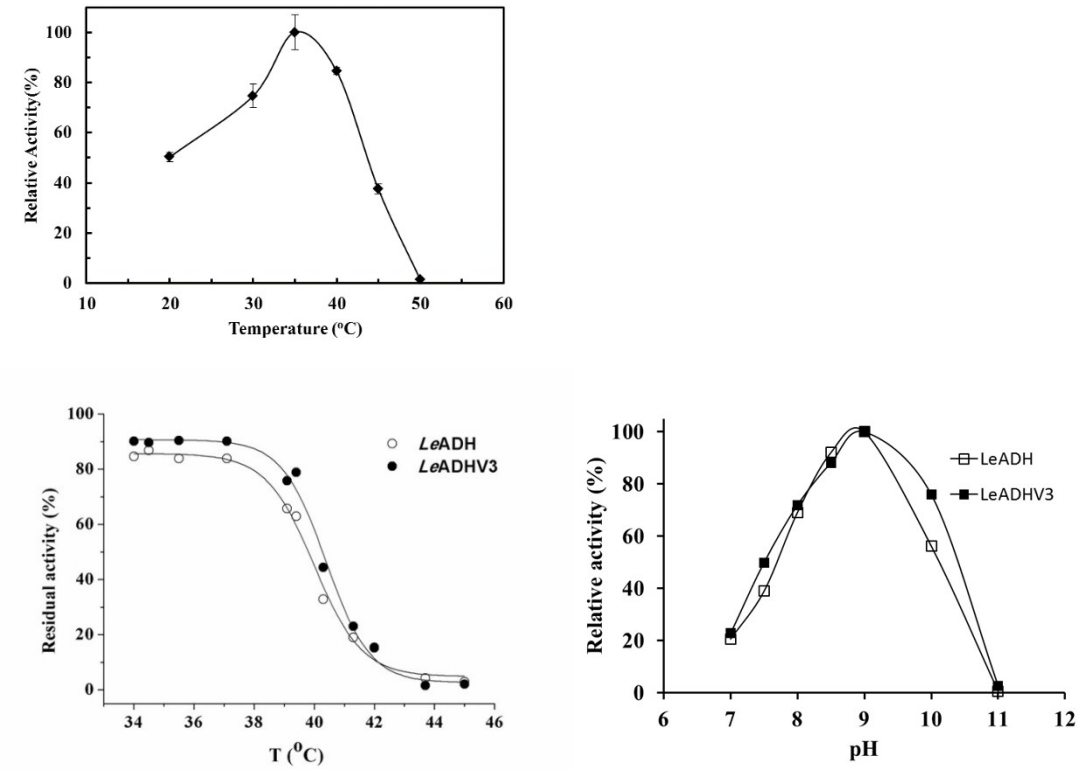

Figure S4. characterization of *Le*ADH. The optimum temperature of *Le*ADH was determined at different temperatures in the range of 20-50°C under standard conditions. The maximum activity was observed at around 35°C. The thermal stability of *Le*ADH was also examined by incubating the purified enzymes (0.1 mg/mL) at different temperatures for 15 min, and the residual activity was assayed. The results showed that T_{15} of *Le*ADH and *Le*ADH_{V3} was 39.6°C and 40.1°C, respectively. The enzyme activity was determined under standard assay condition at various pH (7.0-11.0), the *Le*ADH displayed the highest activity at pH 9.0.

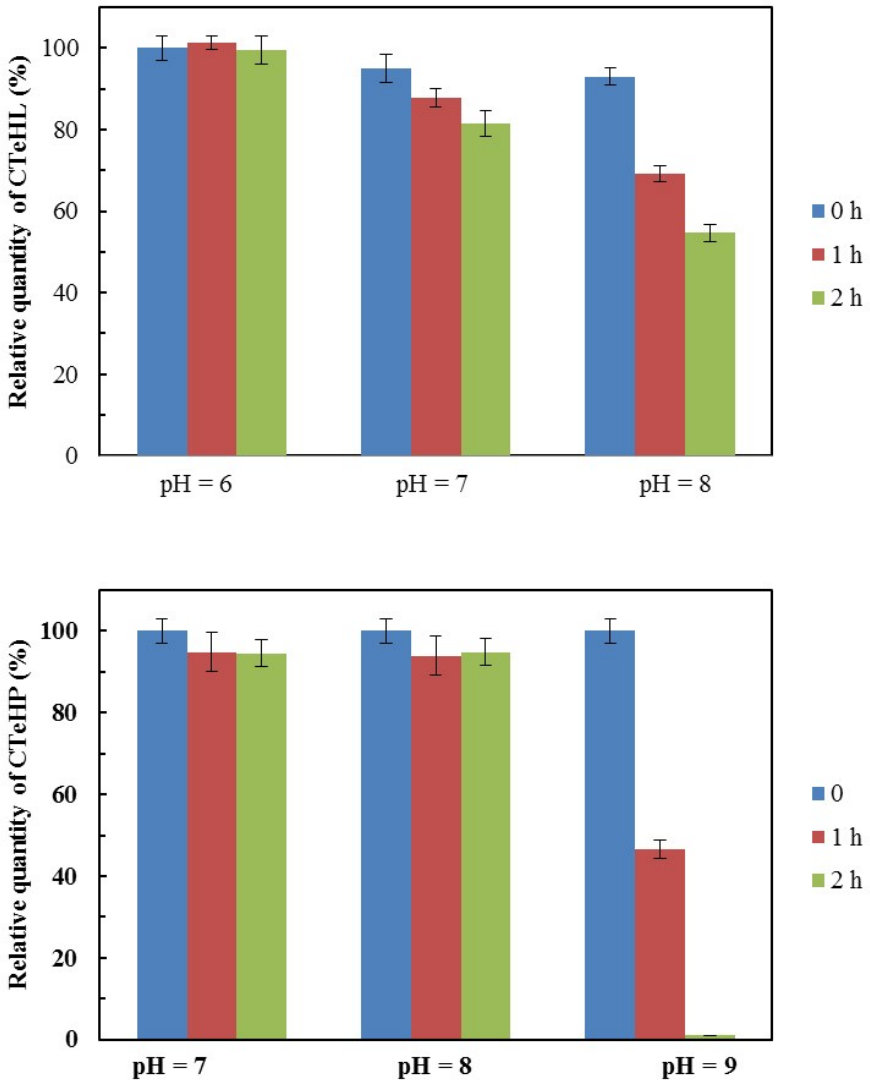

Figure S5. Stability of CTeHP and CTeHL in aqueous medium at various pH. 10 g L^{-1} of CTeHP and CTeHL were incubated at 30 ºC and sampled after 60 and 120 minutes. Data are relative values to the initial GC-FID area of the CTeHP and CTeHL peaks.

Table S1. Primers used in this work.

*Le*ADH_for	ACGGAATTCATGTCACAAACTGTCTTT (*EcoR* I)
*Le*ADH_rev	CCCAAGCTTTTACTTGGCTTCCACAA (*Hind* III)
85&87_for	CTTCACACAGCATCACCCNDTGTTNDTAAGACCACAGATGTTGAA
85&87_rev	TTCAACATCTGTGGTCTTAHNAACAHNGGGTGATGCTGTGTGAAG
127&128_for	ACGTTGTGGTTACTTCTTCTNDTNDTACAATGTTTAATTCCGTAGC
127&128_rev	GCTACGGAATTAAACATTGTAHNAHNAGAAGAAGTAACCACAACGT
129&131_for	TGGTTACTTCTTCTGTTGTCNDTATGNDTAATTCCGTAGCTGCACCCG
129&131_rev	CGGGTGCAGCTACGGAATTAHNCATAHNGACAACAGAAGAAGTAACCA
161&162_for	ACACATTGAAGAATCCTNDTNDTTACGGATACCCAGCATC
161&162_rev	GATGCTGGGTATCCGTAAHNAHNAGGATTCTTCAATGTGT
195&196_for	CTCAGTTGCCACAATCCATCCANDTNDTGTTTTCGGTCCCCAAG
195&196_rev	CTTGGGGACCGAAAACAHNAHNTGGATGGATTGTGGCAACTGAG
213&217_for	ggacaaaagtcagttgaacNDTtcaagtgagNDTataaacaagattttgaagctg
213&217_rev	cagcttcaaaatcttgtttatAHNctcacttgaAHNgttcaactgacttttgtcc
235&236_for	ACAAACTTCCAGCAGGTGCTNDTNDTTTTACTGACGTGAGAGAC
235&236_rev	GTCTCTCACGTCAGTAAAAHNAHNAGCACCTGCTGGAAGTTTGT
V3_for	TTCCATATGATGTCACAAACTGTCTTTGTC (*Nde* I)
V3_rev	TATCTCGAGTTACTTGGCTTCCACAATTTG (*Xho* I)
*Sm*NOX_for	CCGGAATTCGATGTCGAAAATTGTTATCG (*Eco*R I)
*Sm*NOX_rev	CACGTCGACTTATTTCGCTTTCAGAGC (*Sal* I)
V3_for	CCGGAATTCGATGTCACAAACTGTCTTTGTC (*Eco*R I)

V3_rev	CACGTCGACTTACTTGGCTTCCACAATTTG (*Sal* I)
*Sm*NOX_for	TTCCATATGATGTCGAAAATTGTTATCG (*Nde* I)
*Sm*NOX_rev	TATCTCGAGTTATTTCGCTTTCAGAGC (*Xho* I)

Table S2 Effect of metal ions and EDTA on *Le*ADH activity.

Metal ion (1 mM)	Relative activity (%)
Zn^{2+}	93.8 ± 3.1
Ni^{2+}	97.9 ± 3.1
Mg^{2+}	94.8 ± 2.1
Cu^{2+}	89.0 ± 5.8
Fe^{2+}	108.2 ± 0.3
Co^{2+}	98.6 ± 3.1
Mn^{2+}	100.3 ± 2.7
Al^{3+}	98.3 ± 1.4
Ca^{2+}	102.1 ± 0.3
Fe^{3+}	101.4 ± 0.3
EDTA	98.6 ± 1.0

*Le*ADH was pre-incubated with various metal ions or EDTA for 30 min at 25°C and the residual activity was determined using the standard assay. Residual activity was expressed as a percentage of the activity obtained without addition any metal ions or EDTA.

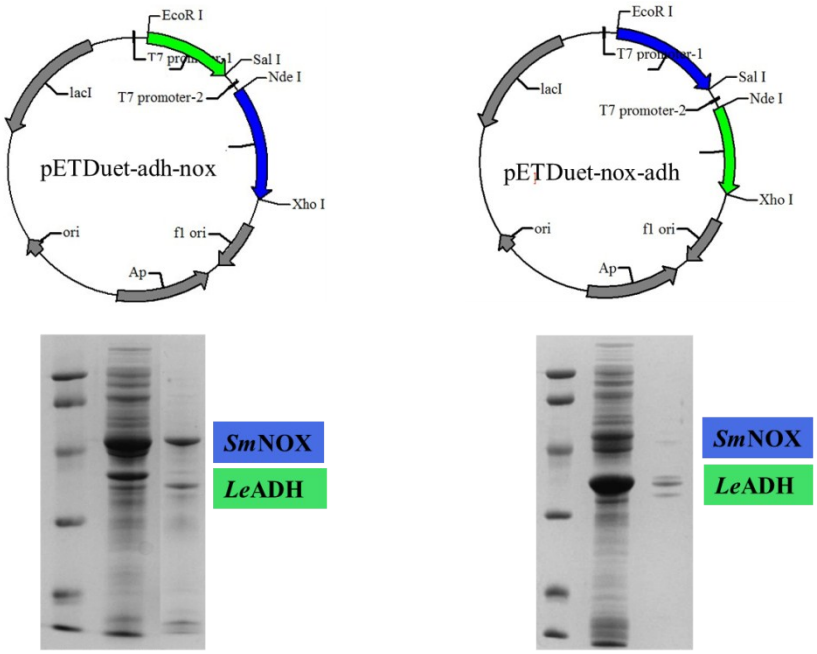

Figure. S6 Schematic presentation of the plasmid containing *adh* and *nox* genes and analysis of expression level by SDS-PAGE. (A) Structure of the coexpression plasmid pETDuet-*adh-nox*. (B) SDS-PAGE analysis of the pETDuet-*adh-nox* expression. Lane 1, protein marker; Lane 2, soluble fraction of cell-free extract from *E. coli* with pETDuet-*adh-nox*; Lane 3, precipitate fraction. (C) Structure of the coexpression plasmid pETDuet-*nox-adh*. (B) SDS-PAGE analysis of the pETDuet-*nox-adh* expression. Lane 1, protein marker; Lane 2, soluble fraction of cell-free extract from *E. coli* with pETDuet-*nox-adh*; Lane 3, precipitate fraction.