Supplementary Material for

Transfer of a Rational Crystal Contact Engineering Strategy between Diverse Alcohol Dehydrogenases

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LkADH      1 TDRLKGKVAIVTGGTLGIGLAIADKFVEEGAKVVITGRHADVGEKAAKSIGGTDVIRFVQ
LbADH      1 SNRLDGKVAIITGGTLGIGLAIATKFVEEGAKVMITGRHSDVGEKAAKSVGTPDQIQFFQ
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LkADH     61 HDASDEAGWTKLFDTTEEAFGPVTTVVNNAGIAVSKSVEDTTTEEWRKLLSVNLDGVFFG
LbADH     61 HDSSDEDGWTKLFDATEKAFGPVSTLVNNAGIAVNKSVEETTTAEWRKLLAVNLDGVFFG
             ** *** ******* ** ***** * ******** **** *** ****** *********

LkADH    121 TRLGIQRMKNKGLGASIINMSSIEGFVGDPTLGAYNASKGAVRIMSKSAALDCALKDYDV
LbADH    121 TRLGIQRMKNKGLGASIINMSSIEGFVGDPSLGAYNASKGAVRIMSKSAALDCALKDYDV
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LkADH    181 RVNTVHPGYIKTPLVDDLEGAEEMMSQRTKTPMGHIGEPNDIAWICVYLASDESKFATGA
LbADH    181 RVNTVHPGYIKTPLVDDLPGAEEAMSQRTKTPMGHIGEPNDIAYICVYLASNESKFATGS
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LkADH    241 EFVVDGGYTAQ
LbADH    241 EFVVDGGYTAQ
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Figure S1. Sequence alignment of *Lk*ADH and *Lb*ADH wild type with an amino acid sequence homology of 88.5 %. The optimal global alignment was generated with the Needleman-Wunsch algorithm (EMBOSS, output format: pair, matrix: BLOSUM62) [1]. Identical amino acids are marked with an asterisk. Crystal contacts (distance between amino acids < 4 Å) are marked for *Lk*ADH (cyan) and *Lb*ADH (green). The positions selected for mutations, T102 and Q126, are underlined. RMSD of the crystal contacts are listed in Supplementary Table S2.

Table S1. Data collection and refinement statistics of X-ray diffraction experiments of *Lk*ADH wild type and mutant Q126K crystals (values in parentheses are for highest resolution shell).

*Lk*ADH Variant	Wild Type	Q126K
Data Collection		
PDB ID	7P36	7P7Y
Beamline	SLS Beamline X06DA	SLS Beamline X06DA
Wavelength, Å	1.000	1.000
Space group	I 2 2 2	I 2 2 2
Cell dimensions, Å	55.87, 79.97, 114.59	55.84, 80.04, 114.93
No. of molecules per asymmetric unit	1	1
Resolution, Å	50–1.14 (1.21–1.14)	50-1.25 (1.32–1.25)
$I/\sigma(I)$	71.4 (26.9)	49.61 (14.32)
CC (1/2)	100.0 (99.8)	100 (99.3)
Completeness, %	100.0 (99.8)	99.2 (95.3)
Redundancy	12.8 (11.9)	11.62 (6.43)

R_{meas}, %	2.3 (8.3)	3.3 (10.9)
Refinement		
Resolution, Å	1.14	1.25
No. unique reflections	93486 (6458)	70930 (4734)
R_{work} / R_{free}	0.093 / 0.107	0.088 / 0.132
No. atoms		
Protein	1865	1865
Water	387	344
Other	23	16
B-factors		
Overall	7.37	9.1
Protein main chain	6.39	7.95
Protein side chain	8.24	10.27
R.m.s. deviations		
Bond lengths, Å	0.012	0.012
Bond angles, °	1.82	1.68
Ramachandran plot		
Most favored, %	98	98
Additional allowed, %	2	2

Table S2. $RMSD_{side\ chain}$ of crystal contacts present in both *Lk*ADH and *Lb*ADH wild type and calculated after side chain and backbone alignment of one tetramer. Crystal contacts were defined by a distance < 4 Å between amino acid residues of neighboring tetramers. The contact $RMSD_{side\ chain}$ was then calculated by alignment of those amino acid residues forming a contact with PyMOL (v.2.3.; Schroedinger). Therefore, a smaller contact RMSD value indicates, that the corresponding crystal contacts in *Lb*ADH and *Lk*ADH wild type crystals are locally more similar.

Amino acid residue	Contacts	Contact $RMSD_{side\ chain}$, Å
H39	D74, E77, R127	1.26
V42	D41, T70	0.95
K45	E66	1.26
D54	T52, D54	4.89
K71	D197	1.58
T102	K48	1.25
Q126	A40/S40, D41, T70, L123, R127	1.13
E203	R208	2.59

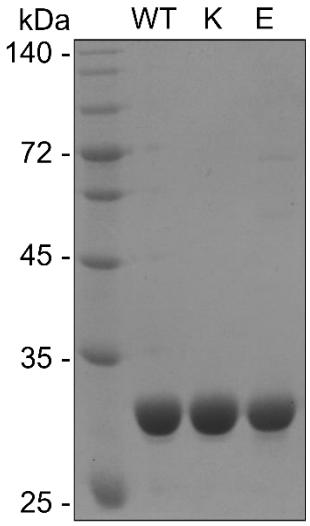


Figure S2. Illustration of protein purity analysis using SDS-PAGE of *Lk*ADH wild type (WT) and mutants Q126K (K) and T102E (E). The proteins purified by IMAC were loaded onto a 12.5% SDS gel (2.5 μg protein) under reducing conditions. Bands between 25–35 kDa correspond to *Lk*ADH monomers.

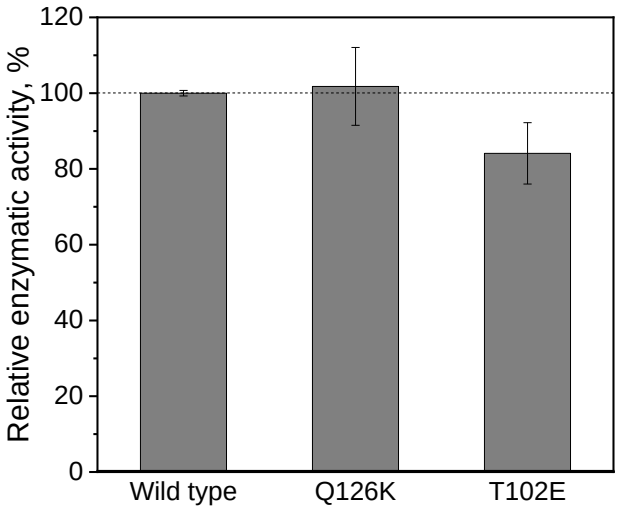
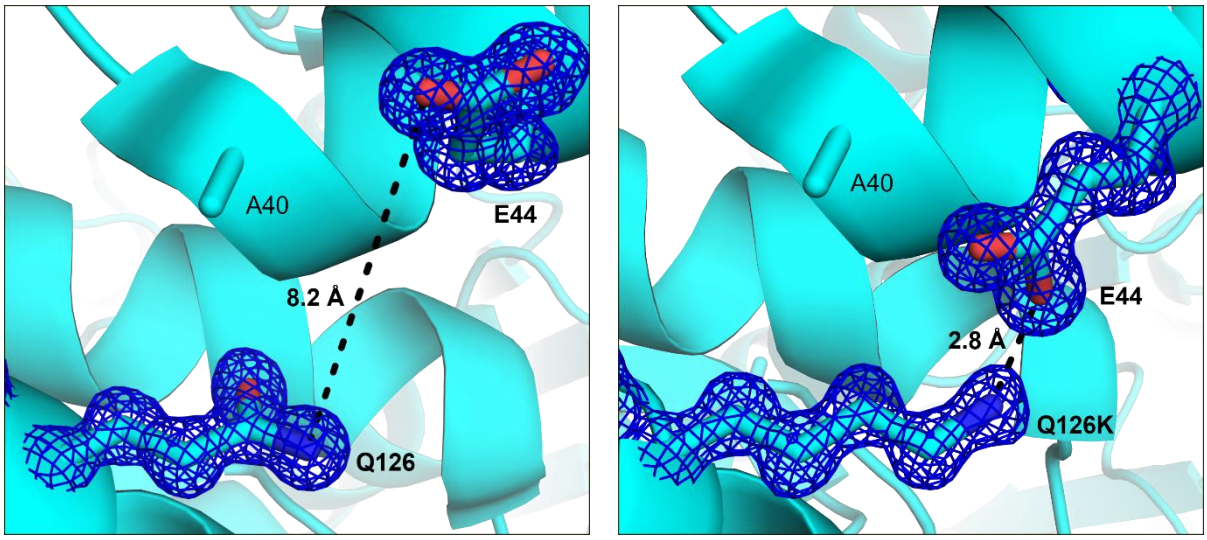


Figure S3. Maximum relative enzymatic activity of *Lk*ADH mutants Q126K and T102E compared to *Lk*ADH wild type. Purified protein solutions of *Lk*ADH variants were adjusted to 6 mg L^{-1}. Enzymatic activity was measured for 10 min at 340 nm (45 °C) with addition of 180 µL buffer (20 mM HEPES, pH 7.0; 1 mM $MgCl_2$) containing 0.5 mM NADPH and 10 mM acetophenone to a final volume of 200 µL (n = 3).



(**a**) (**b**)

Figure S4. Electron density map (blue) of *Lk*ADH crystal contact at position (**a**) Q126 and (**b**) Q126K with well-defined electron density of the lysine and glutamic acid side chain. The map represents the structure factor amplitude difference $2F_o - F_c$ with a contour level of 1.0 σ (calculated with *REFMAC* [2]).

References

1. Madeira, F., Park, Y.M., Lee, J., Buso, N., Gur, T., Madhusoodanan, N., Lopez, R., The EMBL-EBI search and sequence analysis tools APIs in 2019. *Nucleic Acids Res.* **2019**,*47*, W636–W641.
2. Murshudov, G.N., Vagin, A.A., and Dodson, E.J., Refinement of Macromolecular Structures by the Maximum-Likelihood Method. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **1997**. *53*, 240–255.