SUPPLEMENTARY INFORMATION

Structural redesign of lipase B from *Candida antarctica* by circular permutation and incremental truncation.

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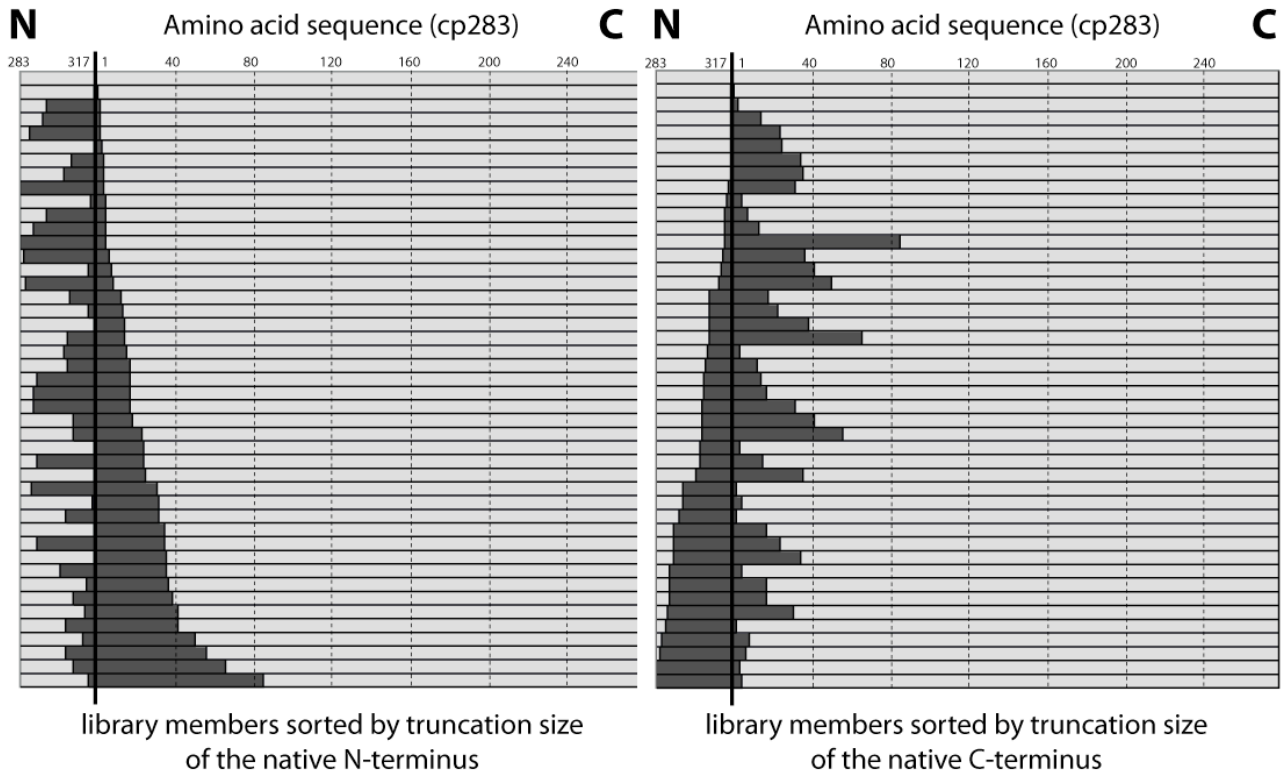
Supplementary figures and text:

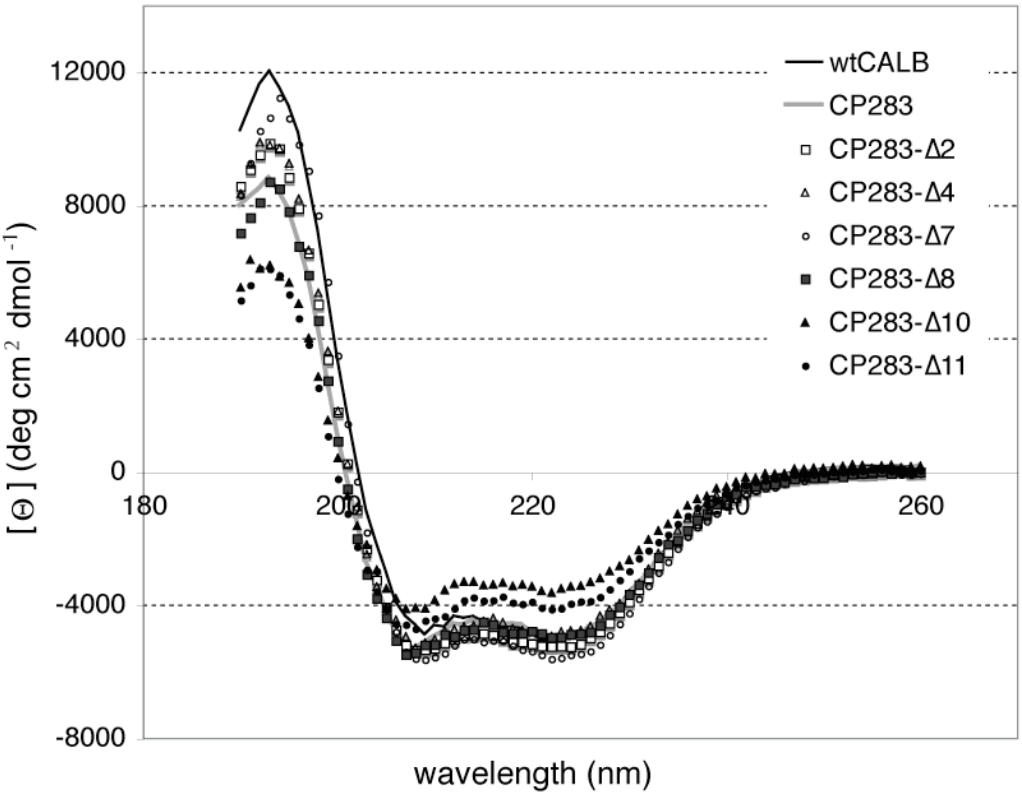
Supplementary figure 1: Size distribution in native loop truncation library.

A.) B.)



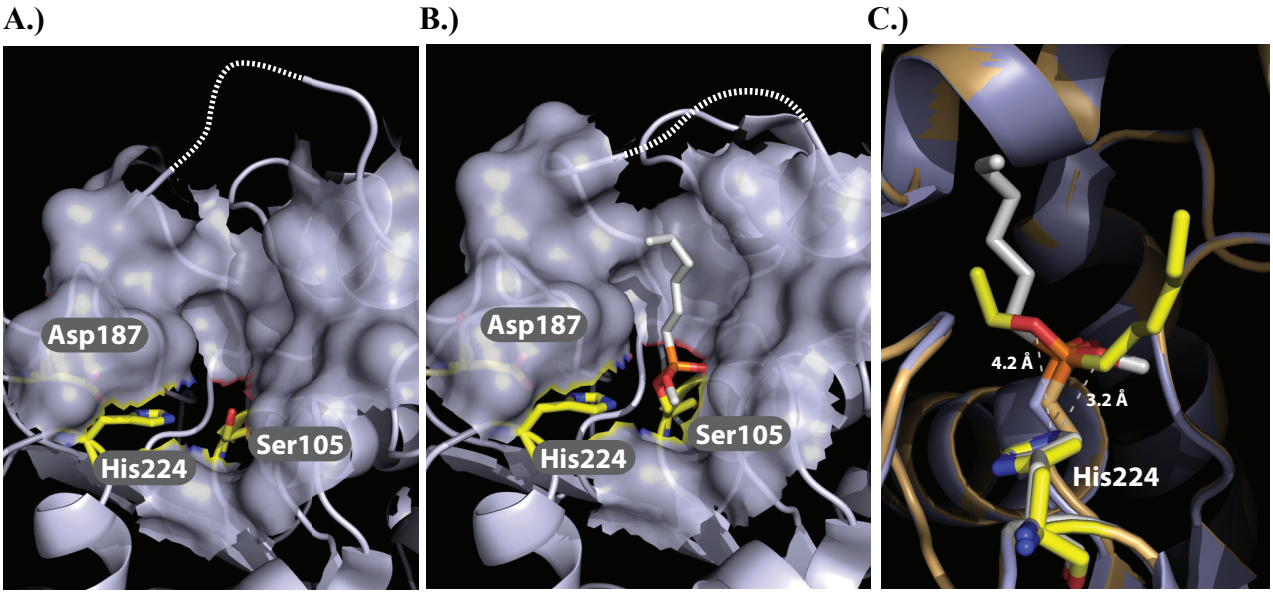
Sequences of 44 randomly selected library members were analyzed by DNA analysis, showing deletion of ≤ 80 amino acids (black bars) that extend in either direction of the starting position in the linker region (marked by line between position 317 and 1). The analyzed sequences were sorted by increasing truncation size at the original N-terminus (A.) or C-terminus (B.). A slight bias in the distribution of truncation sizes is noticeable, resulting from the fragment size selection step.

Supplementary figure 2: Far-UV circular dichroism spectra.



Far-UV circular dichroism spectra for wild type CALB, cp283, and enzyme variants with truncated linkers (cp283Δ2-11). The significant signal decline upon circular permutation of CALB to cp283 corresponds to a drop in the protein's helical content.[1] Incremental truncation of the linker region by 2, 4 or 7 residues correlates directly with an increase in signal intensity (cp283Δ2, Δ4, and Δ7). Further truncation by even a single residue (cp283Δ8) results in a reversion of this trend, causing a decline in helical content that levels out in cp283Δ10 and Δ11. The observed changes in secondary structure content, best seen on the molar ellipticity changes at 195 nm, correlate very well with data from thermal unfolding experiments (T_M values) reported in Table S1.

Supplementary figure 3: Conformation of active site pocket in apo and inhibitor-bound protein.



Comparison of the active site binding pocket in apo-protein (**A**; PDB: 3ICV) and the inhibitor-bound form (**B**; PDB: 3ICW) of cp283Δ7. The residues of the catalytic triad are highlighted in yellow. The methoxy-hexylphosphonate inhibitor is shown in stick representation. Marked with dashed lines, the lid region in the protein structures is invisible due to lacking electron density. No significant conformational changes in the active site in the presence of the substrate analog were observed. **C**) The orientation of the methoxy-hexylphosphonate inhibitor, bound in the active site of cp283Δ7 (shown in grey), is compared to ethoxy-hexylphosphonate in wild type CALB (PDB: 1LBS[2], shown in yellow). In both structures, the oxygen of the phosphonate points towards the oxyanion hole on the backside of the binding pocket. In wild type CALB (yellow), the hexyl moiety sits in the alcohol binding site while the ethoxy group occupies the acyl binding site. This binding conformation represents the unnatural, less-reactive enantiomer. In contrast, cp283Δ7 (grey) shows the substrate analog in the correct orientation, placing the oxygen of the alkoxy group within hydrogen bonding distance (3.2 Å versus 4.2 Å) to Nε of His224.

Supplementary Table 1: Kinetic parameters for selected CALB variants.

enzyme	K_M (μM)	k_{cat} (min^{-1})	k_{cat}/K_M ($min^{-1}\mu M^{-1}$)	relative catalytic efficiency	T_M (˚C)	ΔG_{40} (kcal mol^{-1})	$\Delta\Delta G_{40}$ (kcal mol^{-1})
wild type *	410 ± 40	305 ± 10	0.74	1	53.5 ± 0.2	-1.92	-2.11
cp283 *	410 ± 60	3251 ± 206	7.9	11	39.9 ± 0.3	0.19	0
cp283Δ2	375 ± 40	3100 ± 150	8.3	11	41.0 ± 0.3	-0.08	-0.27
cp283Δ4	160 ± 25	1390 ± 60	8.7	12	41.7 ± 0.4	-0.22	-0.41
cp283Δ7	240 ± 30	1400 ± 65	5.8	8	44.5 ± 0.3	-0.91	-1.10
cp283Δ8	293 ± 36	2645 ± 117	9	12	36.2 ± 0.4	1.00	0.81
cp283Δ10	214 ± 27	1858 ± 68	8.7	12	36.3 ± 0.4	1.02	0.83
cp283Δ11	245 ± 22	1485 ± 42	6.1	8	35.4 ± 0.5	1.21	1.02
cp283Δ4 monomer	230 ± 22	1671 ± 59	7.3	10	40.0 ± 0.4	-0.10	-0.29
cp283Δ4 dimer	204 ± 20	2203 ± 72	10.8	15	42.3 ± 0.3	-0.81	-1.00

Kinetic parameters of CALB variants with *p*-nitrophenol butyrate. CALB nomenclature: cp283Δ2 = circular permutated protein whose N terminus starts at amino acid 283 of the wild type sequence. The Δ2 refers to the number of amino acid deletions in the linker region. Relative catalytic efficiency = [k_{cat}/K_M (variant)] / [k_{cat}/K_M (wild type)]. T_M is the temperature of unfolding, defined as the temperature at which fraction folded = 0.5 as determined by circular dichroism spectroscopy. ΔG_{40} is calculated from thermal denaturation data at 40˚C, a temperature within the transition region of all protein samples.[3] $\Delta\Delta G_{40}$ is the relative free energy of unfolding = [ΔG_{40} (variant)] – [ΔG_{40} (cp283)]. * previously reported.[1]

Supplementary references

1. Qian, Z., Fields, C. J. & Lutz, S. (2007). Investigating the structural and functional consequences of circular permutation on lipase B from *Candida antarctica*. *ChemBioChem* **8**, 1989-1996.
2. Uppenberg, J., Ohrner, N., Norin, M., Hult, K., Kleywegt, G. J., Patkar, S., Waagen, V., Anthonsen, T. & Jones, T. A. (1995). Crystallographic and molecular-modeling studies of lipase B from *Candida antarctica* reveal a stereospecificity pocket for secondary alcohols. *Biochemistry* **34**, 16838-16851.
3. Greenfield, N. J. (2006). Using circular dichroism spectra to estimate protein secondary structure. *Nat Protoc.* **1**, 2876-2890.