Supplementary Figures and Tables

Supplementary Table 1. Optimized synthetic gene encoding SDR oxidoreductase (A0A7C5VFX3).

Synthetic gene ketone-reductase (A0A7C5VFX3)
ATGGACCCTAAATCGCTTGTAGGGAAGCATGCTTTAGTGACGGGTGCTTCACGCGGTA TTGGGGCTGCCATTGCCGAGGCGCTGGCCCAAGCCGGCGCCCGTTTAACGCTGGTAGC CCGTAAACTTCAAGACCTTGAGGAGCGTGTGGAGCGTTTGCGTGCGTGGACGGAAGT GCAAGGAGAGGTCGGAGACGTAGCTGACCCTCAGGCGATGGAGAGCTTAGTACACCG CGCACAGGAACGTTTTGGCCCCGTGGCTATTCTGGTGAATAATGCTGGCTTCGTTTTAA CGGCTCCATTTCTTGAAATCAACGAGGAATTGTGGCAGCGTCATATCGAGGTTAATCT GGGAGCAGCTTATCGCTTGATTCGCTTACTTTTACCGGGCATGTTGGAAATGGGTTGG GGTCGTATTATTAACATTAGCTCCACGGCAGGGCTTACAGGCTATCCCTACGCTGTCC CATATTGCGCAGCAAAGCACGGTCTGATCGGGCTTACACGTGCTTTAGCATTAGAGTT GGCTCAGCGTGGGATCACGGTAAACGCGATTTGTCCCGGTTTTACAGATACCGATTTA CTGCAGGCTTCCGTGTTGCGTGTGGCCCAGCGTACTGCCCGTGCGCCCGAAGCAGTGC TGGAACTTTTTGCCCGCCGCAATCCACAGGGGCGTTTAGTTCGTCCAGAAGAAGTCGC ATGGGCTGTGGTCTGGTTGTGCAGCGAAAAAGCCGCAGCAATTAACGGGCAGGCTAT CGCTGTGGCAGGGGGAGAAGTCATGGTGGGTTAA

Supplementary Table 2. PCR primers for all Tcalid SDRmutants.

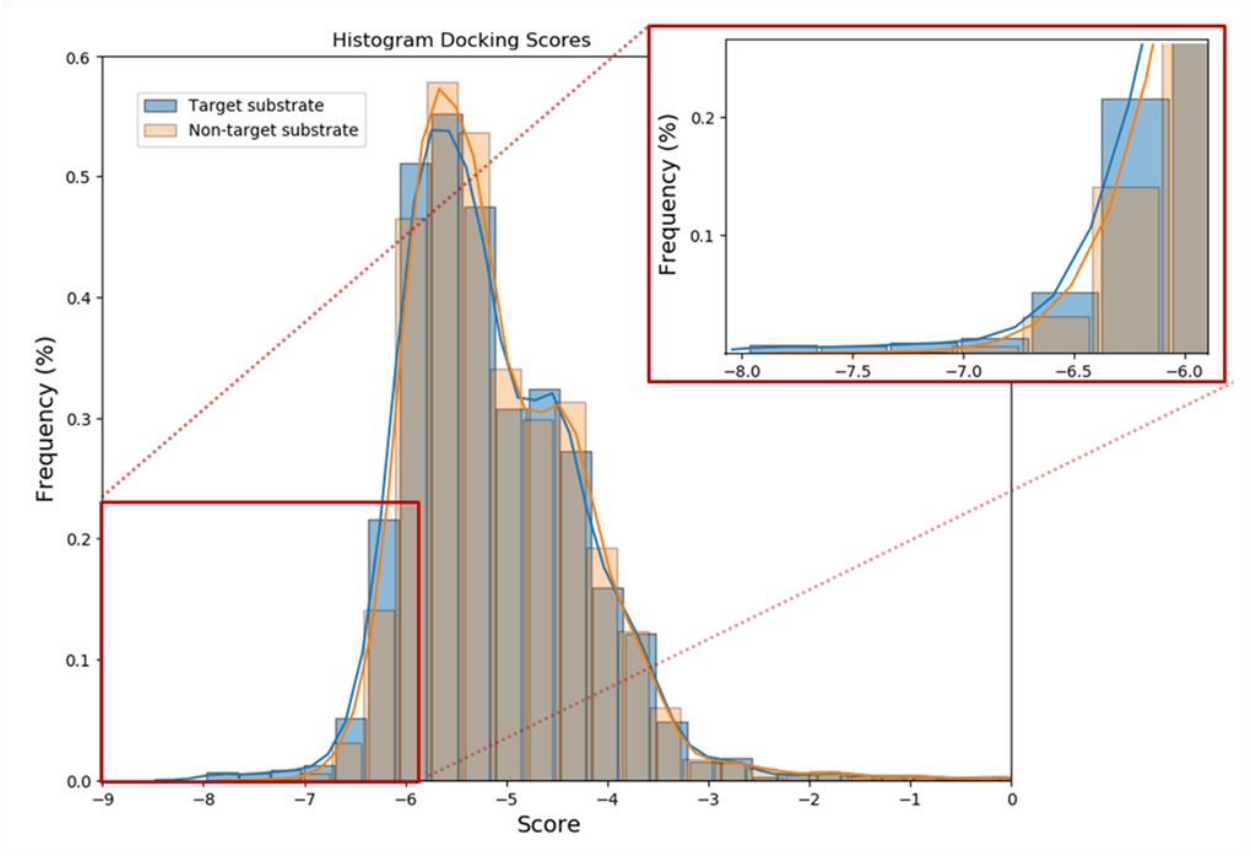
Mutant	**Forward primer (5'-3')**	**Reverse primer (5'-3')**
R40E	cGAAaaacttcaagaccttgaggagcgtgtg	tcttgaagtttTTCggctaccagcgttaaacg
V95F	atgctggcttcTTTttaacggctccatttc	AAgaagccagcattattcaccagaatagccac
V95G	gctggcttcGGCttaacggctccatttc	aGCCgaagccagcattattcaccagaatagc
V95I	atgctggcttcATTttaacggctccatttc	ATgaagccagcattattcaccagaatagccac
V95G	gctggcttcGGCttaacggctccatttc	aGCCgaagccagcattattcaccagaatagc
V95F	atgctggcttcTTTttaacggctccatttc	AAgaagccagcattattcaccagaatagccac
V95I	atgctggcttcATTttaacggctccatttc	ATgaagccagcattattcaccagaatagccac
N115A	cgaggttGCGctgggagcagcttatcg	cccagCGCaacctcgatatgacgctgc
S143A	agcGCGacggcagggcttacagg	ctgccgtCGCgctaatgttaataatacgacc
Y156A	gtcccaGCGtgcgcagcaaagcacg	cgcaCGCtgggacagcgtagggatag
K160A	caGCGcacggtctgatcgggcttac	agaccgtgCGCtgctgcgcaatatgg
D192R	ccCGTttactgcaggcttccgtgttgc	tgcagtaaACGggtatctgtaaaaccgggac
R200A	cgtgttgGCGgtggcccagcgtac	gccacCGCcaacacggaagcctgc
R200K	cgtgttgAAAgtggcccagcgtac	gccacTTTcaacacggaagcctgc
R200N	cgtgttgAACgtggcccagcgtac	gccacGTTcaacacggaagcctgc
R204A	gGCGactgcccgtgcgcccgaag	acgggcagtCGCctgggccacacgcaac
R204K	gAAAactgcccgtgcgcccgaag	acgggcagtTTTctgggccacacgcaac
R204Q	gCAGactgcccgtgcgcccgaag	acgggcagtCTGctgggccacacgcaac
A254D	gGATgggggagaagtcatggtgg	ttctcccccATCcacagcgatagcctg
A254N	cgctgtgAACgggggagaagtcatg	cccccGTTcacagcgatagcctgc

Supplementary Table 3. X-ray diffraction data and structure refinement statistics. Data processing and refinement statistics for Tcalid SDR structures. The values in parentheses represent the high-resolution shell, whereas the values outside the parentheses represent the overall dataset.

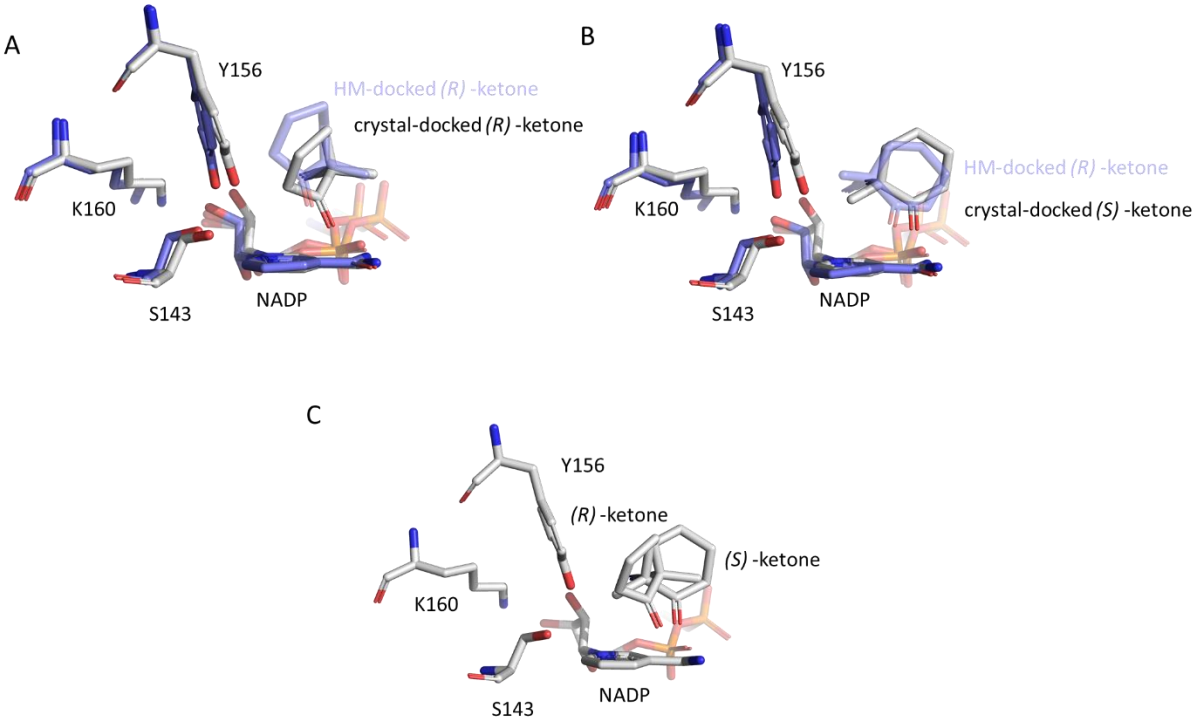
Parameters	**Tcalid SDR** Apoprotein	**Tcalid SDR** NADP bound
<u>Data Processing</u>		
Space Group	$P2_12_12_1$	$P2_12_12_1$
Cell Dimension		
a, b, c (Å)	56.7, 122.9, 163.4	56.6, 121.8, 162.9
α, β, γ (°)	90, 90, 90	90, 90, 90
Wavelength	1.00005	0.97625
Resolution (Å)	98.253-1.875 (2.162-1.875)	97.584-1.698 (1.869-1.698)
Observed Reflections	680772 (35764)	742647 (34962)
Unique Reflections	49723 (2486)	91711 (4586)
Completeness (spherical) (%)	52.6 (7.6)	73.4 (14.9)
Completeness (ellipsoidal) (%)	93.0 (71.8)	94.3 (65.3)
Redundancy	13.7 (14.4)	8.1 (7.6)
R_{merge} (I)	0.140 (1.489)	0.129 (1.250)
R_{pim} (I)	0.039 (0.405)	0.048 (0.483)
I/σ (I)	10.6 (1.9)	8.7 (1.6)
$CC_{1/2}$	0.998 (0.722)	0.997 (0.702)

Refinement		
Resolution (Å)	98.2-1.9	24.8-1.7
Rwork (%)	21.7	17.6
Rfree (%)	24.5	19.8
Bond length RMSD (Å)	0.008	0.009
Bond angle RMSD (°)	0.94	0.98
Mean/Wilson B ($Å^2$)	53/33	31/26
Ramachandran plot		
Favored (%)	96.53	97.69
Outliers (%)	0.19	0.00
Sidechain outliers (%)	0.63	0.38
Clashscore (all atoms)	1.31	2.37
MolProbity	1.08	1.09

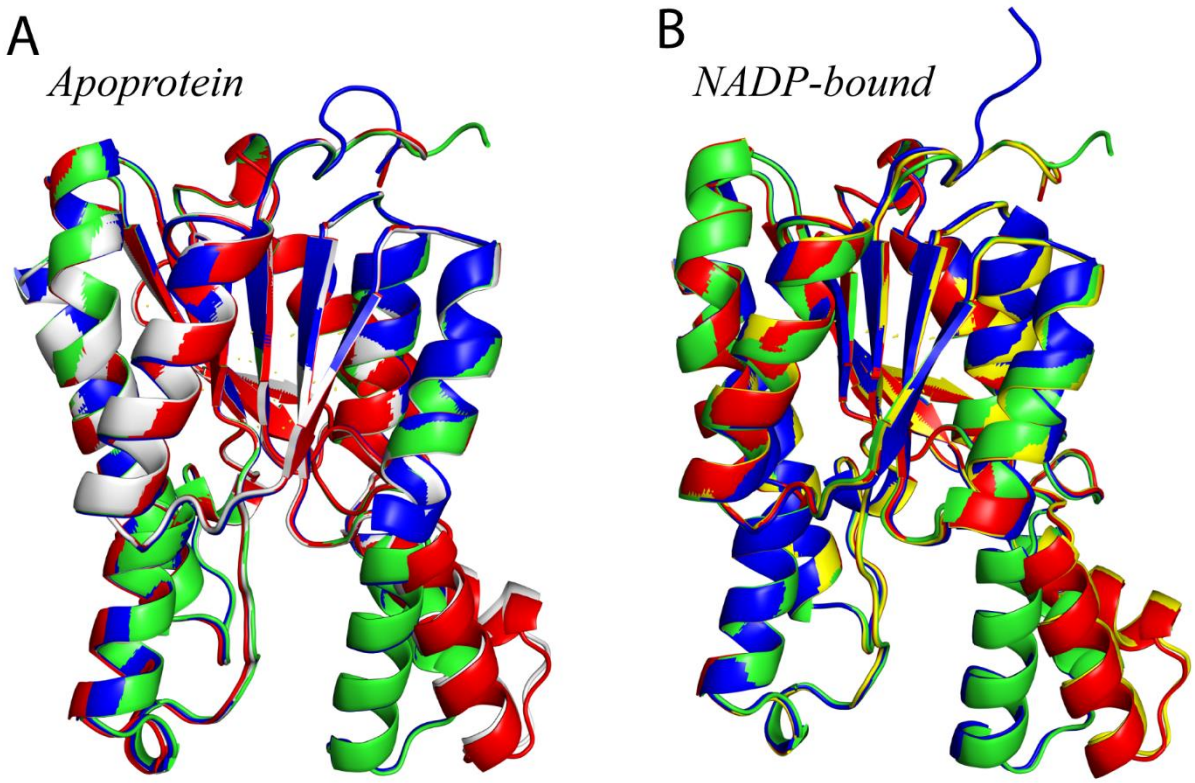
Supplementary Figure 1. Histogram representation of docking scores derived from substrates *S*-ketone (blue) and *R*-ketone (orange) The large overlapping between distributions made the selection of enzymes with full enantio-selectivity for the racemic mixture difficult. The zoom in the top-score section evidences a slightly better affinity for *S*-ketone than *R*-ketone. To circumvent this limitation in the enzyme selection, another filter was applied (*S*-ketone < 3.5Å while *R*-ketone > 3.5, with a minimum difference of 0.5 Å, as previously explained), to preferentially select NACs with more stability for *S*-ketone than *R*-ketone. The selection conducted is conservative and prime conversion of *S*-ketone over *R*-ketone, but it's unlikely that none of the WT sequences will have only preference for *S*-ketone (100%)



Supplementary Figure 2. Comparison of the docking poses between a selected homology model (purple) and crystal structure (white) of Tcalid SDR. Docking poses of the (R)-ketone to both homology model and crystal is shown in panel A and the same is shown for the (S)-ketone is shown in panel B. An overlap of the poses docked into the crystal structure only are shown in panel C. Docking to the crystal structure did not provide additional discriminatory power regarding substrate preference.



Supplementary Figure 3. The variations within conformation of all chains in the Tcalid SDR structures. **(A)** Superposition of apoprotein Tcalid SDR chains. **(B)** Superposition of NADP-bound Tcalid SDR chains. The color scheme is as follows: chain A (white in panel 3A, yellow in panel 3B), chain B (red), chain C (green) and chain D (blue).



Supplementary Table 4. Average conversion (conv%) and enantioselectivity (ee%) ± SEM (n=2) for Tcalid SDR within the target kinetic resolution reaction with NADH or NADPH cofactors at 30 minutes or 6 hours.

Reaction time		**NADH**	**NADPH**
30 min	**conv%**	31.3 ± 5.1	34.2 ± 9.3
	ee%	39.9 ± 11.3	50.6 ± 25.1
6 hr	**conv%**	49.8 ± 2.2	46.7 ± 5.0
	ee%	90.9 ± 12.9	71.4 ± 14.6

Supplementary Table 5. Average conversion (conv%) and enantioselectivity (ee%) ± SEM (n=2) for Tcalid SDR at varying amounts of enzyme concentration (3.5, 5 and 6 μM) within the target kinetic resolution reaction with NADH or NADPH cofactors.

Enzyme concentration (μM)		**NADH**	**NADPH**
3.5	**conv%**	21.4 ± 2.4	30.6 ± 1.7
	ee%	24.8 ± 1.9	34.2 ± 3.1
5	**conv%**	31.4 ± 8.6	43.8 ± 2.2
	ee%	41.7 ± 12.9	69.0 ± 7.1
6	**conv%**	48.2 ± 1.2	45.8 ± 4.0
	ee%	84.1 ± 1.1	73.3 ± 11.0

Supplementary Figure 4. Tcalid SDR mutations and their location within the solved crystal structure of Tcalid SDR (Chain C). Catalytic triad residues are shown in red, the hypothetical catalytic tetrad residue is shown in green, and the residue evaluated for thermostability is shown in blue.