Supplementary Data

Computational design of enhanced detoxification activity of a zearalenone lactonase from *Clonostachys rosea* in acidic medium

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Table S1 Scheme for surface charge redesign in scaffold 3WZL

Scaffold	Catalytic residues	Design positions		Substrate
		Sequence selection residues	Conformation optimization residues	
3WZL	S102, H242, E126	E18, E20, D25, D31, E35, Q37, D40, S41, D57, E70, T76, D89, D92, D117, L131, D133, S136, E142, D143, E144, E145, S147, I149, A151, N152, L155, N156, D157, S162, E163, Q166, D170, E171, T190, A195, D199, E201, D209, A214, T218, E219, F222, D223, I225, I235, L237, D250, V251, Y255, E258	Y16, L33, M38, S62, R63, K79, Y83, C101, S103, H125, T129, K130, L132, H134, L135, N137, L150, V153, M154, V158, M168, V172, R175, L176, H177, N179, Y180, W183, R185, Y187, R189, I191, T216, S220, F221, N224, F243, Y245, R2, R4, L33, Q37, S44, Q45, R52, I93, K94, C101, R118, N121, M123, H125, L132, H134, L135, L150, V153, M154, V158, Q166, R175, Y180, Y187, I191, R204, S220, F221, N234, F243, Y245, K254, Y255, T259, Q261, K262, H263	ZEN

Table S2 Scheme for charge redesign at active site in scaffold 3WZL

Scaffold	Catalytic residues	Design positions		Substrate
		Sequence selection residues	Conformation optimization residues	
3WZL	S102, H242, E126	D31, C101, V153, M154, V158, F221, M241, F243	L28, L33, M38, F39, L80, W99, S103, S106, H125, L132, H134, L135, T138, L150, W165, M168, L176, Y180, W183, Y187, I191, V212, T216, S220, N224, L238, Y245, V246	ZEN

Table S3 Catalytic geometrical constraints for design

Site pair	Type	Atom1[a]	Atom2[a]	Atom3[a]	Atom4[a]	Min[b]	Max[b]
G32-ZEN	DISTANCE	#O12	N			2.6	3.2
	ANGLE	#O12	HN	N		140.0	180.0
	ANGLE	#C12	#O12	HN		80.0	140.0
S103-ZEN	DISTANCE	#O12	N			2.6	3.2
	ANGLE	#O12	HN	N		140.0	180.0
	ANGLE	#C12	#O12	HN		80.0	140.0
H242-S102	DISTANCE	NE2	#OG			2.6	3.2
	ANGLE	NE2	#HG1	#OG		140.0	180.0
	ANGLE	CE1	NE2	#HG1		90.0	150.0
E126-H242[c]	DISTANCE	OE1	#ND1			2.6	3.2
	ANGLE	CD	OE1	#ND1		90.0	150.0
	ANGLE	OE1	#HD1	#ND1		140.0	180.0
E126-H242[c]	DISTANCE	OE2	#ND1			2.6	3.2
	ANGLE	CD	OE2	#HD1		90.0	150.0
	ANGLE	OE2	#HD1	#ND1		140.0	180.0
G213-E126[d]	DISTANCE	N	#OE1			2.6	3.2
	ANGLE	N	HN	#OE1		140.0	180.0
	ANGLE	HN	#OE1	#CD		90.0	150.0
G213-E126[d]	DISTANCE	N	#OE2			2.6	3.2
	ANGLE	N	HN	#OE2		140.0	180.0
	ANGLE	HN	#OE2	#CD		90.0	150.0
T216-E126[e]	DISTANCE	OG1	#OE1			2.6	3.2
	ANGLE	OG1	HG1	#OE1		140.0	180.0
	ANGLE	HG1	#OE1	#CD		90.0	150.0
T216-E126[e]	DISTANCE	OG1	#OE2			2.6	3.2
	ANGLE	OG1	HG1	#OE2		140.0	180.0
	ANGLE	HG1	#OE2	#CD		90.0	150.0
W183-ZEN	DISTANCE	NE1	#O2			2.6	3.2
	ANGLE	#O2	HE1	NE1		130.0	180.0
	ANGLE	#C2	#O2	HE1		90.0	150.0
ZEN-ZEN	DISTANCE	C7P	#C8P			1.5	1.6
	ANGLE	C6P	C7P	#C8P		105.0	115.0
	ANGLE	C7P	#C8P	#C9P		105.0	115.0

[a] Atoms on the latter residue within a site pair are prefixed with '#'.

[b] Distance measurements are given in Å. Angle and torsion measurements are given in degrees.

[c] Either site pair of E126-H242 is needed.

[d] Either site pair of G213-E126 is needed.

[e] Either site pair of T216-E126 is needed.

Table S4 Variation rules for generating transition state rotamers of ZEN

Type	Atom1[a]	Atom2[a]	Atom3[a]	Atom4	Min[b]	Max[b]	Step[b]
Distance	#OG	C12			1.4	1.6	0.1
Angle	#CB	#OG	C12		105.0	115.0	5.0
Torsion	#CA	#CB	#OG	C12	-180.0	179.0	10.0
Distance	C12	O12			1.3	1.3	0.0
Angle	#OG	C12	O12		105.0	115.0	5.0
Torsion	#CB	#OG	C12	O12	-180.0	179.0	10.0
Distance	C12	O10			1.408	1.408	0.0
Angle	O12	C12	O10		109.5	109.5	0.0
Torsion	#OG	O12	C12	O10	120.0	120.0	10.0
Distance	C12	C1			1.500	1.500	0.0
Angle	O12	C12	C1		109.5	109.5	0.0
Torsion	#OG	O12	C12	C1	-120.0	-120.0	10.0
Distance	C1	C2			1.383	1.383	0.0
Angle	C12	C1	C2		117.314	117.314	0.0
Torsion	O12	C12	C1	C2	-86.0	34.0	10.0
Distance	O10	C10			1.449	1.449	0.0
Angle	C12	O10	C10		120.717	120.717	0.0
Torsion	O12	C12	O10	C10	-35.0	85.0	10.0
Distance	C10	C9P			1.498	1.498	0.0
Angle	O10	C10	C9P		107.777	107.777	0.0
Torsion	C12	O10	C10	C9P	120.0	180.0	10.0
Distance	C9P	C8P			1.505	1.505	0.0
Angle	C10	C9P	C8P		110.205	110.205	0.0
Torsion	O10	C10	C9P	C8P	30.0	90.0	10.0
Distance	C3P	C4P			1.511	1.511	0.0
Angle	C2P	C3P	C4P		114.038	114.038	0.0
Torsion	C1P	C2P	C3P	C4P	60.0	120.0	10.0
Distance	C4P	C5P			1.533	1.533	0.0
Angle	C3P	C4P	C5P		106.995	106.995	0.0
Torsion	C2P	C3P	C4P	C5P	-130.0	-70.0	10.0
Distance	C5P	C6P			1.509	1.509	0.0
Angle	C4P	C5P	C6P		101.525	101.525	0.0
Torsion	C3P	C4P	C5P	C6P	100.0	160.0	10.0
Distance	C6P	C7P			1.513	1.513	0.0
Angle	C5P	C6P	C7P		126.282	126.282	0.0
Torsion	C4P	C5P	C6P	C7P	-165.0	-105.0	10.0

[a] Atoms on anchor residue S102 are prefixed with '#'.

[b] Distance measurements are given in Å. Angle and torsion measurements are given in degrees.

Table S5 Predicted pK_a drops and calculated free energy changes for surface charge redesign in scaffold 3WZL

Index	Mutation	pK_a drop of H242	PRODA $\Delta\Delta G^{bind}$ (kcal/mol)	PRODA $\Delta\Delta G^{fold}$ (kcal/mol)
1	D31K	0.221	-0.79	21.09
2	D223K	0.102	-0.42	9.06
3	E219K	0.095	-0.44	0.90
4	D157K	0.079	-0.42	-5.75
5	E35K	0.071	-0.57	21.30
6	D57K	0.068	-0.45	5.96
7	A195K	0.065	-0.23	24.01
8	E145K	0.061	-0.39	-3.66
9	F222K	0.054	-0.23	-23.83
10	L155K	0.052	-0.19	-4.30
11	N152K	0.045	-0.22	-1.84
12	D133K	0.044	-0.40	-2.17
13	E171K	0.037	-0.36	-3.01
14	D209K	0.034	-0.35	-3.93
15	I149K	0.033	-0.38	-4.20
16	E18K	0.032	-0.40	2.04
17	L237K	0.025	-0.21	-2.91
18	T218K	0.024	-0.27	-6.04
19	A214K	0.022	-0.17	-2.56
20	E163K	0.021	-0.34	5.24
21	Q166K	0.021	-0.19	0.81
22	L131K	0.020	-0.24	-6.39
23	S162K	0.019	-0.20	0.30
24	I225K	0.019	-0.22	-1.89
25	D250K	0.018	-0.29	-0.30
26	D143K	0.018	-0.36	2.99
27	S136K	0.018	-0.27	-7.23
28	D40K	0.017	-0.35	3.20
29	I235K	0.016	-0.21	1.11
30	E258K	0.016	-0.34	-0.90
31	D170K	0.014	-0.30	-0.35
32	E70K	0.014	-0.41	1.72
33	T76K	0.014	-0.33	3.87
34	T190K	0.013	-0.34	-0.06
35	Y255K	0.012	-0.22	-2.02
36	D199K	0.011	-0.30	-2.98
37	E144K	0.009	-0.35	-2.67
38	V251K	0.009	-0.18	-3.40
39	E142K	0.008	-0.35	-3.32
40	E20K	0.007	-0.31	3.83
41	D25K	0.006	-0.26	1.21
42	E201K	0.005	-0.31	4.88

43	D89K	0.004	-0.29	-0.65
44	D117K	0.004	-0.27	-2.15
45	D92K	0.002	-0.24	-2.81
46	Q37K	0.000	-0.22	-0.59
47	S147K	0.000	-0.23	2.98
48	N156K	-0.002	-0.21	-3.52
49	S41K	-0.004	-0.21	-2.47
50	A151K	-0.014	-0.20	-5.59

Table S6 Predicted pK_a drops and calculated free energy changes for charge redesign at active site in scaffold 3WZL

Index	Mutation	pK_a drop of H242	PRODA $\Delta\Delta G^{bind}$ (kcal/mol)	PRODA $\Delta\Delta G^{fold}$ (kcal/mol)
1	D31H	0.474	-0.455	15.977
2	F243H	0.297	-5.101	11.047
3	V158H	0.265	-0.350	0.335
4	F221H	0.220	3.116	-1.352
5	M154H	0.201	-1.730	5.119
6	V153H	0.179	1.374	-6.088
7	C101H	0.110	-0.161	23.255
8	M241H	0.090	-0.903	1.091

Fig. S1 Lineweaver-Burk plots for determination of steady-state kinetic parameters of (A) the wild type ZHD101 and variants (B) M2, (C) M8, (D) M9.

Fig. S1 (A)

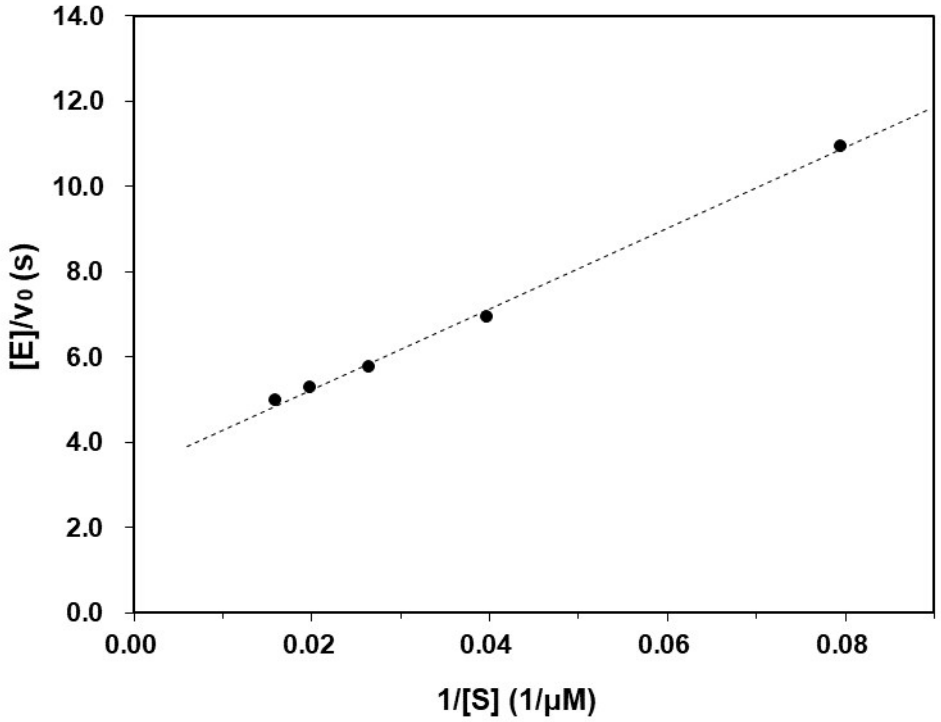

Fig. S1 (B)

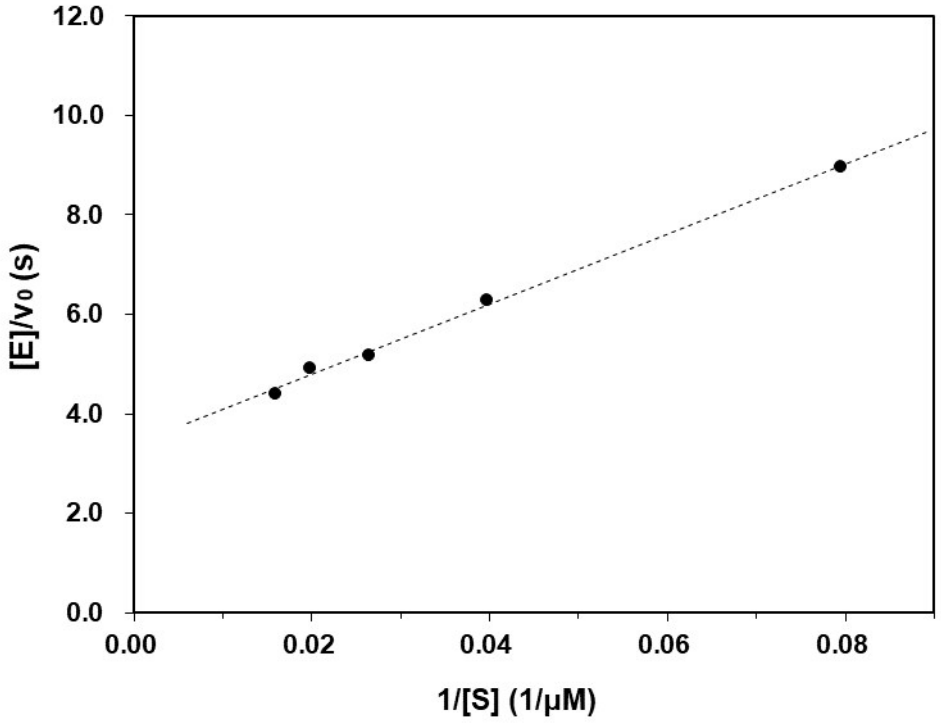

Fig. S1 (C)

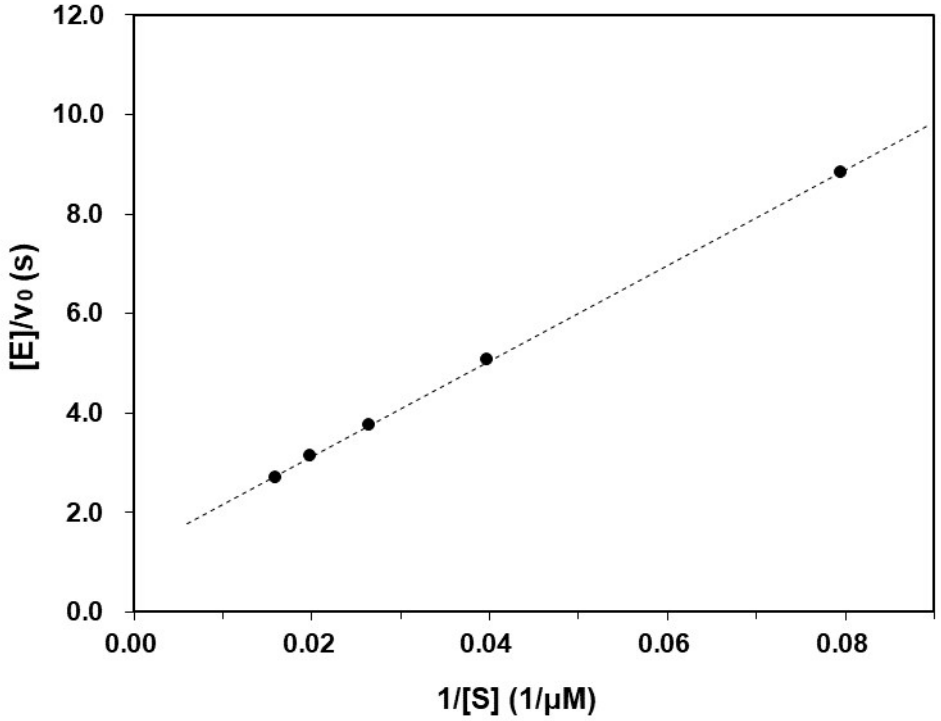

Fig. S1 (D)