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Supporting information for article:

Toward the computational design of protein crystals with improved resolution

Jeliazko R. Jeliazkov, Aaron C. Robinson, Bertrand García-Moreno E., James M. Berger and Jeffrey J. Gray

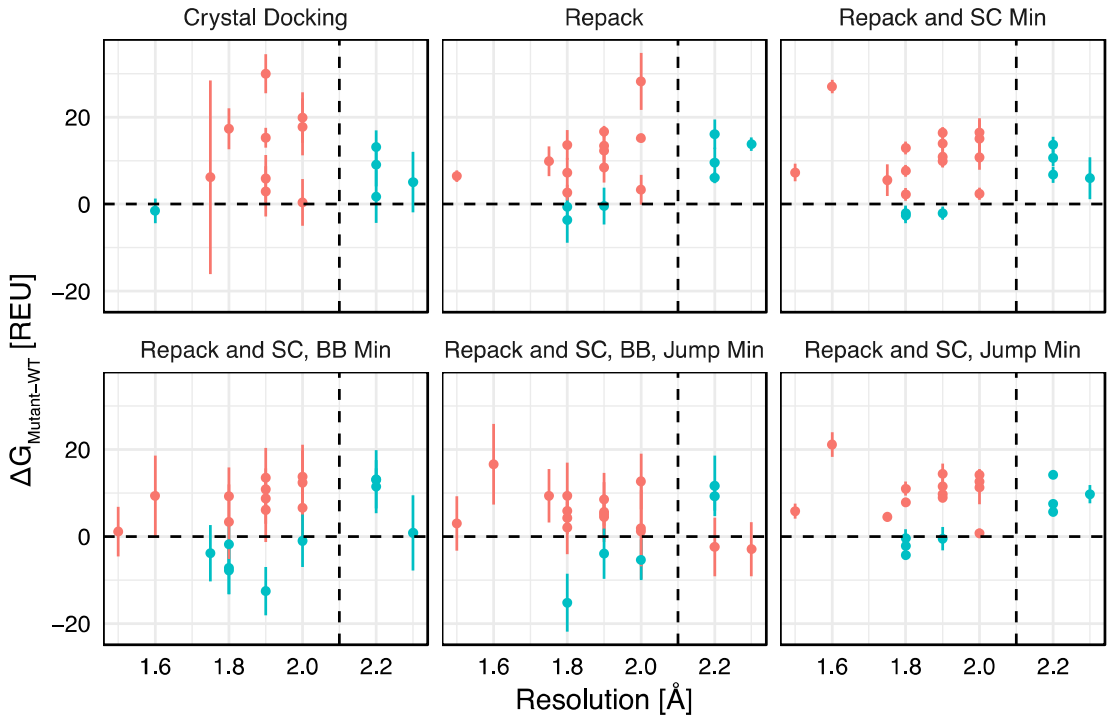

Figure S1 The ability for Rosetta to correctly forward design resolution-enhancing mutations on diphthine synthase depends on the degrees of freedom sampled during the simulation. Each plot here shows the same data as Figure 2 for a particular design strategy. The x-axis indicates the variant resolution and the y-axis shows the difference in energy with respect to the WT (hence the dashed lines represent the WT values for both). Standard deviations are calculated across 10 repeated design simulations. Point color indicates either a correct (*i.e.* a lower score than WT and corresponding higher resolution, blue) or incorrect prediction (red). The strategies are fully detailed in the Results section. Neither the strategy with the most (*Crystal Docking*, #6 in Results) nor the fewest (*Repack*, #1) degrees of freedom sampled was particularly successful. In fact, minimizing on side-chain (*Repack and SC Min*, #2) or rigid-body degrees (*Repack and SC, Jump Min*, #4) of freedom was not sufficient. Rather, successful strategies featured backbone minimization after the point mutation was introduced (*Repack and SC, BB Min*, #3, or *Repack and SC, BB, Jump Min*, #5).

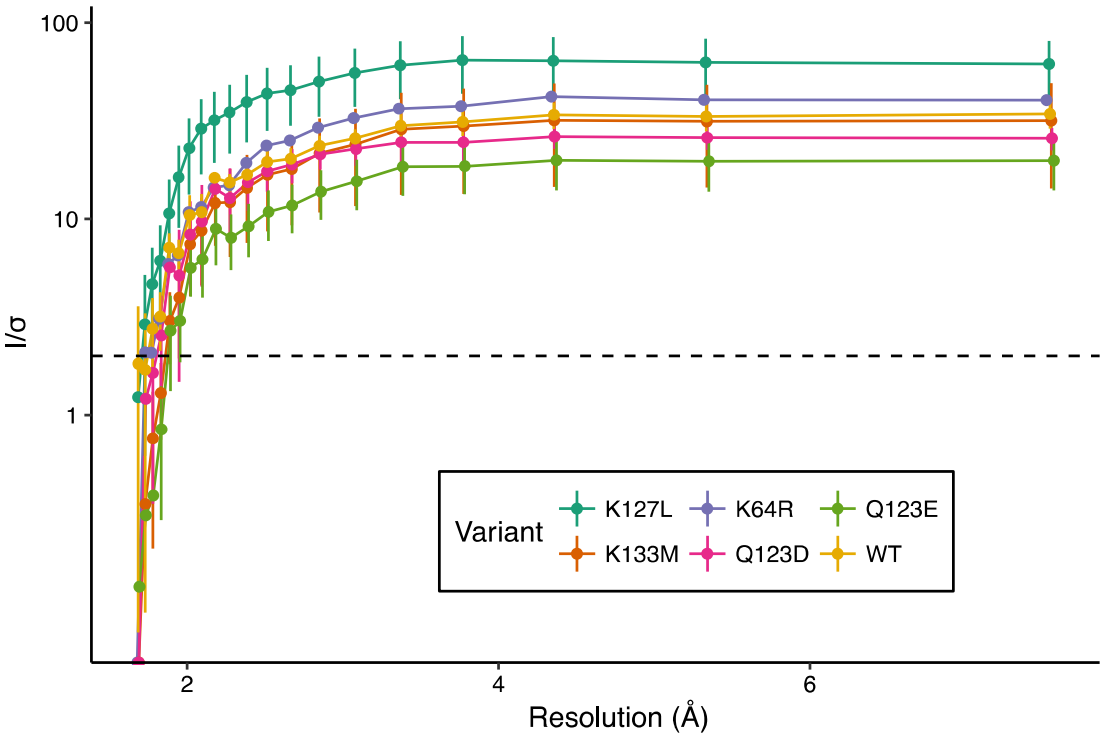

Figure S2 Average I/σ values ± one standard deviation for each resolution shell for each variant. Averages and standard deviations are calculated over all protein crystals for which diffraction data could be collected.

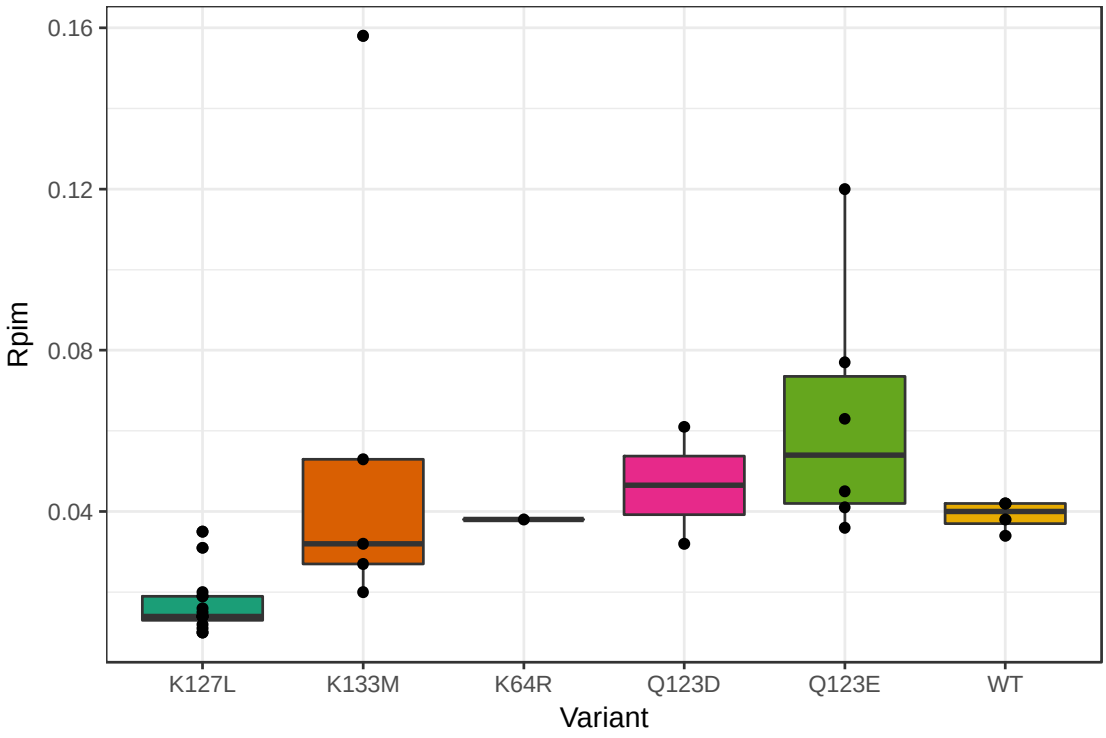

Figure S3 Overall $R_{p.i.m.}$ values calculated for all protein crystals for which diffraction data could be collected. Values show minor differences between all variants, except K127L, which has a slightly lower $R_{p.i.m.}$.

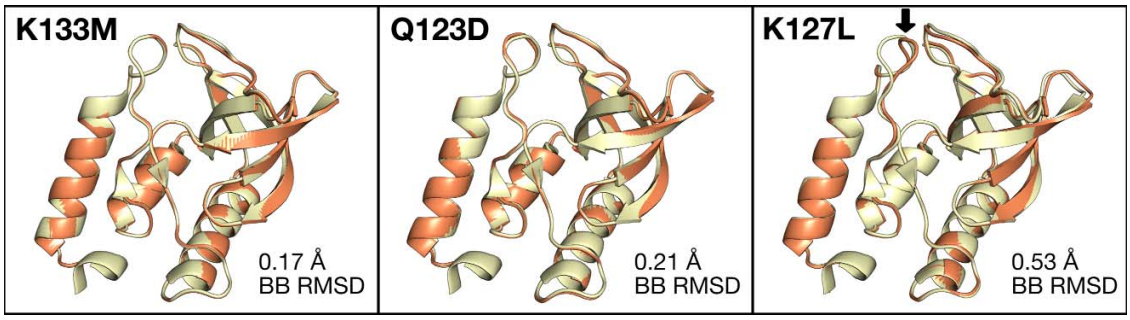

Figure S4 Alignments between design (orange) and crystal structure (yellow) backbones. There are minimal differences, with the greatest backbone RMSD being 0.53 Å for the K127L variant.

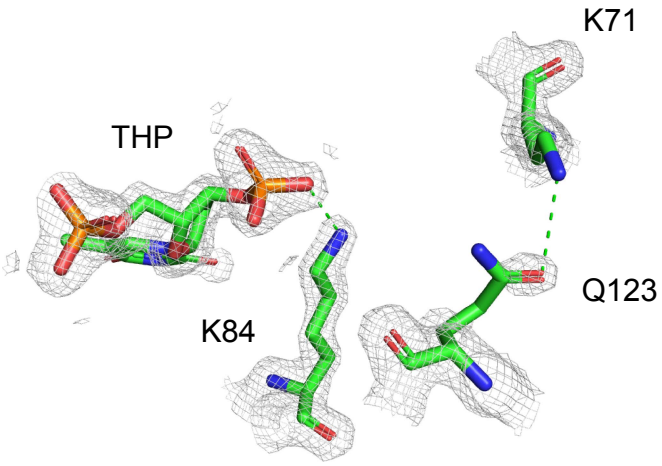

Figure S5 WT interactions between THP and K84, and Q123 and K71. Coordinates for the WT come from PDB ID 3BDC. The 2mFo-DFc map was downloaded from the Uppsala Electron Density Server. The map is contoured at 1.5σ and carved within 2 Å of residues 71, 84, 123, and the THP. Distances below 3.5 Å are highlighted by green dashed lines.

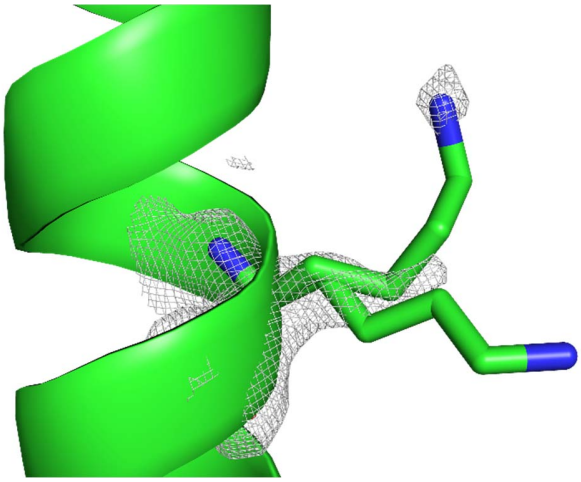

Figure S6 WT density of K64 is missing for some atoms and shows multiple rotameric states. Coordinates for the WT come from PDB ID 3BDC. The 2mFo-DFc map was downloaded from the Uppsala Electron Density Server. The map is contoured at 1.5σ and carved within 2 Å of residue 64.

Table S1 Crystallization conditions and I/σ and completeness values for the highest-resolution shell observed.

Variant	% MPD	pH	Ca2+ ratio	pdTp ratio	Space Group	Resolution	I/¤	Completeness (%)
¤+PHS K127L	40	9	3	2	P41	1.67	3.36	9
¤+PHS K127L	40	9	3	2	P41	1.77	4.74	48.8
¤+PHS K127L	40	8	3	2	P41	1.69	2.37	9.5
¤+PHS K127L	42	9	3	2	P41	1.77	2.23	46.3
¤+PHS K127L	42	9	3	2	P41	1.69	5.1	9
¤+PHS K127L	42	9	3	2	P41	1.68	3.59	7.7
¤+PHS K127L	42	8	3	2	P41	1.73	4.78	25
¤+PHS K127L	44	9	3	2	P41	1.83	2.72	79.6
¤+PHS K127L	44	8	3	2	P41	1.73	4.57	23.2
¤+PHS K127L	44	8	3	2	P41	1.78	2.48	44
¤+PHS K127L	46	9	3	2	P41	1.68	2.98	7.7
¤+PHS K127L	46	9	3	2	P41	1.73	6.77	26.4
¤+PHS K127L	46	8	3	2	P41	1.68	2.29	8.7
¤+PHS K127L	46	8	3	2	P41	1.83	3.05	69.5
¤+PHS K127L	46	8	3	2	P41	1.78	2.34	46.4
¤+PHS WT	21	6	3	2	P21	1.73	3.35	11.4
¤+PHS WT	18	6	3	2	P21	1.77	3.33	33
¤+PHS WT	18	6	3	2	P21	1.78	2.74	25.9
¤+PHS WT	18	6	3	2	P21	1.74	3.6	12.1
¤+PHS WT	18	6	3	2	P21	1.83	2.08	45.4
¤+PHS Q123E	18	6	2	1	P21	1.89	2.79	68.8
¤+PHS Q123E	20	6	2	1	P21	1.94	2.62	66.2
¤+PHS Q123E	20	6	2	1	P21	1.89	3.61	56.5
¤+PHS Q123E	20	6	2	1	P21	1.84	2.37	24.6
¤+PHS Q123E	18	6	2	1	P21	1.89	5.14	60.2
¤+PHS Q123E	18	6	2	1	P21	1.88	2.64	67
¤+PHS Q123E	18	6	2	1	P21	1.96	2.37	87.1
¤+PHS K133M	20	6	2	1	P21	1.84	2.16	43.7
¤+PHS K133M	18	6	2	1	P21	1.89	2.48	67.4
¤+PHS K133M	18	6	2	1	P21	1.9	3.98	81.6
¤+PHS K133M	18	6	2	1	P21	1.79	2.62	26.6
¤+PHS K133M	22	6	2	1	P21	1.89	3.58	74.3
¤+PHS K64R	18	6	3	2	P212121	1.73	2.13	22.2
¤+PHS Q123D	20	6	2	1	P21	1.95	2.39	87.9
¤+PHS Q123D	20	6	2	1	P21	1.78	2.61	25.9

Table S2 Selected statistics for deposited crystal structures.

	K127L (6OK8)	K133M (6U0W)	Q123D (6U0X)
Wavelength	1.54	1.54	1.54
Resolution range	38.26–1.8 (1.865–1.8)	32.21–1.901 (1.968–1.901)	32.26–1.86 (1.927–1.86)
Space group	P 41	P 1 21 1	P 1 21 1
Unit cell	48.097 48.097 63.122 90 90 90	30.927 60.473 38.105 90 93.017 90	30.853 60.715 38.119 90 92.648 90
Total reflections	72360 (1421)	33053 (1498)	34629 (1407)
Unique reflections	12307 (715)	10579 (868)	11156 (680)
Multiplicity	5.9 (2.0)	3.1 (1.9)	3.1 (2.1)
Completeness (%)	91.93 (53.32)	96.24 (79.52)	93.59 (56.06)
Mean I/sigma(I)	35.73 (5.59)	17.08 (2.38)	17.98 (4.54)
Wilson B-factor	22.28	32.22	28.23
R-merge	0.02957 (0.1052)	0.03576 (0.2966)	0.03985 (0.1484)
R-meas	0.03219 (0.1381)	0.0427 (0.3932)	0.04769 (0.1935)
R-pim	0.01254 (0.08851)	0.02308 (0.2552)	0.02591 (0.1225)
CC1/2	1 (0.984)	0.999 (0.937)	0.998 (0.976)
CC*	1 (0.996)	1 (0.984)	1 (0.994)
Reflections used in refinement	12308 (715)	10697 (866)	11125 (680)
Reflections used for R-free	619 (38)	538 (44)	562 (38)
R-work	0.1950 (0.2892)	0.2307 (0.4310)	0.2081 (0.3583)
R-free	0.2331 (0.4364)	0.2728 (0.5641)	0.2717 (0.4044)
CC(work)	0.961 (0.826)	0.922 (0.316)	0.933 (0.491)
CC(free)	0.947 (0.871)	0.913 (0.301)	0.941 (0.125)
Number of non-hydrogen atoms	1147	1097	1129
macromolecules	1032	1032	1032
ligands	26	26	26
solvent	89	39	71

Protein residues	129	129	129
RMS(bonds)	0.022	0.014	0.016
RMS(angles)	2.03	1.62	1.68
Ramachandran favored (%)	92.91	93.70	96.85
Ramachandran allowed (%)	4.72	5.51	2.36
Ramachandran outliers (%)	2.36	0.79	0.79
Rotamer outliers (%)	3.74	0.93	0.00
Clashscore	1.41	3.76	1.41
Average B-factor	25.95	39.27	32.49
macromolecules	25.29	39.36	32.41
ligands	34.24	38.91	28.13
solvent	31.23	37.03	35.38