Supporting Information

Reeve et al. 10.1073/pnas.1411548112

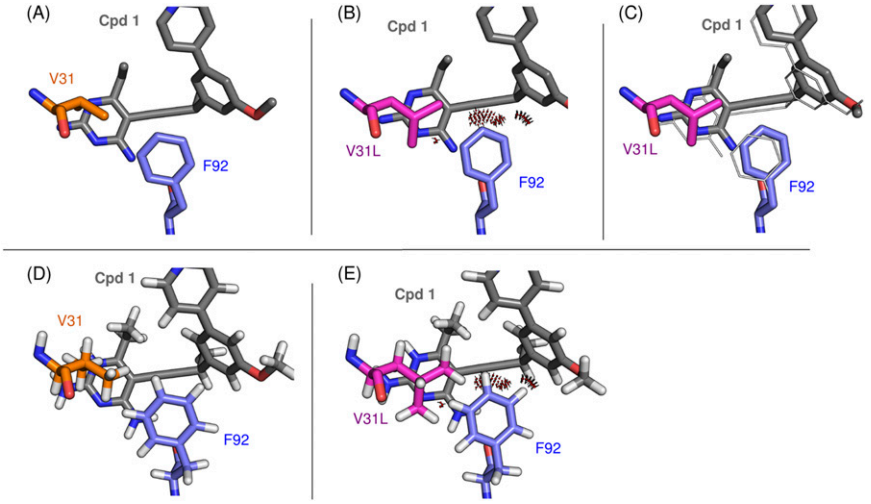

Fig. S1. Computationally predicted effect of the V31L mutation on inhibitor binding. (*A*) Conformations of WT residues F92 (blue), V31 (orange), and compound **1** (gray) in the bound *Staphylococcus aureus* DHFR–inhibitor complex. Oxygen atoms are red; nitrogen atoms are cyan. (*B*) Osprey K*-predicted structure for residue F92 (blue), compound **1** (gray), and the mutation V31L showing the computationally predicted model of the V31L mutant. Important steric clashes between the inhibitor and DHFR that occur in the model after the introduction of the V31L mutation are shown as red probe dots (1) drawn with the Protein Interaction Viewer (2). (*C*) Displacement modeled by Osprey K* of compound **1** and Phe-92 after the introduction of mutation V31L. The model of the V31L mutation is shown in the same colors as in *B*, whereas the structure of the WT is shown in fine white lines. (*D* and *E*) Protonated models of the structures shown in *A* and *B*. Cpd, compound.

1. Davis IW, et al. (2007) MolProbity: All-atom contacts and structure validation for proteins and nucleic acids. *Nucleic Acids Res* 35(web server issue):W375–W383.
2. Roberts K, Donald B (2014) *Protein Interaction Viewer*. Available at www.cs.duke.edu/donaldlab/software/proteinInteractionViewer/. Accessed October 25, 2014.

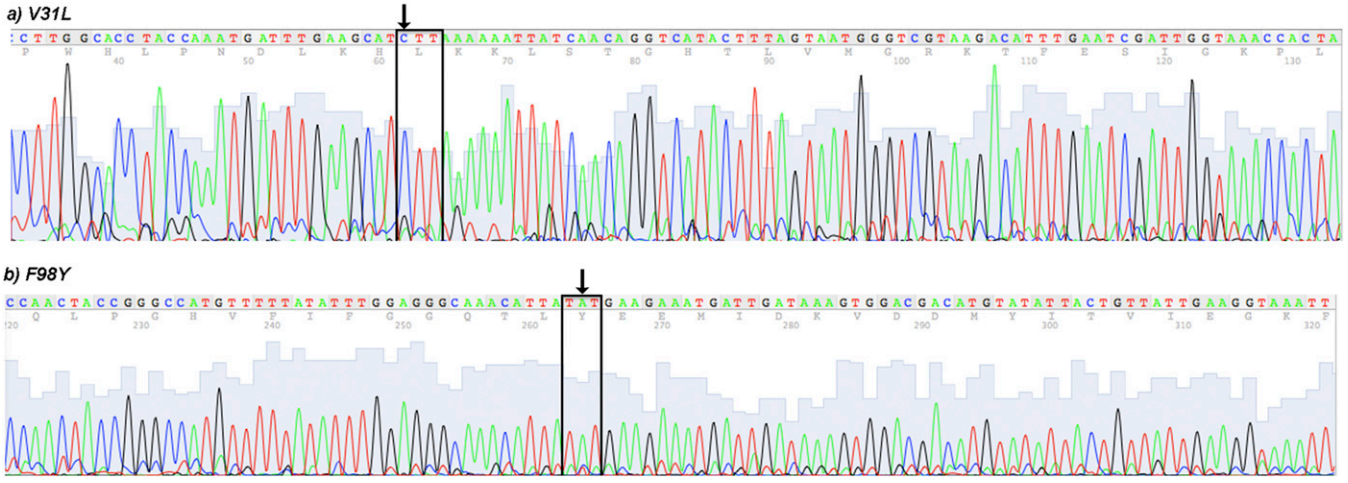

Fig. S2. Sequence chromatographs from the bacterial mutants. *A* shows the region of the chromatograph surrounding the mutation V31L (GTT → CTT), and *B* shows the region of the chromatograph surrounding the mutation F98Y (TTT → TAT). This chromatograph is representative of chromatographs from five colonies sequenced showing the V31L/F98Y mutations.

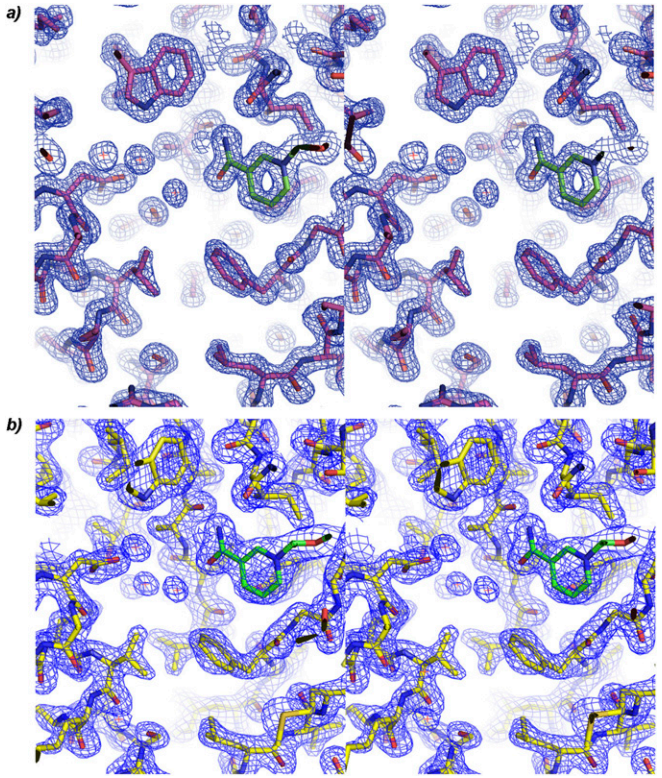

Fig. S3. Stereoview of the electron density (2Fo–Fc) of the active-site residues shown at 1.5σ. *A* shows density for the Sa(F98Y/V31L):NADPH crystal. (*B*) Density for the Sa(F98Y):NADPH crystal.

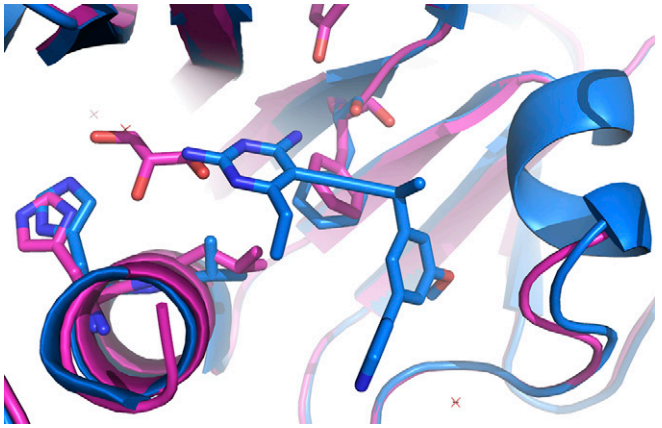

Fig. S4. A molecule of glycerol is observed to bind in the active site of the structure of Sa(F98Y/V31L):NADPH. The Sa(F98Y/V31L) structure is shown in magenta, and the 3SGY structure [Sa(WT):NADPH:**1**] is shown in blue for reference. The molecule of glycerol replaces the two-amino group and a water molecule found in the WT structure.

Table S1. K* rankings by positive-to-negative design ratio

K* rank	Single-nucleotide mutation	Substrate positive design K* score	Negative design compound 1 K* score	Ratio of positive design to negative design
1	V31L	2.16E + 41	3.04E + 19	7.11E + 21
2	V31I	4.87E + 36	8.18E + 14	5.95E + 21
3	L5I	6.06E + 39	3.54E + 24	1.71E + 15
4	L5V	4.01E + 44	3.44E + 30	1.16E + 14
5	L54R	6.31E + 43	1.60E + 30	3.94E + 13
6	L20F	6.06E + 20	1.75E + 07	3.46E + 13
7	V31D	1.54E + 41	5.65E + 32	2.72E + 08
8	L5Q	2.84E + 44	1.20E + 36	2.36E + 08
9	V31A	7.32E + 40	7.86E + 33	9.31E + 06
10	V6G	3.92E + 42	4.27E + 35	9.18E + 06
11	I50L	5.18E + 38	5.69E + 31	9.10E + 06
12	F92S	2.09E + 43	2.52E + 36	8.32E + 06
13	L54Q	2.62E + 42	3.21E + 35	8.14E + 06
14	V31G	2.64E + 40	3.38E + 33	7.80E + 06
15	T46A	2.88E + 42	5.27E + 35	5.46E + 06
16	V61	1.10E + 43	4.01E + 36	2.75E + 06
17	L28M	3.27E + 42	1.58E + 36	2.07E + 06
18	**WT**	**7.16E + 42**	**3.66E + 36**	**1.96E + 06**
19	T46S	4.93E + 42	3.46E + 36	1.42E + 06
20	L28F	2.25E + 42	1.73E + 36	1.30E + 06
21	L28W	1.29E + 43	1.01E + 37	1.28E + 06
22	L20S	3.57E + 41	2.95E + 35	1.21E + 06
23	L20I	3.04E + 42	2.94E + 35	1.03E + 06
24	L20V	7.43E + 41	7.40E + 35	1.01E + 06
25	V6A	4.71E + 42	6.07E + 36	7.76E + 05
26	L54V	1.46E + 41	2.68E + 35	5.44E + 05
27	L28S	1.34E + 41	3.74E + 35	3.59E + 05
28	V6D	1.18E + 43	6.05E + 37	1.95E + 05
29	V6L	7.26E + 42	6.47E + 37	1.12E + 05
30	F92V	8.18E + 40	2.80E + 36	2.29E + 04
31	I50V	2.49E + 42	1.74E + 38	1.43E + 04
32	L28V	3.19E + 39	2.37E + 35	1.35E + 04
33	F92I	8.91E + 40	8.48E + 36	1.05E + 04
34	F92C	1.02E + 40	5.37E + 36	1.90E + 03
35	I50S	3.07E + 41	2.37E + 38	1.30E + 03
36	I50T	1.11E + 42	9.85E + 38	1.13E + 03
37	I50N	6.81E + 41	1.53E + 39	4.46E + 02
38	I50M	1.00E + 40	7.50E + 37	1.33E + 02
39	F92L	4.02E + 37	4.20E + 36	9.59E + 00
40	L5R	4.21E + 34	4.27E + 35	9.88E − 02
41	T46I	5.05E + 36	2.25E + 42	2.25E − 06
42	V6F	0.00E + 00	2.94E + 36	0.00E + 00
43	V31F	0.00E + 00	8.69E − 11	0.00E + 00
44	T46R	0.00E + 00	0.00E + 00	0.00E + 00
45	T56K	0.00E + 00	1.14E + 14	0.00E + 00
46	F92Y	0.00E + 00	3.10E + 32	0.00E + 00
47	I50F	0.00E + 00	3.37E + 35	0.00E + 00

Row 18, showing the scores for the wild-type enzyme, is shown in bold for reference.

Dataset S1. Interactions between residues and ligands are shown with notations for distance and van der Waals (V) or hydrogen bond (H)

Dataset S1