Table S1. Minimal inhibitory concentration values. Values for each indicated compound shown are averages of four biological repeats.

	Cm (μM)	TPP (mM)	Eth (mM)
empty vector	3	0.6	0.5
MdfA	21.7	0.85	2
MdfA(Q131R)	7.7	0.6	0.25

Table S2. Data collection and refinement statistics of MdfA(Q131R/L339E)

Data collection	
PDB accession #	6EUQ
Space group:	*C*2
Cell constants a; b; c (Å)	95.19, 62.87, 100.48
α; β; γ (°)	90.0, 110.3, 90.0
Resolution	44.64– 2.20 (2.27-2.20)
Unique reflections	28294 (2449)
Completeness overall (%)	99.5 (99.9)
Multiplicity (%)	3.5 (3.4)
<I>/<σ(I)>	8.0 (0.9)
*R*meas	0.183 (0.893)
*R*merge	0.135 (0.661)
*R*pim	0.123 (0.596)
CC(1/2)	0.984 (0.491)
Refinement	
No. of reflections	26961
*R*value $_{overall}$ (%) / *R*value $_{free}$ (%)	0.2308 / 0.2483
No. atoms	
Protein	2959
Ligand/ion	128
Water	47
B-factors	
Protein	49.42
Ligand/ion	70.07
Water	48.98
r.m.s. deviations from ideal values	
Bond length (Å)	0.0070
Bond angles (º)	1.0637
Φ, Ψ angle distribution for residues	
In most favoured regions (%)	97.3
In additional allowed regions (%)	2.7
In generously allowed regions (%)	0
In disallowed regions (%)	0

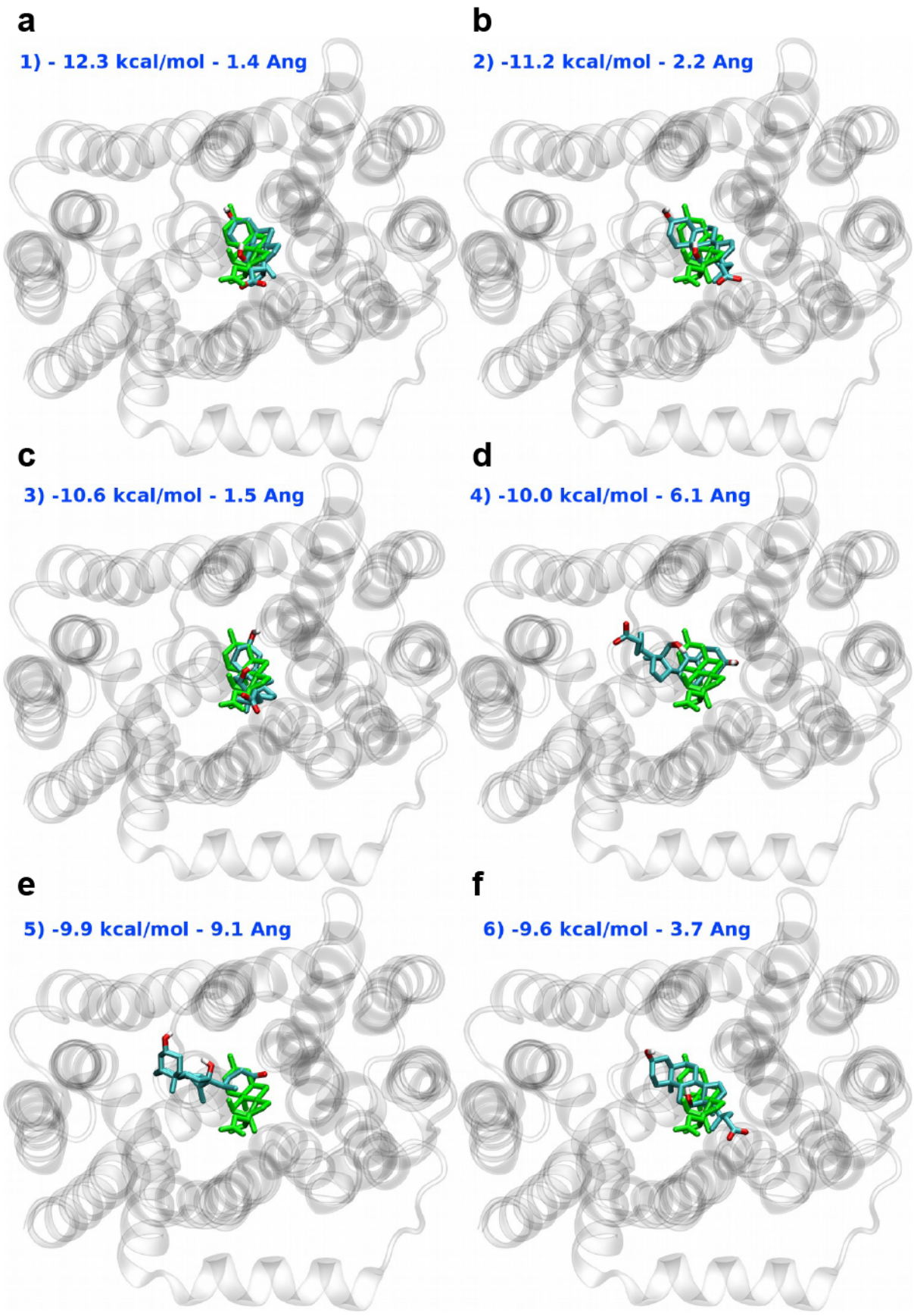

Figure S1. Re-docking deoxycholate to MdfA (Q131R).
MdfA as seen from the cytoplasmic side. Comparison between the first 6 docking poses and the experimental bound configuration of deoxycholate (shown as green sticks). The corresponding "binding affinity" and the RMSD from the experimental pose (expressed in Å) are reported in blue.

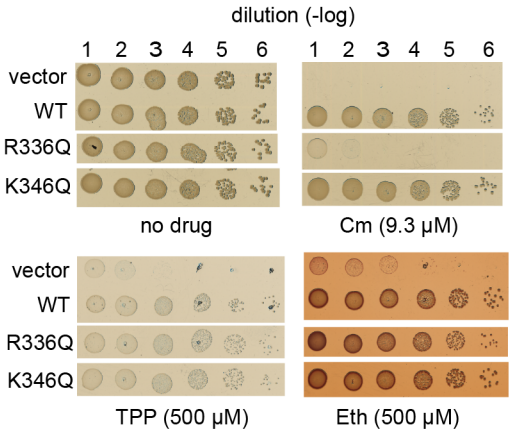

Figure S2. Multidrug resistance activity of the indicated constructs spotted on LB-agar plates containing the indicated drugs.

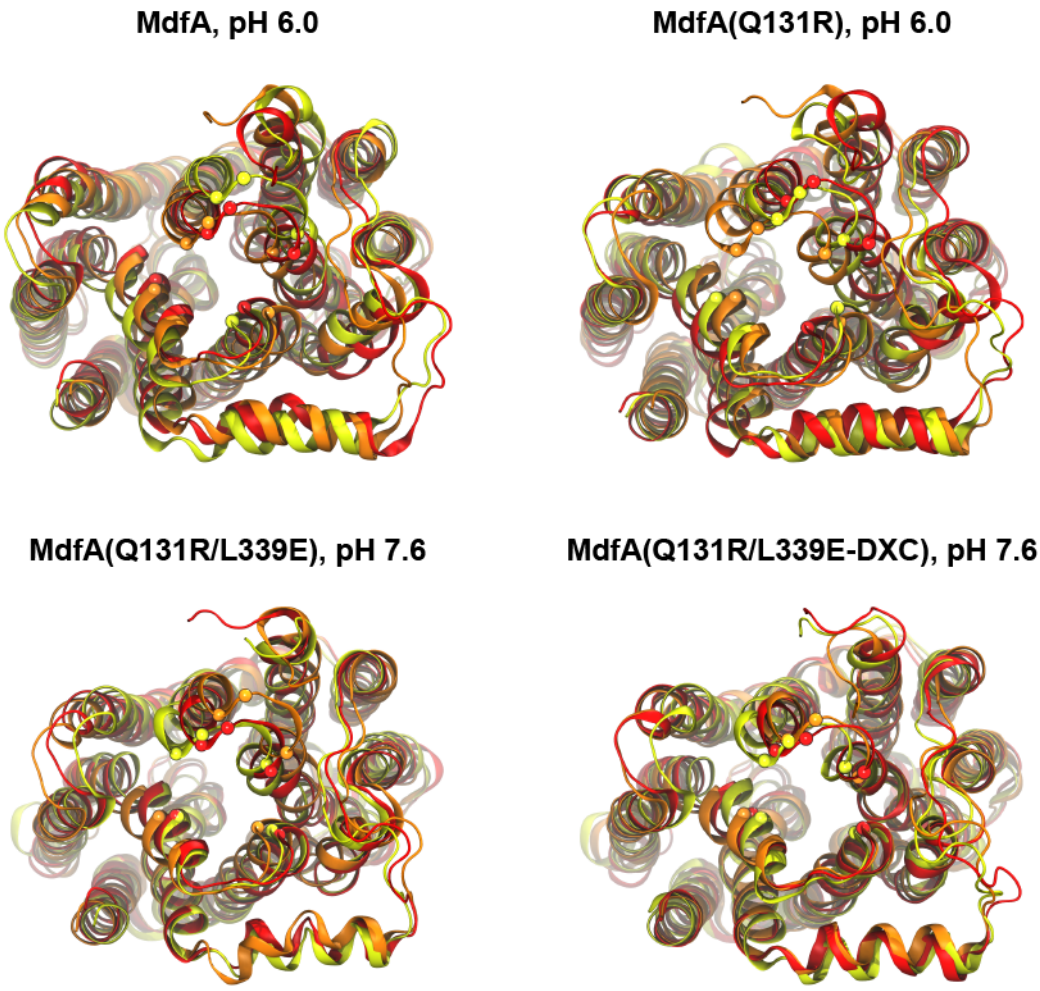

Figure S3. Conformational flexibility of wt MdfA and mutants in all-atom MD simulations
Three representative conformations extracted from the cluster analysis of MD trajectories are shown as ribbons colored red, orange, and yellow. The conformations were chosen arbitrarily from the representatives of the top ten clusters (red ribbons always show the most populated cluster representatives) in order to show maximally dissimilar conformations. Alpha carbons of residues 132, 135, 136, 336 and 346 of the rim are shown as spheres.

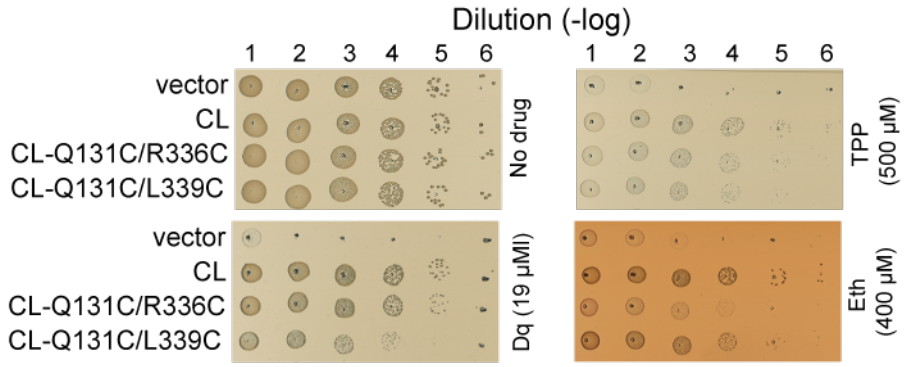

Figure S4. Multidrug resistance activity of the indicated constructs spotted on LB-agar plates containing the indicated drugs.