Supporting Information

Elucidation of the Catalytic Mechanism of a Miniature Zinc Finger Hydrolase

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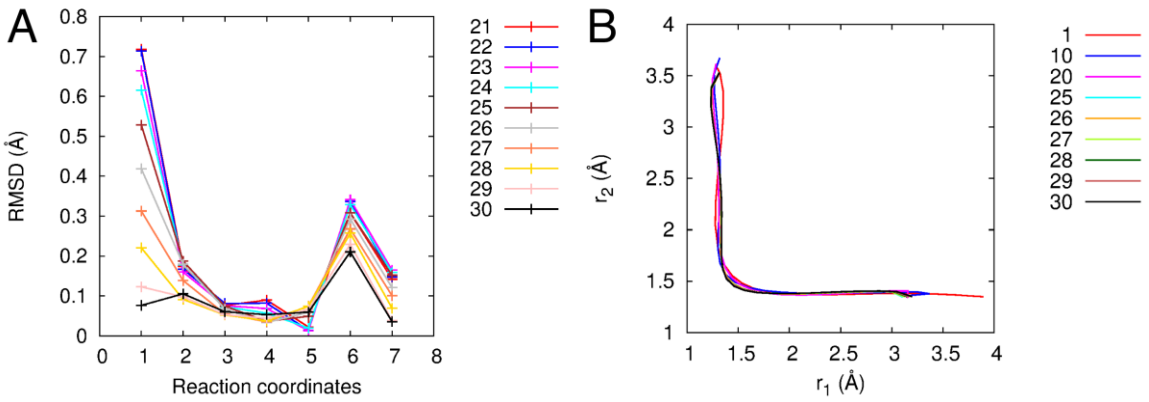

Figure S1. Convergence of the string simulations: (A) RMSDs of all seven reaction coordinates are shown for string iterations 21 to 30. The RMSDs of each reaction coordinate were calculated from their average values in the previous 10 iterations. The figure indicates that the RMSD across all coordinates decreases with string iterations. By iteration 30 all RMSDs are well below 0.5 Å. (B). The strings are plotted in the space of coordinates r1 and r2 for selected iterations, as indicated in the figure legend. The nearly superimposable strings from the various iterations suggest that the string has reached convergence in the space of r1 and r2. Iterations 31 and 32 are excluded from the analysis. This is because in these last two iterations, even after convergence of the string, the number of images was doubled and longer trajectories were propagated to obtain better sampling statistics with higher resolution for the MFEP.

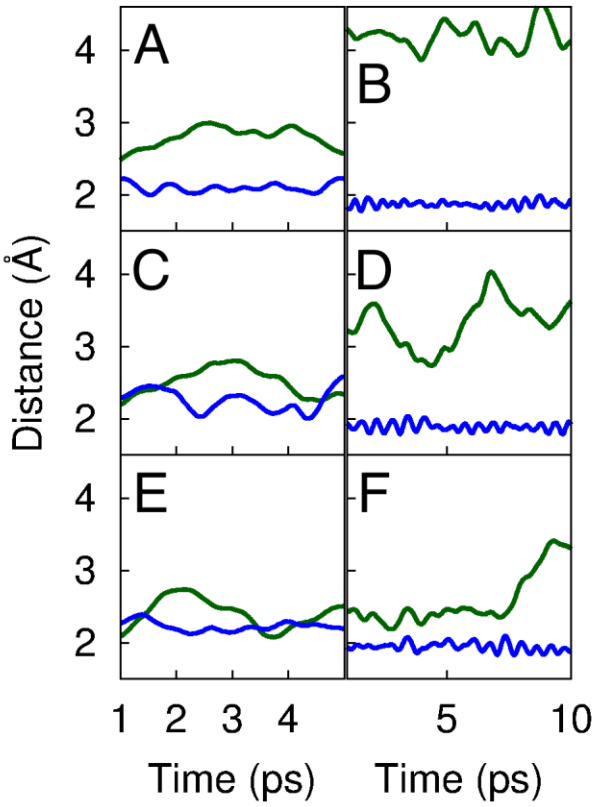

Figure S2. Coordination architecture of the Zn^{2+} center for three additional starting configurations (similar to Fig. 2C and Fig. 2D, manuscript): The time-evolution of the distance between the O atom of water/hydroxide to Zn^{2+} is shown. (A), (C), (E) correspond to QM/MM MD simulations with 2 water molecules (green, blue) in the coordination sphere of Zn^{2+}. (B), (D), (F) correspond to QM/MM MD simulations with 1 water (green) and 1 hydroxide (blue) in the coordination sphere of Zn^{2+}. For (B), (D) and (F) the last 10 ps of the simulations are shown.

Table S1. Relevant structural details and energies of the PES (related to Figure 3).

	R	**TS1**	**I**	**TS2**	**P**
O(OH)-C2(PNPA) (Å)	3.42	2.04	1.41	1.34	1.32
C2(PNPA)-O2(PNPA) (Å)	1.36	1.40	1.48	1.85	3.64
Zn^{2+}-O(OH) (Å)	1.88	1.98	2.86	3.13	3.08
Zn^{2+}-O1(PNPA) (Å)	2.41	2.35	1.92	1.94	1.99
Zn^{2+}-NE2(His10) (Å)	2.07	2.08	2.08	2.07	2.03
Zn^{2+}-NE2(His15) (Å)	2.19	2.16	2.17	2.16	2.10
Zn^{2+}-NE2(His22) (Å)	2.08	2.08	2.11	2.09	2.08
ΔE (kcal/mol)	**0**	**21.9**	**4.1**	**7.40**	**-2.34**

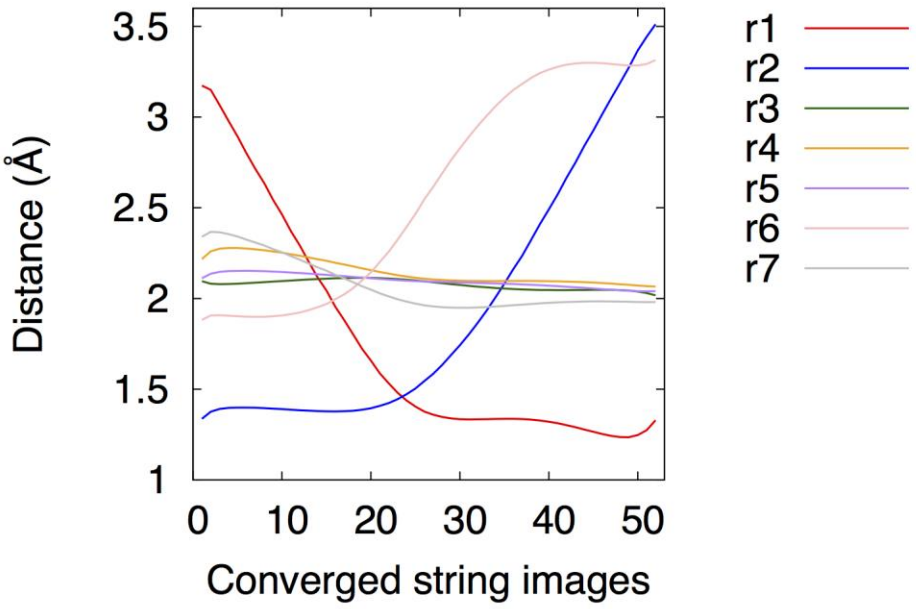

Figure S3. Evolution of all reaction coordinates along the converged string: Except r1 and r2, the remaining coordinates do not change significantly during the course of the reaction. r6, which corresponds to the Zn^{2+}–O_{OH^-} distance, changes during the reaction because OH^-, which is initially strongly bound to Zn^{2+}, binds to the pNPA carbonyl and drifts away from Zn^{2+} as pNPA is hydrolyzed.

Table S2. Parameters derived from Michaelis-Menten kinetics shown in Fig. 6 of the main paper.

MeCN [% (v/v)]	2.66
V_{max} [µM/s * 10^{-3}]	17.0 ± 4.3
k_{cat} [1/s * 10^{-3}]	0.85 ± 0.21
K_M [mM]	5.8 ± 2.1
k_{cat}/K_M [1/M*s]	0.148 ± 0.064
$\Delta G^{\ddagger}$ [kcal/mol]	20.5 ± 0.3

Calculation of reactant and transition state volumes for reaction pathway 1

The volume of the hydrolase catalytic center was determined using the Mol_Volume program available in the MDTools suite of programs.[1] This program determines the volume of a given macromolecule by generating a dense grid around the macromolecule and calculating the number of vertices that appear within a probe radius of the atoms in the molecule. The volume is then expressed as

$$V = \frac{V_{grid} \times N_{near}}{N_{total}} = N_{near} \times V_{per\,node}$$

where V_{grid} is the volume of the grid, N_{near} is the number of vertices within the probe radius of a particular atom, and N_{total} is the total number of vertices.

In our case, we chose the catalytic center to be comprised of the substrate, the Zn^{2+} ion, and either a Zn^{2+} bound water (in the reactant state) or hydroxide ion (in the transition state). The calculations were performed separately on QM/MM optimized reactant and transition state geometries (Figure 3 of the main paper) as well as on an ensemble of reactant and transition state configurations obtained from QM/MM MD simulations. Default values of all parameters were used in the calculations.

Table S3. Volume of the Zn-finger hydrolase catalytic center. The values obtained from QM/MM MD simulations are averaged over 500 snapshots (errors in parentheses). Volumes are reported in $Å^3$

	Reactant state	Transition state	$\Delta V^{\ddagger}$
PES	879	840	-39
QM/MM MD	843.8 (1.7)	792.1 (0.6)	-51.7

Classical MD simulations of the Zn-finger hydrolase

In this study, all calculations were based on the de novo structure reported by Srivastava and Durani.[2] In an effort to identify other catalytically relevant conformations of the Zn-finger hydrolase, we ran five independent 25 ns MD trajectories in which the hydrolase was unrestrained (data not shown). In these simulations, the hydrolase was extremely flexible, as expected, and in some cases partially unfolded. We were unable to identify other conformations of the hydrolase that are significantly different from the originally designed structure and at the same time catalytically relevant.

References

(1) Humphrey, W.; Dalke, A.; Schulten, K. *J. Molec. Graphics.* **1996**, *14*, 33.
(2) Srivastava, K. R.; Durani, S. *PLoS ONE* **2014**, *9*, e96234.

Cartesian coordinates (Å) of optimized QM regions for various stationary points

(a) Reactant state (Figure 3A)

CB SER 6 29.956 26.780 24.749
HB1 SER 6 29.778 26.428 25.778
HB2 SER 6 31.026 26.602 24.537
OG SER 6 29.612 28.139 24.675
HG1 SER 6 30.074 28.526 23.906
ND1 HSD 10 24.517 25.334 27.579
HD1 HSD 10 23.532 25.609 27.575
CG HSD 10 25.096 24.202 27.061
CE1 HSD 10 25.475 26.098 28.116
HE1 HSD 10 25.254 27.013 28.662
NE2 HSD 10 26.658 25.527 27.961
CD2 HSD 10 26.438 24.338 27.311
HD2 HSD 10 27.259 23.671 27.061
ND1 HSD 15 28.476 22.533 31.305
HD1 HSD 15 28.961 21.739 31.733
CG HSD 15 27.135 22.794 31.409
CE1 HSD 15 29.048 23.523 30.595
HE1 HSD 15 30.102 23.540 30.315
NE2 HSD 15 28.139 24.419 30.255
CD2 HSD 15 26.940 23.979 30.745
HD2 HSD 15 26.010 24.524 30.586
ND1 HSD 22 26.753 27.926 32.210
HD1 HSD 22 26.611 27.946 33.223
CG HSD 22 26.156 28.700 31.249
CE1 HSD 22 27.693 27.172 31.623
HE1 HSD 22 28.319 26.474 32.170
NE2 HSD 22 27.737 27.410 30.325
CD2 HSD 22 26.795 28.377 30.083
HD2 HSD 22 26.636 28.768 29.082
C1 PNP 1 30.926 28.604 27.512
C2 PNP 1 29.483 28.957 27.562
O1 PNP 1 28.560 28.191 27.698
O2 PNP 1 29.279 30.295 27.466
C3 PNP 1 27.991 30.766 27.581
C4 PNP 1 27.711 31.639 28.630
C5 PNP 1 26.415 32.122 28.793
C6 PNP 1 25.433 31.692 27.906
C7 PNP 1 25.712 30.840 26.839
C8 PNP 1 27.010 30.379 26.669
N1 PNP 1 24.048 32.126 28.105
O3 PNP 1 23.839 33.023 28.889

O4 PNP 1 23.191 31.531 27.485
H1 PNP 1 31.268 28.787 26.482
H2 PNP 1 31.016 27.534 27.767
H3 PNP 1 31.498 29.254 28.189
H4 PNP 1 28.511 31.918 29.317
H5 PNP 1 26.157 32.802 29.607
H6 PNP 1 24.909 30.572 26.154
H7 PNP 1 27.285 29.721 25.845
ZN ZN2 1 28.433 26.088 28.874
O1 OH 1 30.188 25.698 28.328
H1 OH 1 30.064 24.977 27.674

(b) Intermediate state (Figure 3B)

CB SER 6 30.156 26.632 24.539
HB1 SER 6 29.983 26.295 25.573
HB2 SER 6 31.214 26.431 24.289
OG SER 6 29.835 27.995 24.492
HG1 SER 6 30.215 28.378 23.677
ND1 HSD 10 24.308 24.843 27.825
HD1 HSD 10 23.285 24.887 27.932
CG HSD 10 24.966 23.907 27.072
CE1 HSD 10 25.215 25.624 28.422
HE1 HSD 10 24.949 26.419 29.119
NE2 HSD 10 26.441 25.245 28.076
CD2 HSD 10 26.300 24.171 27.235
HD2 HSD 10 27.164 23.670 26.809
ND1 HSD 15 28.549 22.543 31.524
HD1 HSD 15 29.035 21.761 31.978
CG HSD 15 27.205 22.795 31.587
CE1 HSD 15 29.132 23.522 30.810
HE1 HSD 15 30.196 23.520 30.572
NE2 HSD 15 28.228 24.403 30.419
CD2 HSD 15 27.019 23.962 30.893
HD2 HSD 15 26.090 24.496 30.701
ND1 HSD 22 26.740 27.994 32.196
HD1 HSD 22 26.705 28.084 33.216
CG HSD 22 25.996 28.641 31.246
CE1 HSD 22 27.592 27.167 31.571
HE1 HSD 22 28.309 26.545 32.098
NE2 HSD 22 27.433 27.236 30.265
CD2 HSD 22 26.453 28.165 30.046
HD2 HSD 22 26.135 28.441 29.043
C1 PNP 1 31.396 28.141 27.330
C2 PNP 1 30.276 27.737 28.285

O1 PNP 1 29.316 27.037 27.710
O2 PNP 1 29.791 28.989 28.904
C3 PNP 1 28.787 29.709 28.413
C4 PNP 1 28.306 30.737 29.255
C5 PNP 1 27.173 31.462 28.930
C6 PNP 1 26.510 31.171 27.735
C7 PNP 1 27.038 30.245 26.835
C8 PNP 1 28.181 29.528 27.150
N1 PNP 1 25.239 31.801 27.437
O3 PNP 1 24.889 32.743 28.114
O4 PNP 1 24.572 31.320 26.534
H1 PNP 1 30.970 28.650 26.455
H2 PNP 1 31.903 27.232 26.972
H3 PNP 1 32.121 28.787 27.845
H4 PNP 1 28.830 30.914 30.195
H5 PNP 1 26.772 32.222 29.604
H6 PNP 1 26.539 30.106 25.879
H7 PNP 1 28.584 28.809 26.438
ZN ZN2 1 28.257 25.902 28.848
O1 OH 1 30.796 27.088 29.419
H1 OH 1 31.553 26.540 29.131

(c) Product state (Figure 3C)

CB SER 6 30.498 26.185 24.375
HB1 SER 6 30.373 25.659 25.334
HB2 SER 6 31.467 25.851 23.952
OG SER 6 30.459 27.556 24.632
HG1 SER 6 30.566 28.046 23.791
ND1 HSD 10 24.354 24.929 27.747
HD1 HSD 10 23.333 25.026 27.830
CG HSD 10 24.984 23.930 27.054
CE1 HSD 10 25.278 25.693 28.335
HE1 HSD 10 25.037 26.531 28.989
NE2 HSD 10 26.493 25.237 28.040
CD2 HSD 10 26.324 24.134 27.241
HD2 HSD 10 27.174 23.578 26.858
ND1 HSD 15 28.554 22.468 31.633
HD1 HSD 15 29.055 21.720 32.131
CG HSD 15 27.202 22.680 31.649
CE1 HSD 15 29.128 23.421 30.882
HE1 HSD 15 30.196 23.441 30.664
NE2 HSD 15 28.205 24.251 30.426
CD2 HSD 15 26.995 23.801 30.889
HD2 HSD 15 26.055 24.295 30.648

ND1 HSD 22 26.768 27.898 32.199
HD1 HSD 22 26.671 27.991 33.214
CG HSD 22 26.150 28.620 31.213
CE1 HSD 22 27.624 27.045 31.626
HE1 HSD 22 28.243 26.358 32.196
NE2 HSD 22 27.597 27.172 30.312
CD2 HSD 22 26.697 28.172 30.040
HD2 HSD 22 26.505 28.514 29.024
C1 PNP 1 31.593 28.007 27.533
C2 PNP 1 30.754 27.015 28.228
O1 PNP 1 29.538 26.947 28.041
O2 PNP 1 30.476 30.641 28.441
C3 PNP 1 29.236 30.739 28.224
C4 PNP 1 28.349 31.398 29.155
C5 PNP 1 27.000 31.520 28.936
C6 PNP 1 26.420 30.969 27.773
C7 PNP 1 27.243 30.312 26.833
C8 PNP 1 28.598 30.200 27.043
N1 PNP 1 25.010 31.029 27.579
O3 PNP 1 24.314 31.534 28.449
O4 PNP 1 24.540 30.553 26.547
H1 PNP 1 31.375 27.940 26.456
H2 PNP 1 32.664 27.891 27.737
H3 PNP 1 31.236 29.026 27.839
H4 PNP 1 28.805 31.814 30.055
H5 PNP 1 26.354 32.042 29.645
H6 PNP 1 26.781 29.897 25.940
H7 PNP 1 29.209 29.675 26.305
ZN ZN2 1 28.236 25.737 28.944
O1 OH 1 31.280 26.181 29.100
H1 OH 1 32.272 26.212 29.115