SUPPORTING INFORMATION

Amine Oxidation Mediated by Lysine-Specific-Demethylase 1 (LSD1): QM/MM Insights into Mechanism and Role of Lysine 661

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1. Detailed information on system preparation for MD runs

In the crystal structure (PDB ID: 2V1D), LSD1 binds a substrate-like 21-mer peptide inhibitor, which differs from the wild-type substrate (i.e., the H3 tail of the histone protein) by the K4M mutation. For the simulations, the methionine at the fourth position on the peptide was converted to a dimethyllysine by re-modeling the side chain tail using *Accelrys DS Visualizer* software. The X-Ray structure also lacks crystal water molecules, which were taken from another crystal structure of LSD1 (PDB code 2H94[1]) in complex only with FAD. The two structures were superimposed with respect to their FAD components, and the approximate positions of 20 crystal water molecules were determined. The size of the resulting model system was quite large, and solvation by a water droplet would lead to a system too large to be handled efficiently during the simulations. Therefore, the TOWER domain of LSD1 (i.e., residues 420-518) and the CoREST (chain B) were excluded from the model system, since these parts are far apart from the active-site and have only an indirect allosteric effect on the reaction. No residue with alternating conformation was present among the remaining protein residues. The protonation states of the 90 ionizable residues were determined at pH=8.7 using the H++ web-server[2] and checked by visual inspection. Amongst the titratable residues in the active-site, only the conserved Lys661 and the methylated substrate lysine (H3K4) are predicted to change their protonation state upon substrate binding (see main paper). Therefore, two different oxidation states of K661 and dimethyllysine were considered during the simulations, while FAD was taken to be deprotonated (with a formal charge of -2) and represented by the published force field parameters.[3] The force field parameters for protonated non-methylated lysine are available in the standard CHARMM22 force field,[4] while those for dimethylated lysine were adopted from the literature.[5] The force field parameters were modified accordingly for the deprotonated systems. Missing atoms in the crystal structure

were built with CHARMM package[6] using the standard CHARMM22 force field[4] along with the aforementioned special parameters for FAD and the lysines. The TIP3P model[7] was used for water molecules. After this initial built of the system, a quick minimization with 1000 adapted-basis Newton Raphson (ABNR) steps was applied to remove steric clashes.

The actual system (including the protein, substrate peptide, and FAD) was neutralized with 0.15 M NaCl solution, after being solvated in a pre-equilibrated water droplet with a radius of 35 Å around the center of mass of the protein complex. Following a quick minimization (250 steps of steepest descent and ABNR, respectively) of the solvated system, an iterative procedure (so-called rehydration) was applied. This procedure involves introducing new water molecules in the available voids, i.e., not closer than 2.8 Å to the existing atoms, followed by a 15-ps MD relaxation. During the rehydration procedure and the MD simulations, only the atoms within 15Å of the center of mass were allowed to move, whereas the others were restrained using a harmonic potential. After this rehydration procedure, the system was heated from 50K to 300K with a 10K temperature increase every 100 steps.

2. Coordinates of the optimized geometries for the QM part of the stationary points and corresponding electronic energies

Optimizations were done at QM(B3LYP-D/6-31G*)/CHARMM22 level. Cartesian coordinates are reported only for the QM region of the full QM/MM systems. They correspond to the case of K661 being deprotonated. Total electronic energies are given in au and do not contain dispersion corrections nor zero-point corrections. The stationary points are defined in Figure 1 of the main paper. Link atoms are indicated by a lower-case “h”.

2.1 Upward orientation (using snapshot 1)

2.1.1. Singlet reactant complex (RC1), E= -1528.15727960331 au.

72

C -7.316213 60.907578 74.813017
H -6.346511 61.372035 75.036498
H -7.091796 59.965064 74.294244
C -7.973857 60.580104 76.166657
H -8.129201 61.493396 76.758433
H -7.251655 59.975919 76.731182
C -9.267274 59.768299 76.135109
H -9.149168 58.919296 75.449543
H -10.100710 60.379819 75.758173
N -9.547605 59.214292 77.471724
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H -10.487179 58.815723 77.465210
C -5.697340 53.642016 85.611157
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H -6.544026 53.904271 86.263997
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H -4.559261 54.659771 84.017447
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H -4.848229 55.688651 82.016192
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H -6.604348 55.701997 81.650318
C -5.038543 57.682414 83.886351
H -5.321312 58.032509 84.885356
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N -7.663254 58.732991 81.297354
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C -5.051045 63.684916 80.423031

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H -8.132130 55.747090 79.241990
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O -8.050381 52.276750 80.016930
H -7.420642 52.240992 80.769407
H -8.202174 53.242702 79.883468
h -6.930397 61.682257 85.160504
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h -7.818831 61.555773 74.095184

2.1.2. Triplet reactant complex (RC3), E=-1528.11526211340 au.

72

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H -6.341924 61.368385 75.050976
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C -5.969398 61.847670 82.018451
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H -8.020531 55.729254 79.489163
H -8.835527 55.539295 80.774989
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H -8.227729 53.292369 80.016613
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h -6.036398 52.907529 84.920623
h -7.820577 61.540260 74.118949

2.1.3. Singlet transition state (TS1), E= -1528.12335956990 au.

72

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H -9.540373 59.936116 78.165083
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H -4.803288 57.224380 81.332324
H -6.646842 57.613184 81.303277

H -6.063182 55.841712 81.330602
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H -5.422422 57.997452 84.833972
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H -7.404665 52.238249 80.778639
H -8.179462 53.314287 80.005766
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h -6.063373 52.888075 84.890514
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2.1.4. Triplet transition state (TS3), E= -1528.08961031943 au.

72

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H -7.067201 59.992667 74.264921
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H -9.561194 60.013286 78.125756

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H -4.908695 55.733269 85.273951
C -6.215280 55.518486 83.575033
H -7.054095 55.994426 84.106939
H -6.625761 54.741616 82.920950
N -5.562581 56.502847 82.728542
C -5.728597 56.517708 81.354910
H -4.917862 57.015428 80.818555
H -6.819759 57.533345 81.158309
H -6.107197 55.588735 80.933587
C -4.935732 57.613915 83.427096
H -5.494372 57.830232 84.345332
H -4.942847 58.497821 82.788377
H -3.900549 57.371048 83.696861
C -6.404262 60.891853 84.799439
H -6.225353 60.111479 85.538802
H -5.449319 61.330881 84.501814
N -9.480193 57.074926 84.120149
H -10.211211 56.384733 84.281099
C -9.094621 57.310345 82.817213
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C -8.078890 58.324224 82.686673
C -7.769585 59.109081 83.829909
N -8.135074 58.796342 85.066017
C -8.958044 57.720028 85.263627
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h -7.788523 61.585293 74.060610

2.1.5. Singlet product complex (PC1), E= -1528.15719906242 au.

72

C -7.307019 60.887725 74.823627
H -6.332992 61.344645 75.041602
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H -10.594400 59.009902 77.484482
C -5.785933 53.804469 85.453955
H -5.078948 53.453867 86.213134
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H -4.739960 55.684551 85.432650
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H -6.958380 56.232146 84.523313
H -6.710549 55.157807 83.126699
N -5.548034 56.882681 83.117173
C -5.649510 57.017535 81.814576
H -4.954280 57.674828 81.308122
H -7.700029 58.039492 80.548296
H -6.161970 56.231220 81.266608
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H -5.339815 58.033595 84.848571
H -4.634220 58.751864 83.390265
H -3.807297 57.383932 84.194675
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C -7.661937 59.038540 83.728943
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H -4.491865 62.270356 78.123493
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h -6.912103 61.590128 85.185259
h -6.063150 52.944395 84.844456
h -7.808717 61.537232 74.106335

2.1.6. Triplet product complex (PC3), E= -1528.11454925272 au.

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C -7.320329 60.909528 74.859487
H -6.355286 61.384844 75.079864
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H -5.079959 58.260737 82.794088
H -4.040010 57.211856 83.789133
C -6.327945 60.892878 84.633425
H -6.142548 60.090085 85.346585
H -5.374664 61.347192 84.356306
N -9.448761 57.149681 83.869323
H -10.181982 56.461780 84.031241
C -9.135338 57.459281 82.565950
O -9.756382 56.938091 81.603493
C -8.104974 58.451591 82.424531
C -7.698628 59.151008 83.596806
N -8.031188 58.794832 84.828471
C -8.867471 57.731917 85.018502
O -9.157588 57.276366 86.126705
N -7.628369 58.801936 81.196577
C -6.867034 59.933736 81.028472
C -6.558017 60.739331 82.153376

N -6.904250 60.276259 83.438187
C -6.452025 60.328276 79.742508
H -6.664589 59.659825 78.916055
C -5.829426 61.551802 79.527212
C -5.470722 61.989709 78.128831
H -6.141490 62.788498 77.785340
H -5.556055 61.158978 77.424368
H -4.451592 62.388061 78.069252
C -5.593227 62.399822 80.638744
C -5.053904 63.796543 80.452947
H -4.660481 64.196633 81.395044
H -5.842582 64.476113 80.100755
H -4.269479 63.839701 79.688162
C -5.925371 61.967941 81.924364
H -5.719394 62.637687 82.748443
O -7.756386 57.611522 78.812407
H -8.473276 58.069985 78.271102
H -7.987590 56.673208 78.978655
O -8.116692 55.199446 80.188937
H -7.327569 55.452761 80.739533
H -8.847570 55.651634 80.665923
O -8.004910 52.386095 80.169789
H -7.372263 52.261601 80.911645
H -8.137869 53.361336 80.139687
h -6.943706 61.653978 85.112624
h -6.002705 52.715984 85.111047
h -7.829002 61.549137 74.138225

2.2 Downward orientation (using snapshot 1)

2.2.1. Singlet reactant complex (RC1), E= -1528.10698761189 au.

72

C -7.913561 61.242690 74.517787
H -6.976220 61.624811 74.944023
H -7.687018 60.243542 74.113799
C -8.955358 61.113416 75.639198
H -9.969678 60.930630 75.255750
H -9.007538 62.036561 76.230752
C -8.553286 59.950204 76.547644
H -7.533051 60.110356 76.916388
H -8.502171 59.027505 75.954586
N -9.398088 59.673144 77.708714
H -9.402922 60.453493 78.377441
H -10.360941 59.526320 77.408809
C -5.722645 54.071021 85.096222
H -5.025120 53.926350 85.931464
H -6.617160 54.554219 85.516476
C -5.069392 55.003446 84.037389
H -4.882919 54.510055 83.072642
H -4.085900 55.318215 84.404116
C -5.889267 56.283728 83.757241
H -5.219845 57.129205 83.567246
H -6.477735 56.529456 84.650554
N -6.813234 56.207316 82.622712
C -7.687342 55.046161 82.651412
H -8.507817 55.186594 81.944251
H -8.101836 54.938230 83.660194
H -7.152583 54.124921 82.376505
C -6.166788 56.372429 81.323873
H -5.430667 57.179537 81.381020

H -6.903406 56.648325 80.563436
H -5.641937 55.454357 81.006196
C -5.698482 61.068092 84.722166
H -5.340970 60.280618 85.387211
H -4.843472 61.613348 84.327793
N -9.107512 57.493698 84.289408
H -9.825995 56.811182 84.517439
C -8.891002 57.746527 82.957368
O -9.634777 57.278720 82.084229
C -7.837962 58.744283 82.687278
C -7.247859 59.389423 83.845525
N -7.505363 59.070844 85.103116
C -8.445954 58.118015 85.373382
O -8.787147 57.809155 86.516359
N -7.588014 59.059520 81.447175
C -6.766559 60.120515 81.212968
C -6.237868 60.903765 82.279497
N -6.396874 60.432429 83.586106
C -6.541630 60.508667 79.872804
H -6.895336 59.823265 79.108051
C -5.938517 61.716255 79.568932
C -5.774130 62.160434 78.135517
H -4.773605 62.566114 77.940320
H -6.494991 62.949800 77.881486
H -5.943104 61.323823 77.452199
C -5.540622 62.564080 80.644458
C -5.070061 63.967153 80.364099
H -5.897354 64.581593 79.986248
H -4.307527 63.999022 79.574975
H -4.679829 64.447155 81.269081
C -5.644030 62.137829 81.965724
H -5.334105 62.815684 82.751550
O -9.163680 55.973599 79.645598
H -8.560272 55.238168 79.853678
H -9.114295 56.590077 80.402950
O -8.738020 53.288562 79.604350
H -9.652382 53.613733 79.586977
H -8.733686 52.585539 80.283716
O -7.471452 57.695478 78.223532
H -7.928257 56.922307 78.605146
H -8.218317 58.343411 78.199440
h -6.323814 61.765889 85.279037
h -6.043368 53.071796 84.801685
h -8.194113 61.872393 73.673494

2.2.2. Triplet reactant complex (RC3), E= -1528.07116972744 au.

72

C -7.843430 61.061343 74.643197
H -6.896437 61.450250 75.041168
H -7.609999 60.086289 74.186357
C -8.815800 60.848245 75.816015
H -9.839330 60.623065 75.482166
H -8.880463 61.751331 76.436862
C -8.293709 59.684995 76.668078
H -7.257685 59.896241 76.959535
H -8.248630 58.776446 76.051478
N -9.026658 59.342841 77.886872
H -9.093855 60.136708 78.538418
H -9.981303 59.070779 77.656849
C -5.405325 54.365458 84.959719
H -4.735604 54.173551 85.808627

H -6.256842 54.933063 85.357060
C -4.639821 55.248007 83.942013
H -4.142372 54.686796 83.139688
H -3.846348 55.775436 84.483991
C -5.462819 56.404289 83.304058
H -4.788056 57.165241 82.908557
H -6.109250 56.849748 84.066905
N -6.290292 55.939765 82.209708
C -7.507735 55.215823 82.474845
H -8.317587 55.682851 81.914016
H -7.731511 55.239788 83.541851
H -7.390888 54.190424 82.105036
C -5.867838 56.049508 80.841975
H -4.811021 56.323634 80.796543
H -6.478585 56.818004 80.308579
H -6.061046 55.096903 80.331942
C -5.777178 60.928242 84.870403
H -5.432964 60.165089 85.570203
H -4.909556 61.449500 84.465804
N -9.291393 57.483056 84.487791
H -10.059703 56.852899 84.696257
C -9.038365 57.733574 83.149887
O -9.788006 57.220097 82.274091
C -7.909408 58.569101 82.882133
C -7.354684 59.245751 84.012643
N -7.640120 58.973396 85.289651
C -8.617635 58.071156 85.572484
O -8.970789 57.768563 86.724549
N -7.527548 58.773287 81.585901
C -6.757470 59.873922 81.367716
C -6.306075 60.723200 82.428354
N -6.463423 60.266364 83.756085
C -6.453087 60.257181 80.036673
H -6.747047 59.566496 79.253989
C -5.885728 61.488966 79.728392
C -5.679239 61.899096 78.286777
H -4.730277 62.428160 78.134140
H -6.476175 62.574577 77.942657
H -5.685238 61.022889 77.631607
C -5.575595 62.379189 80.786354
C -5.135125 63.797685 80.516567
H -5.972829 64.419194 80.170167
H -4.383775 63.864225 79.718762
H -4.733825 64.263436 81.425471
C -5.753148 61.966572 82.112560
H -5.499850 62.667725 82.898834
O -9.482725 56.141127 79.775592
H -9.300721 55.254886 80.135983
H -9.416764 56.758773 80.536669
O -8.505397 53.600253 79.911749
H -8.837534 53.505009 79.006239
H -8.670106 52.739839 80.351345
O -7.353246 57.188461 78.469317
H -8.038601 56.608284 78.871209
H -7.901727 58.014411 78.379244
h -6.388508 61.654883 85.405504
h -5.807444 53.389537 84.687792
h -8.171709 61.719025 73.838365

2.2.3. Singlet transition state (TS1), E= -1528.07823822726 au.

72

C -7.877627 61.175447 74.558928
H -6.940834 61.570954 74.974137
H -7.637631 60.186032 74.138609
C -8.890337 61.007215 75.701830
H -9.902618 60.775209 75.339871
H -8.972774 61.930148 76.290279
C -8.408867 59.870131 76.606450
H -7.389477 60.089257 76.947721
H -8.325937 58.946772 76.017326
N -9.207558 59.557515 77.790840
H -9.269283 60.355213 78.437662
H -10.159650 59.321037 77.515118
C -5.510399 54.260727 85.002859
H -4.845179 54.104809 85.861854
H -6.377011 54.822508 85.377832
C -4.760019 55.111285 83.937477
H -4.530193 54.565928 83.011431
H -3.792501 55.409786 84.356266
C -5.472957 56.427258 83.548836
H -4.742479 57.184666 83.255856
H -6.028420 56.814138 84.411543
N -6.400591 56.262043 82.422622
C -7.400999 55.210882 82.542407
H -8.255209 55.436048 81.908617
H -7.738975 55.159429 83.580831
H -6.972676 54.248802 82.240896
C -6.109712 56.762680 81.188484
H -5.092444 57.134213 81.053506
H -6.865155 57.895674 81.049573
H -6.545851 56.237911 80.340260
C -5.705628 60.979794 84.806397
H -5.351776 60.207904 85.491056
H -4.844186 61.520144 84.415968
N -9.168368 57.476673 84.361362
H -9.911874 56.817054 84.572125
C -8.923763 57.743840 83.029971
O -9.633550 57.221062 82.139564
C -7.823209 58.633191 82.783918
C -7.242674 59.286971 83.917736
N -7.535683 58.998233 85.183372
C -8.505345 58.080381 85.453189
O -8.866869 57.780550 86.597275
N -7.478015 58.889436 81.487174
C -6.712397 60.044334 81.276697
C -6.239963 60.821189 82.357104
N -6.377625 60.325101 83.673259
C -6.489108 60.466832 79.957479
H -6.810273 59.800357 79.164488
C -5.912466 61.700525 79.674835
C -5.741065 62.156499 78.244595
H -4.771949 62.642832 78.077018
H -6.515350 62.883278 77.961890
H -5.818510 61.308313 77.557913
C -5.561571 62.541270 80.759908
C -5.115317 63.963360 80.522694
H -5.954065 64.587689 80.186712
H -4.365040 64.039217 79.724912
H -4.712963 64.409880 81.440133

C -5.692755 62.082649 82.070321
H -5.411236 62.752396 82.873230
O -9.180971 56.010590 79.658159
H -8.746433 55.178455 79.920317
H -9.188728 56.592705 80.445585
O -8.747357 53.325849 79.644520
H -9.678679 53.484928 79.423609
H -8.763875 52.601138 80.302614
O -7.317517 57.583033 78.377685
H -7.878454 56.849698 78.702695
H -8.012368 58.285981 78.299506
h -6.331298 61.686956 85.350939
h -5.899391 53.273473 84.753701
h -8.181912 61.811043 73.727366

2.2.4. Triplet transition state (TS3), E= -1528.05401396022 au.

72

C -7.891883 61.240885 74.485564
H -6.959306 61.626370 74.918606
H -7.660627 60.241924 74.083433
C -8.942147 61.109791 75.597869
H -9.951269 60.916496 75.205441
H -9.007241 62.035024 76.183517
C -8.539888 59.953887 76.514530
H -7.536927 60.139317 76.916023
H -8.453665 59.035274 75.917451
N -9.411964 59.655826 77.649245
H -9.398185 60.402348 78.355483
H -10.374524 59.541667 77.335685
C -5.381430 54.330062 84.815475
H -4.669117 54.252891 85.647810
H -6.200520 54.971167 85.168714
C -4.668226 55.018620 83.619189
H -4.392981 54.334238 82.806006
H -3.724813 55.438873 83.985715
C -5.419698 56.211589 82.994874
H -4.705988 56.877271 82.504701
H -5.929087 56.780047 83.789242
N -6.402105 55.827569 81.989673
C -7.517594 54.989658 82.395215
H -8.399803 55.244166 81.814617
H -7.731062 55.148323 83.456926
H -7.273164 53.937947 82.225750
C -6.410714 56.422655 80.739567
H -5.419250 56.734541 80.400233
H -7.046488 57.644918 80.929128
H -7.004343 55.889557 80.004582
C -5.769682 61.020424 84.791589
H -5.413711 60.255191 85.483434
H -4.910707 61.550289 84.380198
N -9.221025 57.482521 84.390897
H -9.975516 56.834012 84.596123
C -8.923249 57.694284 83.059684
O -9.622622 57.172890 82.155649
C -7.812613 58.569936 82.836854
C -7.332991 59.317119 83.948479
N -7.673840 59.084716 85.214954
C -8.612350 58.136749 85.485034
O -8.995387 57.847137 86.626714
N -7.333465 58.752309 81.569973
C -6.651352 59.902299 81.290995

C -6.285215 60.785549 82.348488
N -6.460964 60.354535 83.679285
C -6.367001 60.244420 79.953137
H -6.642268 59.521382 79.187756
C -5.844882 61.492060 79.629514
C -5.662238 61.892282 78.184067
H -4.697811 62.384865 78.006802
H -6.442029 62.601287 77.870139
H -5.725275 61.017192 77.530471
C -5.563752 62.409241 80.675361
C -5.127616 63.821587 80.372598
H -5.957645 64.418503 79.971809
H -4.348572 63.862492 79.600230
H -4.763906 64.324557 81.276855
C -5.753784 62.033760 82.008345
H -5.531923 62.758233 82.782242
O -9.420766 56.010194 79.618529
H -9.076111 55.120087 79.823284
H -9.326850 56.541933 80.434288
O -8.689029 53.357721 79.533512
H -9.455996 53.243414 78.950938
H -8.761591 52.640404 80.196462
O -7.618674 57.608753 78.225043
H -8.164432 56.892450 78.608270
H -8.304845 58.320043 78.140099
h -6.382976 61.740339 85.333493
h -5.807924 53.332201 84.713287
h -8.170109 61.870268 73.640264

2.2.5. Singlet product complex (PC1), E= -1528.12869463715 au.

72

C -7.831154 61.059636 74.622462
H -6.877202 61.435169 75.016186
H -7.620103 60.073514 74.179770
C -8.817507 60.894543 75.789857
H -9.843759 60.690969 75.450421
H -8.864460 61.811720 76.391547
C -8.355420 59.738840 76.683644
H -7.328930 59.927394 77.023508
H -8.316637 58.812832 76.093238
N -9.170113 59.471456 77.872735
H -9.228466 60.301284 78.481904
H -10.124269 59.243951 77.594549
C -5.728938 54.053858 85.137758
H -5.068543 53.654620 85.916222
H -6.588679 54.508531 85.650310
C -4.993517 55.141376 84.312441
H -4.428546 54.724533 83.466758
H -4.244913 55.617098 84.956757
C -5.922840 56.263169 83.769975
H -5.655134 57.212886 84.240572
H -6.965200 56.051023 84.056392
N -5.844835 56.443857 82.318470
C -6.496855 55.429785 81.511130
H -6.380535 55.672990 80.451222
H -7.573367 55.368599 81.734721
H -6.041533 54.451036 81.698409
C -5.621485 57.689242 81.779901
H -4.996058 58.298804 82.429826
H -7.461409 58.236247 80.760618
H -5.265875 57.664841 80.748223

C -5.690617 60.980977 84.863798
H -5.349825 60.220274 85.567205
H -4.817969 61.513154 84.482857
N -9.254383 57.582548 84.318121
H -10.001672 56.917380 84.493195
C -8.840689 57.722353 82.999254
O -9.466384 57.112881 82.084496
C -7.749962 58.597080 82.839115
C -7.267427 59.309525 83.955238
N -7.674808 59.110515 85.206775
C -8.655976 58.193911 85.440106
O -9.080011 57.911678 86.569612
N -6.972490 58.642911 81.620101
C -6.532094 59.997753 81.324260
C -6.179459 60.808704 82.413791
N -6.328976 60.309227 83.727527
C -6.359018 60.424142 80.011486
H -6.601353 59.742750 79.205161
C -5.880994 61.706555 79.726363
C -5.740688 62.164116 78.293083
H -4.772171 62.643672 78.101598
H -6.514832 62.897150 78.028249
H -5.840558 61.318212 77.605031
C -5.567677 62.554586 80.807654
C -5.145318 63.985388 80.571515
H -5.993459 64.603207 80.247480
H -4.403319 64.071266 79.767050
H -4.736801 64.433463 81.485247
C -5.700672 62.094814 82.121467
H -5.459453 62.778360 82.925137
O -9.296513 55.725881 79.747035
H -8.858241 54.854097 79.816204
H -9.305472 56.122556 80.645784
O -8.803873 53.098653 79.290056
H -9.721751 53.222110 79.001501
H -8.851255 52.425127 80.000292
O -7.597990 57.697420 79.206703
H -8.126438 56.862060 79.264787
H -8.239551 58.331301 78.749561
h -6.319227 61.696765 85.393481
h -6.118813 53.207822 84.571819
h -8.151534 61.714555 73.812213

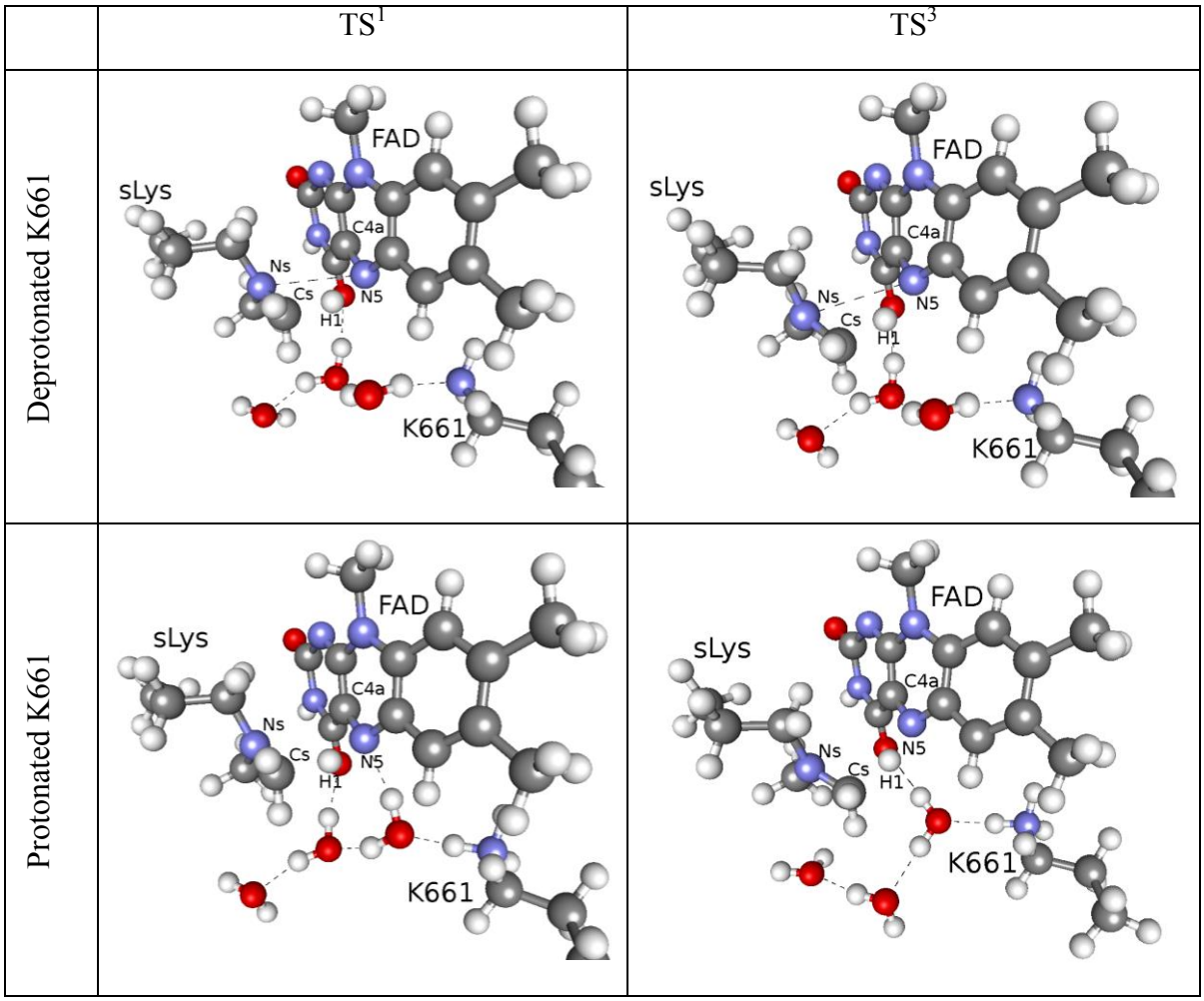
2.2.6. Triplet product complex (PC3), E= -1528.08057857786 au.

72

C -7.849128 61.145789 74.575502
H -6.917643 61.544450 74.999059
H -7.607403 60.155127 74.159594
C -8.886280 60.991014 75.697637
H -9.889425 60.758627 75.310973
H -8.983053 61.921831 76.270522
C -8.458227 59.865555 76.642437
H -7.476685 60.097443 77.074663
H -8.328672 58.938610 76.065636
N -9.370665 59.574807 77.752313
H -9.435676 60.370741 78.402153
H -10.309265 59.399799 77.394821
C -5.988261 53.709920 85.277820
H -5.280299 53.381403 86.047237
H -6.884859 54.078587 85.798751
C -5.377032 54.858669 84.452131

H -4.622676 54.519677 83.727004
H -4.843452 55.545188 85.121078
C -6.440789 55.675973 83.704817
H -7.135008 56.105173 84.438217
H -7.032874 55.034976 83.038077
N -5.873080 56.744933 82.904220
C -5.636942 56.495165 81.492467
H -5.379241 57.435205 80.997185
H -6.553357 56.107104 81.030511
H -4.814183 55.781148 81.331352
C -5.247886 57.767170 83.552222
H -5.168753 57.739264 84.629338
H -7.759707 58.513202 80.654972
H -4.638656 58.441927 82.974441
C -5.800259 61.132687 84.808937
H -5.464236 60.338229 85.477189
H -4.925979 61.662839 84.431337
N -9.196175 57.563020 84.317681
H -9.905062 56.863896 84.518851
C -8.896777 57.786527 82.990405
O -9.531960 57.191341 82.076122
C -7.884306 58.766688 82.769931
C -7.313249 59.425939 83.902895
N -7.671852 59.175590 85.167490
C -8.602488 58.227970 85.423363
O -8.992706 57.918035 86.558500
N -7.479078 59.077265 81.507962
C -6.701962 60.187338 81.274225
C -6.283001 60.989047 82.368202
N -6.495329 60.525870 83.670011
C -6.401274 60.576796 79.955707
H -6.686187 59.906851 79.152866
C -5.816145 61.805080 79.680636
C -5.597713 62.236179 78.249804
H -4.581613 62.613842 78.078335
H -6.285033 63.046521 77.971327
H -5.772111 61.401953 77.561860
C -5.503226 62.665320 80.764419
C -5.042651 64.080218 80.518600
H -5.879121 64.712358 80.190685
H -4.299946 64.144721 79.712955
H -4.625905 64.524841 81.430495
C -5.699281 62.232478 82.075809
H -5.454422 62.914139 82.880722
O -8.965160 55.658907 79.925129
H -8.425623 54.850842 80.009065
H -9.041045 56.088750 80.806751
O -8.740962 53.053706 79.300143
H -9.614036 53.476520 79.271357
H -8.811836 52.373406 80.000588
O -7.750266 57.897158 79.126575
H -8.116390 56.988103 79.235410
H -8.465340 58.421586 78.648348
h -6.417679 61.838819 85.364126
h -6.270928 52.835735 84.691334
h -8.151992 61.781054 73.743168

2.3 Visualization of TS geometries in 'downward' orientation



Singlet and triplet transition states for 'downward' orientation in ball-and-stick representation for deprotonated and protonated K661. Hydrogen bonds are marked by black dashed lines. The chosen atom numbering is also given.

3. Tables

Table S1. QM/MM relative energies ΔE and free energies ΔG (in kcal/mol) of the stationary points (see Fig. 1) for the snapshots with upward and downward orientation. Average values and standard deviations are given in each case. K661 is ***deprotonated***.

	RC1		**TS1**		**PC1**		**RC3**		**TS3**		**PC3**	
Orientation	**ΔE**	**ΔG**	**ΔE**	**ΔG**	**ΔE**	**ΔG**	**ΔE**	**ΔG**	**ΔE**	**ΔG**	**ΔE**	**ΔG**
Upward – 1	0.00	0.00	20.61	19.28	2.04	2.41	28.96	28.58	46.70	43.03	28.87	28.35
Upward – 2	0.00	0.00	24.08	20.75	3.50	3.87	30.03	29.64	46.86	43.12	27.64	27.09
Upward – 3	0.00	0.00	24.12	20.81	2.21	2.57	30.84	30.44	48.98	45.28	29.67	29.19
Upward – 4	0.00	0.00	25.75	22.46	1.53	1.90	32.76	32.39	50.53	46.77	27.37	26.81
Upward – 5	0.00	0.00	24.62	21.21	0.95	1.36	34.70	34.31	46.85	42.97	26.12	25.68
Average:.	0.00	0.00	23.84	20.90	2.05	2.42	31.46	31.07	47.98	44.23	27.93	27.42
Std. Dev.	0.00	0.00	1.93	1.14	0.95	0.94	2.28	2.28	1.71	1.72	1.38	1.37
	RC1		**TS1**		**PC1**		**RC3**		**TS3**		**PC3**	
Orientation	**ΔE**	**ΔG**	**ΔE**	**ΔG**	**ΔE**	**ΔG**	**ΔE**	**ΔG**	**ΔE**	**ΔG**	**ΔE**	**ΔG**
Downward - 1	0.00	0.00	18.52	14.98	-12.87	-12.45	23.48	22.50	37.15	33.15	22.65	21.95
Downward - 2	0.00	0.00	19.96	16.49	-16.32	-15.67	20.14	19.85	37.39	33.45	27.26	26.56
Downward - 3	0.00	0.00	18.25	14.76	-11.58	-11.20	18.41	18.07	34.22	30.47	22.04	21.30
Downward - 4	0.00	0.00	19.74	16.25	-7.03	-6.57	21.80	21.50	39.05	35.28	26.36	25.98
Downward - 5	0.00	0.00	17.80	14.25	-14.87	-14.44	20.73	19.96	37.53	33.59	24.11	23.30
Average:	0.00	0.00	18.85	15.35	-12.53	-12.07	20.91	20.38	37.07	33.19	24.48	23.82
Std. Dev.	0.00	0.00	0.95	0.98	3.57	3.53	1.89	1.70	1.76	1.73	2.27	2.36

Table S2. QM/MM relative energies ΔE and free energies ΔG (in kcal/mol) of the stationary points (see Fig. 1) for the snapshots with upward and downward orientation. Average values and standard deviations are given in each case. K661 is protonated.

	RC1		TS1		PC1		RC3		TS3		PC3	
Orientation	**ΔE**	**ΔG**	**ΔE**	**ΔG**	**ΔE**	**ΔG**	**ΔE**	**ΔG**	**ΔE**	**ΔG**	**ΔE**	**ΔG**
Upward - 1	0.00	0.00	24.79	21.35	15.04	15.20	28.13	27.79	47.79	43.93	30.79	30.74
Upward - 2	0.00	0.00	25.81	22.49	14.86	15.00	28.92	28.59	50.09	46.45	34.86	34.83
Upward - 3	0.00	0.00	21.67	18.23	12.14	12.11	26.41	26.04	47.48	43.80	37.50	37.37
Upward - 4	0.00	0.00	27.03	23.73	13.65	14.02	30.55	30.28	53.88	50.14	38.11	38.00
Upward - 5	0.00	0.00	25.95	22.74	11.67	12.13	33.34	33.05	47.37	43.55	35.23	35.16
Average:.	0.00	0.00	25.05	21.71	13.47	13.69	29.47	29.15	49.32	45.57	35.30	35.22
Std. Dev.	0.00	0.00	2.05	2.12	1.54	1.50	2.63	2.66	2.78	2.81	2.88	2.85
	RC1		**TS1**		**PC1**		**RC3**		**TS3**		**PC3**	
Orientation	**ΔE**	**ΔG**	**ΔE**	**ΔG**	**ΔE**	**ΔG**	**ΔE**	**ΔG**	**ΔE**	**ΔG**	**ΔE**	**ΔG**
Downward - 1	0.00	0.00	22.19	18.77	15.89	15.47	26.57	26.28	43.16	41.25	33.48	32.32
Downward - 2	0.00	0.00	20.33	17.06	16.69	16.28	21.94	21.77	39.81	35.98	36.62	36.19
Downward - 3	0.00	0.00	22.36	19.05	17.39	17.05	26.00	25.61	46.55	42.73	41.69	41.26
Downward - 4	0.00	0.00	28.07	24.28	13.44	13.38	23.70	23.16	47.66	43.48	39.89	39.20
Downward - 5	0.00	0.00	24.69	20.88	15.80	15.88	24.67	24.09	46.42	42.23	37.73	37.21
Average:	0.00	0.00	23.53	20.01	15.84	15.61	24.58	24.18	44.72	41.13	37.88	37.24
Std. Dev.	0.00	0.00	2.97	2.75	1.49	1.38	1.85	1.82	3.22	2.99	3.14	3.37

Table S3. QM/MM relative energies ΔE and free energies ΔG (in kcal/mol) of the stationary points (see Fig. 1) for the two snapshots of the K661M MD simulation (see text for details). Average values and standard deviations are given in each case.

	RC1		**TS1**		**PC1**		**RC3**		**TS3**		**PC3**	
Orientation	**ΔE**	**ΔG**	**ΔE**	**ΔG**	**ΔE**	**ΔG**	**ΔE**	**ΔG**	**ΔE**	**ΔG**	**ΔE**	**ΔG**
K661M - 1	0.00	0.00	21.65	18.24	0.67	-0.19	26.87	26.50	47.73	43.91	31.76	31.42
K661M - 2	0.00	0.00	23.08	19.77	2.93	3.39	27.55	27.21	48.61	44.89	32.67	32.45
Average:.	0.00	0.00	22.37	19.01	1.80	1.60	27.21	26.86	48.17	44.40	32.22	31.94
Std. Dev.	0.00	0.00	1.01	1.08	1.60	2.53	0.48	0.50	0.62	0.69	0.64	0.73

Table S4. QM/MM relative energies (in kcal/mol) of the stationary points (see Fig. 1) in upward and downward orientation. Single-point energies are computed with QM=B3LYP-D/TZVPP at QM/MM geometries optimized with QM=B3LYP-D/6-31G*.

Relative Energy (kcal/mol)	**Deprotonated K661**		**Protonated K661**	
	Upward	**Downward**	**Upward**	**Downward**
Direct Hydride Transfer (HT) (singlet manifold)				
RC^1	0.00	0.00	0.00	0.00
TS^1	20.7	15.6	24.0	18.3
PC^1	0.36	-13.3	7.9	10.1
Radical mechanism (SET) (triplet manifold)				
RC^3	28.2	22.0	25.4	25.7
TS^3	43.3	33.4	44.2	40.4
PC^3	25.8	15.3	27.6	29.0

Table S5. QM/MM relative energies (in kcal/mol) of the stationary points (see Fig. 1) in upward and downward orientation. Single-point energies are obtained with QM=B3LYP-D/6-31G* using the large QM region (173-174 atoms, see text for details).

Relative Energy (kcal/mol)	**Deprotonated K661**		**Protonated K661**	
	Upward	Downward	Upward	Downward
Direct Hydride Transfer (HT) (singlet manifold)				
RC^1	0.00	0.00	0.00	0.00
TS^1	20.7	16.4	25.5	19.6
PC^1	-2.42	-16.8	11.2	12.7
Radical mechanism (SET) (triplet manifold)				
RC^3	27.2	19.1	25.5	23.6
TS^3	43.8	33.3	46.8	40.7
PC^3	29.0	13.9	29.9	30.3

Table S6. Wiberg bond orders for selected atom pairs in the HT and SET transition states in upward and downward orientation, for deprotonated and protonated K661. Values from NBO analysis at the B3LYP-D/6-31G* level, with inclusion of the MM point charges.

Relative Energy (kcal/mol)	**Upward orientation**		**Downward orientation**	
	Depr. K661	Prot. K661	Depr. K661	Prot. K661
Direct Hydride Transfer (HT) (singlet manifold)				
Cs – H1	0.3777	0.3750	0.3896	0.3509
N5 – H1	0.4407	0.4400	0.4236	0.4661
Radical mechanism (SET) (triplet manifold)				
Cs – H1	0.3259	0.3295	**0.4115**	0.3349
N5 – H1	0.4344	0.4344	**0.3625**	0.4355

A late transition state is predicted in each case, except for the proton transfer on the SET pathway in downward orientation (given in bold).

Table S7. Gibbs relative free energies (kcal/mol) of the stationary points obtained from different Kohn-Sham (KS) treatments and for different multiplicities at the QM-only (B3LYP/6-31G*) level. Energies are given relative to the RC minimum energy of the RKS singlet. They are calculated using two model systems with different size of the QM region.

	Standard QM Region			**Extended QM Region**		
Type / Multiplicity	**RC**	**TS**	**PC**	**RC**	**TS**	**PC**
RKS-Singlet	0.00	31.3	16.7	0.00	29.5	14.2
ROKS-Singlet	N/A	31.3	N/A	N/A	29.4	N/A
UKS-Singlet	0.00	31.3	16.9	-0.40	29.4	14.0
UKS-Triplet	35.4	37.5	31.3	35.5	38.1	33.1

RKS, UKS, ROKS: Restricted, Unrestricted, and Restricted-Open-Shell Kohn-Sham, respectively. Each TS geometry and the corresponding RC and PC geometries (determined by IRC following) were optimized at the given level of theory and subjected to vibrational analysis. The standard QM region consisted of sLys, FAD, and two water molecules, while the extended QM region also contained K661 and one more water molecule. The HOMO and LUMO orbitals were mixed at each step of UKS singlet geometry optimizations. In the case of ROKS-Singlet, RC and PC minimum energies are not available from the IRC following procedure due to the lack of analytical second derivatives for ROKS.

The small energy differences among RKS, ROKS, and UKS singlets (observed mainly when using the extended QM region) are due to slight differences in the H-bonding network. The computed spin densities for the singlets show that each KS treatment converges to a closed-shell solution at each stationary point.

Table S8. Selected distances (R, in Å), angles and dihedral angles (θ, in degree) in QM(B3LYP-D/6-31G*)/MM minimum geometries with 'upward' or 'downward' orientation for ***protonated K661***.

	Upward Orientation						**Downward Orientation**					
	RC^1	**TS^1**	**PC^1**	**RC^3**	**TS^3**	**PC^3**	**RC^1**	**TS^1**	**PC^1**	**RC^3**	**TS^3**	**PC^3**
θ(Cs-H1-N5)	119.8	155.9	122.5	91.2	154.2	81.0	85.1	157.6	137.4	92.0	159.7	149.8
R(N5-H1)	2.61	1.25	1.02	3.28	1.22	1.03	2.96	1.21	1.03	2.71	1.22	1.07
R(Cs-H1)	1.10	1.40	2.47	1.09	1.54	3.79	1.10	1.42	2.14	1.09	1.49	1.86
R(Ns-C4a)	2.78	2.87	3.31	3.32	3.18	3.38	2.71	2.79	3.01	2.85	3.19	3.14
R(Cs-N5)	3.29	2.59	3.14	3.48	2.69	3.76	3.07	2.58	2.98	2.96	2.67	2.83
R(Cs-Ns)	1.46	1.35	1.28	1.44	1.39	1.38	1.46	1.35	1.29	1.45	1.38	1.38
R(C4a-N5)	1.31	1.38	1.43	1.37	1.37	1.37	1.31	1.38	1.42	1.37	1.37	1.37
θ(Ns-Cs-N5-C4a)	-18.7	-16.7	-20.8	-22.9	-30.4	-22.9	5.96	4.91	2.98	12.8	-13.0	-14.0

Table S9. Selected distances (R, in Å), angles and dihedral angles (θ, in degree) in QM(B3LYP-D/6-31G*)/MM minimum geometries for the ***K661M mutant***.

	RC^1	**TS^1**	**PC^1**	**RC^3**	**TS^3**	**PC^3**
θ(Cs-H1-N5)	104.2	153.2	50.9	88.6	151.5	80.7
R(N5-H1)	2.62	1.24	1.03	2.83	1.23	1.05
R(Cs-H1)	1.09	1.38	2.60	1.10	1.49	3.56
R(Ns-C4a)	2.72	2.81	2.91	2.87	3.14	3.09
R(Cs-N5)	3.07	2.55	2.10	3.01	2.63	3.54
R(Cs-Ns)	1.46	1.35	1.32	1.45	1.38	1.38
R(C4a-N5)	1.30	1.37	1.42	1.37	1.37	1.36
θ(Ns-Cs-N5-C4a)	-28.1	-19.2	-20.9	-22.0	-28.3	-22.8

Table S10. QM/MM-level ^{15}N and ^{2}H kinetic isotope effect (KIE) values without and with one-dimensional Wigner tunneling corrections for the HT and SET mechanisms. KIE values are computed at pH=7 at the QM(B3LYP-D/6-31G*)/MM level for 'upward' and 'downward' orientations with deprotonated K661 and deprotonated sLys. Experimental and computational KIE values are also given for another closely related amine oxidase (MTOX).

	Experimental KIE	Orientation	Computed KIE (HT/SET)	KIE with tunneling corr. (HT/SET)
LSD1 ($^{15}N/^{14}N$)	N/A	upward:	0.9917 / 0.9959	0.9953 / 0.9961
		downward:	0.9928 / 0.9938	0.9959 / 0.9946
LSD1 ($^{1}H/^{2}H$)	4.0 ± 0.2[a]	upward:	3.80 / 5.05	4.92 / 6.15
		downward:	4.36 / 5.24	5.72 / 6.97
MTOX ($^{15}N/^{14}N$)[b]	0.9952±0.0005[c]		0.9921 / 0.9978[d]	0.9927 / N/A
MTOX ($^{1}H/^{2}H$)[b]	6.8 ± 0.6[e]		4.00 / 1.38[d]	4.77 / N/A

[a] Taken from Ref 8; value at pH=7, all ^{1}H on the two methyl groups of sLys replaced by ^{2}H.

[b] Computed values obtained at the Onsager/B3LYP/6-31+G** level, taken from Ref 9.

[c] Taken from Ref 9; value at pH=7.5.

[d] Equilibrium isotope effects (instead of KIE) from Ref 9 for the radical (SET) mechanism.

[e] Taken from Ref 10; value at pH=8.0.

- Kinetic isotope effects and one-dimensional Wigner corrections were calculated following the procedure described in Ref 11.
- Deuterium KIE values were computed by replacing all hydrogen atoms (^{1}H) on the two methyl groups of sLys by deuterium (^{2}H).

4. Figures

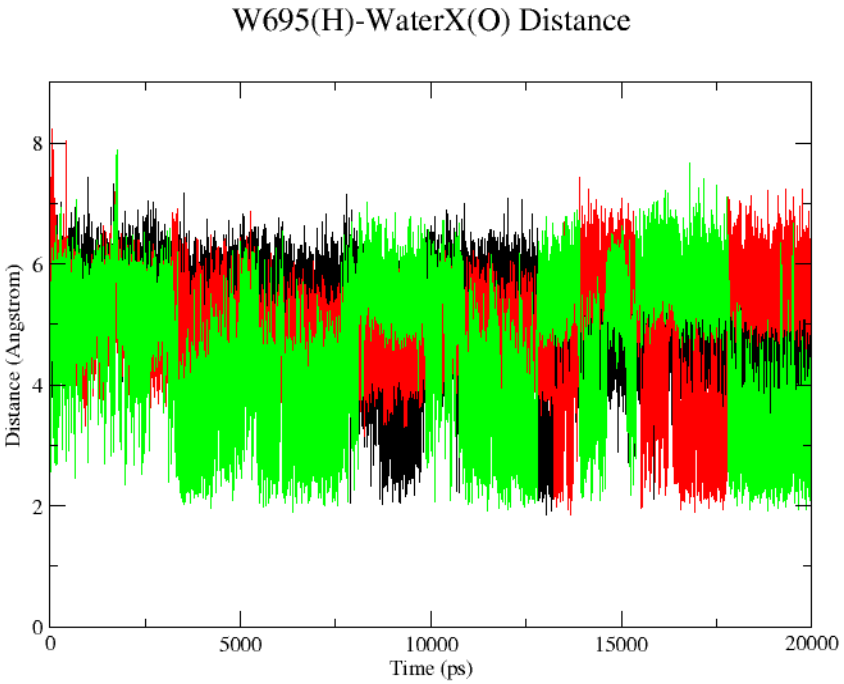


Figure S1. Plot of the distances (in Å) between HE1 of W695 and O of each of the three active-site water molecules throughout the MD simulation run with sLys-NMe_2H^+ and Lys661-NH_2. See text for definition of the MD simulation system.

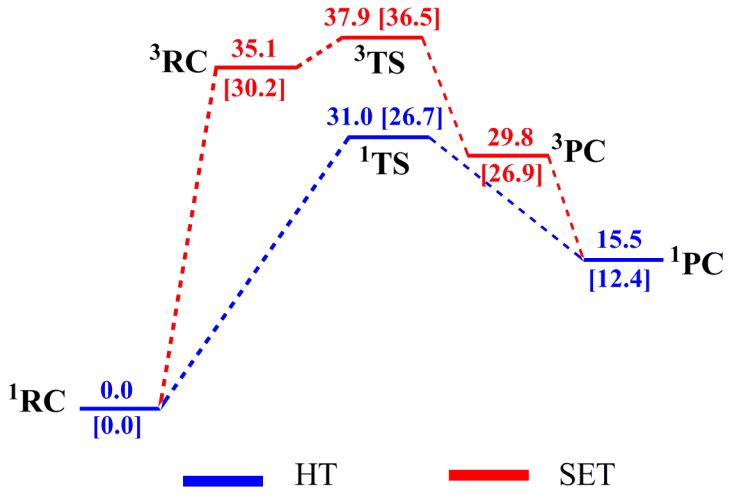


Figure S2. QM-only (B3LYP/6-31G*) Gibbs free energy profile (in kcal/mol) in vacuum and water [values in brackets] for HT and SET pathways (see text in the main paper). See Figure 1 for the definition of the stationary points.

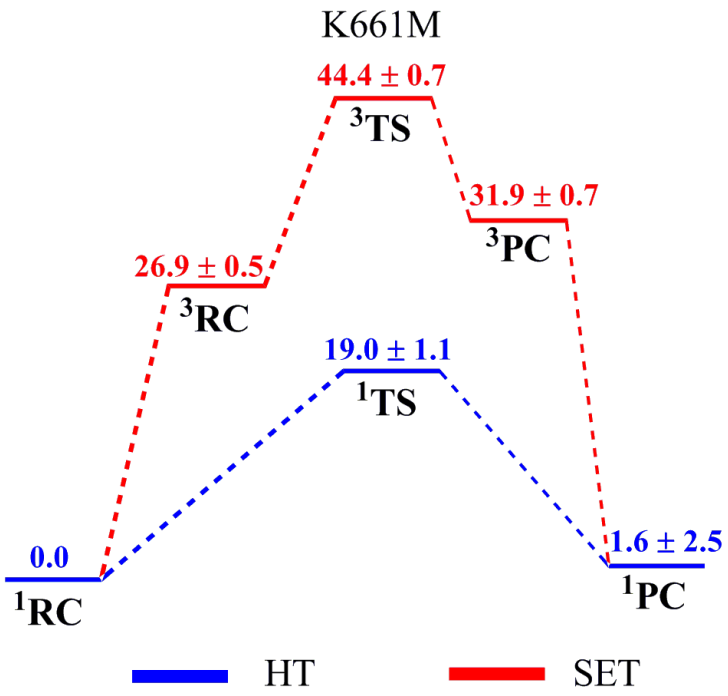


Figure S3. QM(B3LYP-D/6-31G*)/MM Gibbs free energy profiles (in kcal/mol) for HT and SET pathways in the K661 mutant. See Figure 1 for the definition of the stationary points.

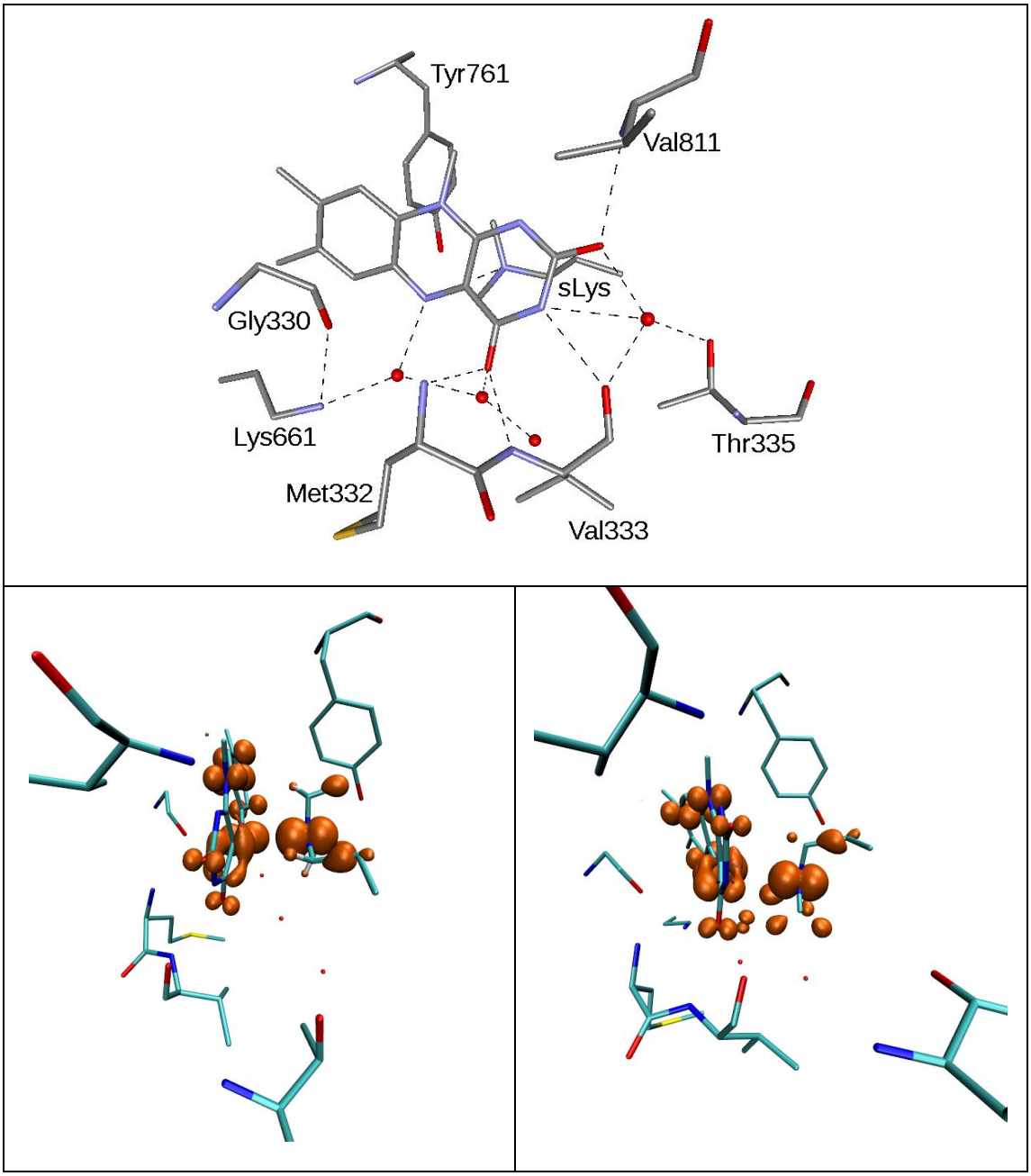
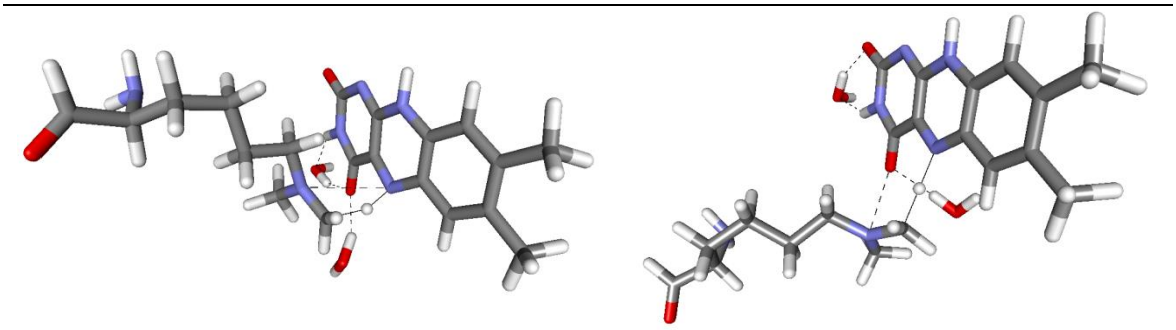


Figure S4. Stick representation of the extended QM model and the corresponding spin densities for triplet reactant (**RC**^{3}) species in upward and downward orientations.

Upper panel: large QM region (173 atoms, see text for explanation) in stick representation; hydrogen bonds are marked with dashed lines. Lower panel: spin density isosurfaces (using an

isocontour value of 0.005) for triplet RC species in upward (lower left panel) and downward (lower right panel) orientation computed at the B3LYP-D/6-31G* level using the large QM region. K661 is deprotonated. Hydrogen atoms are not shown for clarity. The viewing angle is rotated in the lower panel (compared with the upper panel) for a better assessment.



(a) TS^1 (b) TS^3

Figure S5 – Transition state (TS) geometries for (a) HT and (b) SET mechanisms from QM-only (B3LYP/6-31G*) optimizations. H-bonds are marked by black dashed lines.

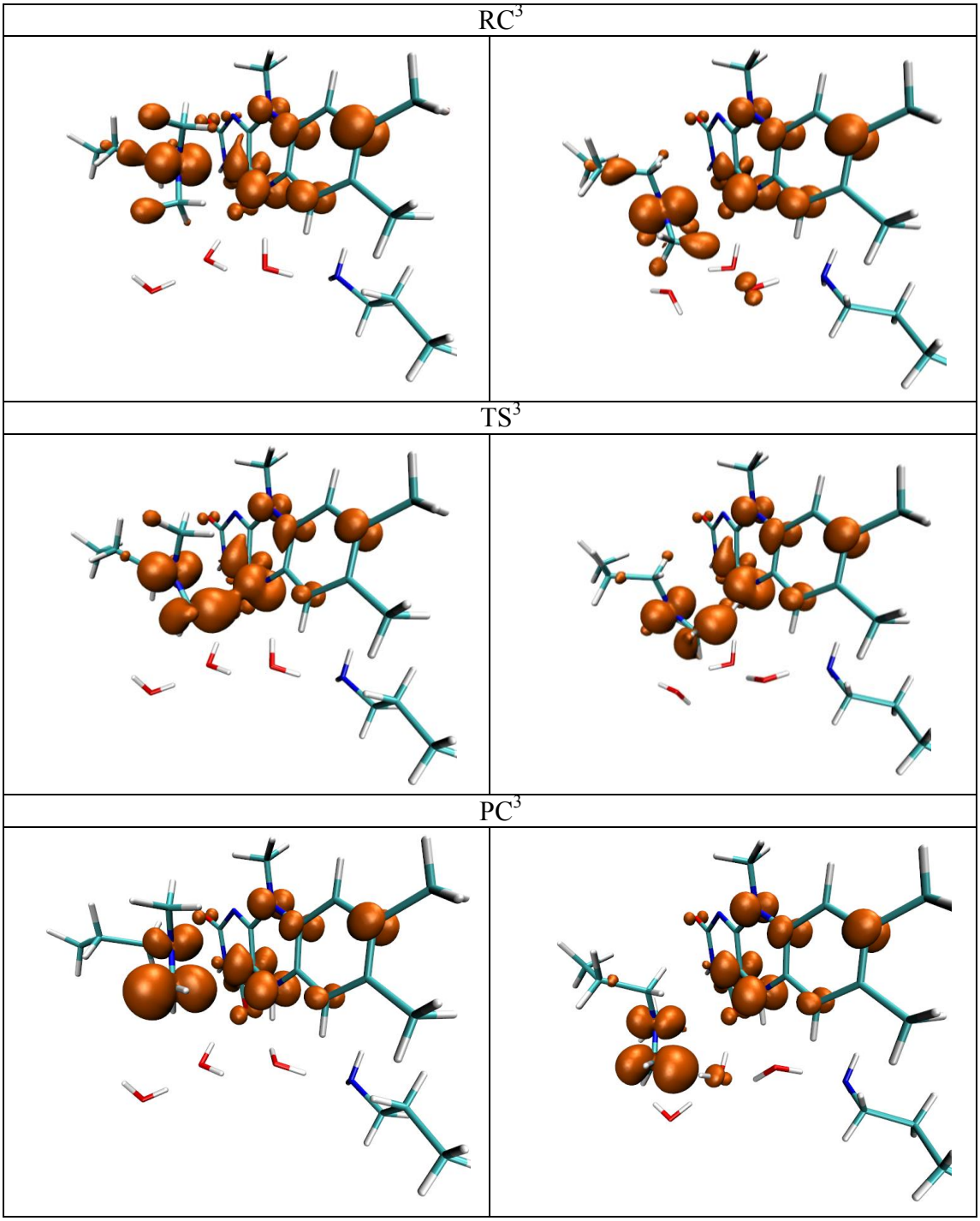
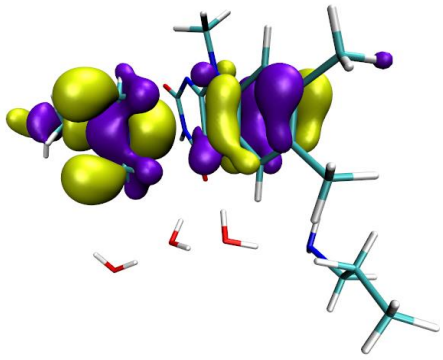
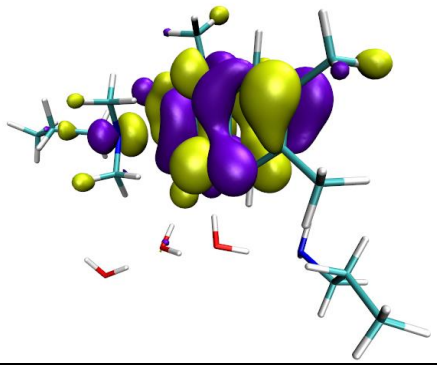
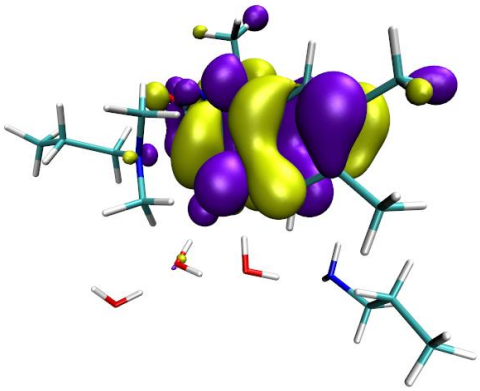
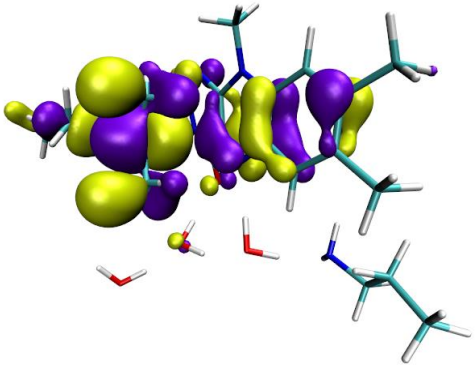
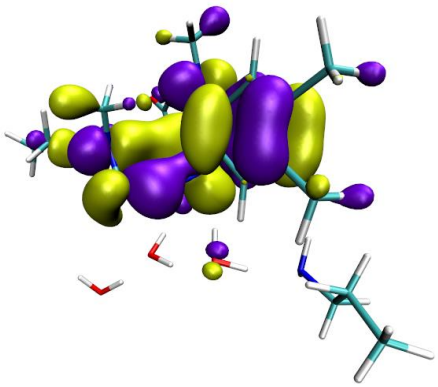
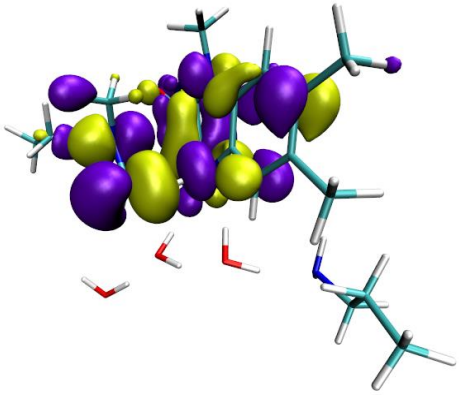


Figure S6. SET mechanism: Spin density isosurfaces (using an isocontour value of 0.005) of all triplet stationary points in upward (left) and downward (right) orientation computed at the B3LYP-D/6-31G* level. K661 is deprotonated.

5. Frontier orbitals of various stationary points in ‘downward’ and ‘upward’ orientation

5.1 Upward orientation

RC^1	
	
HOMO (E= -0.20381870 H)	LUMO (E= -0.10932483 H)
RC^3	
	
SOMO (E= -0.15406782H)	SOMO (E= -0.15953364 H)
TS^1	
	
HOMO (E= -0.20199808 H)	LUMO (E=-0.09272769 H)

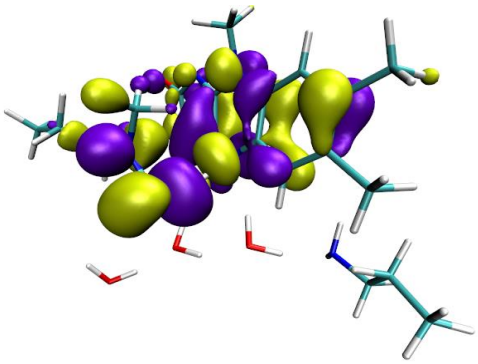
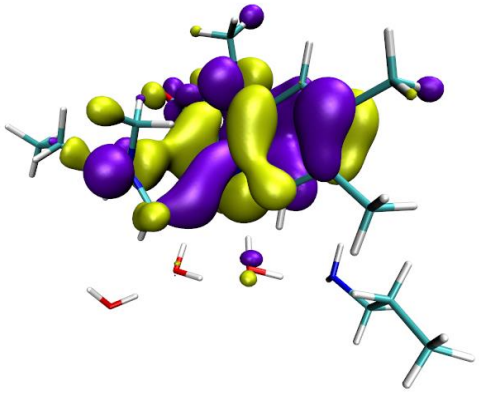
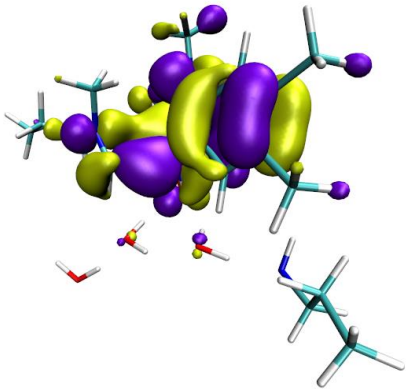
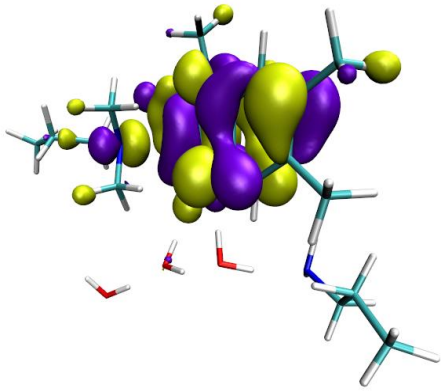
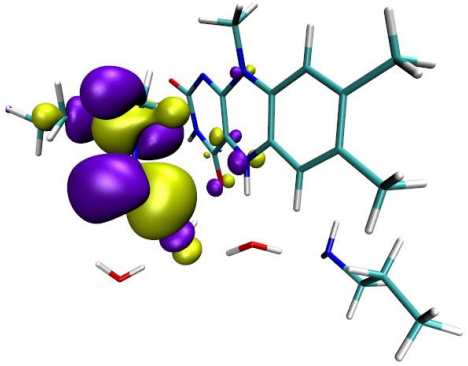
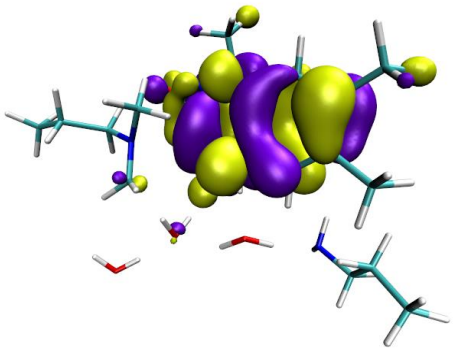
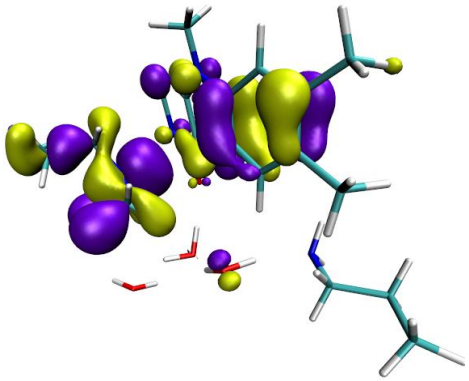
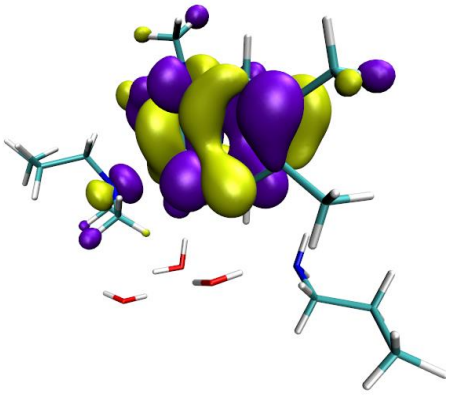
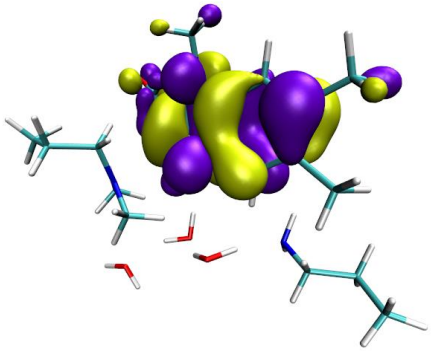
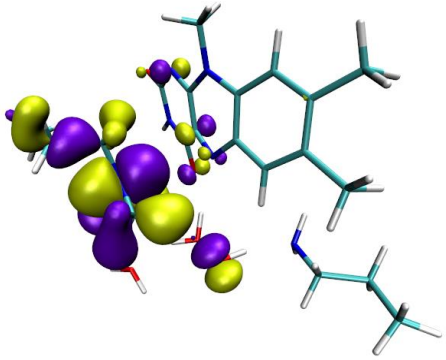
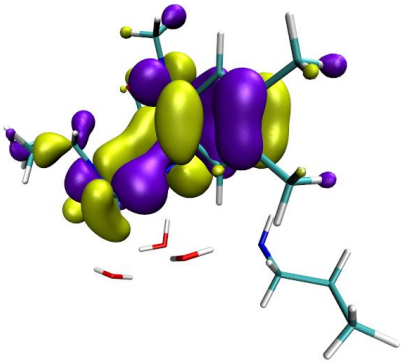
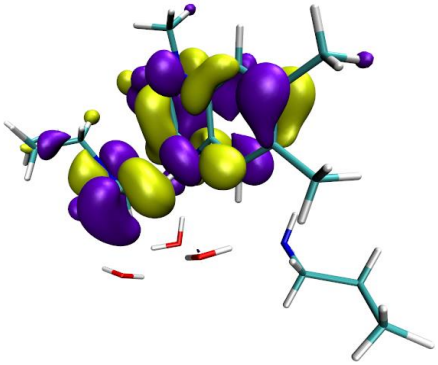
TS^3	
	
SOMO (E= -0.15588660 H)	SOMO (E= -0.12881903H)
PC^1	
	
HOMO (E=-0.17594219 H)	LUMO (E=-0.03081198 H)
PC^3	
	
SOMO (E= -0.13847298 H)	SOMO (E= -0.11112176 H)

Figure S7. HOMO, LUMO, and SOMO isosurfaces (using an isocontour value of 0.2) and corresponding orbital energies computed at the B3LYP-D/6-31G* level at the QM/MM optimized stationary points, in upward orientation. K661 is taken deprotonated.

5.2 Downward orientation

RC[1]	
	
HOMO (E= -0.18217555 H)	LUMO (E= -0.08015034 H)
RC[3]	
	
SOMO (E= -0.11450317H)	SOMO (E= -0.12325397H)
TS[1]	
	
HOMO (E= -0.16837660 H)	LUMO (E= -0.06358632 H)

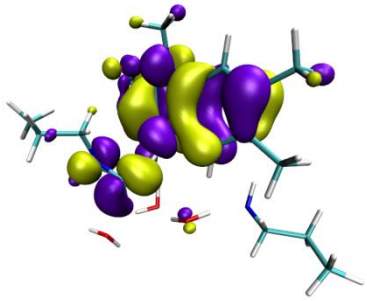
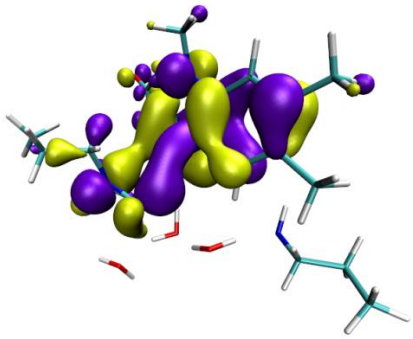
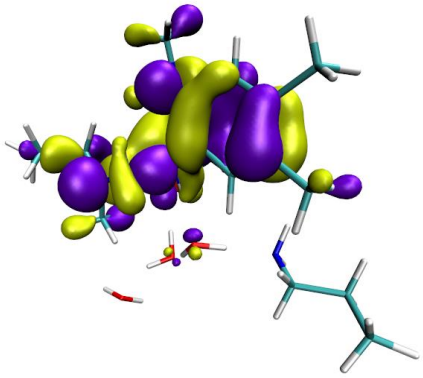
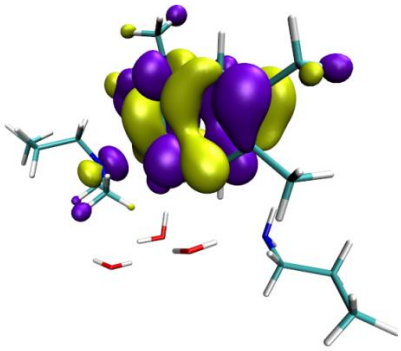
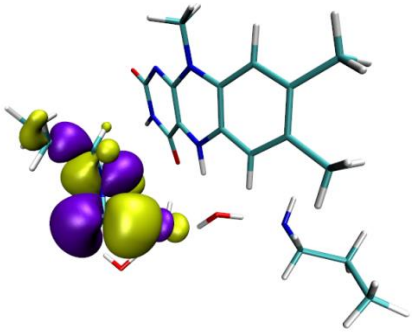
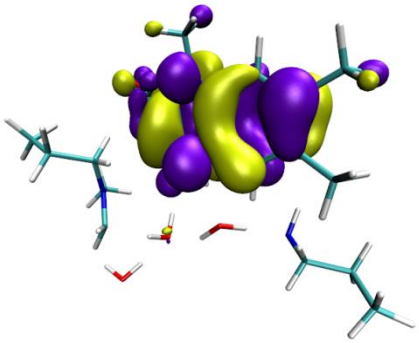
TS^3	
	
SOMO (E= -0.13501707 H)	SOMO (E= -0.09021957 H)
PC^1	
	
HOMO (E= -0.17642505 H)	LUMO (E= +0.00170978 H)
PC^3	
	
SOMO (E= -0.11723343 H)	SOMO (E= -0.09706749 H)

Figure S8. HOMO, LUMO, and SOMO isosurfaces (using an isocontour value of 0.2) and corresponding orbital energies computed at the B3LYP-D/6-31G* level at the QM/MM optimized stationary points, in downward orientation. K661 is taken deprotonated.

6. Energy profile for interconversion of two product minima

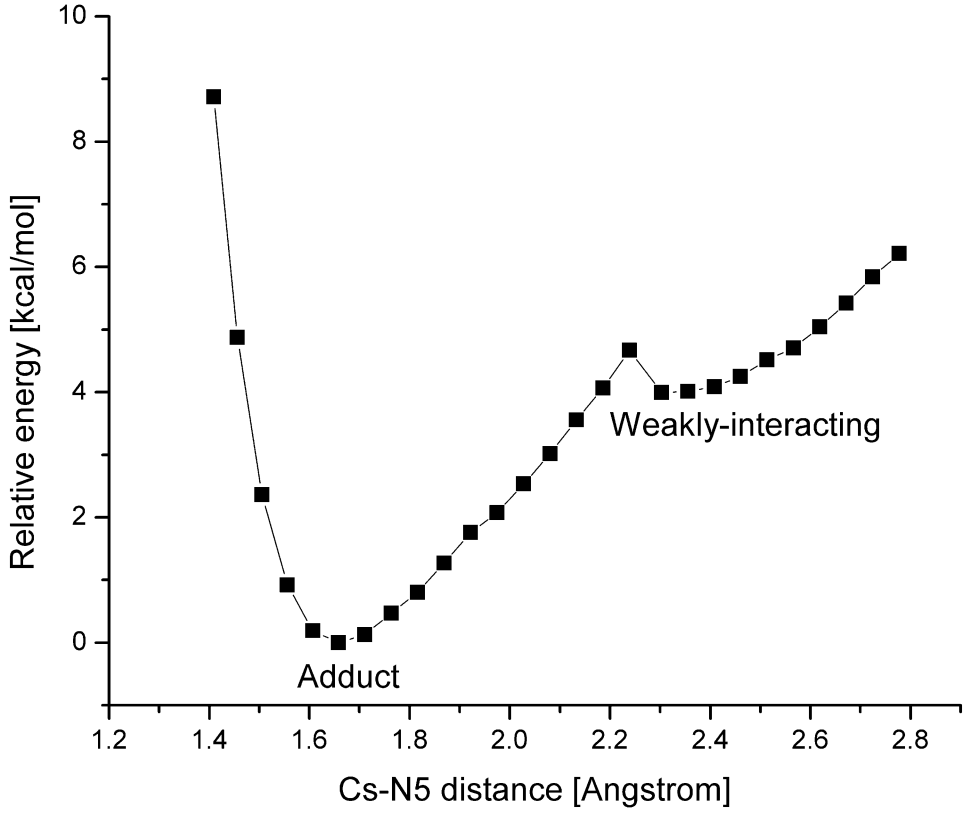


Figure S9. One-dimensional relaxed potential energy scan for a selected snapshot (deprotonated K661/downward-4) at the QM(B3LYP/6-31G*)/CHARMM22 level: Interconversion of the ^{1}PC adduct and the weakly interacting ^{1}PC complex. The Cs-N5 distance was kept fixed at a given value while all the other degrees of freedom were optimized.

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