Supporting Information for

A Combined QM/MM Study on the Reductive Half-reaction of Xanthine Oxidase: Substrate Orientation and Mechanism

*Sebastian Metz and Walter Thiel**

Max-Planck-Institut für Kohlenforschung, D-45470 Mülheim an der Ruhr, Germany

thiel@mpi-muelheim.mpg.de

1. System preparation using CHARMM C29b2 S2

2. QM/MM results (Tables S1-S29, Figures S1-S10) S6

3. Consistency checks (Table S30, Figures S11-S12) S40

4. Setup comparisons (Tables S31-S32, Figures S13-S18) S43

5. CHARMM parameters S50

6. Complete references 75 and 81 S61

1. System preparation using CHARMM C29b2

A. Preparation of the PDB structure.

The PDB structure of the bovin xanthine oxidase dimer (PDB 1FO4) lacks several residues. Using SWISS-MODEL[1-4] we obtained a complete structure after an automatically performed energy minimization. To add the missing residues to the original crystal structure, we overlayed the complete structure with the original crystal structure and glued residues 165 to 192 and 529 to 538 for chains A and B into the crystal structure, i.e. more residues than missing in order to ensure a smooth change over at the interface of the modeled residues with the original crystal structure. For the missing residues 1 and 2 of each chain, we used the standard N-terminus for Ala 3 (positively charged ammonium) and the standard C-terminus (carboxylate) for Val 1332.

As C, N, and O can often not be distinguished unambiguously from X-ray diffraction data, the orientation of Asn and Gln side-chain amides and of the His imidazole rings was adjusted with the program *Reduce*.[5] The automatic assignments of Asn, Gln, and His side-chain orientation and of the His protonation state were reevaluated manually and adjusted where necessary. Flipped residues are listed below (if the residue has been flipped in just one of the chains, the chain ID is given in brackets)

FLIPPED RESIDUES:

ASN 71, GLN 131, ASN 146(B), ASN 351, GLN 473, ASN 556, GLN 626, ASN 650, HSE 683(A), HSD 747, HSD 875, ASN 1145, HSD 1151, GLN 1284, ASN 1287(B) ASN 1324(A)

All Lys and Arg side chains are in the ammonium or guanidinium forms, respectively, and all Asp side chains are in the carboxylate form. For all setups, Glu267 was protonated and Cys992 directly neighboring the doubly protonated histidine Hsp677 was deprotonated. Glu802 was protonated in both chains (except for setup E where it was protonated only in chain B). The chosen protonation states for all histidines are listed below (if the protonation state has been assigned in just one of the two chains, the chain ID is given in brackets)

PROTONATION STATES

GLU 267p, GLU802p (SETUP E: just chain B), CYS 992d

HSD: 67 187 252 292(A) 552(B) 614 747 821 840 863 875 884 1151 1171(B) 1212 (B)

HSE: 81 82 99 109 292(B) 387 552(A) 579 665 683 741 954 1022 1033 1043 1171(A) 1212 (A)1220

HSP: 677 1285

SETUP A, C, D, E, F, G: GLU 1261 (just chain A)

To keep the whole system neutral in charge, Cl^- counterions were added. This was done using the program *ionize*,[6] which puts a three dimensional grid on the enzyme and then iteratively calculates the electrostatic potential on all grid points, adding a single ion at the most favorable position until the system is neutral.

The residues in the direct surrounding of the counterions were reoptimized using CHARMM. As a result of this procedure all counterions were placed on the surface of the enzyme and more than 35Å away from the molybdenum center. Their coordinates are given below for direct use in the original crystal structure.

ADDED COUNTERIONS

```
ATOM  41516  CLA CLA     8       75.360  86.126  20.686
ATOM  41517  CLA CLA     9       28.661 -47.776  50.009
in SETUP A,C,D,F & G
ATOM  41519  CLA CLA    10       93.374  14.979  26.149
```

B. CHARMM Parameters.

To be able to prepare the system by extended classical MD runs prior to the QM/MM optimizations, we needed CHARMM parameters for the full system, including FAD, FEST, MOC and URIC. The FAD parameters were provided by M. Karplus.[7] We used the FEST parameters published in our prior publication.[8] We kept the molybdenum center fixed during MD simulation and used ESP charges for the interaction with the enzymatic environment. The parameters for URIC (uric acid) were adopted from similar molecules like guanine or uracil; the atomic charges were taken from ESP calculations.

Note that our goal was only to obtain reasonable parameters suitable for the preparatory MM-MD simulations. We therefore did not extensively validate the newly derived parameters, nor do we recommend their use in pure MM calculations without further tests. All parameters not provided with the CHARMM package can be found in Section 4 (see pages S50-S60).

C. Hydration Procedure.

The protein structure with added H atoms was hydrated using a sphere of 35 Å radius containing 6840 water molecules cut from an equilibrated simulation of TIP3P water under periodic boundary conditions. The water sphere was superimposed onto the protein structure, centered on MOC(A):C8. All added water molecules whose O atom was within 2.8 Å of any existing non-H atom were deleted. The O atoms of all water molecules (i.e., added and existing ones) within the 35Å sphere were subjected to a spherical quartic boundary potential with parameters FORCE = 0.2, P1 = 2.25, and

DROFF = 33.5. The water molecules were kept rigid using SHAKE constraints. Keeping all other atoms fixed, the water molecules within the sphere were geometry-optimized, performing first 300 steps of steepest descent followed by 300 steps of adapted-basis Newton–Raphson minimization. In the first solvation run, we performed another two minimizations of 300 steps each (steepest descent and adapted-basis Newton Raphson), optimizing the active region without the water molecules, adding a restraint of k=30 mol $Å^{-2}$ on the non-fixed atoms. Finally, the water molecules were subjected to 30 ps molecular dynamics at 300 K with (subsequently decreasing) restaints. Afterward, we restepped to the next solvation cycle. Information on the different solvation cycles is listed below.

HYDRATION CYCLES

Run	number of water molecules added	k/(mol $Å^{-2}$)*
1	1626	fixed
2	64	50
3	69	45
4	51	40
5	44	35
6	30	30
7	32	25
8	26	20
9	21	15
10	52	10
11	27	5
12	24	3
13	34	1

*force constant of harmonic constraints on all residues within 20 Å around C8(URIC), all other residues and the cofactor are fixed

The nonbonded interactions in all CHARMM simulations were treated using

```
NBOND ELEC ATOM FSWITCH CDIE VDW VATOM VFSWITCH CUTNB 17. CTONNB 11.
CTOFNB 15. EPS 1.
NBXMOD 5 E14FAC 1. WMIN 1.5.
```

Active residues
chain A
GLN112, CYS113, CYS148, ARG149, CYS150, THR151, GLY152, HSE579, TYR592, THR646, GLY647, LEU648, PHE649, ASN650, ASP651, GLU652, ILE698, TYR708, LEU712, LYS713, ILE714, ILE736, GLY737, GLY738, GLN739, ASP740, HSE741, PHE742, TYR743, LEU744, GLU745, THR746, HSD747, CYS748, VAL764, SER765, THR766, GLN767, ASN768, ALA769, MET770, LYS771, THR772, GLN773, SER774, PHE775, VAL789, VAL791, LYS792, ARG793, MET794, GLY795, GLY796, GLY797, PHE798, GLY799, GLY800, LYS801, GLU802, THR803, ARG804, SER805, THR806, LEU807, VAL808, SER809, VAL810, LEU827, ARG829, ASP832, MET833, ILE835, THR836, GLY837, GLY838, ARG839, HSD840, PRO841, PHE842, LEU843, ALA844, HSD863, TYR864, SER865, ASN866, ALA867, GLY868, ASN869, SER870, ARG871,

ASP872, LEU873, SER874, HSD875, SER876, ILE877, MET878, GLU879, ARG880, ALA881, LEU882, PHE883, HSD884, MET885, ASP886, ASN887, CYS888, THR897, GLY898, ARG899, LEU900, CYS901, SER906, SER907, ASN908, THR909, ALA910, PHE911, ARG912, GLY913, PHE914, GLY915, GLY916, PRO917, GLN918, ALA919, LEU920, PHE921, ILE922, ALA923, GLU924, TYR947, THR953, HSE954, PHE955, GLN957, LEU959, PHE962, VAL964, ILE1001, PRO1002, THR1003, LYS1004, PHE1005, GLY1006, ILE1007, SER1008, PHE1009, THR1010, VAL1011, PRO1012, PHE1013, LEU1014, ASN1015, GLN1016, ALA1017, GLY1018, ALA1019, HSE1033, GLY1034, GLY1035, THR1036, GLU1037, MET1038, GLY1039, GLN1040, GLY1041, LEU1042, HSE1043, THR1044, LYS1045, MET1046, VAL1049, ILE1063, THR1066, SER1067, THR1068, VAL1071, PRO1072, ASN1073, SER1074, SER1075, PRO1076, THR1077, ALA1078, ALA1079, SER1080, VAL1081, SER1082, THR1083, ASP1084, ILE1085, TYR1086, GLY1087, GLN1088, ALA1089, VAL1090, PHE1132, TYR1133, ARG1134, THR1135, PRO1136, ASN1137, LEU1138, GLY1139, TYR1140, SER1141, PHE1142, GLY1147, ASN1148, ALA1149, PHE1150, HSD1151, TYR1152, PHE1153, THR1154, TYR1155, GLY1156, ILE1178, MET1180, ASP1181, VAL1182, GLY1183, SER1185, LEU1186, ASN1187, ILE1190, ASP1191, ILE1192, GLY1193, GLN1194, VAL1195, GLU1196, GLY1197, ALA1198, PHE1199, VAL1200, GLN1201, GLY1202, LEU1203, GLY1204, LEU1205, PHE1206, GLU1209, LEU1211, TYR1213, TYR1227, ILE1229, PRO1230, LEU1243, ASN1249, LYS1251, ALA1252, ILE1253, SER1256, LYS1257, ALA1258, VAL1259, GLY1260, GLU1261, PRO1262, PRO1263, LEU1264, PHE1265, LEU1266, GLY1267, ALA1268, SER1298, FES2001, MOC2003, URIC2, GOL2, CAL6
chain B
THR1025, TYR1121, GLN1122, ASP1123, ARG1124
crystal water
CRYW3, CRYW4, CRYW5, CRYW7, CRYW9, CRYW11, CRYW12, CRYW13, CRYW15, CRYW19, CRYW23, CRYW24, CRYW25, CRYW40, CRYW42, CRYW45, CRYW48, CRYW49, CRYW52, CRYW56, CRYW58, CRYW61, CRYW63, CRYW65, CRYW71, CRYW74, CRYW77, CRYW85, CRYW89, CRYW91, CRYW93, CRYW94, CRYW96, CRYW97, CRYW101, CRYW108, CRYW109, CRYW114, CRYW122, CRYW125, CRYW126, CRYW128, CRYW143, CRYW157, CRYW160, CRYW168, CRYW174, CRYW177, CRYW189, CRYW191, CRYW196, CRYW199, CRYW200, CRYW203, CRYW211, CRYW213, CRYW218, CRYW222, CRYW224, CRYW225, CRYW227, CRYW230, CRYW240, CRYW243, CRYW244, CRYW248, CRYW274, CRYW277, CRYW283, CRYW287, CRYW300, CRYW308, CRYW317, CRYW343, CRYW369, CRYW373, CRYW382, CRYW405, CRYW408, CRYW428, CRYW429, CRYW447, CRYW498, CRYW536, CRYW558, CRYW563, CRYW565, CRYW570, CRYW571, CRYW575, CRYW596, CRYW615, CRYW632, CRYW636, CRYW706, CRYW712, CRYW751, CRYW753, CRYW798, CRYW847, CRYW947, CRYW1034, CRYW1128, CRYW1205, CRYW1340, CRYW1678
and all solvent water

References:

1. Guex, N.; Peitsch, M. C. *Electrophoresis* **1997**, 18, 2714-2723.
2. Schwede, T.; Kopp, J.; Guex, N.; Peitsch, M. C. *Nucleic Acids Res.* **2003**, 31, 3381-3385.
3. Kopp, J.; Schwede, T. *Nucleic Acids Res.* **2004**, 32, D230-D234.
4. Arnold, K.; Bordoli, L.; Kopp, J.; Schwede, T. *Bioinformatics* **2006**, 22, 195-201.
5. Word, J. M.; Lovell, S. C.; Richardson, J. S.; Richardson, D. C. *J. Mol. Biol.* **1999**, 285, 1735-1747.
6. http://www.ks.uiuc.edu/Development/MDTools/ionize.
7. Luo, G. B.; Andricioaei, I.; Xie, X. S.; Karplus, M. *J. Phys. Chem. B* **2006**, 110, 9363-9367.
8. Metz, S.; Wang, D.; Thiel, W. *J. Am. Chem. Soc.* **2009**, 131, 4628-4640.

2. QM/MM results

SETUP _A, SN100

Table S1. Calculated QM/MM energies of optimized stationary points for snapshot 100 of SETUP A in kcal mol^{-1}.

	BP86/Lanl2DZ			B3LYP/Lanl2DZ			
	E_{QM}	E_{MM}	$E_{QM/MM}$	E_{QM}	E_{MM}	$E_{QM/MM}$	Remark
Reactant[a]	0.0	0.0	0.0	0.0	0.0	0.0	
TS1'	4.0	0.2	4.2	7.1	-0.2	7.0	N3-N9 Proton transfer
IM1'	-2.0	2.3	0.3	-2.8	2.5	-0.4	
TS1	8.5	7.2	15.8	7.9	6.9	14.8	Proton transfer
IM1	6.1	5.6	11.8	6.9	5.3	12.2	
TS2	16.3	0.9	17.2	19.9	0.3	20.2	C-O formation
IM2	14.6	0.5	15.2	17.7	0.1	17.9	
TS3	18.6	0.2	18.8	22.0	-1.2	20.8	Hydride transfer
Product	6.0	6.1	12.1	-5.4	6.5	1.2	

[a] absolute energies (a.u.): -1423.990924 -87.286581 -1511.277505 (BP86)
-1422.892054 -87.284641 -1510.176695 (B3LYP)

active MM region SETUP A SN100 (1588 active atoms)

```
chain A
LEU648 PHE649 HSE741 TYR743 THR766 GLN767 ASN768 LYS771 MET794 GLY797
PHE798 GLY799 GLY800 LYS801 GLU802 THR803 ARG804 SER805 GLY838 ARG839
HSD840 PHE842 ASP872 LEU873 SER874 HSD875 SER876 ILE877 MET878 GLU879
ARG880 ALA881 PHE883 HSD884 ASN908 THR909 ALA910 PHE911 ARG912 GLY913
PHE914 GLY915 GLY916 PRO917 GLN918 LEU920 PHE1005 GLY1006 ILE1007
SER1008 PHE1009 THR1010 VAL1011 PRO1012 LEU1014 ASN1015 GLN1016
GLY1035 THR1036 MET1038 GLN1040 LEU1042 SER1074 SER1075 PRO1076
THR1077 ALA1078 ALA1079 SER1080 VAL1081 SER1082 THR1083 ASP1084
ILE1085 TYR1086 THR1135 LEU1138 ALA1149 PHE1150 HSD1151 THR1154
GLN1194 ALA1198 GLN1201 ALA1258 VAL1259 GLY1260 GLU1261 PRO1262
PRO1263 LEU1264 PHE1265 MOC2003 URIC2 GOL2
crystal water
CRYW19 CRYW40 CRYW42 CRYW45 CRYW48 CRYW56 CRYW58 CRYW74 CRYW126
CRYW168 CRYW174 CRYW189 CRYW191 CRYW211 CRYW222 CRYW224 CRYW240
CRYW244 CRYW248 CRYW287 CRYW288 CRYW343 CRYW369 CRYW373 CRYW405
CRYW428 CRYW447 CRYW498 CRYW570 CRYW571 CRYW573 CRYW575 CRYW706
CRYW753 CRYW847 CRYW947 CRYW1034
solvent water
SOLV250 SOLV376 SOLV771 SOLV845 SOLV1087 SOLV1559 SOLV1845 SOLV1991
```

SETUP_A, SN500

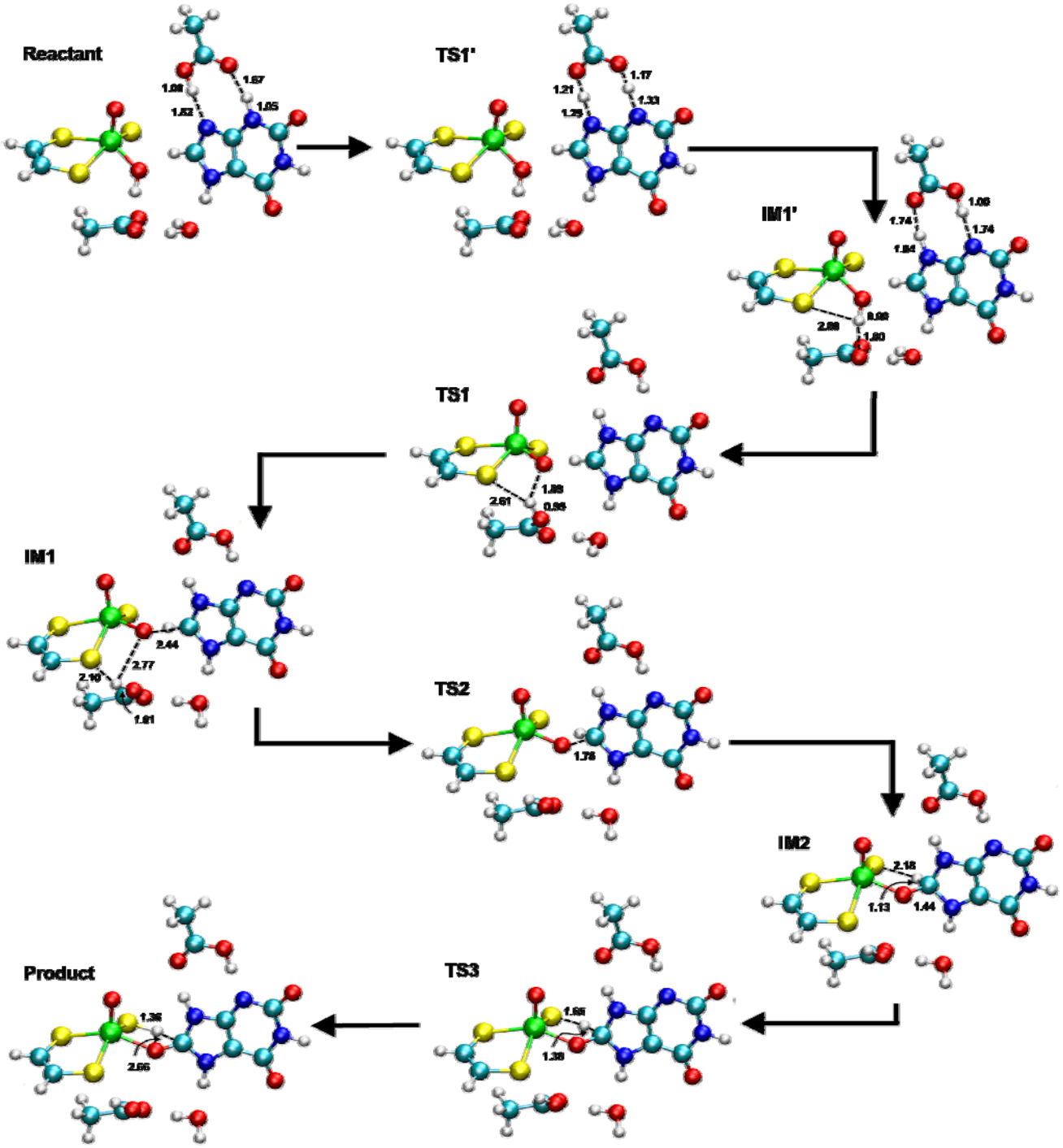

Figure S1. Reaction mechanism for setup A, SN500 from B3LYP/MM calculations. The distances are given in Angstrom.

Table S2. QM/MM energies of optimized stationary points of SETUP A, SN500 in kcal mol^{-1}.

	BP86/Lanl2DZ			B3LYP/Lanl2DZ			
	E_{QM}	E_{MM}	$E_{QM/MM}$	E_{QM}	E_{MM}	$E_{QM/MM}$	Remark
Reactant[a]	0.0	0.0	0.0	0.0	0.0	0.0	
TS1'	3.6	0.0	3.6	6.8	2.1	8.9	N3-N9 Proton transfer
IM1'	-0.4	-0.3	-0.7	1.6	0.8	2.4	
TS1	16.0	2.3	18.3	15.3	3.8	19.1	Proton transfer
IM1	3.0	10.6	13.6	3.5	11.4	14.9	
TS2	9.0	7.0	16.1	12.5	7.8	20.3	C-O formation
IM2	5.6	7.5	13.1	7.1	9.4	16.4	
TS3	10.0	7.8	17.9	12.7	8.9	21.7	Hydride transfer
Product	-0.7	10.2	9.6	-11.7	12.2	0.5	

[a] absolute energies (a.u.): -1423.935494 -87.129750 -1511.065244 (BP86)
-1422.837158 -87.128789 -1509.965947 (B3LYP)

Table S3. QM/MM energies of the rate limiting stationary points of SETUP A, SN500 in kcal mol^{-1} using the L2 link atom scheme, from single point calculations (SP) on the structures obtained with the charge-shift link atom scheme (Figure S1) and on the reoptimized structures (OPT).

	B3LYP/Lanl2DZ (SP)			B3LYP/Lanl2DZ (OPT)			
	E_{QM}	E_{MM}	$E_{QM/MM}$	E_{QM}	E_{MM}	$E_{QM/MM}$	Remark
Reactant[a]	0.0	0.0	0.0	0.0	0.0	0.0	
TS3	13.2	7.2	20.4	13.5	6.9	20.4	Hydride transfer

[a] absolute energies (a.u.): -1422.814695 -87.128789 -1509.943484 (single point)
-1422.815197 -87.129676 -1509.944873 (reoptimized)

Table S4. QM/MM energies of the most important stationary points (reoptimized) of SETUP A, SN500 in kcal mol^{-1} after deprotonation of Glu802.

	B3LYP/Lanl2DZ			
	E_{QM}	E_{MM}	$E_{QM/MM}$	Remark
Reactant_D[a]	0.0	0.0	0.0	
IM1'_D	7.8	2.7	10.5	
IM2_D	28.2	13.5	41.7	
TS3_D	28.8	13.6	42.3	Hydride transfer
Product_D	-6.5	18.4	12.0	

[a] absolute energies (a.u.): -1422.381161 -87.056611 -1509.437771

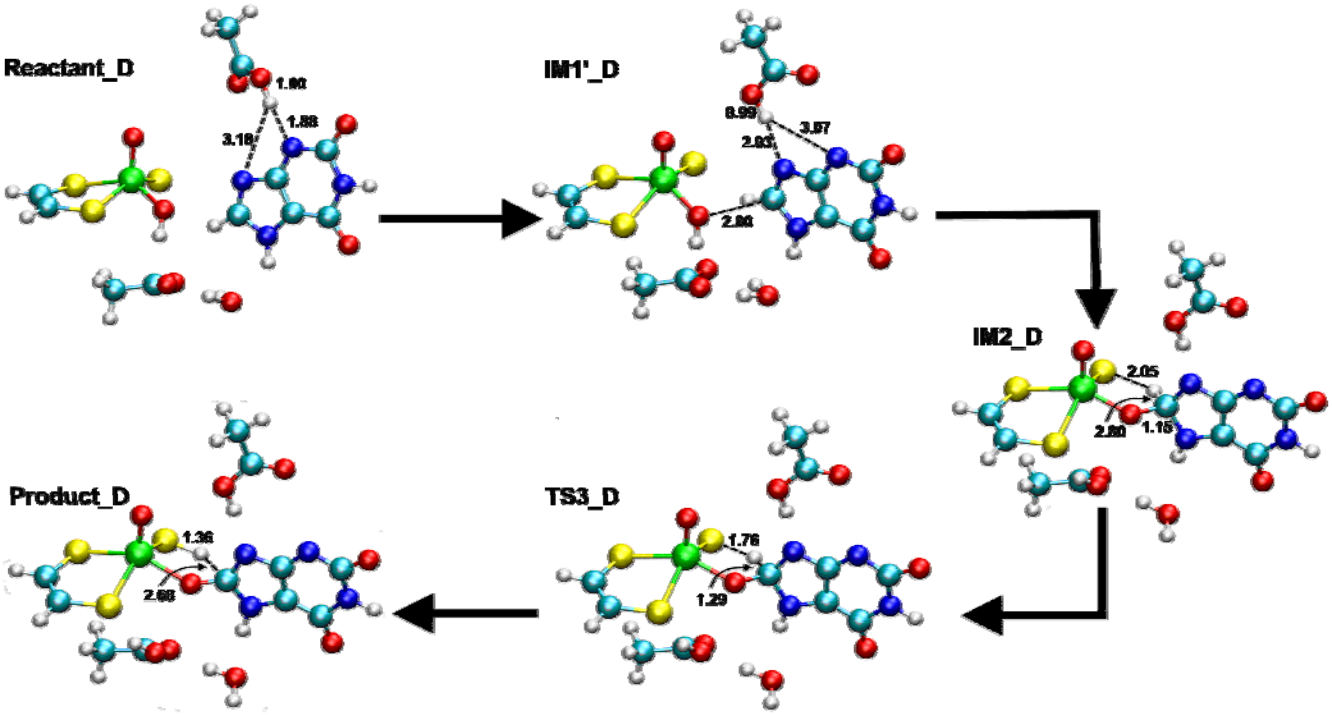

Figure S2. Intermediates of the reaction mechanism for SETUP A, SN500 from B3LYP/MM calculations after deprotonation of Glu802. The distances are given in Angstrom.

active MM region SETUP A SN500 (1594 active atoms)

chain A

LEU648 PHE649 HSE741 TYR743 THR766 GLN767 ASN768 LYS771 MET794 GLY797 PHE798 GLY799 GLY800 LYS801 GLU802 THR803 ARG804 SER805 GLY838 ARG839 HSD840 PHE842 ASP872 LEU873 SER874 HSD875 SER876 ILE877 MET878 GLU879 ARG880 ALA881 PHE883 HSD884 ASN908 THR909 ALA910 PHE911 ARG912 GLY913 PHE914 GLY915 GLY916 PRO917 GLN918 LEU920 PHE1005 GLY1006 ILE1007 SER1008 PHE1009 THR1010 VAL1011 PRO1012 PHE1013 LEU1014 ASN1015 GLN1016 GLY1035 THR1036 MET1038 GLN1040 LEU1042 SER1074 SER1075 PRO1076 THR1077 ALA1078 ALA1079 SER1080 VAL1081 SER1082 THR1083 ASP1084 ILE1085 TYR1086 ALA1149 PHE1150 HSD1151 THR1154 GLN1194 ALA1198 GLN1201 LEU1205 ALA1258 VAL1259 GLY1260 GLU1261 PRO1262 PRO1263 LEU1264 PHE1265 MOC2003 URI2 GOL2

crystal water

CRYW19 CRYW40 CRYW42 CRYW45 CRYW48 CRYW56 CRYW58 CRYW74 CRYW126 CRYW168 CRYW174 CRYW189 CRYW211 CRYW222 CRYW224 CRYW240 CRYW248 CRYW287 CRYW343 CRYW369 CRYW373 CRYW382 CRYW405 CRYW428 CRYW447 CRYW498 CRYW536 CRYW571 CRYW575 CRYW706 CRYW753 CRYW847 CRYW1034

solvent water

SOLV105 SOLV842 SOLV1435 SOLV1559 SOLV1688 SOLV1964 SOLV1991

SETUP _B, SN300

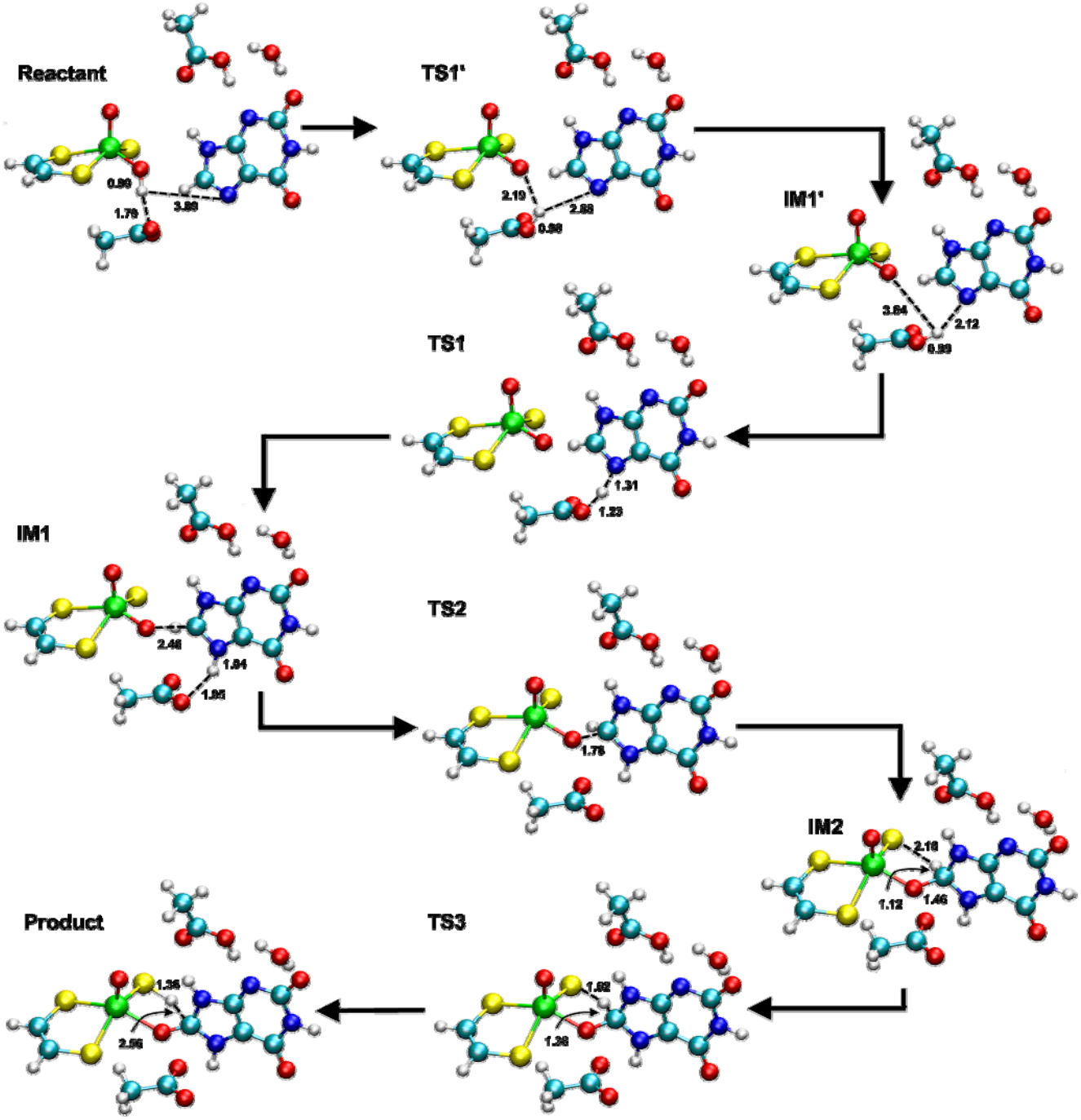

Figure S3. Reaction mechanism for setup B, SN300 from B3LYP/MM calculations. The distances are given in Angstrom. For the sake of clarity, we omitted an additional water molecule close to Glu1261 (cf. Figure 3) that is included in the QM region.

Table S5. Calculated QM/MM energies of optimized stationary points for snapshot 300 of SETUP B in kcal mol^{-1}.

	BP86/Lanl2DZ			B3LYP/Lanl2DZ			
	E_{QM}	E_{MM}	$E_{QM/MM}$	E_{QM}	E_{MM}	$E_{QM/MM}$	Remark
Reactant[a]	0.0	0.0	0.0	0.0	0.0	0.0	
TS1'	17.5	2.2	19.8	18.3	0.1	18.4	Proton transfer 1
IM1'	7.7	2.1	9.8	11.2	0.2	11.5	
TS1	8.2	3.6	11.9	12.7	2.4	15.1	Proton transfer 2
IM1	4.9	3.4	8.4	7.4	3.3	10.8	
TS2	10.2	0.5	10.7	19.2	-1.4	17.9	C-O formation
IM2	9.1	-0.5	8.6	16.6	-2.2	14.4	
TS3	15.8	-1.2	14.5	23.3	-3.4	19.9	Hydride transfer
Product	5.0	3.6	8.6	-3.0	-3.3	0.3	

[a] absolute energies (a.u.): -1500.044484 -82.462433 -1582.506917 (BP86)
-1498.911278 -82.458006 -1581.369284 (B3LYP)

SETUP B, SN300 (1635 active atoms)

```
chain A
LEU648 PHE649 HSE741 TYR743 THR766 GLN767 ASN768 LYS771 MET794 GLY797
PHE798 GLY799 GLY800 LYS801 GLU802 THR803 ARG804 GLY837 GLY838 ARG839
HSD840 PHE842 ASP872 LEU873 SER874 HSD875 SER876 ILE877 MET878 GLU879
ARG880 ALA881 LEU882 PHE883 HSD884 ASN908 THR909 ALA910 PHE911 ARG912
GLY913  PHE914  GLY915  GLY916  PRO917  GLN918  ALA919  LEU920  PHE1005
GLY1006  ILE1007  SER1008  PHE1009  THR1010  VAL1011  PRO1012  LEU1014
ASN1015  GLN1016  GLY1035  THR1036  MET1038  GLN1040  LEU1042  SER1074
SER1075  PRO1076  THR1077  ALA1078  ALA1079  SER1080  VAL1081  SER1082
THR1083  ASP1084  ILE1085  TYR1086  LEU1138  ALA1149  PHE1150  HSD1151
TYR1152  THR1154  GLN1194  ALA1198  GLN1201  LEU1205  ALA1258  VAL1259
GLY1260 GLU1261 PRO1262 PRO1263 LEU1264 PHE1265 MOC2003 URIC2 GOL2
crystal water
CRYW19  CRYW40  CRYW42  CRYW45  CRYW48  CRYW56  CRYW58  CRYW74  CRYW168
CRYW174  CRYW189  CRYW191  CRYW196  CRYW203  CRYW211  CRYW222  CRYW224
CRYW244  CRYW248  CRYW287  CRYW317  CRYW343  CRYW369  CRYW405  CRYW447
CRYW498 CRYW571 CRYW573 CRYW575 CRYW847 CRYW947 CRYW1034
solvent water
SOLV60  SOLV95  SOLV338  SOLV506  SOLV589  SOLV737  SOLV1423  SOLV1559
SOLV1991
```

SETUP _B, SN500

Table S6. Calculated QM/MM energies of optimized stationary points for Snapshot 500 of SETUP B in kcal mol^{-1}.

	BP86/Lanl2DZ			B3LYP/Lanl2DZ			
	E_{QM}	E_{MM}	$E_{QM/MM}$	E_{QM}	E_{MM}	$E_{QM/MM}$	Remark
Reactant[a]	0.0	0.0	0.0	0.0	0.0	0.0	
TS1'	17.2	1.4	18.6	20.0	0.2	20.2	Proton transfer1
IM1'	11.7	2.7	14.3	13.0	1.0	14.1	
TS1	11.9	4.4	16.3	16.5	4.0	20.4	Proton transfer2
IM1	10.1	4.3	14.4	12.5	3.5	16.0	
TS2	14.1	0.2	14.3	18.9	-0.9	18.0	C-O formation
IM2	12.5	-1.6	10.9	16.6	-2.3	14.2	
TS3	17.5	-1.6	15.9	22.3	-2.8	19.5	Hydride transfer
Product	10.1	2.6	12.7	0.7	2.8	3.5	

[a] absolute energies (a.u.): -1500.045305 -82.390174 -1582.435479 (BP86)
-1498.909442 -82.386832 -1581.296274 (B3LYP)

SETUP B SN500 (1613 active atoms)

```
chain A
LEU648 HSE741 TYR743 THR766 GLN767 ASN768 LYS771 MET794 GLY797 PHE798
GLY799 GLY800 LYS801 GLU802 THR803 ARG804 SER805 GLY837 GLY838 ARG839
HSD840 PHE842 ASP872 LEU873 SER874 HSD875 SER876 ILE877 MET878 GLU879
ARG880 ALA881 LEU882 PHE883 HSD884 ASN908 THR909 ALA910 PHE911 ARG912
GLY913 PHE914 GLY915 GLY916 PRO917 GLN918 LEU920 PHE1005 GLY1006
ILE1007 SER1008 PHE1009 THR1010 VAL1011 PRO1012 LEU1014 ASN1015
GLN1016 GLY1035 THR1036 MET1038 GLN1040 LEU1042 SER1074 SER1075
PRO1076 THR1077 ALA1078 ALA1079 SER1080 VAL1081 SER1082 THR1083
ASP1084 ILE1085 TYR1086 LEU1138 ALA1149 PHE1150 HSD1151 TYR1152
THR1154 GLN1194 ALA1198 GLN1201 LEU1205 ALA1258 VAL1259 GLY1260
GLU1261 PRO1262 PRO1263 LEU1264 PHE1265 MOC2003 URIC2 GOL2
crystal water
CRYW19 CRYW40 CRYW42 CRYW45 CRYW48 CRYW56 CRYW58 CRYW168 CRYW174
CRYW189 CRYW191 CRYW196 CRYW203 CRYW211 CRYW222 CRYW224 CRYW244
CRYW248 CRYW287 CRYW317 CRYW343 CRYW369 CRYW405 CRYW447 CRYW498
CRYW571 CRYW575 CRYW847 CRYW947 CRYW1034
solvent
SOLV60 SOLV106 SOLV141 SOLV503 SOLV736 SOLV737 SOLV1139 SOLV1559
SOLV1902 SOLV1991
```

SETUP_C, SN200

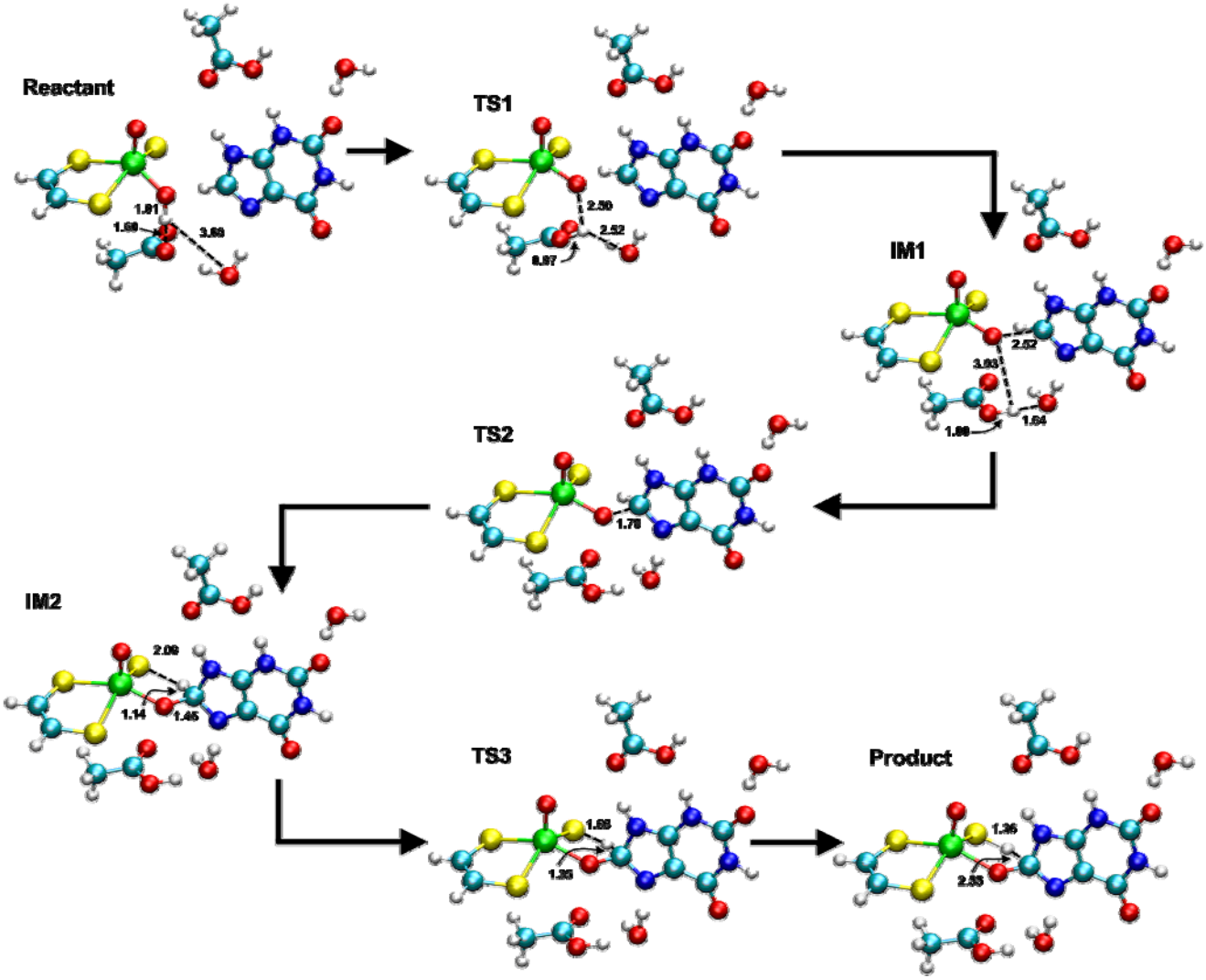

Figure S4. Reaction mechanism for setup C, SN200 from B3LYP/MM calculations. The distances are given in Angstrom.

Table S7. Calculated QM/MM energies of optimized stationary points for Snapshot 200 of SETUP C in kcal mol^{-1}.

	BP86/Lanl2DZ			B3LYP/Lanl2DZ			
	E_{QM}	E_{MM}	$E_{QM/MM}$	E_{QM}	E_{MM}	$E_{QM/MM}$	Remark
Reactant[a]	0.0	0.0	0.0	0.0	0.0	0.0	
TS1	12.5	-2.9	9.6	14.1	-3.2	10.9	Proton transfer
IM1	5.5	0.0	5.5	7.8	0.1	7.9	
TS2	15.3	-4.8	10.5	23.4	-5.4	17.9	C-O formation
IM2	13.7	-5.4	8.3	22.6	-6.3	16.3	
TS3	17.7	-5.0	12.7	24.7	-5.5	19.1	Hydride transfer
Product	6.8	-1.2	5,6	0.8	-0.5	0.3	

[a] absolute energies (a.u.): -1500.366679 -93.136949 -1593.503628 (BP86)
-1499.232154 -93.134585 -1592.366739 (B3LYP)

SETUP C, SN200 (1581 active atoms)

```
chain A
LEU648 PHE649 HSE741 TYR743 THR766 GLN767 ASN768 LYS771 MET794 GLY797
PHE798 GLY799 GLY800 LYS801 GLU802 THR803 ARG804 SER805 GLY837 GLY838
ARG839 HSD840 PHE842 ASP872 LEU873 SER874 HSD875 SER876 ILE877 MET878
GLU879 ARG880 ALA881 PHE883 HSD884 ASN908 THR909 ALA910 PHE911 ARG912
GLY913 PHE914 GLY915 GLY916 PRO917 GLN918 LEU920 LYS1004 PHE1005
GLY1006 ILE1007 SER1008 PHE1009 THR1010 VAL1011 PRO1012 LEU1014
ASN1015 GLN1016 GLY1035 THR1036 MET1038 GLN1040 LEU1042 SER1074
SER1075 PRO1076 THR1077 ALA1078 ALA1079 SER1080 VAL1081 SER1082
THR1083 ASP1084 ILE1085 TYR1086 ALA1149 PHE1150 HSD1151 THR1154
GLN1194 ALA1198 GLN1201 ALA1258 VAL1259 GLY1260 GLU1261 PRO1262
PRO1263 LEU1264 PHE1265 MOC2003 URIC2 GOL2
crystal water
CRYW19 CRYW40 CRYW42 CRYW45 CRYW48 CRYW56 CRYW58 CRYW126 CRYW168
CRYW174 CRYW189 CRYW191 CRYW211 CRYW222 CRYW224 CRYW248 CRYW343
CRYW369 CRYW373 CRYW405 CRYW429 CRYW447 CRYW498 CRYW571 CRYW573
CRYW575 CRYW706 CRYW753 CRYW847 CRYW947 CRYW1034 CRYW1911
solvent
SOLV154 SOLV506 SOLV735 SOLV845 SOLV1107 SOLV1228 SOLV1559 SOLV1566
SOLV1589
```

SETUP _C, SN400

Table S8. Calculated relative QM/MM energies of optimized stationary points for Snapshot 400 of SETUP C in kcal mol^{-1}.

	BP86/Lanl2DZ			B3LYP/Lanl2DZ			
	E_{QM}	E_{MM}	$E_{QM/MM}$	E_{QM}	E_{MM}	$E_{QM/MM}$	Remark
Reactant[a]	0.0	0.0	0.0	0.0	0.0	0.0	
TS1	15.1	-1.9	13.2	18.0	-3.1	14.9	Proton transfer
IM1	12.0	-1.0	11.0	15.8	-2.4	13.4	
TS2	25.9	-7.6	18.2	35.5	-8.6	26.6	C-O formation
IM2	19.8	-6.2	13.5	25.9	-7.4	18.5	
TS3	28.4	-8.6	19.8	37.1	-9.2	27.9	Hydride transfer
Product	13.9	-2.9	11.0	6.9	-4.0	2.9	

[a] absolute energies (a.u.): -1500.442697 -83.783296 -1584.225993 (BP86)
-1499.311013 -83.779643 -1583.090656 (B3LYP)

SETUP C, SN400 (1612 active atoms)

```
chain A
LEU648 PHE649 HSE741 TYR743 THR766 GLN767 ASN768 ALA769 LYS771 MET794
GLY797 PHE798 GLY799 GLY800 LYS801 GLU802 THR803 ARG804 SER805 GLY837
GLY838 ARG839 HSD840 PHE842 ASP872 LEU873 SER874 HSD875 SER876 ILE877
MET878 GLU879 ARG880 ALA881 PHE883 HSD884 ASN908 THR909 ALA910 PHE911
ARG912 GLY913 PHE914 GLY915 GLY916 PRO917 GLN918 LEU920 PHE1005
GLY1006 ILE1007 SER1008 PHE1009 THR1010 VAL1011 PRO1012 LEU1014
ASN1015 GLN1016 GLY1035 THR1036 MET1038 GLN1040 LEU1042 SER1074
SER1075 PRO1076 THR1077 ALA1078 ALA1079 SER1080 VAL1081 SER1082
THR1083 ASP1084 ILE1085 TYR1086 LEU1138 ALA1149 PHE1150 HSD1151
TYR1152 THR1154 GLN1194 ALA1198 GLN1201 ALA1258 VAL1259 GLY1260
GLU1261 PRO1262 PRO1263 LEU1264 PHE1265 MOC2003 URIC2 GOL2
crystal water
CRYW19 CRYW40 CRYW42 CRYW45 CRYW48 CRYW56 CRYW58 CRYW74 CRYW168
CRYW174 CRYW189 CRYW191 CRYW199 CRYW211 CRYW222 CRYW224 CRYW248
CRYW287 CRYW343 CRYW369 CRYW373 CRYW405 CRYW428 CRYW447 CRYW498
CRYW571 CRYW573 CRYW575 CRYW706 CRYW753 CRYW847 CRYW947 CRYW1034
solvent
SOLV506 SOLV686 SOLV799 SOLV1139 SOLV1187 SOLV1559 SOLV1591 SOLV1976
SOLV1991
```

SETUP_D, SN200

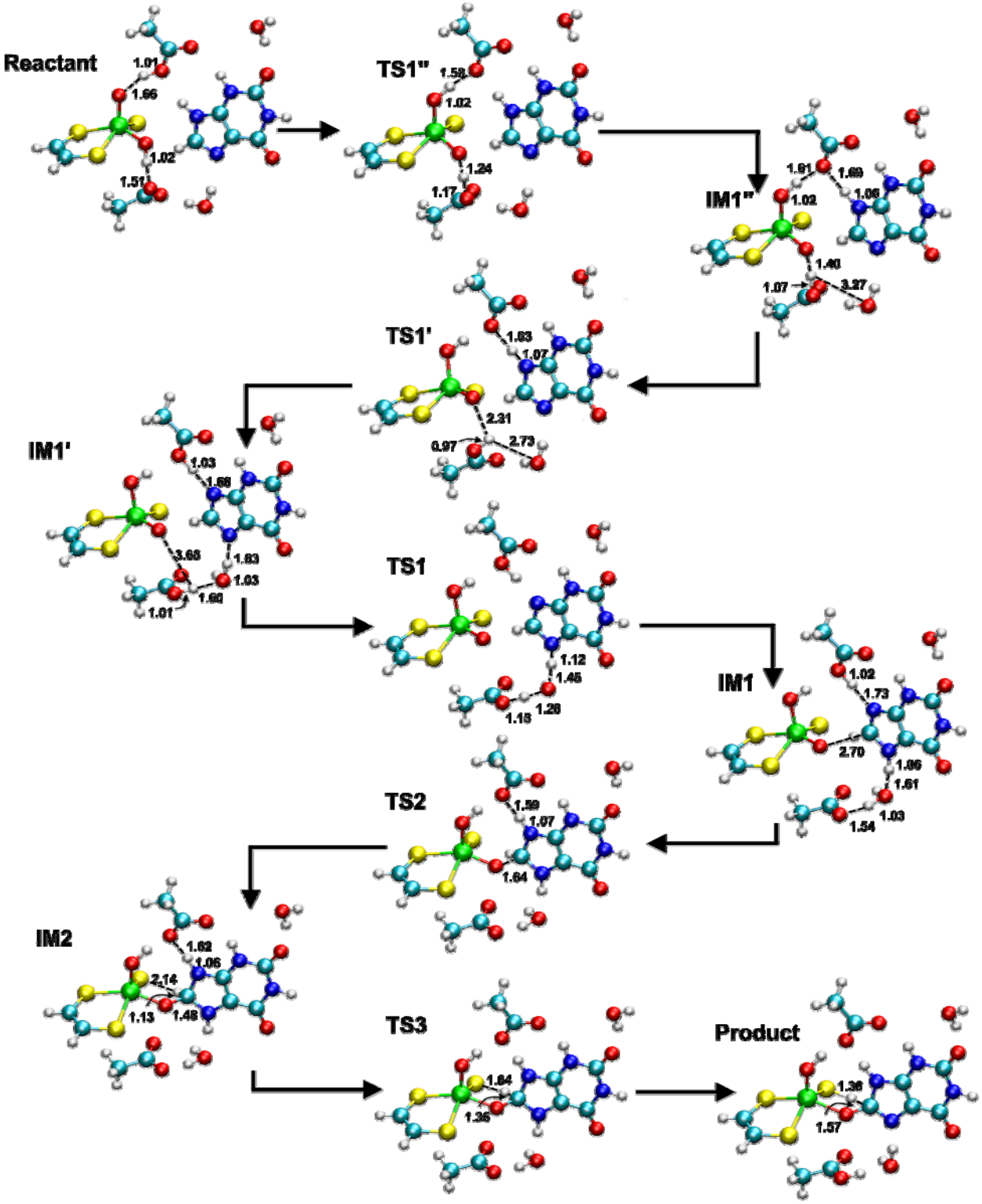

Figure S5. Reaction mechanism for setup D, SN200 from B3LYP/MM calculations. The distances are given in Angstrom.

Table S9. Calculated relative QM/MM energies of optimized stationary points for Snapshot 200 of SETUP D in kcal mol^{-1}.

	BP86/Lanl2DZ			B3LYP/Lanl2DZ			
	E_{QM}	E_{MM}	$E_{QM/MM}$	E_{QM}	E_{MM}	$E_{QM/MM}$	Remark
Reactant[a]	0.0	0.0	0.0	0.0	0.0	0.0	
TS1"	4.5	-1.8	1.0	11.0	-5.6	5.4	Proton transfer1
IM1"	2.4	-3.5	-1.0	10.5	-5.8	4.8	
TS1'	4.5	-3.5	1.0	11.8	-5.2	6.6	OH-rotation
IM1'	3.0	-5.2	-2.2	10.2	-6.4	3.8	
TS1	5.4	-5.2	0.2	12.7	-5.1	7.6	Proton transfer2
IM1	5.6	-5.4	0.2	12.8	-6.0	6.7	
TS2	22.3	-8.4	13.9	37.1	-10.1	27.0	C-O formation
IM2	22.4	-8.7	13.7	35.8	-9.9	25.9	
TS3	25.9	-7.9	18.0	37.6	-9.1	28.5	Hydride transfer
Product	13.0	-5.5	7.5	11.9	-5.9	6.0	

[a] absolute energies (a.u.): -1500.418458 -94.061978 -1594.480436 (BP86)
-1499.290110 -94.058351 -1593.348461 (B3LYP)

SETUP D, SN200 (1589 active atoms)

```
chain A
LEU648 HSE741 TYR743 THR766 GLN767 ASN768 ALA769 LYS771 MET794 GLY797
PHE798 GLY799 GLY800 LYS801 GLU802 THR803 ARG804 SER805 GLY837 GLY838
ARG839 HSD840 PHE842 ASP872 LEU873 SER874 HSD875 SER876 ILE877 MET878
GLU879 ARG880 ALA881 PHE883 HSD884 ASN908 THR909 ALA910 PHE911 ARG912
GLY913 PHE914 GLY915 GLY916 PRO917 GLN918 LEU920 PHE1005 GLY1006
ILE1007 SER1008 PHE1009 THR1010 VAL1011 PRO1012 PHE1013 LEU1014
ASN1015 GLN1016 GLY1035 THR1036 MET1038 GLN1040 LEU1042 SER1074
SER1075 PRO1076 THR1077 ALA1078 ALA1079 SER1080 VAL1081 SER1082
THR1083 ASP1084 ILE1085 TYR1086 LEU1138 ALA1149 PHE1150 HSD1151
THR1154 ALA1198 GLN1201 ALA1258 VAL1259 GLY1260 GLU1261 PRO1262
PRO1263 LEU1264 PHE1265 MOC2003 URIC2 GOL2
crystal water
CRYW19 CRYW40 CRYW42 CRYW45 CRYW48 CRYW56 CRYW58 CRYW126 CRYW168
CRYW174 CRYW189 CRYW191 CRYW203 CRYW211 CRYW222 CRYW224 CRYW244
CRYW248 CRYW287 CRYW343 CRYW369 CRYW373 CRYW405 CRYW418 CRYW447
CRYW498 CRYW571 CRYW575 CRYW706 CRYW753 CRYW847 CRYW947 CRYW1034
solvent
SOLV60 SOLV504 SOLV676 SOLV779 SOLV973 SOLV1559 SOLV1575 SOLV1991
```

SETUP _D, SN400

Table S10. Calculated relative QM/MM energies of optimized stationary points for Snapshot 400 of SETUP C in kcal mol^{-1}.

	BP86/Lanl2DZ			B3LYP/Lanl2DZ			
	E_{QM}	E_{MM}	$E_{QM/MM}$	E_{QM}	E_{MM}	$E_{QM/MM}$	Remark
Reactant[a]	0.0	0.0	0.0	0.0	0.0	0.0	
TS1	6.8	-6.2	0.6	10.9	-7.8	3.0	Proton transfer1
IM1	6.4	-11.9	-5.4	10.2	-13.4	-3.3	
TS1"	9.1	-11.4	-2.3	14.9	-12.6	2.3	Proton transfer2
IM1"	3.4	-8.0	-4.6	6.1	-7.8	-1.7	
TS2	27.9	-12.2	15.7	37.9	-13.2	24.8	C-O formation
IM2	25.9	-11.4	14.5	36.1	-12.1	23.9	
TS3	30.1	-11.6	18.5	40.9	-12.3	28.5	Hydride transfer
Product	13.7	-9.0	4.7	9.5	-9.7	-0.1	
TS2'	24.4	-9.7	14.7	34.6	-10.5	24.0	Proton transfer3
IM2'	22.7	-8.2	14.5	26.6	-8.3	18.3	
TS3'	28.1	-8.4	19.7	34.8	-8.5	26.3	Hydride transfer
Product'	12.2	-4.6	7.6	4.9	-5.0	-0.1	

[a] absolute energies (a.u.): -1500.422839 -93.115010 -1593.537849 (BP86)
-1499.289170 -93.111652 -1592.400822 (B3LYP)

SETUP D, SN400 (1575 active atoms)

```
chain A
LEU648 HSE741 TYR743 THR766 GLN767 ASN768 ALA769 LYS771 MET794 GLY797
PHE798 GLY799 GLY800 LYS801 GLU802 THR803 ARG804 GLY838 ARG839 HSD840
PHE842 ASP872 LEU873 SER874 HSD875 SER876 ILE877 MET878 GLU879 ARG880
ALA881 PHE883 HSD884 ASN908 THR909 ALA910 PHE911 ARG912 GLY913 PHE914
GLY915 GLY916 PRO917 GLN918 LEU920 PHE1005 GLY1006 ILE1007 SER1008
PHE1009 THR1010 VAL1011 LEU1014 ASN1015 GLN1016 GLY1034 GLY1035
THR1036 MET1038 GLN1040 LEU1042 SER1074 SER1075 PRO1076 THR1077
ALA1078 ALA1079 SER1080 VAL1081 SER1082 THR1083 ASP1084 ILE1085
TYR1086 THR1135 LEU1138 ALA1149 PHE1150 HSD1151 TYR1152 THR1154
GLN1194 ALA1198 GLN1201 ALA1258 VAL1259 GLY1260 GLU1261 PRO1262
PRO1263 LEU1264 PHE1265 MOC2003 URIC2 GOL2
crystal water
CRYW19 CRYW40 CRYW42 CRYW45 CRYW48 CRYW56 CRYW58 CRYW74 CRYW126
CRYW168 CRYW174 CRYW189 CRYW211 CRYW222 CRYW224 CRYW248 CRYW287
CRYW343 CRYW369 CRYW373 CRYW405 CRYW418 CRYW447 CRYW498 CRYW571
CRYW575 CRYW706 CRYW847 CRYW947 CRYW1034
solvent
SOLV60 SOLV97 SOLV245 SOLV359 SOLV660 SOLV814 SOLV1046 SOLV1559
SOLV1585 SOLV1991
```

SETUP _E, SN100

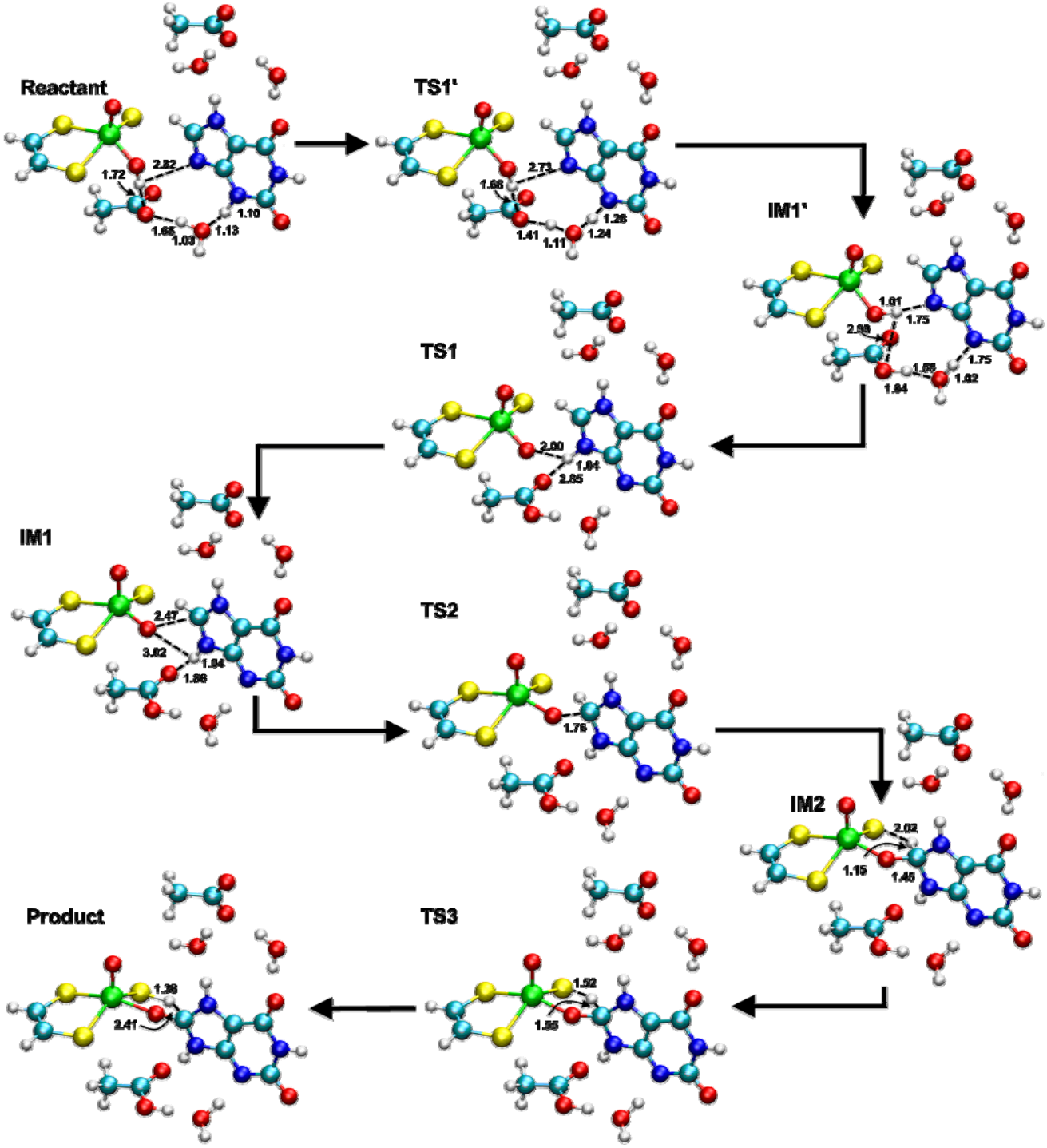

Figure S6. Reaction mechanism for setup E, SN100 from B3LYP/MM calculations. The distances are given in Angstrom.

Table S11. Calculated relative QM/MM energies of optimized stationary points for Snapshot 100 of SETUP E in kcal mol^{-1}.

	BP86/Lanl2DZ			B3LYP/Lanl2DZ			
	E_{QM}	E_{MM}	$E_{QM/MM}$	E_{QM}	E_{MM}	$E_{QM/MM}$	Remark
Reactant[a]	0.0	0.0	0.0	0.0	0.0	0.0	
TS1'	1.2	0.0	1.2	2.6	0.2	2.8	Proton transfer 1
IM1'	-4.9	-0.2	-5.1	-6.0	0.1	-5.9	
TS1	0.5	1.8	2.4	0.9	1.6	2.5	Proton transfer 2
IM1	-5.0	4.2	-0.9	-4.6	4.1	-0.4	
TS2	4.7	1.7	6.3	8.7	1.3	10.1	C-O formation
IM2	3.5	1.3	4.8	6.8	1.2	8.0	
TS3	7.4	0.7	8.1	10.5	0.5	11.0	Hydride transfer
Product	0.8	4.3	5.1	-11.4	5.6	-5.8	

[a] absolute energies (a.u.): -1576.550884 -90.891787 -1667.442671 (BP86)
-1575.372245 -90.889416 -1666.261661 (B3LYP)

SETUP E, SN100 (1590 active atoms)

```
chain A
LEU648 PHE649 HSE741 TYR743 THR766 GLN767 ASN768 ALA769 LYS771 MET794
GLY797 PHE798 GLY799 GLY800 LYS801 GLU802 THR803 ARG804 SER805 GLY837
GLY838 ARG839 HSD840 PHE842 ASP872 LEU873 SER874 HSD875 SER876 ILE877
MET878 GLU879 ARG880 ALA881 PHE883 HSD884 ASN908 THR909 ALA910 PHE911
ARG912 GLY913 PHE914 GLY915 GLY916 PRO917 GLN918 LEU920 PHE1005
GLY1006 ILE1007 SER1008 PHE1009 THR1010 VAL1011 PRO1012 PHE1013
LEU1014 ASN1015 GLN1016 GLY1034 GLY1035 THR1036 MET1038 GLN1040
LEU1042 SER1074 SER1075 PRO1076 THR1077 ALA1078 ALA1079 SER1080
VAL1081 SER1082 THR1083 ASP1084 ILE1085 TYR1086 ALA1149 PHE1150
THR1154 GLN1194 ALA1198 GLN1201 ALA1258 VAL1259 GLY1260 GLU1261
PRO1262 PRO1263 LEU1264 PHE1265 MOC2003 URIC2 GOL2
crystal water
CRYW19 CRYW40 CRYW42 CRYW45 CRYW48 CRYW56 CRYW58 CRYW74 CRYW126
CRYW168 CRYW174 CRYW189 CRYW191 CRYW203 CRYW211 CRYW222 CRYW224
CRYW244 CRYW248 CRYW343 CRYW369 CRYW405 CRYW428 CRYW429 CRYW447
CRYW498 CRYW570 CRYW571 CRYW573 CRYW575 CRYW706 CRYW753 CRYW847
CRYW943 CRYW947 CRYW1034
solvent water
SOLV250 SOLV342 SOLV430 SOLV676 SOLV869 SOLV1207 SOLV1423 SOLV1559
SOLV1991
```

SETUP _E, SN300

Table S12. Calculated relative QM/MM energies of optimized stationary points for Snapshot 300 of SETUP E in kcal mol^{-1}, containing one water molecule less in the QM region than Snapshot 100.

	BP86/Lanl2DZ			B3LYP/Lanl2DZ			
	E_{QM}	E_{MM}	$E_{QM/MM}$	E_{QM}	E_{MM}	$E_{QM/MM}$	Remark
Reactant[a]	0.0	0.0	0.0	0.0	0.0	0.0	
TS1'	-1.0	1.5	0.5	2.9	1.1	4.0	Proton transfer 1
IM1'	-7.6	1.8	-5.8	-8.2	1.1	-7.1	
TS1	-1.4	4.1	2.6	1.8	2.5	4.4	Proton transfer 2
IM1	-7.9	7.1	-0.8	-7.3	9.6	2.3	
TS2	-0.8	6.8	6.0	6.3	6.2	12.5	C-O formation
IM2	-1.1	6.8	5.6	3.5	6.3	9.8	
TS3	3.2	6.8	10.1	7.7	6.8	14.5	Hydride transfer
Product	-8.1	10.5	2.4	-17.7	11.4	-6.3	

[a] absolute energies (a.u.): -1500.067181 -91.057880 -1591.125061 (BP86)
-1498.932206 -91.055535 -1589.987742 (B3LYP)

SETUP E, SN300 (1585 active atoms)

```
chain A
LEU648 PHE649 HSE741 TYR743 THR766 GLN767 ASN768 LYS771 MET794 GLY797
PHE798 GLY799 GLY800 LYS801 GLU802 THR803 ARG804 SER805 GLY837 GLY838
ARG839 HSD840 PHE842 ASP872 LEU873 SER874 HSD875 SER876 ILE877 MET878
GLU879 ARG880 ALA881 PHE883 HSD884 ASN908 THR909 ALA910 PHE911 ARG912
GLY913 PHE914 GLY915 GLY916 PRO917 GLN918 LEU920 PHE1005 GLY1006
ILE1007 SER1008 PHE1009 THR1010 VAL1011 PRO1012 LEU1014 ASN1015
GLN1016 GLY1035 THR1036 MET1038 GLN1040 LEU1042 SER1074 SER1075
PRO1076 THR1077 ALA1078 ALA1079 SER1080 VAL1081 SER1082 THR1083
ASP1084 ILE1085 TYR1086 ALA1149 PHE1150 HSD1151 TYR1152 THR1154
GLN1194 ALA1198 GLN1201 ALA1258 VAL1259 GLY1260 GLU1261 PRO1262
PRO1263 LEU1264 PHE1265 MOC2003 URIC2 GOL2
crystal water
CRYW19 CRYW40 CRYW42 CRYW45 CRYW48 CRYW56 CRYW58 CRYW74 CRYW126
CRYW168 CRYW174 CRYW189 CRYW203 CRYW211 CRYW222 CRYW224 CRYW244
CRYW248 CRYW343 CRYW369 CRYW382 CRYW405 CRYW428 CRYW429 CRYW447
CRYW498 CRYW571 CRYW575 CRYW706 CRYW753 CRYW817 CRYW847 CRYW943
CRYW947 CRYW1034
solvent water
SOLV260 SOLV498 SOLV676 SOLV869 SOLV1317 SOLV1559 SOLV1927 SOLV1991
```

SETUP _E, SN500

Table S13. Calculated relative QM/MM energies of optimized stationary points for Snapshot 500 of SETUP E in kcal mol^{-1}, containing one water molecule less in the QM region than Snapshot 100.

	BP86/Lanl2DZ			B3LYP/Lanl2DZ			
	E_{QM}	E_{MM}	$E_{QM/MM}$	E_{QM}	E_{MM}	$E_{QM/MM}$	Remark
Reactant[a]	0.0	0.0	0.0	0.0	0.0	0.0	
TS1'	1.2	0.8	2.0	2.1	0.7	2.8	Proton transfer 1
IM1'	-8.7	0.7	-8.1	-10.6	1.3	-9.3	
TS1	-0.2	4.2	4.0	0.9	4.3	5.2	Proton transfer 2
IM1	-7.4	9.1	1.7	-6.5	9.1	2.6	
TS2	0.2	6.5	6.7	4.0	6.1	10.1	C-O formation
IM2	-2.2	5.8	3.6	1.5	5.9	7.3	
TS3	2.5	5.7	8.2	5.3	5.7	11.0	Hydride transfer
Product	-2.8	8.6	5.8	-14.4	9.3	-5.0	

[a] absolute energies (a.u.): -1500.120054 -91.178763 -1591.298818 (BP86)
-1498.982528 -91.176664 -1590.159193 (B3LYP)

SETUP E, SN500 (1624 active atoms)

```
chain A
LEU648 PHE649 HSE741 TYR743 THR766 GLN767 ASN768 LYS771 MET794 GLY797
PHE798 GLY799 GLY800 LYS801 GLU802 THR803 ARG804 SER805 GLY837 GLY838
ARG839 HSD840 PHE842 ASP872 LEU873 SER874 HSD875 SER876 ILE877 MET878
GLU879 ARG880 ALA881 PHE883 HSD884 ASN908 THR909 ALA910 PHE911 ARG912
GLY913 PHE914 GLY915 GLY916 PRO917 GLN918 LEU920 PHE1005 GLY1006
ILE1007 SER1008 PHE1009 THR1010 VAL1011 PRO1012 PHE1013 LEU1014
ASN1015 GLN1016 GLY1034 GLY1035 THR1036 MET1038 GLN1040 LEU1042
SER1074 SER1075 PRO1076 THR1077 ALA1078 ALA1079 SER1080 VAL1081
SER1082 THR1083 ASP1084 ILE1085 TYR1086 THR1135 LEU1138 ALA1149
PHE1150 HSD1151 THR1154 GLN1194 ALA1198 GLN1201 ALA1258 VAL1259
GLY1260 GLU1261 PRO1262 PRO1263 LEU1264 PHE1265 MOC2003 URIC2 GOL2
crystal water
CRYW19 CRYW40 CRYW42 CRYW45 CRYW48 CRYW56 CRYW58 CRYW74 CRYW168
CRYW174 CRYW189 CRYW191 CRYW203 CRYW211 CRYW222 CRYW224 CRYW244
CRYW248 CRYW343 CRYW369 CRYW405 CRYW428 CRYW429 CRYW447 CRYW498
CRYW571 CRYW575 CRYW706 CRYW847 CRYW947 CRYW1034 CRYW1556 SOLV498
solvent water
SOLV676 SOLV869 SOLV1057 SOLV1168 SOLV1314 SOLV1323 SOLV1559 SOLV1662
SOLV1927 SOLV1991
```

SETUP _F, SN200

Table S14. Calculated relative QM/MM energies of optimized stationary points for Snapshot 200 of SETUP F in kcal mol^{-1}.

	BP86/Lanl2DZ			B3LYP/Lanl2DZ			
	E_{QM}	E_{MM}	$E_{QM/MM}$	E_{QM}	E_{MM}	$E_{QM/MM}$	Remark
Reactant[a]	0.0	0.0	0.0	0.0	0.0	0.0	
TS1'	0.5	0.6	1.1	2.5	0.2	2.7	Proton transfer 1
IM1'	-7.4	-0.1	-7.5	-9.3	0.3	-9.2	
TS1	-1.6	-3.4	-5.0	1.0	-3.7	-2.7	Proton transfer 2
IM1	-14.2	-2.9	-17.1	-14.0	-2.8	-16.8	
TS2	-2.3	0.3	-2.0	1.6	-0.3	1.3	C-O formation
IM2	-5.2	1.3	-4.0	-1.1	1.4	0.3	
TS3	1.8	1.7	3.5	4.0	-0.3	3.7	Hydride transfer
Product	-3.3	4.5	1.2	13.7	5.5	-8.2	
TS_P'	3.3	3.3	6.6	-7.7	3.9	-3.8	Reorientation
Product'	-3.2	2.0	-1.2	-14.3	2.1	-12.1	

[a] absolute energies (a.u.): -1500.381518 -87.771978 -1588.153496 (BP86)
-1499.245314 -87.769858 -1587.015173 (B3LYP)

SETUP F, SN200 (1565 active atoms)

```
chain A
LEU648 PHE649 HSE741 TYR743 THR766 GLN767 ASN768 ALA769 LYS771 MET794
GLY797 PHE798 GLY799 GLY800 LYS801 GLU802 THR803 ARG804 SER805 GLY837
GLY838 ARG839 HSD840 PHE842 ASP872 LEU873 SER874 HSD875 SER876 ILE877
MET878 GLU879 ARG880 ALA881 PHE883 HSD884 ASN908 THR909 ALA910 PHE911
ARG912 GLY913 PHE914 GLY915 GLY916 PRO917 GLN918 PHE1005 GLY1006
ILE1007 SER1008 PHE1009 THR1010 VAL1011 PRO1012 PHE1013 LEU1014
ASN1015 GLN1016 GLY1034 GLY1035 THR1036 MET1038 GLN1040 LEU1042
SER1074 SER1075 PRO1076 THR1077 ALA1078 ALA1079 SER1080 VAL1081
SER1082 THR1083 ASP1084 ILE1085 TYR1086 LEU1138 ALA1149 PHE1150
THR1154 GLN1194 ALA1198 GLN1201 ALA1258 VAL1259 GLY1260 GLU1261
PRO1262 PRO1263 LEU1264 MOC2003 URIC2 GOL2
crystal water
CRYW19 CRYW40 CRYW42 CRYW45 CRYW48 CRYW56 CRYW58 CRYW126 CRYW157
CRYW168 CRYW174 CRYW189 CRYW191 CRYW211 CRYW222 CRYW224 CRYW248
CRYW343 CRYW369 CRYW373 CRYW405 CRYW447 CRYW498 CRYW570 CRYW571
CRYW573 CRYW575 CRYW685 CRYW706 CRYW842 CRYW847 CRYW1034
solvent water
SOLV319 SOLV538 SOLV662 SOLV686 SOLV763 SOLV857 SOLV1061 SOLV1301
SOLV1641 SOLV1753 SOLV1991
```

SETUP _F, SN400

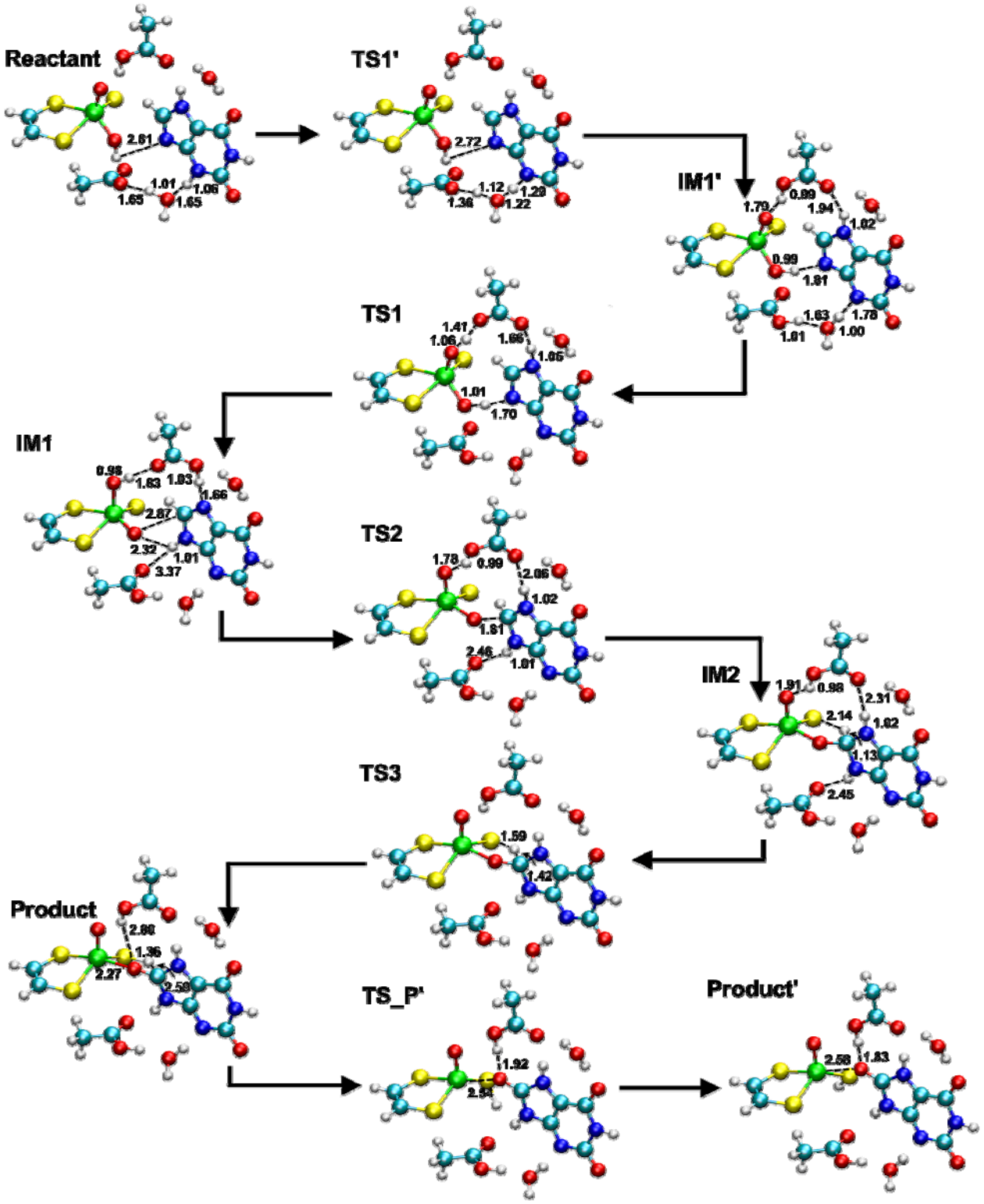

Figure S7. Reaction mechanism for setup F, SN400 from B3LYP/MM calculations. The distances are given in Angstrom.

Table S15. Calculated relative QM/MM energies of optimized stationary points for Snapshot 400 of SETUP F in kcal mol^{-1}.

	BP86/Lanl2DZ			B3LYP/Lanl2DZ			
	E_{QM}	E_{MM}	$E_{QM/MM}$	E_{QM}	E_{MM}	$E_{QM/MM}$	Remark
Reactant[a]	0.0	0.0	0.0	0.0	0.0	0.0	
TS1'	1.6	0.5	2.0	3.6	0.7	4.2	Proton transfer 1
IM1'	-8.5	-0.1	-8.6	-9.6	0.2	-9.4	
TS1	0.1	-3.5	-3.3	4.6	-4.9	-0.4	Proton transfer 2
IM1	-15.6	2.7	-12.9	-15.5	2.4	-13.1	
TS2	0.8	1.7	2.5	5.1	0.9	6.0	C-O formation
IM2	-2.1	0.5	-1.6	1.7	0.2	2.0	
TS3	4.3	0.3	4.6	7.1	0.1	7.2	Hydride transfer
Product	-1.4	2.3	0.8	-13.0	3.9	-9.1	
TS_P'	2.9	1.8	4.7	-7.4	2.0	-5.3	Reorientation
Product'	-3.5	-1.2	-4.7	-13.7	-0.9	-14.6	

[a] absolute energies (a.u.): -1500.35366417 -87.22039847 -1587.57406265 (BP86)
-1499.21756940 -87.21864060 -1586.43621000 (B3LYP)

SETUP F, SN400 (1523 active atoms)

```
chain A
LEU648 PHE649 HSE741 TYR743 THR766 GLN767 ASN768 ALA769 LYS771 MET794
GLY797 PHE798 GLY799 GLY800 LYS801 GLU802 THR803 ARG804 SER805 GLY837
GLY838 ARG839 HSD840 PHE842 ASP872 LEU873 SER874 HSD875 SER876 ILE877
MET878 GLU879 ARG880 ALA881 PHE883 HSD884 ASN908 THR909 ALA910 PHE911
ARG912 GLY913 PHE914 GLY915 GLY916 PRO917 GLN918 PHE1005 GLY1006
ILE1007 SER1008 PHE1009 THR1010 VAL1011 PRO1012 LEU1014 ASN1015
GLN1016 GLY1034 GLY1035 THR1036 MET1038 GLN1040 LEU1042 SER1074
SER1075 PRO1076 THR1077 ALA1078 ALA1079 SER1080 VAL1081 SER1082
THR1083 ASP1084 ILE1085 TYR1086 THR1135 ALA1149 PHE1150 GLN1194
ALA1198 GLN1201 ALA1258 VAL1259 GLY1260 GLU1261 PRO1262 PRO1263
LEU1264 MOC2003 URIC2 GOL2
crystal water
CRYW19 CRYW40 CRYW42 CRYW45 CRYW48 CRYW56 CRYW58 CRYW96 CRYW157
CRYW168 CRYW174 CRYW189 CRYW191 CRYW211 CRYW222 CRYW224 CRYW248
CRYW343 CRYW369 CRYW373 CRYW405 CRYW447 CRYW498 CRYW571 CRYW573
CRYW575 CRYW685 CRYW706 CRYW1034
solvent water
SOLV43 SOLV86 SOLV318 SOLV319 SOLV686 SOLV763 SOLV857 SOLV1061
SOLV1077 SOLV1331 SOLV1641 SOLV1753 SOLV1991
```

SETUP _G

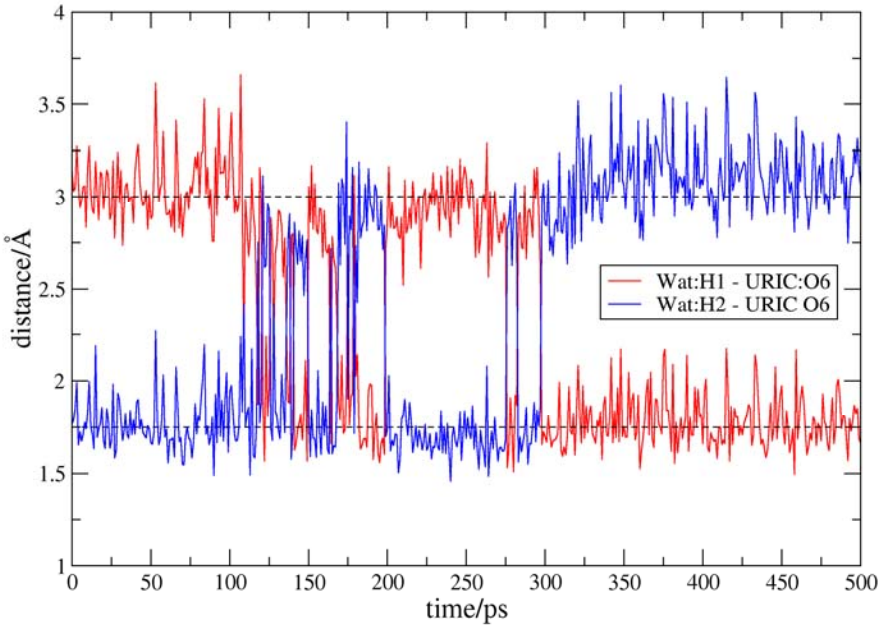

Figure S8. Distance plot for the water molecule coordinating towards O6 of xanthine. The water molecule rotates and thereby changes the coordination mode from H2 to H1 coordinating toward URIC:O6.

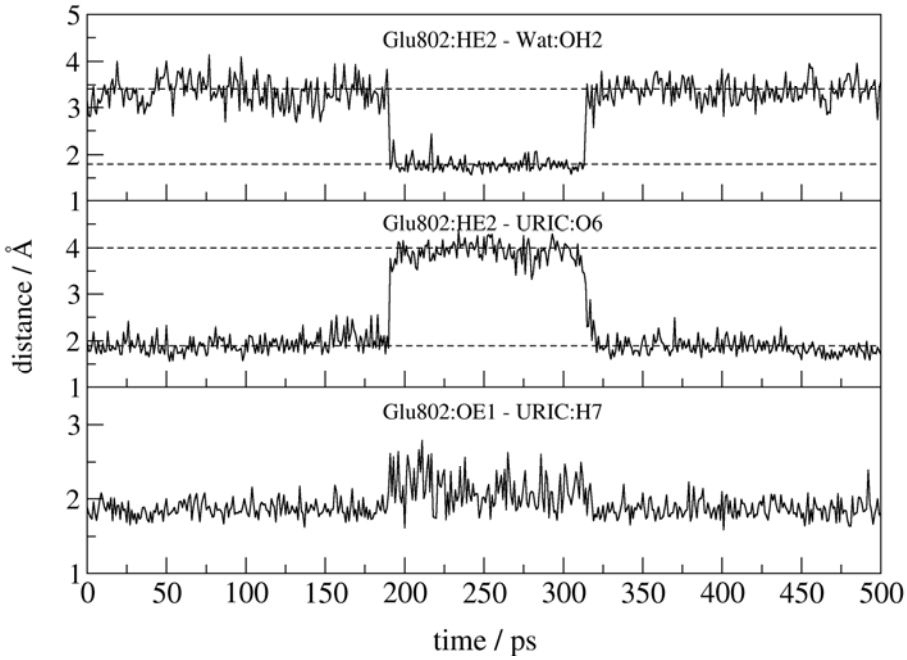

Figure S9. Distance plot for the coordination mode of Glu802: While the coordination of OE1 towards H7 stays intact, we find two different features for the Glu802:HE2 - Uric:O6 interaction: one direct interaction (1- 180 ps and 350 - 500 ps) and a water bridged interaction (180 - 350 ps). We investigated the first one in snapshots SN100 and SN400, and the latter one in SN200.

SETUP _G, SN100

Table S16. Calculated relative QM/MM energies of optimized stationary points for Snapshot 100 of SETUP G in kcal mol^{-1}.

	BP86/Lanl2DZ			B3LYP/Lanl2DZ			
	E_{QM}	E_{MM}	$E_{QM/MM}$	E_{QM}	E_{MM}	$E_{QM/MM}$	Remark
Reactant[a]	0.0	0.0	0.0	0.0	0.0	0.0	
TS1'	-0.7	1.6	0.9	3.1	1.5	4.5	Proton transfer 1
IM1'	-2.8	-3.1	-5.9	-1.9	-3.7	-5.6	
TS1"	-1.9	-3.5	-5.4	-1.3	-4.0	-5.3	OH-Rotation
IM1"	-5.4	-5.1	-10.5	-4.3	-5.6	-9.8	
TS1	1.0	-0.4	0.6	0.8	-0.9	-0.1	Proton transfer 2
IM1	0.1	-0.4	-0.3	0.2	-0.4	-0.2	
TS2	1.0	-0.4	0.6	8.5	-5.1	3.4	C-O formation
IM2	2.3	-7.0	-4.7	6.9	-8.1	-1.2	
TS3	8.6	-5.5	3.1	12.8	-8.0	4.8	Hydride transfer
Product	4.7	-2.8	1.9	-5.2	-2.0	-7.2	

[a] absolute energies (a.u.): -1500.367965 -89.818817 -1590.186781 (BP86)
-1499.232671 -89.815870 -1589.048541 (B3LYP)

SETUP G, SN100 (1550 active atoms)

```
chain A
LEU648 PHE649 HSE741 TYR743 THR766 GLN767 ASN768 ALA769 LYS771 MET794
GLY796 GLY797 PHE798 GLY799 GLY800 LYS801 GLU802 THR803 ARG804 SER805
GLY837 GLY838 ARG839 HSD840 PHE842 ASP872 LEU873 SER874 HSD875 SER876
ILE877 MET878 GLU879 ARG880 ALA881 PHE883 HSD884 ASN908 THR909 ALA910
PHE911  ARG912  GLY913  PHE914  GLY915  GLY916  PRO917  GLN918  PHE1005
GLY1006  ILE1007  SER1008  PHE1009  THR1010  VAL1011  LEU1014  ASN1015
GLN1016  GLY1035  THR1036  MET1038  GLN1040  LEU1042  SER1074  SER1075
PRO1076  THR1077  ALA1078  ALA1079  SER1080  VAL1081  SER1082  THR1083
ASP1084  ILE1085  TYR1086  ALA1149  PHE1150  TYR1152  THR1154  GLN1194
ALA1198  GLN1201  ALA1258  VAL1259  GLY1260  GLU1261  PRO1262  PRO1263
LEU1264 PHE1265 MOC2003 URIC2 GOL2
crystal water
CRYW19  CRYW40  CRYW42  CRYW45  CRYW48  CRYW56  CRYW58  CRYW74  CRYW114
CRYW126  CRYW168  CRYW174  CRYW189  CRYW191  CRYW211  CRYW222  CRYW224
CRYW248  CRYW343  CRYW369  CRYW382  CRYW447  CRYW498  CRYW571  CRYW575
CRYW632 CRYW706 CRYW847 CRYW1034 CRYW1205
solvent water
SOLV421  SOLV455  SOLV491  SOLV546  SOLV573  SOLV751  SOLV857  SOLV1321
SOLV1572 SOLV1641 SOLV1753 SOLV1803
```

SETUP _G, SN200

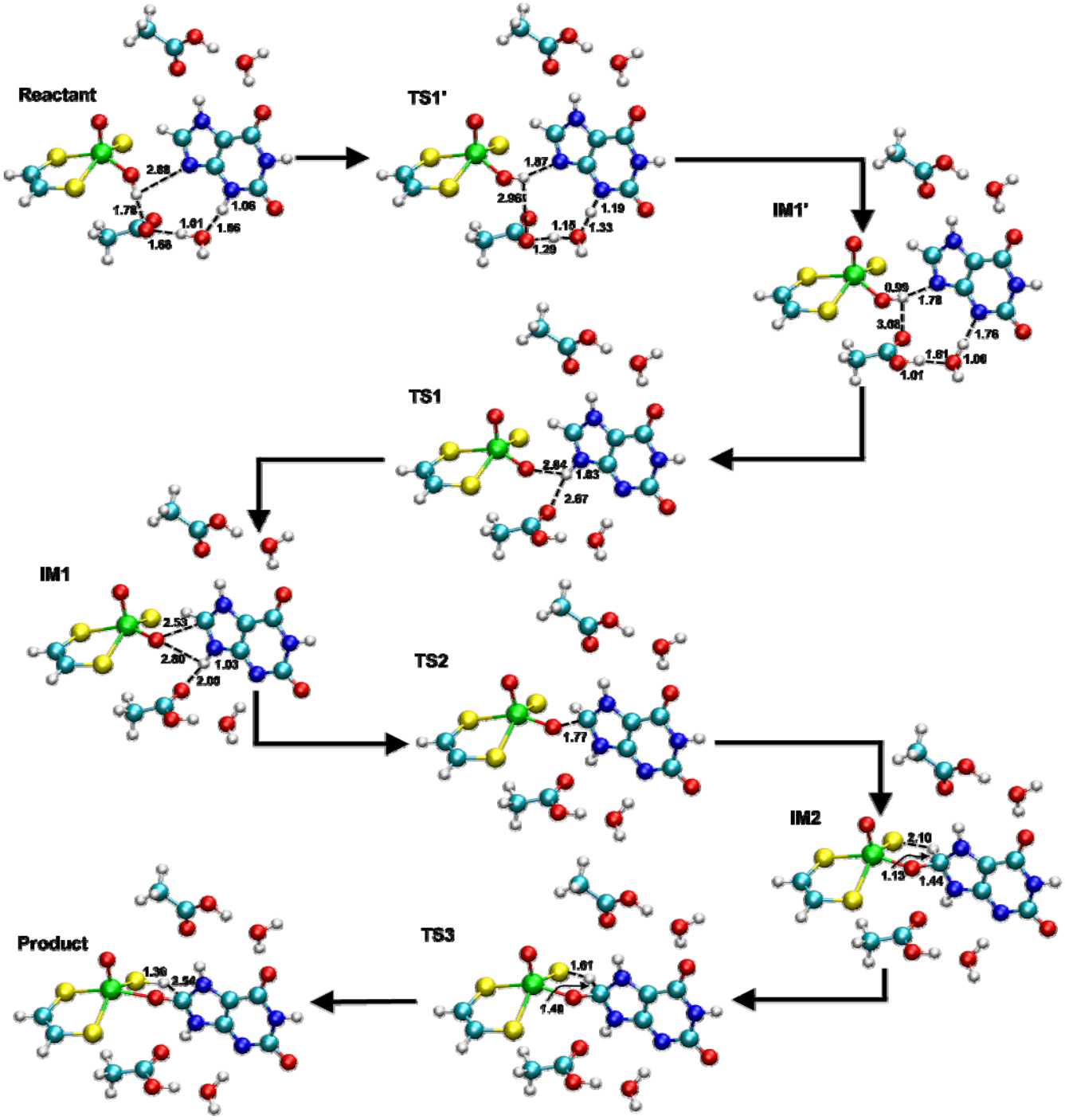

Figure S10. Reaction mechanism for setup G, SN200 from B3LYP/MM calculations. The distances are given in Angstrom.

Table S17. Calculated relative QM/MM energies of optimized stationary points for Snapshot 200 of SETUP G in kcal mol^{-1}.

	BP86/Lanl2DZ			B3LYP/Lanl2DZ			
	E_{QM}	E_{MM}	$E_{QM/MM}$	E_{QM}	E_{MM}	$E_{QM/MM}$	Remark
Reactant[a]	0.0	0.0	0.0	0.0	0.0	0.0	
TS1'	3.9	0.3	4.2	5.5	-0.4	5.1	Proton transfer 1
IM1'	-5.2	0.6	-4.6	-5.1	0.3	-4.8	
TS1	-1.3	4.5	3.1	-0.7	3.9	3.3	Proton transfer 2
IM1	-1.3	3.9	2.6	-0.8	4.0	3.2	
TS2	8.5	-0.7	7.8	13.4	-0.9	12.5	C-O formation
IM2	6.8	-2.5	4.4	12.5	-2.8	9.7	
TS3	11.1	-1.1	10.1	14.2	-0.7	13.5	Hydride transfer
Product	1.7	3.2	4.9	-8.0	3.9	-4.1	

[a] absolute energies (a.u.): -1500.354732 -43.210292 -1543.565024 (BP86)
-1499.218480 -43.208278 -1542.426758 (B3LYP)

SETUP G, SN200 (1588 active atoms)

```
chain A
LEU648 PHE649 HSE741 TYR743 THR766 GLN767 ASN768 ALA769 LYS771 MET794
GLY796 GLY797 PHE798 GLY799 GLY800 LYS801 GLU802 THR803 ARG804 SER805
GLY837 GLY838 ARG839 HSD840 PHE842 ASP872 LEU873 SER874 HSD875 SER876
ILE877 MET878 GLU879 ARG880 ALA881 PHE883 HSD884 ASN908 THR909 ALA910
PHE911  ARG912  GLY913  PHE914  GLY915  GLY916  PRO917  GLN918  LEU920
PHE1005  GLY1006  ILE1007  SER1008  PHE1009  THR1010  VAL1011  PHE1013
LEU1014  ASN1015  GLN1016  GLY1035  THR1036  MET1038  GLN1040  LEU1042
SER1074  SER1075  PRO1076  THR1077  ALA1078  ALA1079  SER1080  VAL1081
SER1082  THR1083  ASP1084  ILE1085  TYR1086  PHE1150  TYR1152  THR1154
GLN1194  ALA1198  GLN1201  ALA1258  VAL1259  GLY1260  GLU1261  PRO1262
PRO1263 LEU1264 PHE1265 MOC2003 URIC2 GOL2
crystal water
CRYW189  CRYW191  CRYW211  CRYW222  CRYW224  CRYW244  CRYW248  CRYW343
CRYW369  CRYW382  CRYW447  CRYW498  CRYW571  CRYW575  CRYW632  CRYW706
CRYW847 CRYW1034 CRYW1205
solvent water
SOLV138 SOLV421 SOLV455 SOLV491 SOLV546 SOLV573 SOLV857 SOLV893
SOLV1272 SOLV1321 SOLV1572 SOLV1641 SOLV1752 SOLV1753
```

SETUP _G, SN400

Table S18. Calculated relative QM/MM energies of optimized stationary points for Snapshot 400 of SETUP G in kcal mol^{-1}.

	BP86/Lanl2DZ			B3LYP/Lanl2DZ			
	E_{QM}	E_{MM}	$E_{QM/MM}$	E_{QM}	E_{MM}	$E_{QM/MM}$	Remark
Reactant[a]	0.0	0.0	0.0	0.0	0.0	0.0	
TS1'	2.8	0.1	2.9	9.4	-0.9	8.5	Proton transfer 1
IM1'	-7.0	1.0	-8.0	-6.0	-1.5	-7.5	
TS1"	-6.7	-0.7	-7.4	-5.3	-1.9	-7.2	OH-Rotation
IM1"	-7.2	-1.7	-9.0	-6.2	-2.4	-8.6	
TS1	0.0	1.0	1.0	3.1	-0.6	2.5	Proton transfer 2
IM1	-13.5	10.1	-3.4	-10.8	9.0	-1.8	
TS2	-6.7	4.5	-2.1	-0.2	2.6	2.5	C-O formation
IM2	-1.5	-6.3	-7.8	-2.2	-0.2	-2.5	
TS3	-1.9	2.6	0.7	3.2	1.3	4.5	Hydride transfer
Product	-8.6	7.9	-0.8	-17.9	8.7	-9.3	

[a] absolute energies (a.u.): -1500.413756 -49.980759 -1550.394515 (BP86)
-1499.281279 -49.976648 -1549.257927 (B3LYP)

SETUP G, SN400 (1595 active atoms)

chain A

LEU648 HSE741 TYR743 THR766 GLN767 ASN768 ALA769 LYS771 MET794 GLY796
GLY797 PHE798 GLY799 GLY800 LYS801 GLU802 THR803 ARG804 SER805 GLY837
GLY838 ARG839 HSD840 PHE842 ASP872 LEU873 SER874 HSD875 SER876 ILE877
MET878 GLU879 ARG880 ALA881 PHE883 HSD884 ASN908 THR909 ALA910 PHE911
ARG912 GLY913 PHE914 GLY915 GLY916 PRO917 GLN918 LEU920 PHE1005
GLY1006 ILE1007 SER1008 PHE1009 THR1010 VAL1011 PHE1013 LEU1014
ASN1015 GLN1016 GLY1035 THR1036 MET1038 GLN1040 LEU1042 SER1074
SER1075 PRO1076 THR1077 ALA1078 ALA1079 SER1080 VAL1081 SER1082
THR1083 ASP1084 ILE1085 TYR1086 ALA1149 PHE1150 HSD1151 TYR1152
THR1154 GLN1194 ALA1198 GLN1201 ALA1258 VAL1259 GLY1260 GLU1261
PRO1262 PRO1263 LEU1264 PHE1265 MOC2003 URIC2 GOL2

crystal water

CRYW19 CRYW40 CRYW42 CRYW45 CRYW48 CRYW56 CRYW58 CRYW74 CRYW114
CRYW168 CRYW174 CRYW189 CRYW191 CRYW211 CRYW213 CRYW222 CRYW224
CRYW244 CRYW248 CRYW343 CRYW369 CRYW382 CRYW429 CRYW447 CRYW498
CRYW571 CRYW575 CRYW632 CRYW706 CRYW847 CRYW1034

solvent water

SOLV163 SOLV228 SOLV419 SOLV421 SOLV573 SOLV574 SOLV857 SOLV893
SOLV1276 SOLV1321 SOLV1641 SOLV1752 SOLV1753 SOLV1991

Table S19. Calculated QM/MM energies of the rate limiting stationary points for snapshot 400 of SETUP G in kcal mol^{-1} using the L2 link atom scheme, from single point calculations (SP) on the structures obtained with the charge-shift link atom scheme and for the reoptimized structures (OPT).

	B3LYP/Lanl2DZ (SP)			B3LYP/Lanl2DZ (OPT)			
	E_{QM}	E_{MM}	$E_{QM/MM}$	E_{QM}	E_{MM}	$E_{QM/MM}$	Remark
IM1" [a,b]	0.0	0.0	0.0	0.0	0.0	0.0	
TS3	9.9	3.8	13.6	10.1	3.4	13.5	Hydride transfer

[a] IM1" and TS3 are the lowest and highest point on the energy profile, respectively. The corresponding energy differences for the charge-shift link atom scheme are 9.4, 3.2, and 13.1 kcal mol^{-1} (Table S18).
[b] absolute energies (a.u.): -1499.268497 -49.980544 -1549.249042 (single point)
-1499.268638 -49.981706 -1549.250344 (optimized)

Table S20. Calculated relative QM/MM energies of optimized stationary points for Snapshot 400 of SETUP G in kcal mol^{-1} using BP86 as functional, values for B3LYP are given in the paper. The QM and MM contributions are given in Tables S21-S23, see below.

	BP86/Lanl2DZ		BP86/def2-SVP		BP86/def2-TZVP		BP86/TZVP-all_electron	
Reactant	0.0		0.0		0.0		0.0	
TS1'	2.9	(2.9)	2.4	(2.4)	3.1	(3.1)	2.5	(2.5)
IM1'	-8.0		-7.2		-7.9		-8.5	
TS1"	-7.4	(0.6)	-7.0	(0.2)	-7.7	(0.2)	-8.0	(0.5)
IM1"	-9.0		-7.9		-8.7		-9.4	
TS1	1.0	(10.0)	1.5	(9.4)	1.3	(9.0)	2.9	(12.3)
IM1	-3.4		-4.4		-2.8		-1.6	
TS2	-2.1	(1.3)	-3.4	(1.0)	-0.9	(1.9)	-0.5	(1.0)
IM2	-7.8		-9.1		-5.7		-7.9	
TS3	0.7	(8.5)	-0.6	(8.5)	4.3	(10.0)	-1.3	(6.6)
Product	-0.8		-1.3		2.4		-5.7	
ΔE_{max}	10.0		9.4		13.0		12.3	

Table S21. Calculated relative QM/MM energies of optimized stationary points for Snapshot 400 of SETUP G in kcal mol^{-1}. Energies for single point calculation with the SVPalls2 basis set for B3LYP are given in parentheses.

	BP86/def2-SVP			B3LYP/def2-SVP		
	E_{QM}	E_{MM}	$E_{QM/MM}$	E_{QM}	E_{MM}	$E_{QM/MM}$
Reactant	0.0	0.0	0.0	0.0(0.0)	0.0(0.0)	0.0(0.0)
TS1'	2.8	-0.4	2.4	5.6(4.7)	-0.4(-0.4)	5.3(4.3)
IM1'	-6.4	-0.8	-7.2	-8.9(-10.3)	-0.1(-0.1)	-9.0(-10.4)
TS1"	-6.1	-0.9	-7.0	-7.9(-8.9)	-0.4(-0.4)	-8.3(-9.3)
IM1"	-5.8	-2.1	-7.9	-8.1(-8.4)	-1.6(-1.6)	-9.7(9.9)
TS1	0.6	0.9	1.5	0.2(2.2)	0.6(0.6)	0.8(2.8)
IM1	-14.4	10.0	-4.4	-15.3(-12.2)	10.5(10.5)	-4.8(-1.7)
TS2	-8.3	4.9	-3.4	-5.4(-5.0)	4.2(4.2)	-1.2(0.8)
IM2	-3.2	-5.9	-9.1	-0.6(-3.1)	-6.3(6.3)	-6.8(9.4)
TS3	-3.5	2.9	-0.6	-1.3(-7.6)	2.2(2.2)	0.9(-5.3)
Product	-9.4	8.1	-1.3	-20.5(-30.7)	10.3(10.3)	-10.3(-20.4)

Table S22. Calculated relative QM/MM energies of optimized stationary points for Snapshot 400 of SETUP G in kcal mol^{-1}. Energies for single point calculation with the TZVPalls2 basis set for B3LYP are given in parentheses.

	BP86/def2-TZVP			B3LYP/def2-TZVP		
	E_{QM}	E_{MM}	$E_{QM/MM}$	E_{QM}	E_{MM}	$E_{QM/MM}$
Reactant	0.0	0.0	0.0	0.0(0.0)	0.0(0.0)	0.0(0.0)
TS1'	4.2	-1.1	3.1	8.9(9.1)	-1.2(-1.2)	7.7(8.0)
IM1'	-6.1	-1.8	-7.9	-7.4(-7.2)	-1.5(-1.5)	-8.9(-8.7)
TS1"	-6.0	-1.7	-7.7	-6.5(-6.2)	-1.8(-1.8)	-8.2(-8.0)
IM1"	-5.9	-2.8	-8.7	-6.7(-7.0)	-2.5(-2.5)	-9.2(-9.5)
TS1	1.6	-0.4	1.3	3.0(5.4)	-0.7(-0.7)	2.4(4.7)
IM1	-12.1	9.3	-2.8	-10.6(-8.6)	9.4(9.4)	-1.3(0.8)
TS2	-4.7	3.8	-0.9	0.3(0.7)	2.8(2.8)	3.1(3.6)
IM2	1.4	-7.0	-5.7	5.9(4,1)	-7.3(-7.3)	-1.3(-3.1)
TS3	2.1	2.2	4.3	6.5(1.2)	1.3(1.3)	7.7(2.5)
Product	-4.8	7.3	2.4	-13.5(-23.2)	8.5(8.5)	-5.0(-14.7)

Table S23. Calculated relative QM/MM energies of optimized stationary points for Snapshot 400 of SETUP G in kcal mol^{-1}.

	BP86/all-electron-TZVP			B3LYP/all-electron-TZVP		
	E_{QM}	E_{MM}	$E_{QM/MM}$	E_{QM}	E_{MM}	$E_{QM/MM}$
Reactant	0.0	0.0	0.0	0.0	0.0	0.0
TS1'	3.2	-0.7	2.5	9.1	-1.1	8.0
IM1'	-7.1	-1.5	-8.5	-7.0	-1.7	-8.7
TS1"	-6.8	-1.2	-8.0	-6.5	-1.5	-8.0
IM1"	-6.6	-2.8	-9.4	-6.7	-2.8	-9.5
TS1	2.8	0.0	2.9	5.3	-0.6	4.7
IM1	-11.0	9.4	-1.6	-8.6	9.3	0.8
TS2	-5.3	4.9	-0.5	0.8	3.0	3.8
IM2	-1.0	-6.9	-7.9	4.2	-7.5	-3.3
TS3	-3.6	2.3	-1.3	1.6	1.2	2.7
Product	-13.7	8.0	-5.7	-23.3	8.7	-14.7

Table S24. Results for the complete enzyme (setup G): QM/MM energies in kcal mol^{-1} for different basis sets, relative to the energy of the reactant. Activation barriers relative to the preceding minima are given in parentheses. BP86 results.

	ALL-BP86/Lanl2DZ		ALL-BP86/def2-SVP		ALL-BP86/def2-SVP-all_electron (SP)		ALL-BP86/def2-TZVP		ALL-BP86/def2-TZVP-all_electron (SP)		ALL-BP86/TZVP-all_electron	
Reactant	0.0		0.0		0.0		0.0		0.0		0.0	
TS1'	3.5	(3.5)	1.8	(1.8)	1.6	(1.6)	2.6	(2.6)	2.8	(2.8)		
IM1'	-7.9		-6.9		-7.2		-7.9		-8.1			
TS1"	-7.8	(0.1)	-6.4	(0.5)	-6.3	(0.9)	-7.4	(0.5)	-7.7	(0.4)		
IM1"	-9.0		-8.1		-8.1		-8.9		-9.5		-9.5	
TS1	1.0	(10.0)	0.9	(9.0)	2.9	(11.0)	1.1	(10.0)	2.5	(12.0)	2.6	(12.1)
IM1	-2.7		-4.0		-1.2		-3.1		-0.6		-0.6	
TS2	-2.1	(0.6)	-3.7	(0.3)	-2.3	(-1.1)	-0.7	(2.4)	-0.5	(0.1)	-0.1	(0.5)
IM2	-8.1		-9.3		-11.2		-5.7		-8.1		-8.2	
TS3	0.4	(8.5)	-0.9	(8.6)	-5.4	(5.8)	3.5	(9.2)	-1.7	(6.4)	-1.4	(6.8)
Product	-1.2		-1.1		-7.5		1.3		-5.5		-5.5	
ΔE_{max}	10.0		9.0		11.0		12.4		12.0		12.1	

Table S25. Results for the complete enzyme (setup G): QM/MM energies in kcal mol^{-1} for different basis sets, relative to the energy of the reactant. Activation barriers relative to the preceding minima are given in parentheses. B3LYP results.

	ALL-B3LYP/Lanl2DZ		ALL-B3LYP/def2-SVP		ALL-B3LYP/def2-SVP-all_electron (SP)		ALL-B3LYP/def2-TZVP		ALL-B3LYP/def2-TZVP-all_electron (SP)		ALL-B3LYP/def2-TZVP-all_electron	
Reactant	0.0		0.0		0.0		0.0		0.0		0.0	
TS1'	7.7	(7.7)	4.7	(4.7)	4.5	(4.5)	8.1	(8.1)	7.9	(7.9)		
IM1'	-8.4		-9.4		-9.2		-8.1		-7.7			
TS1"	-7.6	(0.8)	-8.4	(1.0)	-8.3	(0.9)	-7.4	(0.7)	-7.6	(0.1)		
IM1"	-9.2		-9.5		-9.0		-8.9		-9.2		-9.1	
TS1	1.6	(10.8)	0.2	(9.7)	2.9	(11.9)	0.9	(9.8)	3.5	(12.7)	3.6	(12.7)
IM1	-2.2		-3.8		-1.0		-1.4		1.4		1.5	
TS2	1.4	(3.6)	-1.3	(2.5)	0.0	(1.0)	3.7	(5.1)	3.6	(2.2)	3.9	(2.4)
IM2	-3.6		-7.0		-8.6		-1.2		-3.2		-3.2	
TS3	2.9	(6.5)	0.4	(7.4)	-4.7	(3.9)	7.8	(9.0)	2.6	(5.8)	2.9	(6.2)
Product	-10.8		-10.6		-20.3		-5.8		-14.7		-14.5	
ΔE_{max}	12.1		9.9		11.9		16.7		12.7		13.0	

Table S26. Results for the complete enzyme (setup G): QM, MM and QM/MM energies in kcal mol^{-1} for the Lanl2DZ basis set, relative to the energy of the reactant.

	ALL-BP86/LANL2DZ			ALL-B3LYP/LANL2DZ		
	E_{QM}	E_{MM}	$E_{QM/MM}$	E_{QM}	E_{MM}	$E_{QM/MM}$
Reactant	0.0	0.0	0.0	0.0	0.0	0.0
TS1'	3.7	-0.2	3.5	9.6	-1.9	7.7
IM1'	-6.8	-1.1	-7.9	-5.6	-2.7	-8.4
TS1"	-7.0	-0.8	-7.8	-4.6	-2.9	-7.6
IM1"	-6.8	-2.2	-9.0	-5.4	-3.9	-9.2
TS1	0.0	1.0	1.0	2.6	-1.0	1.6
IM1	-12.4	9.7	-2.7	-10.0	7.8	-2.2
TS2	-6.9	4.8	-2.1	0.0	1.5	1.4
IM2	-1.9	-6.2	-8.1	-1.4	-2.3	-3.6
TS3	-2.1	2.4	0.4	3.2	-0.3	2.9
Product	-8.8	7.5	-1.2	-18.0	7.2	-10.8

Table S27. Results for the complete enzyme (setup G): QM, MM and QM/MM energies in kcal mol^{-1} for the def2-SVP basis set, relative to the energy of the reactant. The values in parentheses are from single point calculations with the all-electron-SVP basis set.

	ALL-BP86/def2-SVP			ALL-B3LYP/def2-SVP		
	E_{QM}	E_{MM}	$E_{QM/MM}$	E_{QM}	E_{MM}	$E_{QM/MM}$
Reactant	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)
TS1'	2.3(2.1)	-0.5(-0.5)	1.8(1.6)	5.3(5.1)	-0.7(-0.7)	4.7(4.5)
IM1'	-6.0(-6.3)	-0.9(-0.9)	-6.9(-7.2)	-8.7(-8.6)	-0.6(-0.6)	-9.4(-9.2)
TS1"	-5.4(-5.3)	-1.0(-1.0)	-6.4(-6.3)	-7.6(-7.5)	-0.8(-0.8)	-8.4(-8.3)
IM1"	-4.7(-4.6)	-3.5(-3.5)	-8.1(-8.1)	-7.2(-6.8)	-2.2(-2.2)	-9.5(-9.0)
TS1	-0.3(1.8)	1.1(1.1)	0.9(2.9)	-0.4(2.3)	0.6(0.6)	0.2(2.9)
IM1	-13.9(-11.1)	9.9(9.9)	-4.0(-1.2)	-13.7(10.9)	9.9(9.9)	-3.8(-1.0)
TS2	-9.0(-7.6)	5.3(5.3)	-3.7(-2.3)	-5.0(-3.7)	3.7(3.7)	-1.3(0.0)
IM2	-3.3(-5.2)	-6.0(-6.0)	-9.3(-11.2)	-0.3(-1.9)	-6.7(-6.7)	-7.0(-8.6)
TS3	-3.2(-7.6)	2.3(2.3)	-0.9(-5.4)	-1.2(-6.3)	1.5(1.5)	0.4(-4.7)
Product	-9.0(-15.3)	7.9(7.9)	-1.1(7.5)	-20.2(-29.8)	9.6(9.6)	-10.6(-20.3)

Table S28. Results for the complete enzyme (setup G): QM, MM and QM/MM energies in kcal mol^{-1} for the def2-TZVP basis set, relative to the energy of the reactant. The values in parentheses are from single point calculations with the all-electron-SVP basis set.

	ALL-BP86/def2-TZVP			ALL-B3LYP/def2-TZVP		
	E_{QM}	E_{MM}	$E_{QM/MM}$	E_{QM}	E_{MM}	$E_{QM/MM}$
Reactant	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)	0.0(0.0)
TS1'	4.0(4.1)	-1.4(-1.4)	2.6(2.8)	10.2(10.0)	-2.0(-2.0)	8.1(7.9)
IM1'	-6.2(-6.4)	-1.7(-1.7)	-7.9(-8.1)	-5.8(-5.3)	-2.3(-2.3)	-8.1(-7.7)
TS1"	-5.7(-6.1)	-1.6(-1.6)	-7.4(-7.7)	-5.0(-5.1)	-2.5(-2.5)	-7.4(-7.6)
IM1"	-5.8(-6.5)	-3.0(-3.0)	-8.9(-9.5)	-5.4(-5.7)	-3.5(-3.5)	-8.9(-9.2)
TS1	0.5(1.9)	0.6(0.6)	1.1(2.5)	1.5(4.1)	-0.6(-0.6)	0.9(3.5)
IM1	-12.3(-9.7)	9.2(9.2)	-3.1(-0.6)	-10.1(-7.3)	8.7(8.7)	-1.4(1.4)
TS2	-4.3(-4.0)	3.5(3.5)	-0.7(-0.5)	1.7(1.7)	1.9(1.9)	3.7(3.6)
IM2	1.1(-1.2)	1.9(1.9)	-5.7(-8.1)	6.8(4.9)	-8.1(-8.1)	-1.2(-3.2)
TS3	1.6(-3.5)	1.9(1.9)	3.5(-1.7)	7.6(2.4)	0.2(0.2)	7.8(2.6)
Product	-5.7(-12.5)	7.0(7.0)	1.3(-5.5)	-13.7(-22.6)	7.9(7.9)	-5.8(-14.7)

Table S29. Results for the complete enzyme (setup G): Calculated relative QM, MM and QM/MM energies of a subset of optimized stationary points for Snapshot 400 in kcal mol^{-1}.

	ALL-BP86/all-electron-TZVP			ALL-B3LYP/all-electron-TZVP		
	E_{QM}	E_{MM}	$E_{QM/MM}$	E_{QM}	E_{MM}	$E_{QM/MM}$
Reactant	0.0	0.0	0.0	0.0	0.0	0.0
IM1"	-6.3	-3.2	-9.5	-4.5	-4.7	-9.1
TS1	2.0	0.6	2.6	5.2	-1.6	3.6
IM1	-9.6	9.0	-0.6	-6.2	7.7	1.5
TS2	-4.5	4.5	-0.1	2.7	1.2	3.9
IM2	-1.1	-7.0	-8.2	6.0	-9.3	-3.2
TS3	-3.0	1.6	-1.4	4.0	-1.2	2.9
Product	-13.1	7.6	-5.5	-21.5	7.0	-14.5

3. Consistency checks

Meaningful energy profiles can be obtained only if the calculated stationary points are connected by contiguous reaction paths. For example, there must not be any distant conformational changes such as side chain flips and water reorientations that are unrelated to the reaction under investigation. In our QM/MM work, these requirements are routinely checked by visual inspection. Here we report some additional quantitative checks that involve the RMSD (root-mean square deviation) between the optimized QM/MM structures of the reactant complex and the rate-limiting transition state TS3. For a given setup and snapshot, these structures are easily superimposed because they share the same fixed outer region. Table S30 presents the computed RMSD values for all snapshots considered, in particular those for the active optimized region (typically around 1600 atoms) and for the active region without the QM atoms. The latter RMSD values are most relevant for assessing the structural consistency of the protein environment during the reaction. They are generally found to be about 0.1 Å in all cases which indicates a high degree of overall structural consistency. A residue-specific analysis was performed for setup A (SN500) and setup G (SN400). Figures S11 and S12 show the RMSD values of individual residues that exceed 0.1 Å, respectively. The main structural changes trivially occur in the QM region (colored in green) where the reaction takes place. The side chains of the MM residues next to the QM region (colored in red) also show some appreciable changes because they have to adapt to the reactive events in the QM region. The same holds to a lesser extent for the next shell of residues (colored in yellow). In residues farther away from the QM region (colored in blue), the changes are getting less and less pronounced (RMSD values mostly below 0.2 Å). It should be noted that Figure S11 (S12) shows the RMSD data for only 38 (40) residues out of a total of 135 (141) residues in the active region (see pages S9 and S30) since the RMSD values for the remaining residues are below the threshold of 0.1 Å. The plots in Figures S11 and S12 confirm the absence of structural inconsistencies in distant residues.

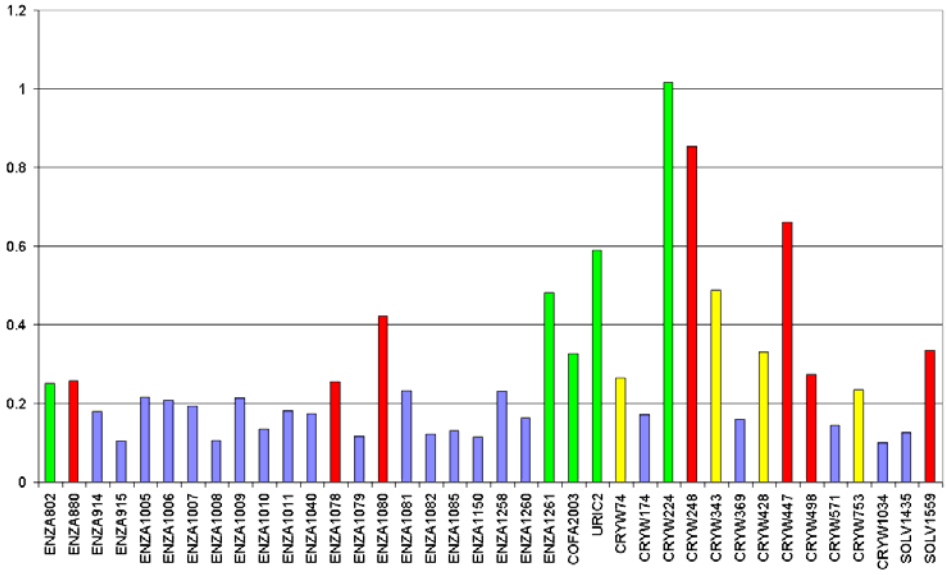

Figure S11. Residue-specific RMSD values (Å) in Setup A, SN500. Shown are only residues with RMSD values above 0.1 Å. Color code: green, QM region; red, neighboring first-shell residues; yellow, second-shell residues; blue, other more distant residues.

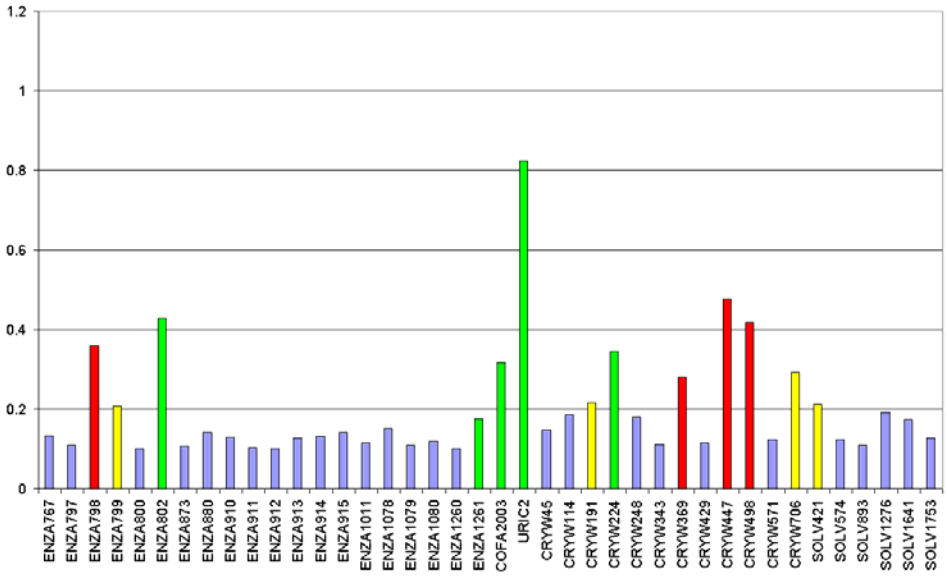

Figure S12. Residue-specific RMSD values (Å) in Setup G, SN400. Shown are only residues with RMSD values above 0.1 Å. Color code see Figure S11.

Table S30. RMSD values (in Å) between the optimized reactant complex[a] and transition state TS3 for all snapshots considered (see text).

	complete system	active region	active region without QM atoms	QM atoms
Setup A (SN100)	0.042	0.156	0.120	0.639
Setup A (SN500)	0.042	0.157	0.128	0.588
Setup B (SN300)	0.046	0.167	0.098	0.846
Setup B (SN500)	0.041	0.150	0.088	0.757
Setup C (SN200)	0.032	0.123	0.065	0.636
Setup C (SN400)	0.044	0.159	0.097	0.774
Setup D (SN200)	0.036	0.139	0.065	0.751
Setup D (SN400)	0.046	0.178	0.089	0.940
Setup E (SN100)	0.037	0.145	0.094	0.661
Setup E (SN300)	0.040	0.156	0.118	0.635
Setup E (SN500)	0.033	0.125	0.073	0.637
Setup F (SN200)	0.036	0.136	0.084	0.651
Setup F (SN400)	0.039	0.149	0.098	0.673
Setup G (SN100)	0.034	0.131	0.072	0.661
Setup G (SN200)	0.033	0.127	0.073	0.635
Setup G (SN400)[b]	0.035	0.134	0.087	0.621

[a] The reactant complex is defined as the lowest lying minimum preceding TS3, i.e. IM1' for setup E, IM1 for setup F, IM1" for setup G and the starting structure "Reactant" for all other setups.

[b] For Setup G (SN400), we reoptimized the two appropriate structures using the same QM region as in the gas-phase QM study, see Section III.J and Figure 9 of the paper. The RMSD value from the QM/MM study is 0.741 Å (for the QM atoms including the link atoms), much lower than the RMSD value from the gas-phase QM study which is 1.876 Å after structural alignment.

4. Setup Comparisons

Table S31. Energy differences for snapshots where the QM region has been replaced. All values refer to IM2 structures and are given in kcal mol^{-1} using the B3LYP functional.

	ΔE_{QM}(LANL)[a]	$\Delta E_{QM/MM}$(LANL)	ΔE_{QM}(TZVP)[a]	$\Delta E_{QM/MM}$ (TZVP)
G2A	9.6	18.7	9.3	18.4
G2C	-	-	-	-
G2D	-	-	-	-
G2F	9.4	12.6	9.0	12.2
A2G	-5.7	-1.0	-5.8	-1.1
C2G	-5.9	0.7	-5.6	0.8
D2G	4.9	1.6	5.5	2.1
F2G	5.1	6.1	4.5	5.5

[a] including the QM-MM interaction

Conventions used in the following Figures S13-S16:

The lowest point on the energy profile of setup G, SN100 is assigned an energy of zero. The other energy profiles are shifted such that the relative energy of one selected species is equal to the value obtained from COSMO (ε=8) single-point calculations on the extended 66-atom QM region (see Table 10 of the paper for the corresponding numerical data). Selected species are the reactant in Figure S13, the intermediate complex IM1 in Figure S14, the tetrahedral intermediate in Figure S15 and the product in Figure S16. Their relative energies are represented by squares in Figures S13-S16.

The resulting four sets of energy profiles are quite similar. A representative overall set can be obtained by averaging each of the energy values in the four individual sets, which is feasible since they all refer to the same energy scale (having the same zero energy, see above). The resulting set of energy profiles is shown in Figure 8 of the paper.

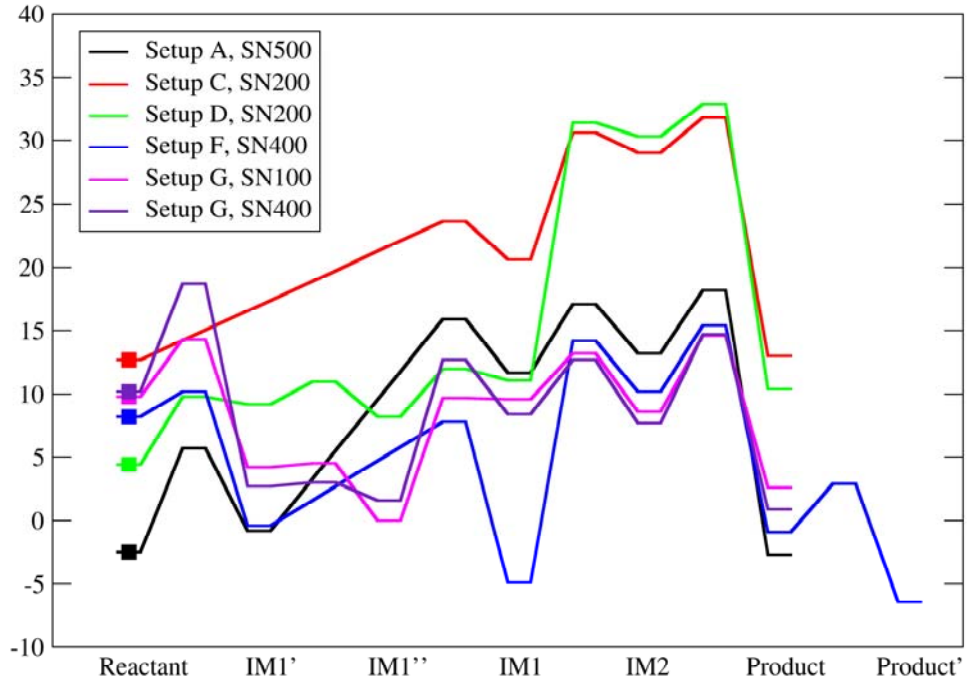

Figure S13. Reaction profiles scaled on the relative energies of the **reactant**, represented by the squares (B3LYP-COSMO, ε=8, see preceding text and Section III.I of the paper).

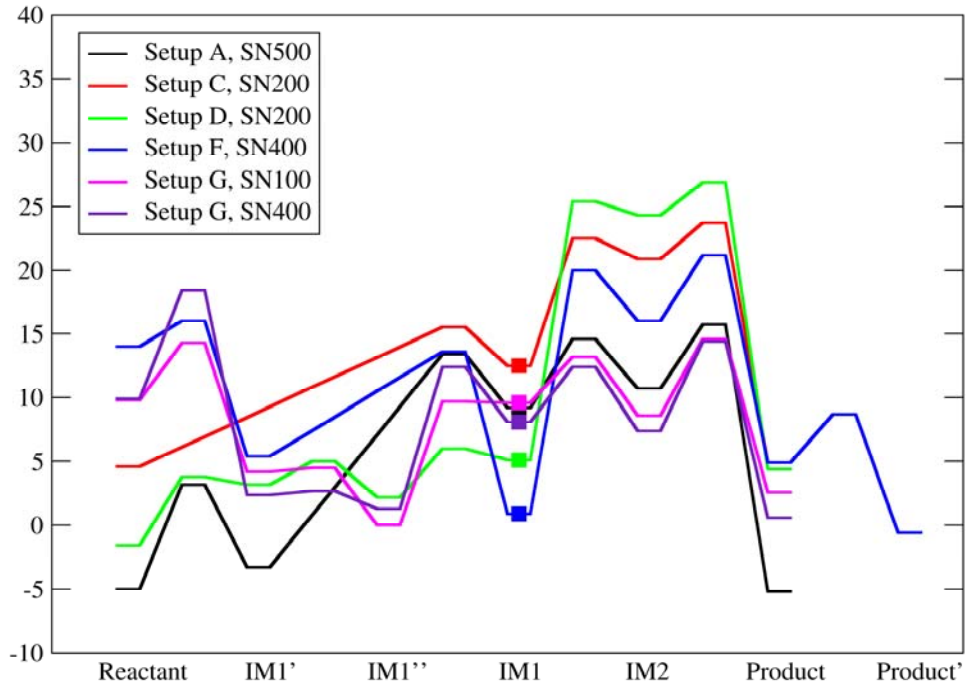

Figure S14. Reaction profiles scaled on the relative energies of **IM1**, represented by the squares (B3LYP-COSMO, ε=8, see preceding text and Section III.I of the paper).

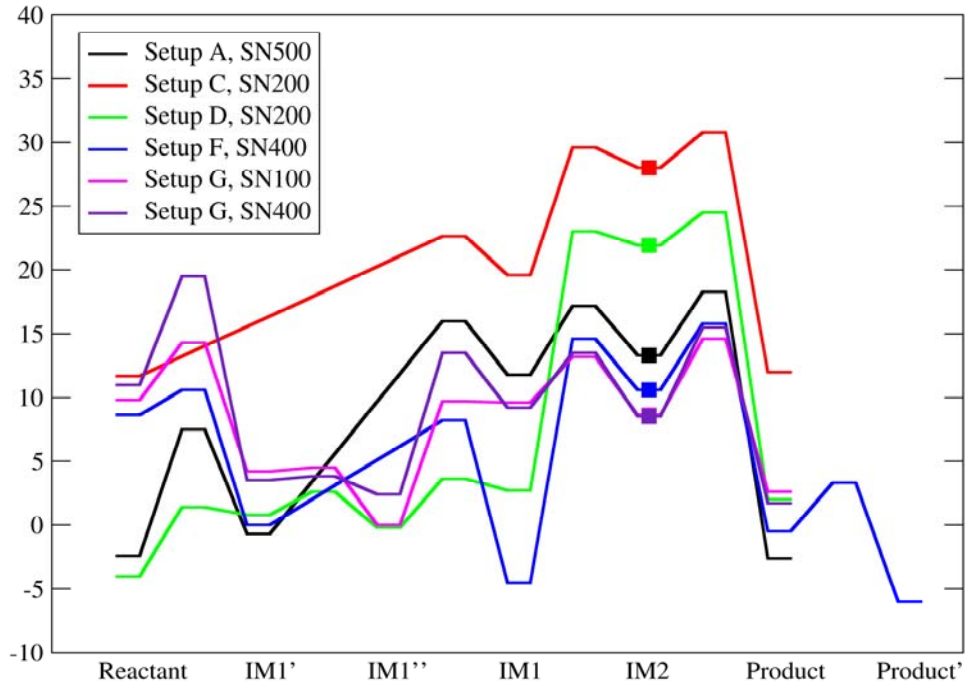

Figure S15. Reaction profiles scaled on the relative energies of **IM2**, represented by the squares (B3LYP-COSMO, ε=8, see preceding text and Section III.I of the paper).

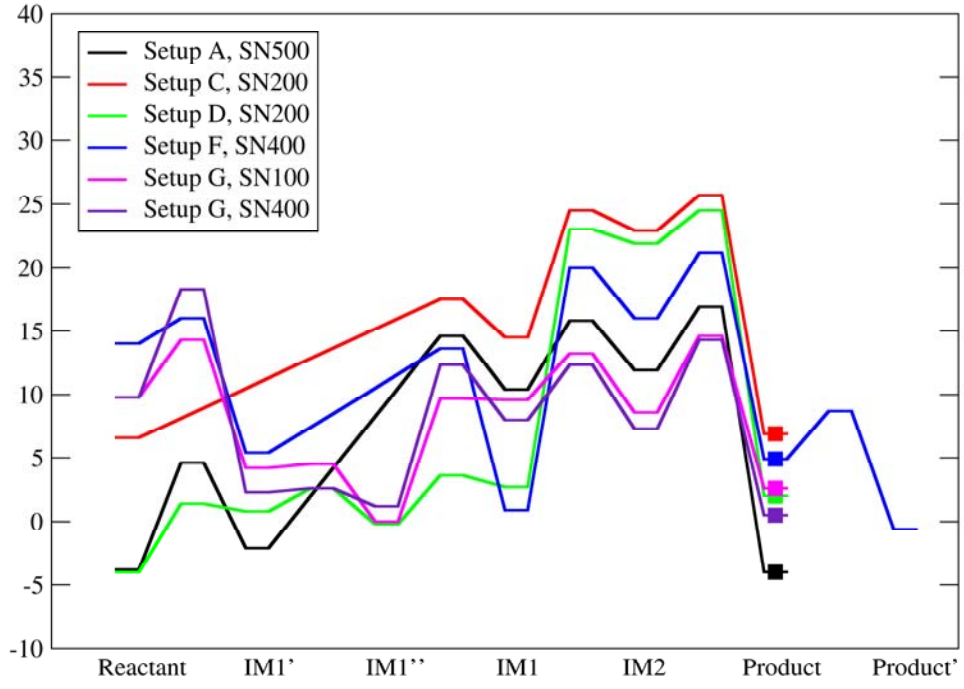

Figure S16. Reaction profiles scaled on the relative energies of the **product**, represented by the squares (B3LYP-COSMO, ε=8, see preceding text and Section III.I of the paper).

ESP charges and key electrostatic interactions

As discussed in the paper (Section III.I), we have analyzed the charge distributions obtained in the single-point B3LYP calculations for the extended QM region (Figure 5 of the paper). ESP charges were determined for four species (reactant, IM1, IM2, product) in three snapshots of setup A (SN500) and setup G (SN100 and SN400). Figure S17 shows the overall ESP charges of the residues included in the QM region. It is obvious that these charges are quite similar for the different snapshots and often also for the different species, except for the change between the reactant and IM1 that is due to the proton transfer from the cofactor (setup A) or the substrate (setup G) to Glu1261.

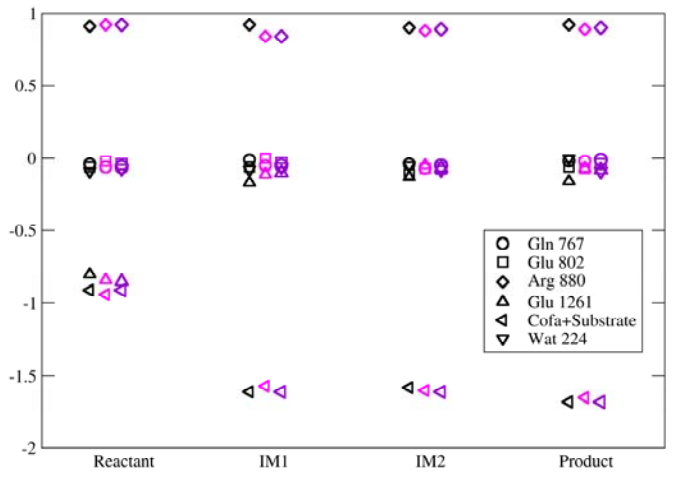

Figure S17: ESP charges (*e*) for the residues included in the extended QM region (see Figure 5 in the paper). Each triple represents the data for snapshots A-SN500, G-SN100, and G-SN-400, from left to right.

Using the computed ESP charges and the relevant distances from the QM/MM optimized structures, individual electrostatic interactions were calculated for key atom pairs in the reactant and in IM2. Table S32 lists the corresponding ESP charges and distances as well as the difference (IM2-reactant) of the computed Coulomb interaction energies between the two point charges (a positive value indicates that the interaction is stronger in the reactant than in IM2).

Table S32. Selected ESP charges (*e*), distances (Å), and differences ΔE (kcal mol^{-1}) of the corresponding Coulomb interaction energies in IM2 and the reactant (IM2-reactant, see text). The notation conforms to Scheme 6 of the paper.

interaction	Reactant			IM2			
	charge 1	charge 2	distance	charge 1	charge 2	distance	ΔE
Setup A, SN500							
H_{Glu}-N_{acc} [a)]	0.45	-0.67	1.80	0.43	-0.61	1.69	4.1
H_{Subs}-O_{acc} [b)]	0.51	-0.71	1.65	0.43	-0.68	1.85	20.4
H:Arg880-O6	0.51	-0.74	1.74	0.52	-0.84	1.60	-18.6
H:Arg880-H7	0.51	0.67	3.14	0.52	0.50	3.46	-11.2
Setup G, SN100							
H7-O:Glu802	0.58	-0.63	1.72	0.55	-0.67	2.06	11.1
H:Glu802-O6	0.50	-0.58	1.80	0.52	-0.47	1.61	3.1
H:Arg880-O2	0.46	-0.68	1.90	0.50	-0.77	1.70	-20.5
H:Arg880-H3	0.46	0.40	2.59	0.50	0.36	2.78	2.1
H:Arg880-N3	0.46	-0.40	3.01	0.50	-0.74	2.64	-26.2
Setup G, SN400							
H7-O:Glu802	0.64	-0.67	1.72	0.55	-0.68	1.96	19.4
H:Glu802-O6	0.47	-0.60	1.83	0.50	-0.66	1.66	-14.8
H:Arg880-O2	0.48	-0.70	1.90	0.51	-0.78	1.70	-19.0
H:Arg880-H3	0.48	0.50	2.47	0.51	0.37	2.79	-9.8
H:Arg880-N3	0.48	-0.66	2.90	0.51	-0.73	2.63	-10.7

[a)] Reactant: H9-N9, IM2:H3-N3
[b)] Reactant: H7-O:Glu802, IM2: H3-O:Glu802

The data in Table S32 address the key interactions between the substrate and the active-site residues Arg880 and Glu802, both for the "upside" (setup A) and "upside down" (setup G) orientation of the substrate. For setup A, the binding mode of Glu802 changes from the reactant to IM2, whereas the interaction of Arg880 with the substrate remains similar. By contrast, for setup G, the binding mode of Glu802 remains the same, whereas the interaction of Arg880 changes due to the proton transfer that leads to a deprotonated N3 center in IM2 (see Scheme 6 in the paper).

We find that Arg880 is provides significant stabilization when going from the reactant to IM2 in all three snapshots (see Table S32): In setup A, this stabilization of about 30 kcal mol^{-1} is caused by a higher negative charge at and a shorter distance to O6 as well as less repulsion with H7. In setup G, this stabilization is even larger (ca. 45 kcal mol^{-1} for SN100 and 40 kcal mol^{-1} for SN400) since the substrate-Arg880 interactions in IM2 benefit from shorter distances and higher negative charges of O2 and N3. Additionally, the repulsive interaction of Arg880 with H3, which is part of a water molecule in IM2, is reduced in SN400.

For Glu802, there is again a common trend for all three snapshots: When going from the reactant state to IM2, the substrate-Glu802 interactions become less stabilizing. This can be attributed to lower charges on the interacting atoms and/or increased distances. There is one exception to this trend, namely setup G SN400, where the interaction H:Glu802-O6 becomes stronger which is however overcompensated by the weakening of H7-O:Glu802.

The data in Table S32 also offer an explanation why the "upside" orientation is better stabilized in the reactant state than the "upside down" orientation: In setup A we find the interaction of Arg880 with O6 to be about 13-19 kcal mol^{-1} stronger than the interaction of Arg880 with O2 in setup G. Hence, the substrate-Arg880 interaction (with O6) favors the "upside" orientation in the reactant state. On the other hand, the substrate-Arg880 interaction (primarily with O2 and N3) is also the origin of the stronger stabilization of the "upside down" orientation in IM2, which is the catalytically active conformation.

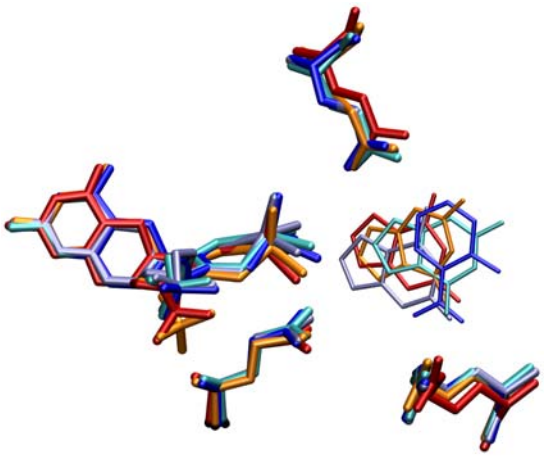

Figure S18. Aligned structures for setup A (orange), setup G (red), and the crystal structures 1FO4 (dark blue), 3EUB (ice blue), and 3B9J (cyan).

Intuitively, it seems strange that a binding pocket containing residues with quite polar side chains should bind two different orientations of the same substrate equally or at least comparably well. On the other hand one should keep in mind that XO is not uniquely designed to transform xanthine to uric acid, but also hypoxanthine to xanthine. The binding pocket may thus be a compromise for performing both tasks. Therefore, the induced fit with different substrates may result in rather different orientations in the binding pocket, as can be seen in Figure S18 which shows crystal structures with three different substrates (salicylic acid - dark blue, 2,8-dioxo-6-methylpurin - ice blue, and xanthine - cyan). Obviously, the orientation of the substrates varies and the enzymatic side chains adopt specific orientations. The optimized QM/MM structures (orange and red) differ slightly more, particularly with respect to the position of the molybdopterin phosphate (which is however known from experiment to be very floppy, with different orientations found in different crystal structures, see e.g. Schindelin, H.; Kisker, C.; Rees, D. C. *J. Biol. Inorg. Chem.* **1997**, 2, 773-781); also, the geometry of the molybdenum center is less tilted than in the experimental structures. However, all substrates conserve the important interaction with Arg880 in the binding pocket.

5. CHARMM parameters

```
!=====================================================================
! CHARMM PARAMETERS for FAD provided by M. Karplus,
!                       cf. J. Phys. Chem. B, 110, 9363-9367 (2006)
!=====================================================================


!!!!!!!!!!!!  FAD TOPOLOGY !!!!!!!!!!!!!!!!

MASS 304   NX1     14.00700  N ! FAD-specific
MASS 305   HX1      1.00800  H ! FAD-specific
MASS 306   CX1     12.011000 C ! FAD-specific
MASS 307   OX1     15.999400 O ! FAD-specific
MASS 308   CX2     12.011000 C ! FAD-specific
MASS 309   CX3     12.011000 C ! FAD-specific
MASS 310   NX2     14.00700  N ! FAD-specific
MASS 311   CX4     12.011000 C ! FAD-specific
MASS 312   OX2     15.999400 O ! FAD-specific
MASS 313   NY1     14.00700  N ! FAD-specific
MASS 314   CY1     12.011000 C ! FAD-specific
MASS 315   CY2     12.011000 C ! FAD-specific
MASS 316   NY2     14.00700  N ! FAD-specific
MASS 317   CZ1     12.011000 C ! FAD-specific
MASS 318   HZ1      1.00800  H ! FAD-specific
MASS 319   CZ2     12.011000 C ! FAD-specific
MASS 320   CZ3     12.011000 C ! FAD-specific
MASS 321   CZ4     12.011000 C ! FAD-specific
MASS 322   HZ4      1.00800  H ! FAD-specific

RESI FAD  -2.000        ! cf. J. Phys. Chem. B, 110, 9363-9367 (2006)
GROUP
ATOM C4'  CN7     0.16
ATOM H4'  HN7     0.09
ATOM O4'  ON6B   -0.50
ATOM C1'  CN7B    0.16
ATOM H1'  HN7     0.09
GROUP
ATOM C5   CN5     0.28
ATOM N7   NN4    -0.71
ATOM C8   CN4     0.34
ATOM H8   HN3     0.12
ATOM N9   NN2    -0.05
ATOM N1   NN3A   -0.74
ATOM C2   CN4     0.50
ATOM H2   HN3     0.13
ATOM N3   NN3A   -0.75
ATOM C4   CN5     0.43
ATOM C6   CN2     0.46
ATOM N6   NN1    -0.77
ATOM H61  HN1     0.38
ATOM H62  HN1     0.38
```

```
GROUP
ATOM C2'  CN7     0.14  !                       H61  H62
ATOM H2'' HN7     0.09  !                         \  /
ATOM O2'  ON5    -0.66  !                          N6
ATOM H2'  HN5     0.43  !                          |
GROUP                   !                          C6
ATOM C3'  CN7           !                        //  \
ATOM H3'  HN7     0.09  !                        N1   C5--N7\\
ATOM O3'  ON5    -0.66  !                        |    ||     C8-H8
ATOM H3T  HN5     0.43  !                        C2   C4--N9/
GROUP                   !                      / \\ /     \
ATOM C5'  CN8    -0.08  !                    H2   N3       \
ATOM H5'  HN8     0.09  !                                   \
ATOM H5'' HN8     0.09  !                                    \
ATOM O5'  ON2    -0.62  !                                     \
ATOM PA   P       1.50  !           O1B    O1A    H5' H4'  O4'   \
ATOM O1A  ON3    -0.82  !           |      |      |    \ /   \   \
ATOM O2A  ON3    -0.82  !      O3B-PB-O3A--PA-O5'-C5'---C4'    C1'
ATOM O3A  ON2    -0.74  !       |   |       |      |     \     / \
ATOM PB   P       1.50  !       |   O2B    O2A    H5''   C3'--C2' H1'
ATOM O1B  ON3    -0.90  ! HS51-CS5-HS52                 / \   / \
ATOM O2B  ON3    -0.90  !       |                    O3' H3' O2' H2''
ATOM O3B  ON2    -0.74  ! HS41-CS4-OS4-HS42          |       |
GROUP                   !       |                    H3'     H2'
ATOM CS1  CT2     0.21  ! HS31-CS3-OS3-HS32
ATOM HS11 HA      0.09  !       |
ATOM HS12 HA      0.09  ! HS21-CS2-OS2-HS22
ATOM CS2  CT1     0.17  !       |
ATOM HS21 HA      0.09  ! HS11-CS1-HS12
ATOM OS2  OH1    -0.66           \
ATOM HS22 H       0.43  !         \
ATOM CS3  CT1     0.17  !          \
ATOM HS31 HA      0.09  !           \
ATOM OS3  OH1    -0.66  !            \
ATOM HS32 H       0.43  !             \
ATOM CS4  CT1     0.17  !              \        HZ4 HZ61 HZ62
ATOM HS41 HA      0.09  !               \       |    |  /
ATOM OS4  OH1    -0.66  !  OX2   NX2     NY2   CZ4   CZ6--HZ63
ATOM HS42 H       0.43  !   \\  /   \   /   \ /   \\ /
ATOM CS5  CT2     0.22  !    CX4     CX3    CY2   CZ3   HZ53
ATOM HS51 HA      0.09  !     |       |     ||     |    |
ATOM HS52 HA      0.09  !    NX1     CX2    CY1   CZ2---CZ5--HZ52
GROUP                   !    /  \   /   \  // \   //    |
ATOM NX1  NX1    -0.89  !  HX1   CX1     NY1   CZ1      HZ51
ATOM HX1  HX1     0.43  !        ||             |
ATOM CX1  CX1     0.82  !        OX1           HZ1
ATOM OX1  OX1    -0.61
ATOM CX2  CX2     0.24
ATOM CX3  CX3     0.37
ATOM NX2  NX2    -0.81
ATOM CX4  CX4     1.09
ATOM OX2  OX2    -0.66
ATOM NY1  NY1    -0.60
ATOM CY1  CY1     0.60
ATOM CY2  CY2    -0.30
ATOM NY2  NY2     0.20
```

```
ATOM CZ1  CZ1    -0.46
ATOM HZ1  HZ1     0.24
ATOM CZ2  CZ2     0.19
ATOM CZ5  CT3    -0.44
ATOM HZ51 HA      0.09
ATOM HZ52 HA      0.09
ATOM HZ53 HA      0.09
ATOM CZ3  CZ3     0.18
ATOM CZ6  CT3    -0.48
ATOM HZ61 HA      0.09
ATOM HZ62 HA      0.09
ATOM HZ63 HA      0.09
ATOM CZ4  CZ4    -0.28
ATOM HZ4  HZ4     0.19
BOND PB  O3A    PB  O1B    PB  O2B    PB  O3B    O3A PA
BOND PA  O1A    PA  O2A    PA  O5'    O3' H3T
BOND O5' C5'    C5' C4'    C4' O4'    C4' C3'    O4' C1'
BOND C1' N9     C1' C2'    N9  C4     N9  C8     C4  N3
BOND C2  N1     C6  N6
BOND N6  H61    N6  H62    C6  C5     C5  N7
BOND C2' C3'    C2' O2'    O2' H2'    C3' O3'
BOND C1' H1'    C2' H2''   C3' H3'    C4' H4'    C5' H5'
BOND C5' H5''   C8  H8     C2  H2
BOND CS1 HS11   CS1 HS12   CS1 CS2    CS2  HS21  CS2 OS2
BOND OS2 HS22   CS2 CS3    CS3 HS31   CS3  OS3   OS3 HS32
BOND CS3 CS4    CS4 HS41   CS4 OS4    OS4  HS42  CS4 CS5
BOND CS5 HS51   CS5 HS52   CS5 O3B    CS1  NY2
BOND NX1 HX1    NX1 CX1    CX1 OX1    CX1  CX2   CX2 CX3
BOND CX3 NX2    NX2 CX4    CX4 OX2    CX2  NY1   NY1 CY1
BOND CY1 CY2    CY2 NY2    NY2 CX3    NX1  CX4
BOND CY1 CZ1    CZ1 HZ1    CZ1 CZ2    CZ2  CZ5   CZ5 HZ51
BOND CZ5 HZ52   CZ5 HZ53   CZ2 CZ3    CZ3  CZ6   CZ6 HZ61
BOND CZ6 HZ62   CZ6 HZ63   CZ3 CZ4    CZ4  HZ4   CZ4 CY2
DOUBLE          N1  C6     N3  C2     C4   C5    N7  C8
IMPR N6  C6  H61 H62
IMPR C6  N1  C5  N6
IMPR CZ2 CZ1 CZ3 CZ5
IMPR CZ3 CZ2 CZ4 CZ6
IMPR CY1 CZ1 NY1 CY2
IMPR NY2 CY2 CX3 CS1
IMPR CX2 NY1 CX1 CX3
PATC  FIRS NONE LAST NONE
```

```
!!!!!!!!!!!!  FAD PARAMETER  !!!!!!!!!!!!!!!!
BONDS
CX4  NX2    350.000     1.3700  ! FAD
CX4  NX1    350.000     1.4000  ! FAD
CX4  OX2    640.000     1.1900  ! FAD
NX2  CX3    350.000     1.2900  ! FAD
CX3  CX2    282.000     1.4700  ! FAD
CX2  CX1    282.000     1.5000  ! FAD
CX3  NY2    350.000     1.3600  ! FAD
CX2  NY1    350.000     1.2700  ! FAD
CX1  OX1    640.000     1.1900  ! FAD
CX1  NX1    350.000     1.3600  ! FAD
NX1  HX1    465.600     1.0000  ! FAD
NY2  CT2    310.000     1.4600  ! FAD
NY2  CY2    390.000     1.3900  ! FAD
CY2  CZ4    440.000     1.4000  ! FAD
CY2  CY1    440.000     1.4000  ! FAD
CY1  NY1    390.000     1.3700  ! FAD
CY1  CZ1    440.000     1.4000  ! FAD
CZ4  HZ4    370.000     1.0700  ! FAD
CZ4  CZ3    440.000     1.3800  ! FAD
CZ3  CZ2    440.000     1.4100  ! FAD
CZ2  CZ1    440.000     1.3700  ! FAD
CZ1  HZ1    370.000     1.0700  ! FAD
CZ2  CT3    345.000     1.5100  ! FAD
CZ3  CT3    345.000     1.5100  ! FAD
CT2  ON2    340.000     1.4400  ! FAD
ANGLES
OX2  CX4  NX1      98.00    118.6    ! FAD
OX2  CX4  NX2      98.00    123.1    ! FAD
NX1  CX4  NX2      70.00    118.3    ! FAD
CX3  NX2  CX4      60.00    120.5    ! FAD
NX2  CX3  CX2      65.00    124.1    ! FAD
NX2  CX3  NY2      90.00    120.1    ! FAD
CX2  CX3  NY2      65.00    115.8    ! FAD
CX1  CX2  CX3      65.00    116.8    ! FAD
CX1  CX2  NY1      65.00    119.1    ! FAD
CX3  CX2  NY1      65.00    124.1    ! FAD
CX2  CX1  OX1      80.00    124.0    ! FAD
CX2  CX1  NX1      65.00    112.9    ! FAD
NX1  CX1  OX1      98.00    123.2    ! FAD
CX1  NX1  HX1      18.00    117.0    ! FAD
CX4  NX1  CX1      60.00    127.4    ! FAD
CX4  NX1  HX1      18.00    117.0    ! FAD
CX3  NY2  CT2      44.00    119.5    ! FAD
CT2  NY2  CY2      80.00    119.6    ! FAD
CX3  NY2  CY2      60.00    120.9    ! FAD
NY2  CY2  CZ4      65.00    122.7    ! FAD
NY2  CY2  CY1      65.00    118.9    ! FAD
CZ4  CY2  CY1      70.00    118.4    ! FAD
CY2  CY1  NY1      65.00    121.2    ! FAD
CY2  CY1  CZ1      70.00    120.1    ! FAD
NY1  CY1  CZ1      65.00    118.7    ! FAD
CY1  NY1  CX2      60.00    119.2    ! FAD
CY2  CZ4  HZ4      31.00    120.2    ! FAD
```

```
CZ3  CZ4  HZ4      31.00    118.6    ! FAD
CY2  CZ4  CZ3      70.00    121.1    ! FAD
CZ4  CZ3  CZ2      70.00    120.5    ! FAD
CZ4  CZ3  CT3      70.00    119.3    ! FAD
CZ2  CZ3  CT3      70.00    120.2    ! FAD
CZ1  CZ2  CZ3      70.00    118.1    ! FAD
CZ3  CZ2  CT3      70.00    121.2    ! FAD
CZ1  CZ2  CT3      70.00    120.7    ! FAD
CZ2  CZ1  CY1      70.00    121.8    ! FAD
CZ2  CZ1  HZ1      31.00    121.2    ! FAD
CY1  CZ1  HZ1      31.00    117.1    ! FAD
CZ2  CT3  HA       35.00    115.0    ! FAD
CZ3  CT3  HA       35.00    115.0    ! FAD
NY2  CT2  HA       46.50    106.2    ! FAD
NY2  CT2  CT1      65.00    109.5    ! FAD
CN8  CN7  ON6B    110.00    109.0    ! FAD
ON6B CN7B CN7     110.00    105.0    ! FAD
NN2  CN7B CN7      70.00    113.7    ! FAD
CN7  CN7  CN7      53.35    111.0  8.0   2.561   ! FAD
CN7  CN8  ON2      75.70    110.1    ! FAD
P    ON2  CT2      20.0     120.0  35.   2.33    ! FAD
CT2  CT1  OH1      75.700   110.1000 ! FAD
ON2  CT2  CT1      75.700   110.1000 ! FAD
ON2  CT2  HA       45.900   108.8900 ! FAD
```

DIHEDRAL

```
ON3  P    ON2  CT2       0.10    3     0.0    ! FAD
X    CX4  NX2  X         2.05    2     180.0  ! FAD
X    NX2  CX3  X         2.05    2     180.0  ! FAD
X    CX2  CX3  X         0.50    2     180.0  ! FAD
X    CX1  CX2  X         0.50    2     180.0  ! FAD
X    CX1  NX1  X         2.05    2     180.0  ! FAD
X    CX4  NX1  X         2.05    2     180.0  ! FAD
X    CX3  NY2  X         2.05    2     180.0  ! FAD
X    CY2  NY2  X         2.05    2     180.0  ! FAD
X    CY1  CY2  X         3.10    2     180.0  ! FAD
X    NY1  CY1  X         2.05    2     180.0  ! FAD
X    NY1  CX2  X         2.05    2     180.0  ! FAD
X    CY2  CZ4  X         3.10    2     180.0  ! FAD
X    CZ4  CZ3  X         3.10    2     180.0  ! FAD
X    CZ2  CZ3  X         3.10    2     180.0  ! FAD
X    CZ1  CZ2  X         3.10    2     180.0  ! FAD
X    CY1  CZ1  X         3.10    2     180.0  ! FAD
X    CZ2  CT3  X         0.10    3       0.0  ! FAD
X    CZ3  CT3  X         0.10    3       0.0  ! FAD
X    CT2  NY2  X         0.01    3       0.0  ! FAD
X    CN7  CN7  X         0.15    3       0.0  ! FAD
X    CT2  ON2  X         0.25    3       0.0  ! FAD
X    ON6B CN7B X         0.10    3       0.0  ! FAD
X    CN7  CN8  X         0.200   3       0.0  ! FAD
X    CN7  CN7B X         0.15    3       0.0  ! FAD
X    CN7  ON6B X         0.10    3       0.0  ! FAD
X    CN8  ON2  X        -0.10    3       0.0  ! FAD
ON2  P    ON2  CT2       0.95    2       0.0  ! FAD
```

IMPROPER

CZ1	X	X	CZ4	15.0000	0	0.0000	! FAD
CZ2	X	X	CY2	15.0000	0	0.0000	! FAD
CZ3	X	X	CY1	15.0000	0	0.0000	! FAD
NY1	X	X	NY2	65.0000	0	0.0000	! FAD
CY1	X	X	CX3	100.0000	0	0.0000	! FAD
CX2	X	X	CY2	100.0000	0	0.0000	! FAD
CX2	X	X	CX4	100.0000	0	0.0000	! FAD
NY2	X	X	CX4	60.0000	0	180.0000	! FAD
NY2	X	X	CX1	60.0000	0	180.0000	! FAD
CX3	X	X	NX1	60.0000	0	0.0000	! FAD
CX1	X	X	NX2	60.0000	0	0.0000	! FAD
CZ3	X	X	CT3	130.0000	0	0.0000	! FAD
CY1	X	X	CY2	15.0000	0	0.0000	! FAD
CZ2	X	X	CT3	130.0000	0	0.0000	! FAD
NY2	X	X	CT2	45.0000	0	0.0000	! FAD
CX2	X	X	CX3	100.0000	0	0.0000	! FAD
CX4	X	X	OX2	147.0000	0	0.0000	! FAD
NX1	X	X	HX1	18.0000	0	0.0000	! FAD
CX1	X	X	OX1	147.0000	0	0.0000	! FAD

NONBONDED

CX1	0.000000	-0.141000	1.870000	! FAD
CX2	0.000000	-0.141000	1.870000	! FAD
CX3	0.000000	-0.141000	1.870000	! FAD
CX4	0.000000	-0.141000	1.870000	! FAD
CY1	0.000000	-0.141000	1.870000	! FAD
CY2	0.000000	-0.141000	1.870000	! FAD
CZ1	0.000000	-0.141000	1.870000	! FAD
CZ2	0.000000	-0.141000	1.870000	! FAD
CZ3	0.000000	-0.141000	1.870000	! FAD
CZ4	0.000000	-0.141000	1.870000	! FAD
HX1	0.000000	-0.049800	0.800000	! FAD
HZ1	0.000000	-0.042000	1.330000	! FAD
HZ4	0.000000	-0.042000	1.330000	! FAD
NX1	0.000000	-0.090000	1.830000	! FAD
NX2	0.000000	-0.090000	1.830000	! FAD
NY1	0.000000	-0.090000	1.830000	! FAD
NY2	0.000000	-0.090000	1.830000	! FAD
OX1	0.000000	-0.120000	1.700000	! FAD
OX2	0.000000	-0.120000	1.700000	! FAD

```
!=====================================================================
! CHARMM topology for molybdenum-bond uric acid
!=====================================================================

!!!!!!!!!!!!  TOPOLOGY !!!!!!!!!!!!!!!!

MASS 353   UN2U    14.00700  N ! URI-specific angles assigned
MASS 354   UHN2    1.00800   H ! URI-specific angles assigned
MASS 355   UC1T   12.011000  C ! URI-specific angles assigned
MASS 356   UON1   15.999400  O ! URI-specific angles assigned
MASS 357   UN2B   14.00700   N ! URI-specific angles assigned
MASS 358   UCN5   12.011000  C ! URI-specific angles assigned
MASS 359   UC5G   12.011000  C ! URI-specific angles assigned
MASS 360   UCN1   12.011000  C ! URI-specific angles assigned
MASS 361   UC8T   12.011000  C ! URI-specific angles assigned
MASS 362   UN7B   14.00700   N ! URI-specific angles assigned
MASS 363   UN9B   14.00700   N ! URI-specific angles assigned

RESI URIC         -1.00  ! CHARGES are UNREVISED ESP CHARGES!!!!!!!
GROUP
ATOM N1   UN2U    -0.94  !
ATOM H1   UHN2     0.41  !                      O6
ATOM C2   UC1T     1.10  !             H7       ||      H1
ATOM O2   UON1    -0.76  !             |      /C6\    /
ATOM N3   UN2B    -0.94  !            N7---C5/     \N1
ATOM C4   UCN5     0.68  !           /     ||        |
ATOM C5   UC5G    -0.32  !      O8==C8     ||        |
ATOM C6   UCN1     0.82  !           \     ||        |
ATOM O6   UON1    -0.70  !            N9---C4\     /C2
ATOM N7   UN7B    -0.61  !            |       \N3/    \\
ATOM H7   UHN2     0.40  !            H9       (-)     O2
ATOM C8   UC8T     0.81  !
ATOM O8   UON1    -0.71  !
ATOM N9   UN9B    -0.63  !
ATOM H9   UHN2     0.39  !
BOND    H1 N1   N1 C2   C2 N3   N3 C4   C4 N9
BOND    N9 H9   C8 N7   N7 C5   C5 C6   C6 N1
BOND    N7 H7   N9 C8
DOUBLE  C2 O2   C4 C5   C6 O6   C8 O8
!Improper and DONO/ACC from URA (& patch PYRU) and GUA (& patch PURG)
IMPR C2 N3 N1 O2    C6 N1 C5 O6    N9 C4 H9 C8    N7 C5 C8 H7
DONO H1  N1
DONO H7  N7
DONO H9  N9
ACCE O2  C2
ACCE O6  C6
ACCE O8  C8
AUTO ANGLES DIHE
PATC FIRS NONE LAST NONE
```

!!!!!!!!!!!! URIC PARAMETER !!!!!!!!!!!!!!!!

BONDS

```
UHN2 UN2U  474.0  1.00  !U, JWK, adm jr. 7/24/91
UN2U UC1T  340.0  1.44  !U,T adm jr. 11/97
UC1T UON1  860.0  1.19  !nad/ppi, jjp1/adm jr. 7/95
UC1T UN2B  302.0  1.40  !U,T adm jr. 11/97
UHN2 UN2B  474.0  1.00  !G, adm jr. 11/97 AS IN PYRO and PURG patch
UCN5 UN2B  320.0  1.37  !U,T adm jr. 11/97 from URA: CN3 NN2B
UCN5 UC5G  320.0  1.30  !G, adm jr. 11/97
UC5G UCN1  302.0  1.41  !G, adm jr. 11/97
UCN1 UN2U  340.0  1.44  !U,T adm jr. 11/97 from URA: CN1 NN2U
UC5G UN7B  302.0  1.40  !G, adm jr. 11/97 from GUA: CN5 NN2B
UC8T UN7B  302.0  1.38  !U,T adm jr. 11/97
UCN5 UN9B  302.0  1.39  !G, adm jr. 11/97  from GUA: CN5 NN2B
UHN2 UN7B  474.0  1.00  !G, adm jr. 11/97 AS IN PYRO and PURG patch
UC8T UON1  860.0  1.28  !nad/ppi, jjp1/adm jr. 7/95
UHN2 UN9B  474.0  1.00  !G, adm jr. 11/97 AS IN PYRO and PURG patch
UC8T UN9B  302.0  1.33  !U,T adm jr. 11/97
UCN1 UON1  660.0  1.20  !U,A,G par_a4 adm jr. 10/2
```

ANGLES

```
UN2B UCN5 UN9B    140.0  126.6  ! from NN2B CN5 NN3G
UC5G UCN5 UN9B    100.0  111.9  ! G
UC5G UN7B UC8T    100.0  106.6  ! from CN4 NN2B CN5
UHN2 UN7B UC8T     40.5  128.8  ! U, h1
UN7B UC8T UON1    100.0  123.2  ! U, o2
UON1 UC8T UN9B    100.0  124.2  ! U, o2
UCN5 UN9B UC8T     70.0  103.9  ! from CN1T NN2B CN3
UCN5 UN9B UHN2     40.5  121.0  ! U, h1
UC8T UN9B UHN2     40.5  135.1  ! U, h1
UC1T  UN2U  UHN2   40.5  114.4  ! U, h3
UCN1  UN2U  UHN2   40.5  116.2  ! U
UC1T  UN2U  UCN1   50.0  129.4  ! U
UN2U  UC1T  UON1  100.0  122.1  ! U
UN2B  UC1T  UON1  100.0  123.1  ! U, o2
UN2B  UC1T  UN2U   50.0  114.9  ! U
UC1T  UN2B  UCN5   70.0  121.0  ! from URA  CN1T NN2B CN3
UC1T  UN2B  UHN2   40.5  119.5  ! U, h1
UCN5  UN2B  UHN2   32.0  120.5  ! U  !!! from URA CN3  NN2B HN2
UC5G  UCN5  UN2B  100.0  121.4  ! G
UHN2  UN7B  UC5G   30.0  125.1  ! from CN5 NN2B HN2
UCN5  UC5G  UN7B  100.0  104.6  ! from CN5G CN5 NN2B
UCN1  UC5G  UN7B  125.0  127.4  ! from CN1  CN5G NN4
UCN1  UC5G  UCN5   70.0  122.9  !
UC5G  UCN1  UN2U  100.0  110.3  ! from : CN5G CN5 NN2B
UC5G  UCN1  UON1   50.0  125.3  !
UN2U  UCN1  UON1   50.0  122.1  ! from NN2G CN1 ON1
UN7B  UC8T  UN9B   50.0  113.5  ! U
```

DIHEDRALS

```
UON1 UCN1 UC5G UN7B  0.0  2   180.0  ! from GUA  ON1  CN1  CN5G NN4
UN2U UCN1 UC5G UN2B  2.0  2   180.0  ! from GUA  NN2G CN1  CN5G NN4
UN2U UCN1 UC5G UN7B  2.0  2   180.0  ! from GUA  NN2G CN1  CN5G NN4
UC1T UN2B UCN5 UCN1  2.0  2   180.0  ! from GUA  CN2  NN3G CN5  NN2B
```

```
UCN5 UN2B UC1T UN2U  1.5  2   180.0  ! from URA  CN3  NN2B CN1T NN2U
UON1 UCN1 UC5G UCN5 14.0  2   180.0  ! from GUA  ON1  CN1  CN5G CN5
UCN5 UC5G UCN1 UN2U  0.2  2   180.0  ! from GUA  CN5  CN5G CN1  NN2G
UC5G UCN1 UN2U UC1T  1.5  2   180.0  ! from URA  CN1T NN2U CN1  CN3
UN2B UCN5 UC5G UCN1  2.0  2   180.0  ! from GUA  NN3G CN5  CN5G CN1
UON1 UCN1 UN2U UHN2  0.0  2   180.0  ! from URA  ON1  CN1T NN2U HN2
UN2B UC1T UN2U UCN1  1.5  2   180.0  ! from URA  NN2B CN1T NN2U CN1
UC5G UCN5 UN2B UHN2  1.6  2   180.0  ! from URA  CN3  CN3  NN2B HN2
UN2U UC1T UN2B UHN2  1.6  2   180.0  ! from URA  NN2U CN1T NN2B HN2
UON1 UC1T UN2B UHN2  0.0  2   180.0  ! from URA  ON1  CN1T NN2B HN2
UON1 UC1T UN2U UHN2  0.0  2   180.0  ! from URA  ON1  CN1T NN2U HN2
UN2B UC1T UN2U UHN2  3.8  2   180.0  ! from URA  NN2B CN1T NN2U HN2
UC5G UCN1 UN2U UHN2  4.8  2   180.0  ! from URA  CN3T CN1  NN2U HN2
UC5G UCN5 UN2B UC1T  1.5  2   180.0  ! from URA  CN3  CN3  NN2B CN1T
UON1 UCN1 UN2U UC1T 14.0  2   180.0  ! from GUA  ON1  CN1  NN2G CN2
UHN2 UN2B UC5G UCN5  1.2  2   180.0  ! from Gua  HN2  NN2B CN5  CN5G
UHN2 UN7B UC5G UCN5  1.2  2   180.0  ! from Gua  HN2  NN2B CN5  CN5G
UCN1 UC5G UN7B UHN2  1.2  2   180.0  ! from XHN2  XN2B  XCN4  XNN4
UC1T UN2B UCN5 UN9B  2.0  2   180.0  ! from GUA CN2  NN3G CN5  NN2B
UN2B UCN5 UN9B UC8T  2.0  2   180.0  ! from GUA NN3G CN5  NN2B CN4
UN2B UCN5 UN9B UHN2  1.2  2   180.0  ! from GUA HN2  NN2B CN4  NN4
UHN2 UN2B UCN5 UN9B  1.2  2   180.0  ! from XHN2  XN2B  XCN4  XNN4
UCN5 UN9B UC8T UN2B 16.0  2   180.0  ! from GUA CN5G NN4  CN4  NN2B
UCN5 UC5G UN2B UC8T  6.0  2   180.0  ! from GUA CN4  NN2B CN5  CN5G
UCN5 UC5G UN7B UC8T  6.0  2   180.0  ! from GUA CN4  NN2B CN5  CN5G
UC5G UCN5 UN9B UC8T  6.0  2   180.0  ! from GUA CN5  CN5G NN4  CN4
UC5G UCN5 UN9B UHN2  1.6  2   180.0  ! from URA CN3  CN3  NN2B HN2
UCN1 UC5G UN7B UC8T  2.0  2   180.0  ! from GUA CN1  CN5G NN4  CN4
X     UC1T  UN2U  X  0.9  2   180.0  !
X     UC1T  UN2B  X  0.9  2   180.0  ! From X CN1 NN2 X, for thymines
X     UC5G  UCN5  X  0.0  2   180.0  !
X     UC8T  UN2U  X  0.9  2   180.0  !
X     UC8T  UN7B  X  0.9  2   180.0  ! From X CN1 NN2 X, for thymines
X     UC8T  UN9B  X  0.9  2   180.0  ! From X CN1 NN2 X, for thymines
IMPROPER
UC1T  X   X  UON1 90.0  0   0.0   !U
UC8T  X   X  UON1 90.0  0   0.0   !U
UCN1  X   X  UON1 90.0  0   0.0   !U
UN9B  X   X  UC8T  7.0  0   0.0   !G, adm jr. 11/97
UN2B  X   X  UHN2  7.0  0   0.0   !G, adm jr. 11/97 NN2B CN4 CN5 HN2
UN7B  X   X  UHN2  7.0  0   0.0   !G, adm jr. 11/97 NN2B CN4 CN5 HN2
NONBONDED
UN2U    0.0    -0.20       1.85
UHN2    0.0    -0.0460     0.2245
UC1T    0.0    -0.10       1.9000
UON1    0.0    -0.1200     1.70
UN2B    0.0    -0.20       1.85 ! From NN2, for N9 in guanines
UCN5    0.0    -0.075      1.9000
UC5G    0.0    -0.075      1.9000
UCN1    0.0    -0.10       1.9000
UC8T    0.0    -0.10       1.9000
UN7B    0.0    -0.20       1.85 ! From NN2, for N9 in guanines
UN9B    0.0    -0.20       1.85 ! From NN2, for N9 in guanines
```

```
!=====================================================================
! CHARMM Parameter Molypdopterin, NOT RECOMMENDED FOR CLASSICAL MD
!=====================================================================
!!!!!!!!!!!!  TOPOLOGY !!!!!!!!!!!!!!!!
MASS   210 MO   95.94000 MO ! FOR MOLYBDENUM
MASS   211 OMO  15.99900 O  ! MO==O
MASS   212 SMO  32.06000 S  ! MO==S

RESI MOC          -3.00   ! Mo-cofactor: -1 (Mo) + -2 (phosphate)
GROUP     ! charge -1 Part 1 (on which PATCH MOPS is applied)
ATOM   Mo    MO    0.947  !
ATOM   OM1   OMO  -0.552  !                OM1
ATOM   OM2   OH1  -0.923  !                ||
ATOM   HOM2  H     0.503  !          S7'---Mo==SR1
ATOM   SR1   SMO  -0.271  !          /    / \
ATOM   S8'   SS   -0.172  ! (C6)---C7' S8'  OM2
ATOM   S7'   SS   -0.055  !          \\ /    |
ATOM   C8'   CE1  -0.235  !  (C3')---C8'   HOM2
ATOM   C7'   CE1  -0.242  !
!
GROUP     ! charge: 0.00 Part 2
ATOM N1      NN3G  -0.74
ATOM C2      CN2    0.75  !
ATOM N2      NN1   -0.68  !
ATOM H21'    HN1    0.35  !
ATOM H22'    HN1    0.32  !                     H6'
ATOM N3      NN2G  -0.34  !           O4    H5'|
ATOM H3      HN2    0.26  !           ||    |  |  (C7')
ATOM C4      CN1    0.54  !           C4    N5 |  /
ATOM O4      ON1   -0.51  !          /  \ /  \ |/  (C8')
ATOM C9      CN5G   0.00  !      H3-N3   C9   C6    |
ATOM C10     CN5    0.26  !         |    ||   |     |
ATOM N8      N2    -0.61  !         C2   C10  C7   C3'---(C4')
ATOM H8'     HC     0.40  !        / \\ / \  / |\  / \
GROUP                     !  H21'-N2   N1   N8 | O3' H3'
ATOM N5      N2    -0.61  !       |         |  |
ATOM H5'     HC     0.40  !       H22'      H8'|
ATOM C6      CNT2   0.12  !                    H7
ATOM H6'     HA     0.09
ATOM C7      CN7B   0.16
ATOM H7      HN7    0.09
ATOM O3'     ON6B  -0.50
ATOM C3'     CN7    0.16
ATOM H3'     HN7    0.09
!
GROUP      ! charge: -2.0 Part 3, from RESI MP_2
ATOM C4'  CN9 -0.18     !        (C3')
ATOM H101 HN9  0.09     !         |
ATOM H102 HN9  0.09     !   H102--C4'--H101
GROUP                   !         |
ATOM O4'  ON2 -0.40     !        O4'
ATOM P    P    1.10     !         |
ATOM O1P  ON3 -0.90     ! O3P(-)--P(+)--O1P(-)
ATOM O2P  ON3 -0.90     !         |
ATOM O3P  ON3 -0.90     !        O2P(-)
```

```
!!!!!!!!!!!!  TOPOLOGY PATCH !!!!!!!!!!!!!!!!
PRES MOPS -1.000          ! patch to MO(IV) species
GROUP                     !            OM1
ATOM   Mo    MO    0.775  !            ||
ATOM   OM1   OMO  -0.498  !      S7'---Mo---SR1---HSR
ATOM   SR1   SMO  -0.576  !      /    /
ATOM   HSR   HS    0.133  !     /    /
ATOM   S8'   SS   -0.514  ! ---C7' S8'
ATOM   S7'   SS   -0.261  !     \\ /
ATOM   C8'   CE1   0.072  !    ---C8'
ATOM   C7'   CE1  -0.131  !
DELETE ATOM OM2
DELETE ATOM HOM2
BOND SR1 HSR
ANGLE HSR SR1 MO
IC SR1 MO OM1 S8'   2.1700    107.51 -100.79  90.00    0.0000
IC HSR SR1 MO OM1   1.3900    101.66  84.64  000.00    0.0000

!!!!!!!!!!!!  MOCO PARAMETER  !!!!!!!!!!!!!!!!
BONDS               !=======dummy parameters==================
SMO HS  275.000 1.385   ! from S HS
MO  OMO  00.00  1.73    ! from 1vdv
OC  MO   00.00  2.15
MO  SMO  00.00  2.17    ! from 1vdv
MO  OH1 250.0   1.97    ! from 1vdv K from QM !!!!!!!
MO  SS    0.0   0.0
SS  CE1   0.0   0.0
ANGLES              !=======dummy parameters==================
OMO  MO   OH1     50.000   105.495 ! From QM
OMO  MO   OMO     00.000   107.507 ! x-ray
OMO  MO   SMO     00.000   107.827 ! From QM
OMO  MO   SS      00.000   101.542 ! From QM
OH1  MO   SMO     70.000   100.000 !
OH1  MO   SS      70.000   075.000 !
SMO  MO   SS      00.000   077.239 !
SS   MO   SS      00.000   079.321 ! From QM
MO   OH1  H       35.000   119.659 ! From QM
MO   SS   CE1     00.000   107.736 ! From QM
SS   CE1  CE1     00.000   122.122 ! From QM
HS   SMO  MO      30.000   100.000 ! FROM QM
CC   OC   MO      35.000   120.000 !
DIHEDRAL            !=======dummy parameters==================
MO   SS   CE1  CE1   00.00       1     3.908 ! from 1vlb
MO   SS   CE1  CN7   00.00       1  -165.552 ! from 1vlb
MO   SS   CE1  CNT2  00.00       1   178.353 ! from 1vlb
OH1  MO   SS   CE1   20.0000     4   180.0
SMO  MO   SS   CE1    0.0000     4     0.00
H    OH1  MO   SS     4.5000     2     0.00
OMO  MO   OH1  H      4.5000     2     0.00
SMO  MO   OH1  H      4.5000     2     0.00
OMO  MO   SS   CE1   00.00       1    89.770  ! from 1vlb
SS   MO   SS   CE1    0.0000     1     0.00
NONBONDED           !======dummy parameters==================
MO   0.0100  0.0000   0.6500 !
OMO  0.0000 -0.1200   1.7000   0.000000  -0.120000     1.400000 !
SMO  0.0000 -0.4700   2.200000
```

6. Complete references 75 and 81

[75] CHARMM27 force field:

(a) MacKerell, A.D., Jr.; Feig, M.; Brooks, C.L., III *J. Comput. Chem.* **2004**, 25, 1400-1415.

(b) Foloppe, N.; Mackerell, A. D., Jr. *J. Comput. Chem.* **2000**, 21, 86-104.

(c) MacKerell, Jr., A. D.; Bashford, D.; Bellott, M.; Dunbrack Jr., R.L.; Evanseck, J.D.; Field, M.J.; Fischer, S.; Gao, J.; Guo, H.; Ha, S.; Joseph-McCarthy, D.; Kuchnir, L.; Kuczera, K.; Lau, F.T.K.; Mattos, C.; Michnick, S.; Ngo, T.; Nguyen, D.T.; Prodhom, B.; Reiher, W.E., III; Roux, B.; Schlenkrich, M.; Smith, J.C.; Stote, R.; Straub, J.; Watanabe, M.; Wiorkiewicz-Kuczera, J.; Yin, D.; Karplus, M. *J. Phys. Chem. B* **1998**, 102, 3586-3616.

[81] (a) Sherwood, P.; de Vries, A. H.; Guest, M. F.; Schreckenbach, G.; Catlow, C. R. A.; French, S. A.; Sokol, A. A.; Bromley, S. T.; Thiel, W.; Turner, A. J.; Billeter, S.; Terstegen, F.; Thiel, S.; Kendrick, J.; Rogers, S. C.; Casci, J.; Watson, M.; King, F.; Karlsen, E; Sjovoll, M.; Fahmi, A.; Schäfer, A.; Lennartz, C. *J. Mol. Struc. (Theochem)* **2003**, 632, 1-28.

(b) ChemShell is a modular QM/MM program developed in the European QUASI project under the coordination of P. Sherwood (see http://www.chemshell.org).