Highlighting the Problems With the Gas Phase Modeling of Oxyanion Holes and Illustrating the Need for Complete Enzyme Models

Supporting Information

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EXAMINING THE EFFECT OF TWO "IDEALLY POSITIONED" WATER MOLECULES ON AN OXYANION IN THE GAS PHASE

In order to examine the effect of two water molecules on an oxyanion in the gas-phase, we created a model oxyanion hole (which is broadly similar to the model used by the authors of Ref. [1]), comprised of two water molecules placed at either 5Å or 1.6Å (as measured by $O_{wat}H$ --- O distance) from the oxygen atoms of both a simple ketone (representing the RS) and its corresponding oxyanion (representing the TS) respectively. Subsequently, the energetics of each species was obtained by means of a single point QM/MM calculation, using the MOLARIS software package[2,3] interfaced with Gaussian 03[4]. Here, the QM part was treated at the B3LYP/6-31G* level of theory, and the classical part (which was comprised of the two water molecules that create our model oxyanion hole) was represented using the ENZYMIX forcefield[3]. In the case of the species with the water molecules at large separation from the relevant oxygen, the water molecules were simply taken as being planar to the oxygen. However, in the case of the species were the water molecules were close to the oxygen, the hydrogen atom interacting with the oxygen (either C=O or $C\text{-}O^-$) was minimized to its optimal position. This resulted in the thermodynamic cycle presented in Fig. 6 of the main text. Shown below are the Cartesian coordinates of the relevant species, and the absolute energies of each of the species are provided in Table S1.

Structure	E_{QM}	$E_{classical}$
Ketone, 5.0Å Separation	-268.972764	0.009242
Ketone, 1.9Å Separation	-268.982154	-0.007032
Oxyanion, 5.0Å Separation	-269.008787	0.018486
Oxyanion, 1.9Å Separation	-269.062778	-0.003108

Table S1: Absolute quantum (E_{QM}) and classical ($E_{classical}$) energies corresponding to each of the species shown in the thermodynamic cycle of Fig. 6 of the main text, in Hartrees. Here, the quantum energy also includes the interaction between the ab initio and the classical regions.

Ketone with 2 Water Molecules at 5Å Separation from the C=O

C	0.000000	0.799000	0.000000
O	0.000000	-0.429000	0.000000
C	1.282000	1.576000	-0.085000
C	-1.282000	1.575000	0.085000
H	1.262000	2.434000	0.593000
H	2.146000	0.942000	0.120000
H	1.375000	1.978000	-1.102000
H	-1.375000	1.978000	1.102000
H	-1.262000	2.434000	-0.593000
H	-2.146000	0.942000	-0.120000
O	0.286000	3.298000	4.321000
H	0.274000	4.281000	4.139000
O	5.383720	-2.959727	0.028056
H	4.433661	-2.740282	0.025002
H	5.441948	-3.921465	0.036163
O	-5.385522	-2.960466	-0.028996
H	-4.432522	-2.742466	-0.024996
H	-5.444816	-3.922496	-0.033910

Ketone with 2 Water Molecules at 1.9Å Separation from the C=O

C	-0.7300000000	-0.3920000000	-0.0480000000
O	0.3160000000	-0.0200000000	-0.7490000000
C	-2.1570000000	-0.0700000000	-0.5560000000
C	-0.5370000000	-1.1730000000	1.2750000000
H	-2.9550000000	-0.4040000000	0.1230000000
H	-2.2320000000	1.0130000000	-0.6850000000
H	-2.3100000000	-0.5450000000	-1.5290000000
H	-1.4780000000	-1.3970000000	1.8000000000
H	-0.0250000000	-2.1180000000	1.0660000000
H	0.0980000000	-0.5740000000	1.9320000000
O	-0.3240000000	1.8880000000	4.3840000000
H	-1.1210000000	1.6740000000	4.8830000000
O	0.1950000000	2.2340000000	-2.5680000000
H	0.0474283360	1.5380009500	-1.8244355260
H	1.1900000000	2.0960000000	-2.7690000000
O	2.9210000000	-1.2540000000	-0.3820000000
H	1.9599118100	-0.8985533500	-0.2718435090
H	3.2910000000	-0.6040000000	-1.0680000000

Oxyanion with 2 Water Molecules at 5Å Separation from the C=O

C	-0.688000	-0.378000	-0.070000
O	0.367000	0.011000	-0.762000
C	-2.019000	0.037000	-0.738000
C	-0.683000	-1.892000	0.231000
H	-2.907000	-0.255000	-0.157000
H	-2.020000	1.123000	-0.868000
H	-2.087000	-0.431000	-1.726000
H	-1.538000	-2.211000	0.846000
H	-0.709000	-2.451000	-0.711000
H	0.243000	-2.142000	0.756000
O	-0.674000	0.306000	1.276000
H	-1.498000	0.078000	1.729000
O	-0.036043	4.640810	-4.477302
H	0.014878	3.903213	-3.880764
H	0.810778	4.776692	-4.885873
O	5.635854	-2.618854	0.088943
H	4.788974	-2.207324	-0.037306
H	6.273340	-2.215038	-0.488156

Oxyanion with 2 Water Molecules at 1.9Å Separation from the C=O

C	-0.7300000000	-0.3920000000	-0.0480000000
O	0.3160000000	-0.0200000000	-0.7490000000
C	-2.0650000000	0.0190000000	-0.7160000000
C	-0.7270000000	-1.9100000000	0.2560000000
H	-2.9560000000	-0.2580000000	-0.1340000000
H	-2.0540000000	1.1030000000	-0.8520000000
H	-2.1360000000	-0.4520000000	-1.7010000000
H	-1.5720000000	-2.2400000000	0.8790000000
H	-0.7530000000	-2.4680000000	-0.6850000000
H	0.2050000000	-2.1490000000	0.7740000000
O	-0.7170000000	0.2910000000	1.2870000000
H	-1.5330000000	0.0410000000	1.7380000000
O	0.2050000000	2.0360000000	-2.7690000000
H	-0.0118325790	1.2902852540	-2.0795751570
H	1.2120000000	2.1080000000	-2.6050000000
O	2.8400000000	-1.3810000000	-0.6160000000
H	1.8333654190	-1.1374150750	-0.5472708890
H	3.1800000000	-0.4890000000	-0.9650000000

EXAMINING THE EFFECT OF DEFORMING THE MODEL OXYANION HOLE

Having established the effect of two water molecules in an ideal orientation relative to the oxyanion in the gas-phase, we then proceeded to examine the effect of distorting these water molecules relative to the oxygen of either the ketone or its oxyanion counterpart, in order to establish how sensitive the system is to the orientation of the two water molecules. The structures of each of the deformed species are given below, and the absolute energetics for each of the species given in Table S2. The energetics relative to having the two water molecules at 5Å separation from either the ketone or the oxyanion can be found in Table 2 of the main text. All QM/MM energies were obtained by the procedure outlined in the previous section of the Supporting Information.

Structure	*Ketone*		*Oxyanion*	
	E_{QM}	$E_{classical}$	E_{QM}	$E_{classical}$
Deformed Structure 1	-268.960145	0.020557	-269.024067	0.020398
Deformed Structure 2	-268.957872	0.153942	-269.021498	0.158404
Deformed Structure 3	-268.977268	0.045577	-269.047004	0.047011
Deformed Structure 4	-268.975605	0.006374	-269.047407	0.006215
Deformed Structure 5	-268.975000	0.026932	-269.043812	0.026773
Deformed Structure 6	-268.973327	0.034262	-269.045601	0.032828

Table S2: Absolute quantum (E_{QM}) and classical ($E_{classical}$) energies, in Hartrees, corresponding to six structures in which the model oxyanion hole has been "deformed" by altering the conformation of the two water molecules from their ideal position relative to the oxygen atom of either the ketone or the oxyanion. Here, the quantum energy also includes the interaction between the ab initio and the classical regions. The energetics relative to the corresponding species with the water molecules at 5Å separation from either the ketone or oxyanion are given in Table 2 of the main text.

Ketone, Deformed Structure 1

C	-0.7300000000	-0.3920000000	-0.0480000000
O	0.3160000000	-0.0200000000	-0.7490000000
C	-2.1570000000	-0.0700000000	-0.5560000000
C	-0.5370000000	-1.1730000000	1.2750000000
H	-2.9550000000	-0.4040000000	0.1230000000
H	-2.2320000000	1.0130000000	-0.6850000000
H	-2.3100000000	-0.5450000000	-1.5290000000
H	-1.4780000000	-1.3970000000	1.8000000000
H	-0.0250000000	-2.1180000000	1.0660000000
H	0.0980000000	-0.5740000000	1.9320000000
O	-0.3240000000	1.8880000000	4.3840000000
H	-1.1210000000	1.6740000000	4.8830000000
O	0.1950000000	2.2340000000	-2.5680000000
H	-0.0240000000	1.34600000000	-2.5350000000
H	1.1900000000	2.09600000000	-2.7690000000
O	2.9210000000	-1.25400000000	-0.3820000000
H	2.1360000000	-1.54500000000	-0.7400000000
H	3.2910000000	-0.60400000000	-1.0680000000

Ketone, Deformed Structure 2

C	-0.7300000000	-0.3920000000	-0.0480000000
O	0.3160000000	-0.0200000000	-0.7490000000
C	-2.1560000000	-0.0700000000	-0.5560000000
C	-0.5370000000	-1.1540000000	1.2850000000
H	-2.9550000000	-0.4030000000	0.1220000000
H	-2.2310000000	1.0130000000	-0.6860000000
H	-2.3090000000	-0.5450000000	-1.5300000000
H	-1.4780000000	-1.3790000000	1.8080000000
H	-0.0160000000	-2.0970000000	1.0920000000
H	0.0900000000	-0.5410000000	1.9370000000
O	-0.3260000000	1.8880000000	4.3830000000
H	-1.1240000000	1.6740000000	4.8830000000
O	-0.6370000000	1.8940000000	-2.3820000000
H	-1.2140000000	1.8920000000	-1.5530000000
H	0.3350000000	1.8210000000	-2.6960000000
O	2.4130000000	-0.7250000000	-0.3460000000
H	1.5420000000	-1.0480000000	-0.7430000000
H	2.7830000000	-0.0750000000	-1.0320000000

Ketone, Deformed Structure 3

C	-0.7300000000	-0.3920000000	-0.0480000000
O	0.3160000000	-0.0200000000	-0.7490000000
C	-2.1560000000	-0.0700000000	-0.5560000000
C	-0.5370000000	-1.1540000000	1.2850000000
H	-2.9550000000	-0.4030000000	0.1220000000
H	-2.2310000000	1.0130000000	-0.6860000000
H	-2.3090000000	-0.5450000000	-1.5300000000
H	-1.4780000000	-1.3790000000	1.8080000000
H	-0.0160000000	-2.0970000000	1.0920000000
H	0.0900000000	-0.5410000000	1.9370000000
O	-0.3260000000	1.8880000000	4.3830000000
H	-1.1240000000	1.6740000000	4.8830000000
O	0.1950000000	2.2340000000	-2.5680000000
H	-0.0240000000	1.3460000000	-2.5350000000
H	1.1900000000	2.0960000000	-2.7690000000
O	2.9210000000	-1.2540000000	-0.3820000000
H	2.0850000000	-0.4200000000	-0.3350000000
H	3.2910000000	-0.6040000000	-1.0680000000

Ketone, Deformed Structure 4

C	-0.7300000000	-0.3920000000	-0.0480000000
O	0.3160000000	-0.0200000000	-0.7490000000
C	-2.1560000000	-0.0700000000	-0.5560000000
C	-0.5370000000	-1.1540000000	1.2850000000
H	-2.9550000000	-0.4030000000	0.1220000000
H	-2.2310000000	1.0130000000	-0.6860000000
H	-2.3090000000	-0.5450000000	-1.5300000000
H	-1.4780000000	-1.3790000000	1.8080000000
H	-0.0160000000	-2.0970000000	1.0920000000
H	0.0900000000	-0.5410000000	1.9370000000
O	-0.3260000000	1.8880000000	4.3830000000
H	-1.1240000000	1.6740000000	4.8830000000
O	0.3300000000	2.4260000000	-2.6700000000
H	-0.1980000000	1.6750000000	-2.2730000000
H	1.3250000000	2.2880000000	-2.8710000000
O	2.9210000000	-1.2540000000	-0.3820000000
H	2.0020000000	-0.7410000000	-0.3620000000
H	3.2910000000	-0.6040000000	-1.0680000000

Ketone, Deformed Structure 5

C	-0.7300000000	-0.3920000000	-0.0480000000
O	0.3160000000	-0.0200000000	-0.7490000000
C	-2.1560000000	-0.0700000000	-0.5560000000
C	-0.5370000000	-1.1540000000	1.2850000000
H	-2.9550000000	-0.4030000000	0.1220000000
H	-2.2310000000	1.0130000000	-0.6860000000
H	-2.3090000000	-0.5450000000	-1.5300000000
H	-1.4780000000	-1.3790000000	1.8080000000
H	-0.0160000000	-2.0970000000	1.0920000000
H	0.0900000000	-0.5410000000	1.9370000000
O	-0.3260000000	1.8880000000	4.3830000000
H	-1.1240000000	1.6740000000	4.8830000000
O	0.1950000000	2.4000000000	-2.5390000000
H	0.1940000000	1.4160000000	-2.7120000000
H	1.1900000000	2.2620000000	-2.7400000000
O	2.9210000000	-1.2540000000	-0.3820000000
H	2.0640000000	-0.5730000000	-0.3650000000
H	3.2910000000	-0.6040000000	-1.0680000000

Ketone, Deformed Structure 6

C	-0.7300000000	-0.3920000000	-0.0480000000
O	0.3160000000	-0.0200000000	-0.7490000000
C	-2.1560000000	-0.0700000000	-0.5560000000
C	-0.5370000000	-1.1540000000	1.2850000000
H	-2.9550000000	-0.4030000000	0.1220000000
H	-2.2310000000	1.0130000000	-0.6860000000
H	-2.3090000000	-0.5450000000	-1.5300000000
H	-1.4780000000	-1.3790000000	1.8080000000
H	-0.0160000000	-2.0970000000	1.0920000000
H	0.0900000000	-0.5410000000	1.9370000000
O	-0.3260000000	1.8880000000	4.3830000000
H	-1.1240000000	1.6740000000	4.8830000000
O	-0.1970000000	2.3470000000	-2.7290000000
H	-0.7780000000	1.8750000000	-2.0360000000
H	0.7750000000	2.2740000000	-3.0440000000
O	2.9210000000	-1.2540000000	-0.3820000000
H	1.9180000000	-0.7590000000	-0.2920000000
H	3.2910000000	-0.6040000000	-1.0680000000

Oxyanion, Deformed Structure 1

C	-0.7300000000	-0.3920000000	-0.0480000000
O	0.3160000000	-0.0200000000	-0.7490000000
C	-2.0650000000	0.0190000000	-0.7160000000
C	-0.7270000000	-1.9100000000	0.2560000000
H	-2.9560000000	-0.2580000000	-0.1340000000
H	-2.0540000000	1.1030000000	-0.8520000000
H	-2.1360000000	-0.4520000000	-1.7010000000
H	-1.5720000000	-2.2400000000	0.8790000000
H	-0.7530000000	-2.4680000000	-0.6850000000
H	0.2050000000	-2.1490000000	0.7740000000
O	-0.7170000000	0.2910000000	1.2870000000
H	-1.5330000000	0.0410000000	1.7380000000
O	0.2830000000	2.2890000000	-2.6480000000
H	-0.4030000000	1.8610000000	-2.0270000000
H	1.2780000000	2.1510000000	-2.8490000000
O	2.9210000000	-1.2540000000	-0.3820000000
H	1.9180000000	-0.7590000000	-0.2920000000
H	3.2910000000	-0.6040000000	-1.0680000000

Oxyanion, Deformed Structure 2

C	-0.7300000000	-0.3920000000	-0.0480000000
O	0.3160000000	-0.0200000000	-0.7490000000
C	-2.0650000000	0.0190000000	-0.7160000000
C	-0.7270000000	-1.9100000000	0.2560000000
H	-2.9560000000	-0.2580000000	-0.1340000000
H	-2.0540000000	1.1030000000	-0.8520000000
H	-2.1360000000	-0.4520000000	-1.7010000000
H	-1.5720000000	-2.2400000000	0.8790000000
H	-0.7530000000	-2.4680000000	-0.6850000000
H	0.2050000000	-2.1490000000	0.7740000000
O	-0.7170000000	0.2910000000	1.2870000000
H	-1.5330000000	0.0410000000	1.7380000000
O	-0.2220000000	1.8680000000	-2.3530000000
H	-0.8890000000	1.9090000000	-1.5960000000
H	0.7730000000	1.7300000000	-2.5550000000
O	2.4130000000	-0.7250000000	-0.3460000000
H	1.5420000000	-1.0480000000	-0.7430000000
H	2.7830000000	-0.0750000000	-1.0320000000

Oxyanion, Deformed Structure 3

C	-0.7300000000	-0.3920000000	-0.0480000000
O	0.3160000000	-0.0200000000	-0.7490000000
C	-2.0650000000	0.0190000000	-0.7160000000
C	-0.7270000000	-1.9100000000	0.2560000000
H	-2.9560000000	-0.2580000000	-0.1340000000
H	-2.0540000000	1.1030000000	-0.8520000000
H	-2.1360000000	-0.4520000000	-1.7010000000
H	-1.5720000000	-2.2400000000	0.8790000000
H	-0.7530000000	-2.4680000000	-0.6850000000
H	0.2050000000	-2.1490000000	0.7740000000
O	-0.7170000000	0.2910000000	1.2870000000
H	-1.5330000000	0.0410000000	1.7380000000
O	0.1950000000	2.2340000000	-2.5680000000
H	-0.0240000000	1.3460000000	-2.5350000000
H	1.1900000000	2.0960000000	-2.7690000000
O	2.9210000000	-1.2540000000	-0.3820000000
H	2.0850000000	-0.4200000000	-0.3350000000
H	3.2910000000	-0.6040000000	-1.0680000000

Oxyanion, Deformed Structure 4

C	-0.7300000000	-0.3920000000	-0.0480000000
O	0.3160000000	-0.0200000000	-0.7490000000
C	-2.0650000000	0.0190000000	-0.7160000000
C	-0.7270000000	-1.9100000000	0.2560000000
H	-2.9560000000	-0.2580000000	-0.1340000000
H	-2.0540000000	1.1030000000	-0.8520000000
H	-2.1360000000	-0.4520000000	-1.7010000000
H	-1.5720000000	-2.2400000000	0.8790000000
H	-0.7530000000	-2.4680000000	-0.6850000000
H	0.2050000000	-2.1490000000	0.7740000000
O	-0.7170000000	0.2910000000	1.2870000000
H	-1.5330000000	0.0410000000	1.7380000000
O	0.3300000000	2.4260000000	-2.6700000000
H	-0.1980000000	1.6750000000	-2.2730000000
H	1.3250000000	2.2880000000	-2.8710000000
O	2.9210000000	-1.2540000000	-0.3820000000
H	2.0020000000	-0.7410000000	-0.3620000000
H	3.2910000000	-0.6040000000	-1.0680000000

Oxyanion, Deformed Structure 5

C	-0.7300000000	-0.3920000000	-0.0480000000
O	0.3160000000	-0.0200000000	-0.7490000000
C	-2.0650000000	0.0190000000	-0.7160000000
C	-0.7270000000	-1.9100000000	0.2560000000
H	-2.9560000000	-0.2580000000	-0.1340000000
H	-2.0540000000	1.1030000000	-0.8520000000
H	-2.1360000000	-0.4520000000	-1.7010000000
H	-1.5720000000	-2.2400000000	0.8790000000
H	-0.7530000000	-2.4680000000	-0.6850000000
H	0.2050000000	-2.1490000000	0.7740000000
O	-0.7170000000	0.2910000000	1.2870000000
H	-1.5330000000	0.0410000000	1.7380000000
O	0.1950000000	2.4000000000	-2.5390000000
H	0.1940000000	1.4160000000	-2.7120000000
H	1.1900000000	2.2620000000	-2.7400000000
O	2.9210000000	-1.2540000000	-0.3820000000
H	2.0640000000	-0.5730000000	-0.3650000000
H	3.2910000000	-0.6040000000	-1.0680000000

Oxyanion, Deformed Structure 6

C	-0.7300000000	-0.3920000000	-0.0480000000
O	0.3160000000	-0.0200000000	-0.7490000000
C	-2.0650000000	0.0190000000	-0.7160000000
C	-0.7270000000	-1.9100000000	0.2560000000
H	-2.9560000000	-0.2580000000	-0.1340000000
H	-2.0540000000	1.1030000000	-0.8520000000
H	-2.1360000000	-0.4520000000	-1.7010000000
H	-1.5720000000	-2.2400000000	0.8790000000
H	-0.7530000000	-2.4680000000	-0.6850000000
H	0.2050000000	-2.1490000000	0.7740000000
O	-0.7170000000	0.2910000000	1.2870000000
H	-1.5330000000	0.0410000000	1.7380000000
O	0.2830000000	2.2890000000	-2.6480000000
H	-0.4030000000	1.8610000000	-2.0270000000
H	1.2780000000	2.1510000000	-2.8490000000
O	2.9210000000	-1.2540000000	-0.3820000000
H	1.9180000000	-0.7590000000	-0.2920000000
H	3.2910000000	-0.6040000000	-1.0680000000

EXAMINING THE EFFECT OF CONSTRAINTS ON THE DEFORMATION OF THE OXYANION HOLE

Having examined the energetics of various “deformed” model oxyanion holes, the next question is to elucidate how strong a constraint is required to maintain this deformation. In order to address this issue, we selected two structures (Structures 1 and 6 from Table S2), and fixed all atoms with the exception of the two hydrogen atoms that are with hydrogen bonding distance of the C=O or C-O$^-$ as relevant, and then applied positional constraints of various magnitudes (i.e. 5000, 500, 50, 5, 0.5 and 0 kcal mol^{-1} Å^{-2}) to the positions of these two hydrogen atoms, while performing 1000 steps of QM/MM steepest descent minimization. As discussed in the main text, we found that in order to maintain the deformation, an unphysical force constant of > 50 kcal mol^{-1} Å^{-2} is required (note that protein force constants are typically at most 5 kcal mol^{-1} Å^{-2} for

small deformations). The energies of the structures at the end point of each minimization run relative to the corresponding structure with the two water molecules at 5Å separation have been presented in Table 3 of the main text. For illustration purposes, the absolute energies of the end points of each minimization run when starting from Structures 1 and 6 of Table 2 of the main text are shown in Table S3 and S4, and the coordinates of the structures after 1000 steps of QM/MM minimization with positional constraints of 5000 and 0.5 kcal mol^{-1} $Å^{-2}$ (i.e. the two extremes) are shown below for both the ketone and oxyanion species.

K	***Oxyanion***		***Ketone***	
	E_{QM}	$E_{classical}$	E_{QM}	$E_{classical}$
5000	-268.962049	0.018422	-269.024588	0.018342
500	-268.961499	0.009546	-269.027554	0.009896
50	-268.967473	0.002534	-269.042349	0.006358
5	-268.980252	-0.001020	-269.058691	0.010518
0.5	-268.982141	-0.005020	-269.060174	-0.003665
0	-268.981904	-0.005912	-269.060222	-0.004462

Table S3: The effect of constraints of different magnitude on the relaxation of the "deformed" water molecules of Structure 1 of Table 2 of the main text, relative to either the C=O or C-O^- species, after 1000 steps of QM/MM minimization. Shown here are the absolute quantum (E_{QM}) and classical ($E_{classical}$), in Hartrees. The magnitude of the constraint, K, is presented in kcal mol^{-1} $Å^{-2}$. Note that here, the quantum energy also includes the interaction between the ab initio and the classical regions. The energetics relative to the corresponding species with the water molecules at 5Å separation from either the ketone or oxyanion are given in Table 3 of the main text.

Ketone, K = 5000 kcal mol^{-1} Å^{-2}

C	-0.7300000000	-0.3920000000	-0.0480000000
O	0.3160000000	-0.0200000000	-0.7490000000
C	-2.1570000000	-0.0700000000	-0.5560000000
C	-0.5370000000	-1.1730000000	1.2750000000
H	-2.9550000000	-0.4040000000	0.1230000000
H	-2.2320000000	1.0130000000	-0.6850000000
H	-2.3100000000	-0.5450000000	-1.5290000000
H	-1.4780000000	-1.3970000000	1.8000000000
H	-0.0250000000	-2.1180000000	1.0660000000
H	0.0980000000	-0.5740000000	1.9320000000
O	-0.3240000000	1.8880000000	4.3840000000
H	-1.1210000000	1.6740000000	4.8830000000
O	0.1950000000	2.2340000000	-2.5680000000
H	-0.0275962750	1.3354803340	-2.5329201010
H	1.1900000000	2.0960000000	-2.7690000000
O	2.9210000000	-1.2540000000	-0.3820000000
H	2.1257216370	-1.5473940850	-0.7441731140
H	3.2910000000	-0.6040000000	-1.0680000000

Oxyanion, K = 5000 kcal mol^{-1} Å^{-2}

C	-0.7300000000	-0.3920000000	-0.0480000000
O	0.3160000000	-0.0200000000	-0.7490000000
C	-2.0650000000	0.0190000000	-0.7160000000
C	-0.7270000000	-1.9100000000	0.2560000000
H	-2.9560000000	-0.2580000000	-0.1340000000
H	-2.0540000000	1.1030000000	-0.8520000000
H	-2.1360000000	-0.4520000000	-1.7010000000
H	-1.5720000000	-2.2400000000	0.8790000000
H	-0.7530000000	-2.4680000000	-0.6850000000
H	0.2050000000	-2.1490000000	0.7740000000
O	-0.7170000000	0.2910000000	1.2870000000
H	-1.5330000000	0.0410000000	1.7380000000
O	0.1950000000	2.2340000000	-2.5680000000
H	-0.0275434970	1.3348810010	-2.5321465900
H	1.1900000000	2.0960000000	-2.7690000000
O	2.9210000000	-1.2540000000	-0.3820000000
H	2.1249172880	-1.5468746510	-0.7441837470
H	3.2910000000	-0.6040000000	-1.0680000000

Ketone, K = 0.5 kcal mol^{-1} Å^{-2}

C	-0.7300000000	-0.3920000000	-0.0480000000
O	0.3160000000	-0.0200000000	-0.7490000000
C	-2.1570000000	-0.0700000000	-0.5560000000
C	-0.5370000000	-1.1730000000	1.2750000000
H	-2.9550000000	-0.4040000000	0.1230000000
H	-2.2320000000	1.0130000000	-0.6850000000
H	-2.3100000000	-0.5450000000	-1.5290000000
H	-1.4780000000	-1.3970000000	1.8000000000
H	-0.0250000000	-2.1180000000	1.0660000000
H	0.0980000000	-0.5740000000	1.9320000000
O	-0.3240000000	1.8880000000	4.3840000000
H	-1.1210000000	1.6740000000	4.8830000000
O	0.1950000000	2.2340000000	-2.5680000000
H	0.0405503650	1.5202880190	-1.8437376260
H	1.1900000000	2.0960000000	-2.7690000000
O	2.9210000000	-1.2540000000	-0.3820000000
H	1.9524673120	-0.9160060190	-0.2901276340
H	3.2910000000	-0.6040000000	-1.0680000000

Oxyanion, K = 0.5 kcal mol^{-1} Å^{-2}

C	-0.7300000000	-0.3920000000	-0.0480000000
O	0.3160000000	-0.0200000000	-0.7490000000
C	-2.0650000000	0.0190000000	-0.7160000000
C	-0.7270000000	-1.9100000000	0.2560000000
H	-2.9560000000	-0.2580000000	-0.1340000000
H	-2.0540000000	1.1030000000	-0.8520000000
H	-2.1360000000	-0.4520000000	-1.7010000000
H	-1.5720000000	-2.2400000000	0.8790000000
H	-0.7530000000	-2.4680000000	-0.6850000000
H	0.2050000000	-2.1490000000	0.7740000000
O	-0.7170000000	0.2910000000	1.2870000000
H	-1.5330000000	0.0410000000	1.7380000000
O	0.1950000000	2.2340000000	-2.5680000000
H	0.0468858780	1.4764108690	-1.8735755200
H	1.1900000000	2.0960000000	-2.7690000000
O	2.9210000000	-1.2540000000	-0.3820000000
H	1.9317519820	-0.9355801280	-0.3536859110
H	3.2910000000	-0.6040000000	-1.0680000000

K	*Oxyanion*		*Ketone*	
	E_{QM}	$E_{classical}$	E_{QM}	$E_{classical}$
5000	-268.974194	0.002196	-269.045336	0.031123
500	-268.973889	0.023728	-269.045355	0.024032
50	-268.977873	0.082549	-269.052934	0.009562
5	-268.981214	-0.003904	-269.058067	-0.002518
0.5	-268.981610	-0.006167	-269.058612	-0.004861
0	-268.981652	-0.008892	-269.058668	-0.005147

Table S4: The effect of constraints of different magnitude on the relaxation of the "deformed" water molecules of Structure 6 of Table 2 of the main text, relative to either the C=O or C-O$^-$ species, after 1000 steps of QM/MM minimization. Shown here are the absolute quantum (E_{QM}) and classical ($E_{classical}$), in Hartrees. The magnitude of the constraint, K, is presented in kcal mol^{-1} Å^{-2}. Note that here, the quantum energy also includes the interaction between the ab initio and the classical regions. The energetics relative to the corresponding species with the water molecules at 5Å separation from either the ketone or oxyanion are given in Table 3 of the main text.

Ketone, K = 5000 kcal mol^{-1} Å^{-2}

C	-0.7300000000	-0.3920000000	-0.0480000000
O	0.3160000000	-0.0200000000	-0.7490000000
C	-2.1560000000	-0.0700000000	-0.5560000000
C	-0.5370000000	-1.1540000000	1.2850000000
H	-2.9550000000	-0.4030000000	0.1220000000
H	-2.2310000000	1.0130000000	-0.6860000000
H	-2.3090000000	-0.5450000000	-1.5300000000
H	-1.4780000000	-1.3790000000	1.8080000000
H	-0.0160000000	-2.0970000000	1.0920000000
H	0.0900000000	-0.5410000000	1.9370000000
O	-0.3260000000	1.8880000000	4.3830000000
H	-1.1240000000	1.6740000000	4.8830000000
O	0.2830000000	2.2890000000	-2.6480000000
H	-0.3985557050	1.8567164530	-2.0244007320
H	1.2780000000	2.1510000000	-2.8490000000
O	2.9210000000	-1.2540000000	-0.3820000000
H	1.9275979700	-0.7645100480	-0.2915993340
H	3.2910000000	-0.6040000000	-1.0680000000

Oxyanion, K = 5000 kcal mol^{-1} Å^{-2}

C	-0.7300000000	-0.3920000000	-0.0480000000
O	0.3160000000	-0.0200000000	-0.7490000000
C	-2.0650000000	0.0190000000	-0.7160000000
C	-0.7270000000	-1.9100000000	0.2560000000
H	-2.9560000000	-0.2580000000	-0.1340000000
H	-2.0540000000	1.1030000000	-0.8520000000
H	-2.1360000000	-0.4520000000	-1.7010000000
H	-1.5720000000	-2.2400000000	0.8790000000
H	-0.7530000000	-2.4680000000	-0.6850000000
H	0.2050000000	-2.1490000000	0.7740000000
O	-0.7170000000	0.2910000000	1.2870000000
H	-1.5330000000	0.0410000000	1.7380000000
O	0.2830000000	2.2890000000	-2.6480000000
H	-0.3984350770	1.8559445100	-2.0239361040
H	1.2780000000	2.1510000000	-2.8490000000
O	2.9210000000	-1.2540000000	-0.3820000000
H	1.9263618150	-0.7640382550	-0.2919965490
H	3.2910000000	-0.6040000000	-1.0680000000

Ketone, K = 0.5 kcal mol^{-1} Å^{-2}

C	-0.7300000000	-0.3920000000	-0.0480000000
O	0.3160000000	-0.0200000000	-0.7490000000
C	-2.1560000000	-0.0700000000	-0.5560000000
C	-0.5370000000	-1.1540000000	1.2850000000
H	-2.9550000000	-0.4030000000	0.1220000000
H	-2.2310000000	1.0130000000	-0.6860000000
H	-2.3090000000	-0.5450000000	-1.5300000000
H	-1.4780000000	-1.3790000000	1.8080000000
H	-0.0160000000	-2.0970000000	1.0920000000
H	0.0900000000	-0.5410000000	1.9370000000
O	-0.3260000000	1.8880000000	4.3830000000
H	-1.1240000000	1.6740000000	4.8830000000
O	0.2830000000	2.2890000000	-2.6480000000
H	0.1295986300	1.5667708550	-1.9219637040
H	1.2780000000	2.1510000000	-2.8490000000
O	2.9210000000	-1.2540000000	-0.3820000000
H	1.9629132040	-0.8918478510	-0.2654440610
H	3.2910000000	-0.6040000000	-1.0680000000

Oxyanion, K = 0.5 kcal mol^{-1} Å^{-2}

C	-0.7300000000	-0.3920000000	-0.0480000000
O	0.3160000000	-0.0200000000	-0.7490000000
C	-2.0650000000	0.0190000000	-0.7160000000
C	-0.7270000000	-1.9100000000	0.2560000000
H	-2.9560000000	-0.2580000000	-0.1340000000
H	-2.0540000000	1.1030000000	-0.8520000000
H	-2.1360000000	-0.4520000000	-1.7010000000
H	-1.5720000000	-2.2400000000	0.8790000000
H	-0.7530000000	-2.4680000000	-0.6850000000
H	0.2050000000	-2.1490000000	0.7740000000
O	-0.7170000000	0.2910000000	1.2870000000
H	-1.5330000000	0.0410000000	1.7380000000
O	0.2830000000	2.2890000000	-2.6480000000
H	0.1360308690	1.5307761560	-1.9416628330
H	1.2780000000	2.1510000000	-2.8490000000
O	2.9210000000	-1.2540000000	-0.3820000000
H	1.9367870920	-0.9203353120	-0.3386668910
H	3.2910000000	-0.6040000000	-1.0680000000

SAMPLE INPUT SCRIPT

In order to facilitate the reproduction of our data, we have provided here a sample python script to run our QM/MM minimization protocol. However, in order to run the program, the reader will require copies of MOLARIS and Gaussian. In order to obtain a copy of MOLARIS, which is available on request, as well as the relevant library files, please contact the corresponding author of this manuscript.

```
#!/bin/bash
#source /usr/usc/python/2.5/setup.csh
#source /usr/usc/gaussian/default/setup.csh

# Set MOLARIS path
MOLARIS=/auto/rcf-proj3/aw/molaris/bin/molaris_hpc9.09

# Set home directory
hdir=/auto/rcf-proj3/aw/skamerli/rcf96/goodman/model/outplane/h2o_planar_mini

# Working directory = home directory
wdir=$hdir

#Define important variables, i.e.:

# Number of QM atoms in system
num=$1

# Reference quantum energy (in Hartrees)
e_ref=-269.0106727

# Charge and multiplicity on the system
chrg="-1 1"

# Basis set / functional
method="MPW1PW91/6-311++G**"

# PDB filename
pdb="$num.pdb"

# QM atoms (i.e. atom numbers)
reg1_atm="1 to 12"

# Number of minimization steps
nstep=1000
```

```
cd $hdir

#Define output directory name as well as directory on /tmp to run the job on
ttype=get_ener$num
temp=/tmp/skamerli

###############
# All subsequent parts of script are not to be changed unless you know exactly what you are
#doing. If the above variables have been correctly defined, script will run as is. However, you
#need to skip to the end of the script, which generates and runs a MOLARIS input file to set
#paths again.
###############

OUT_DIR=$temp/$ttype
molarisdir=$wdir/$ttype
g98dir=$OUT_DIR/g98scr
szemet=$OUT_DIR/szemet


if [ ! -e $temp ]; then mkdir $temp; fi
if [ ! -e $wdir ]; then mkdir $wdir; fi
if [ ! -e $OUT_DIR ]; then mkdir $OUT_DIR ; fi
if [ ! -e $szemet ]; then mkdir $szemet ; fi
if [ ! -e $g98dir ]; then mkdir $g98dir ; fi
if [ ! -e $molarisdir ]; then mkdir $molarisdir ; fi

rs="majom"
qminp=$g98dir/$rs.inp #g98 input file
qmmmin=$g98dir/mol.in #molaris generated input for qm script
qmmmres=$g98dir/d.o #qm output for molaris

# create qm.csh: file qm_script.py has to be in $g98dir
cp ~/bin/sc/qm_script.py $g98dir/qm_script.py
cp ~/bin/sc/smear.bsh $g98dir/smear.bsh
cp ~/bin/sc/smearcharge $g98dir/smearcharge

#Copy restart from parent run + counter to output directory
cp $hdir/$rest_in $OUT_DIR
cp counter.dat $OUT_DIR

chmod +x $g98dir/smear.bsh
chmod +x $g98dir/smearcharge
chmod +x $g98dir/qm_script.py
echo "#!/bin/csh
if (! -d /tmp/skamerli/g98scr) then
        if (! -d /tmp/skamerli) mkdir /tmp/skamerli
```

```
      mkdir /tmp/skamerli/g98scr
endif

setenv GAUSS_SCRDIR /tmp/skamerli/g98scr
cd $g98dir
touch $OUT_DIR/qm.csh
#.-smear.bsh $qmmmin
./qm_script.py --qm_prog='g03'\
            --qm_method='$method'\
            --qm_inp='$qminp'\
            --file_in='$qmmmin'\
            --file_out='$qmmmres'\
            --e_ref='$e_ref'\
            --CHRG-MULT='$chrg'\
            --xmov='qm.$type.xyz'
">$OUT_DIR/qm.csh
chmod +x $OUT_DIR/qm.csh

chmod +x $OUT_DIR/qm.csh

cp $g98dir/qm_script.py $OUT_DIR

cp $g98dir/qm_script.py $OUT_DIR
#       create molaris file:


###################################
# This part of the script generates a MOLARIS input file
###################################

echo "
$pdb keepallh
enzymix
ac
     reg1_atm $reg1_atm
     map_pf 1 1 2
     map_lambda 1.0
     md_parm
#       fix_region1
       gas_phase 1
       steep_mini 1
       ss 0.001
       nsteps $nstep
       temp 0.0
       fix_atom  1 to 13
       fix_atom  15 16 18
```

```
qmmm
        qmmm_interval 1
        script $OUT_DIR/qm.csh
        qmmm_in $qmmmin
        qmmm_out $qmmmres
        red_lk_crg
        qmmm_noaver
        qmmm_steep_stepsize 0.9 1.1 0.0001
           skip_step0_qmmm
      end
    end
  end
end
end
end
">$OUT_DIR/mini.inp

#run molaris:

cp $hdir/$pdb $OUT_DIR
cp $hdir/$pdb $szemet

molout=$wdir/$ttype/out

#exesave


$MOLARIS <$OUT_DIR/mini.inp >$OUT_DIR/mini.out


cp -r $OUT_DIR/* $molarisdir

################ End of script
```

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