

Supporting Information for

**⁵¹V NMR Crystallography of Vanadium Chloroperoxidase and its
Directed Evolution P395D/L241V/T343A Mutant: Protonation
Environments of the Active Site**

Rupal Gupta¹, Guangjin Hou¹, Rokus Renirie², Ron Wever², and Tatyana Polenova^{1}*

¹*Department of Chemistry and Biochemistry, University of Delaware, Newark, DE 19716, United States;* ²*Van't Hoff Institute for Molecular Science, University of Amsterdam, POSTBUS 94157, 1090 GD Amsterdam, The Netherlands*

E-mail: tpolenov@udel.edu

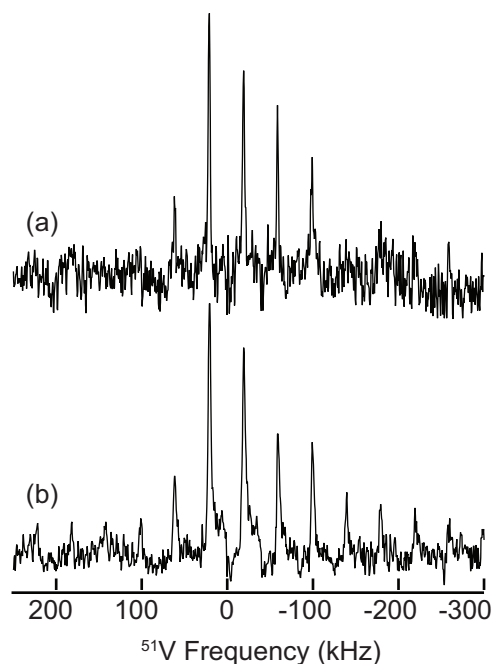


Figure S1. 20.0 T ^{51}V NMR spectrum acquired at MAS of 40 kHz of wild type VCPO at pH 8.3 purified from (a) *E. coli*; and (b) *S. cerevisiae*. The relative intensities of the spinning sidebands are identical for the two spectra suggesting that the vanadate cofactor remains the same.

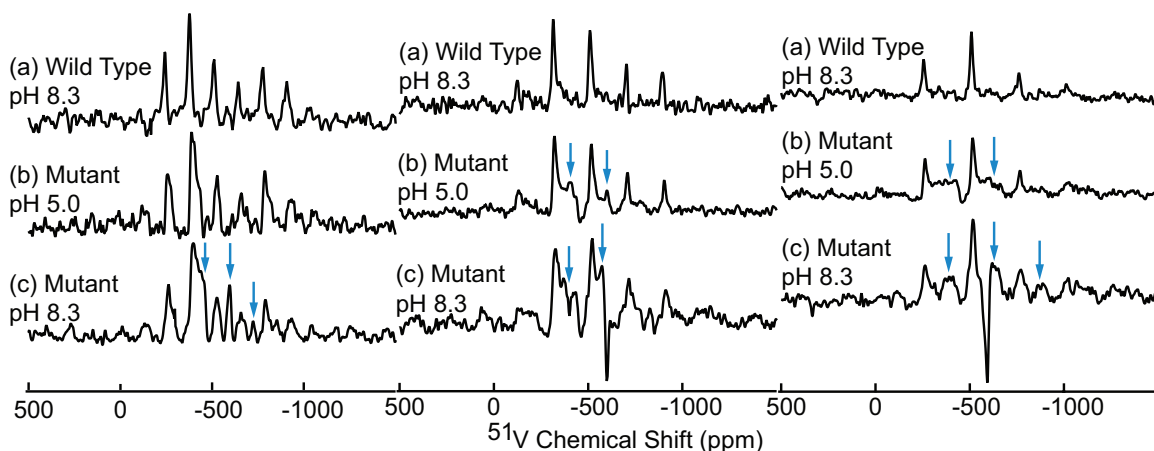


Figure S2. ^{51}V MAS spectrum of VCPO and its mutant at 14.1 T and 21, 31 and 40 kHz MAS (a) wild type enzyme at pH 8.3; (b) P395D/L241V/T343A mutant at pH 5.0 and; (c) P395D/L241V/T343A mutant at pH 8.3. The arrows indicate the spinning sidebands associated with the second species in the triple mutant.

At 31 and 40 kHz, spinning sidebands originating from the second species can also be observed in the spectra from the P395D/L241V/T343A mutant prepared at pH 5.0

(Figure S2, sidebands marked with an arrow). This comparison of the spectra of the P395D/L241V/T343A mutant at pH 8.3, which clearly exhibits two species, and the mutant at pH 5.0 clarifies the poor agreement between the lineshapes of the numerical simulations and experimental data at these spinning frequencies. It is noteworthy that there is a presence of an impurity species in the mutant sample at pH 8.3 which gives rise to an out of phase resonance near -599 ppm. This out of phase resonance is more easily detectable at 31 and 40 kHz and further makes the characterization of the second species difficult. The origin of this resonance is not understood, and it is always detected in multiple preparations for this enzyme.

Determination of the Relative Orientation of the CSA and Quadrupolar Tensors in VCPO. Magic angle spinning NMR spectra are not only sensitive to the magnitudes of the CSA and the quadrupolar interactions, but also bear information on the relative orientation of the two tensors. It has been shown by us and by others¹ that numerical simulations of the full spectral envelope including the spinning sidebands from the satellite transitions permit the determination of the quadrupolar and the CSA tensors as well as their relative orientations. For small vanadium(V)-containing inorganic complexes, the spectra containing both central and satellite transitions can be easily obtained using frequency-stepped acquisition or broadband excitation pulses.² Figure S3 shows one such example of a vanadium inorganic complex where the full spectrum using frequency-stepped acquisition can be acquired in approximately 10 hours, and the numerical simulations yielded the accurate NMR parameters.³ However, for large biological systems, such as VCPO, where the vanadium constitutes only 0.07% of the sample weight, acquiring a spectrum with full spinning sideband manifold from all satellite transitions, in a frequency-stepped manner, is challenging due to a much lower sensitivity. Figure S4 (left panel) shows the experimental spectrum of VCPO recorded at the MAS 21 kHz along with the numerical simulations. The simulations reveal the entire spectral envelope including the regions where satellite transitions are expected but not observed experimentally due to the limited excitation and detection bandwidth of the NMR probe. Consequently, information regarding the orientation between the CSA and

quadrupolar tensors is difficult to extract in VCPO and, in general, in biological molecules.

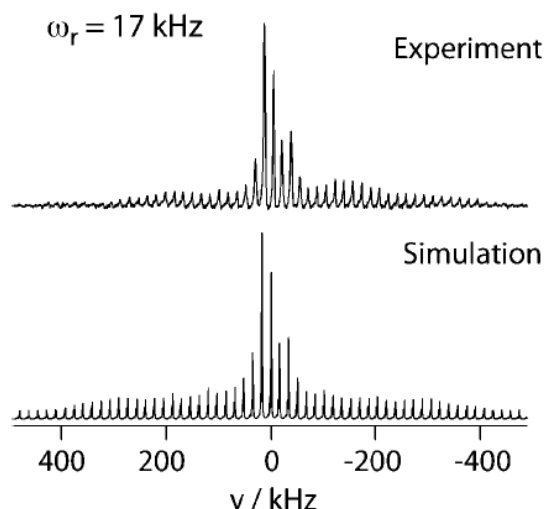


Figure S3. An example of ^{51}V MAS spectra from an inorganic complex, VO(enSAL)TBCat acquired using frequency-stepped acquisition.³ (a) A summation of 10 frequency-stepped ^{51}V NMR spectra acquired 9.4 T and MAS of 17 kHz. Each individual spectrum took approximately 1 hour for acquisition. (b) Simulation of the experimental data using the parameters $C_Q = 5.8$ MHz, $\eta_Q = 0.95$, $\delta_\sigma = 485$ ppm, $\eta_\sigma = 0.25$.

While determination of relative CSA and quadrupolar tensor orientations is challenging for low-sensitivity biological samples, for systems with large C_Q , an estimate of tensor orientations can be obtained using fast MAS frequencies. For example, under fast MAS conditions (31 and 40 kHz), both the lineshape and the position of the spinning sidebands are sensitive to the Euler angles for $C_Q = 14$ MHz (the value for the P395D/L241V/T343A mutant) but not for $C_Q = 10$ MHz (the value for the wild type), shown in Figure S4 (right panel). As is clear from the Figure, for the Euler angles of $(\alpha, \beta, \gamma) = (0, 0, 0)$, numerical simulations do not correctly recapitulate either the lineshape or the position of the spinning sidebands for the P395D/L241V/T343A mutant sample, indicating that the two tensors are not collinear. However, if $(\alpha, \beta, \gamma) = (0, 30, 0)$ are used in the simulations, a shift in the spinning sidebands position of approximately 2.5 kHz is observed under fast MAS frequencies, producing a reasonable agreement between the simulations and the experiment.

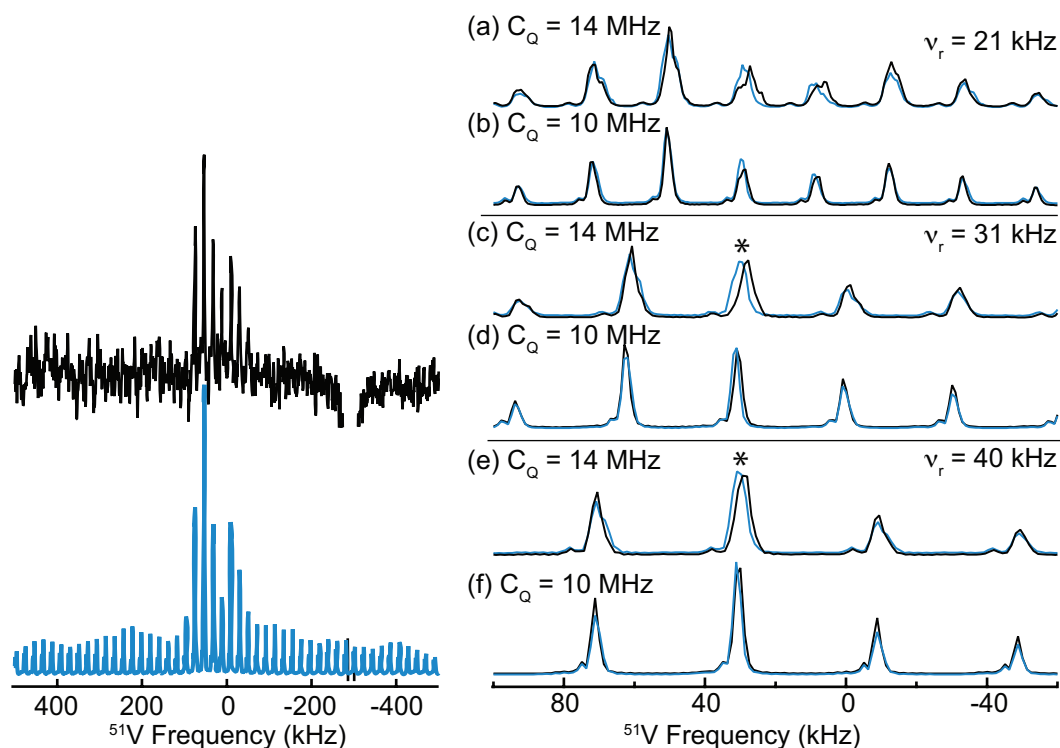


Figure S4. Left: An expanded view of 14.1 T ^{51}V NMR spectrum of the wild type enzyme at pH 7.3 acquired at MAS of 21 kHz (black) and the corresponding simulation (blue) showing all the satellite transitions that are not observed in the experimental data due to the limited probe excitation bandwidth. Right: Simulations showing the effect of the relative orientation of the CSA and quadrupolar tensors on the position of the spinning sideband for $C_Q = 10$ and 14 MHz. The colored traces are the simulations with Euler angles of $(\alpha, \beta, \gamma) = (0, 30, 0)$ and the corresponding simulations with the same parameters and $(\alpha, \beta, \gamma) = (0, 0, 0)$ are the black traces. Simulations were performed at the MAS frequencies of 21 kHz (a, b); 31 kHz (c, d), and; 40 kHz (e, f). The spinning sidebands marked with asterisks indicate the observable shifts due to the relative orientations of the CSA and the quadrupolar tensors.

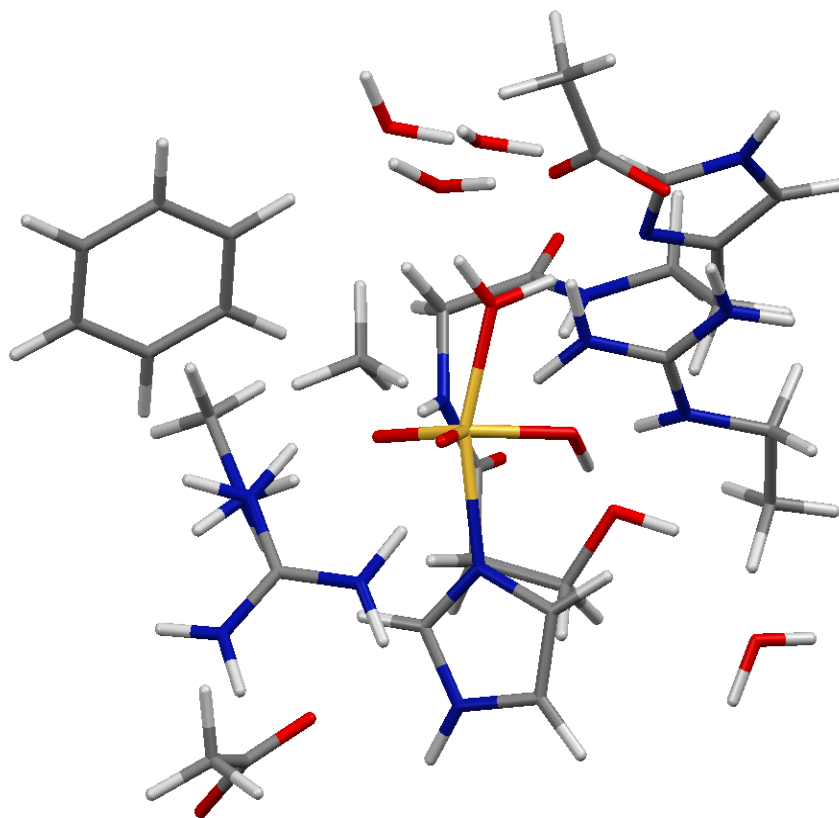


Figure S5. An example of a model of the active site considered for DFT calculations.

Gaussian input command line for NMR parameter calculation

#b3lyp/6-311G(d,p) scf=tight nmr prop=efg

Input Coordinates for VOD44 model:

| | | | |
|---|----------|---------|---------|
| C | -18.1380 | 10.7310 | -4.8710 |
| C | -18.2600 | 11.1730 | -3.4240 |
| O | -18.6070 | 12.3430 | -3.2080 |
| O | -18.0830 | 10.3670 | -2.4870 |
| C | -20.1770 | 21.8250 | -0.8680 |
| N | -19.1390 | 21.4180 | 0.1330 |
| N | -13.9130 | 19.2810 | -0.3730 |
| C | -14.9870 | 18.5100 | -0.4800 |
| N | -15.1500 | 17.4410 | 0.3030 |
| N | -15.9110 | 18.8310 | -1.3720 |
| C | -15.5740 | 15.2240 | -2.9790 |
| C | -17.2960 | 20.7170 | -4.5510 |
| C | -17.8620 | 20.3390 | -3.3410 |
| C | -17.4030 | 19.8440 | -5.6320 |
| C | -18.5280 | 19.1130 | -3.2160 |
| C | -18.0630 | 18.6360 | -5.5080 |

| | | | |
|---|----------|---------|---------|
| C | -18.6270 | 18.2720 | -4.2970 |
| C | -15.3190 | 21.9350 | 2.1930 |
| C | -16.5420 | 22.3660 | 3.0040 |
| O | -17.4848 | 21.5558 | 3.1994 |
| C | -20.6650 | 19.5450 | 3.2950 |
| C | -21.9780 | 19.4950 | 2.4900 |
| O | -23.0340 | 19.8070 | 3.0070 |
| C | -20.5470 | 18.3040 | 4.2150 |
| O | -21.1790 | 17.1530 | 3.6550 |
| N | -21.8760 | 19.1990 | 1.1950 |
| C | -23.0580 | 19.0830 | 0.3430 |
| C | -23.8830 | 17.8910 | 0.8040 |
| O | -25.0920 | 17.9910 | 0.9380 |
| N | -23.2260 | 16.7730 | 1.1040 |
| C | -23.9260 | 15.5860 | 1.5730 |
| C | -22.9420 | 14.4780 | 1.9510 |
| C | -22.5700 | 13.5680 | 0.8210 |
| C | -22.8740 | 12.2680 | 0.5940 |
| N | -21.7240 | 13.9430 | -0.2020 |
| C | -21.5200 | 12.9140 | -1.0030 |
| N | -22.2040 | 11.8860 | -0.5410 |
| C | -19.6600 | 13.3820 | 3.8000 |
| C | -19.5240 | 12.4620 | 2.6140 |
| N | -18.8650 | 13.1380 | 1.5040 |
| C | -18.5370 | 12.5610 | 0.3570 |
| N | -18.7960 | 11.2560 | 0.1400 |
| N | -18.0770 | 13.3120 | -0.6440 |
| C | -16.9840 | 17.2390 | 4.1000 |
| C | -17.6420 | 16.5610 | 3.1370 |
| N | -16.8630 | 18.5360 | 3.6490 |
| C | -17.4220 | 18.6340 | 2.4560 |
| N | -17.8990 | 17.4480 | 2.1240 |
| O | -17.8380 | 16.3900 | -0.3010 |
| O | -19.2760 | 18.4650 | -0.0560 |
| O | -19.9320 | 16.0970 | 1.6040 |
| O | -20.2280 | 16.3660 | -0.8770 |
| V | -19.0970 | 16.9670 | 0.5000 |
| O | -20.5650 | 15.8510 | 6.2810 |
| O | -19.5680 | 15.0930 | -3.2640 |
| O | -18.1320 | 14.1470 | -5.2050 |
| O | -22.3280 | 16.0810 | -2.4190 |
| H | -17.7337 | 9.7410 | -4.9087 |
| H | -17.4893 | 11.4013 | -5.3952 |

| | | | |
|---|----------|---------|---------|
| H | -21.1180 | 21.9516 | -0.3746 |
| H | -20.2664 | 21.0666 | -1.6175 |
| H | -19.4093 | 20.5559 | 0.5617 |
| H | -19.0554 | 22.1268 | 0.8335 |
| H | -18.2596 | 21.2997 | -0.3281 |
| H | -14.1486 | 20.2183 | -0.6300 |
| H | -14.4557 | 17.2099 | 0.9846 |
| H | -15.9664 | 16.8714 | 0.2079 |
| H | -16.7291 | 18.2645 | -1.4704 |
| H | -15.7908 | 19.6400 | -1.9474 |
| H | -16.3736 | 15.5511 | -2.3477 |
| H | -15.9700 | 14.6356 | -3.7802 |
| H | -17.7888 | 20.9906 | -2.4955 |
| H | -16.9678 | 20.1129 | -6.5718 |
| H | -18.9596 | 18.8318 | -2.2781 |
| H | -18.1383 | 17.9803 | -6.3502 |
| H | -19.1390 | 17.3371 | -4.2037 |
| H | -15.6396 | 21.4558 | 1.2916 |
| O | -16.6141 | 23.6897 | 3.5402 |
| H | -21.0920 | 18.5399 | 5.1051 |
| H | -19.5037 | 18.0732 | 4.2718 |
| H | -20.9491 | 16.3780 | 4.1727 |
| H | -19.8363 | 19.5632 | 2.6184 |
| H | -23.6515 | 19.9568 | 0.5139 |
| H | -22.7096 | 18.8574 | -0.6432 |
| H | -21.4764 | 18.2824 | 1.1846 |
| H | -23.4560 | 13.8637 | 2.6605 |
| H | -22.0377 | 14.9767 | 2.2311 |
| H | -23.5168 | 11.6533 | 1.1888 |
| H | -22.2303 | 10.9746 | -0.9516 |
| H | -20.9044 | 12.9141 | -1.8782 |
| H | -24.5727 | 15.2312 | 0.7979 |
| H | -22.6406 | 17.0317 | 1.8723 |
| H | -20.4061 | 12.9992 | 4.4646 |
| H | -19.9475 | 14.3565 | 3.4644 |
| H | -18.8714 | 11.6703 | 2.9175 |
| H | -20.5182 | 12.2400 | 2.2867 |
| H | -18.6444 | 14.1069 | 1.6158 |
| H | -19.2413 | 10.7099 | 0.8495 |
| H | -18.5428 | 10.8340 | -0.7305 |
| H | -17.9824 | 14.3004 | -0.5250 |
| H | -17.8265 | 12.8869 | -1.5138 |
| H | -17.9123 | 15.5260 | 3.1597 |

| | | | |
|---|----------|---------|---------|
| H | -16.4223 | 19.2837 | 4.1458 |
| H | -17.4786 | 19.5216 | 1.8611 |
| H | -20.5664 | 15.4677 | -0.8625 |
| H | -19.9648 | 16.6003 | 6.2810 |
| H | -20.0586 | 15.0354 | 6.2810 |
| H | -18.9678 | 15.8423 | -3.2640 |
| H | -19.0616 | 14.2774 | -3.2640 |
| H | -17.5318 | 14.8963 | -5.2050 |
| H | -17.6256 | 13.3314 | -5.2050 |
| H | -21.7278 | 16.8303 | -2.4190 |
| H | -21.8216 | 15.2654 | -2.4190 |
| H | -16.6268 | 16.8476 | 5.0296 |
| H | -13.5852 | 19.2668 | 0.5717 |
| H | -18.7230 | 13.4435 | 4.3130 |
| H | -19.1049 | 10.7391 | -5.3291 |
| H | -15.0648 | 16.0755 | -3.3797 |
| H | -14.8877 | 14.6338 | -2.4085 |
| H | -16.7905 | 21.6547 | -4.6511 |
| H | -19.8878 | 22.7474 | -1.3267 |
| H | -14.7310 | 22.7953 | 1.9499 |
| H | -14.7308 | 21.2524 | 2.7701 |
| H | -20.6515 | 20.4314 | 3.8941 |
| H | -24.5131 | 15.8410 | 2.4304 |
| H | -20.4669 | 16.9574 | -1.5944 |

Example of the vanadate coordinates for some of the models considered:

VOS4

| | | | |
|---|----------|---------|---------|
| O | -17.8380 | 16.3900 | -0.3010 |
| O | -19.2760 | 18.4650 | -0.0560 |
| O | -19.9320 | 16.0970 | 1.6040 |
| O | -20.2280 | 16.3660 | -0.8770 |
| V | -19.0970 | 16.9670 | 0.5000 |
| H | -20.5664 | 15.4677 | -0.8625 |

VOD14

| | | | |
|---|----------|---------|---------|
| O | -17.8380 | 16.3900 | -0.3010 |
| O | -19.2760 | 18.4650 | -0.0560 |
| O | -19.9320 | 16.0970 | 1.6040 |
| O | -20.2280 | 16.3660 | -0.8770 |
| V | -19.0970 | 16.9670 | 0.5000 |
| H | -20.4669 | 16.9574 | -1.5944 |
| H | -17.0277 | 16.6908 | 0.1167 |

| | | | |
|-------|-----------|---------|---------|
| VOD24 | | | |
| O | -17.8380 | 16.3900 | -0.3010 |
| O | -19.2760 | 18.4650 | -0.0560 |
| O | -19.9320 | 16.0970 | 1.6040 |
| O | -20.2280 | 16.3660 | -0.8770 |
| V | -19.0970 | 16.9670 | 0.5000 |
| H | -20.4669 | 16.9574 | -1.5944 |
| H | -18.41215 | 18.8576 | -0.2017 |

| | | | |
|-------|----------|---------|---------|
| VOD34 | | | |
| O | -17.8380 | 16.3900 | -0.3010 |
| O | -19.2760 | 18.4650 | -0.0560 |
| O | -19.9320 | 16.0970 | 1.6040 |
| O | -20.2280 | 16.3660 | -0.8770 |
| V | -19.0970 | 16.9670 | 0.5000 |
| H | -20.5664 | 15.4677 | -0.8625 |

| | | | |
|--------|----------|---------|---------|
| VOT124 | | | |
| O | -17.8380 | 16.3900 | -0.3010 |
| O | -19.2760 | 18.4650 | -0.0560 |
| O | -19.9320 | 16.0970 | 1.6040 |
| O | -20.2280 | 16.3660 | -0.8770 |
| V | -19.0970 | 16.9670 | 0.5000 |
| H | -18.4121 | 18.8576 | -0.2017 |
| H | -17.0277 | 16.6908 | 0.1167 |
| H | -20.5664 | 15.4677 | -0.8625 |

| | | | |
|--------|----------|---------|---------|
| VOT134 | | | |
| O | -17.8380 | 16.3900 | -0.3010 |
| O | -19.2760 | 18.4650 | -0.0560 |
| O | -19.9320 | 16.0970 | 1.6040 |
| O | -20.2280 | 16.3660 | -0.8770 |
| V | -19.0970 | 16.9670 | 0.5000 |
| H | -20.5664 | 15.4677 | -0.8625 |
| H | -17.027 | 16.6908 | 0.1167 |
| H | -19.3172 | 15.6405 | 2.1831 |

| | | | |
|--------|----------|---------|---------|
| VOT234 | | | |
| O | -17.8380 | 16.3900 | -0.3010 |
| O | -19.2760 | 18.4650 | -0.0560 |
| O | -19.9320 | 16.0970 | 1.6040 |
| O | -20.2280 | 16.3660 | -0.8770 |
| V | -19.0970 | 16.9670 | 0.5000 |
| H | -20.5664 | 15.4677 | -0.8625 |
| H | -19.3172 | 15.6405 | 2.1831 |

H -18.4121 18.8576 -0.2017

Complete list of all input coordinated used for the calculations are available upon request.

References

(1) (a) Pooransingh, N.; Pomerantseva, E.; Ebel, M.; Jantzen, S.; Rehder, D.; Polenova, T., *Inorg. Chem.* **2003**, 42, 1256. (b) Skibsted, J.; Nielsen, N. C.; Bildsøe, H.; Jakobsen, H. J., *Chem. Phys. Lett.* **1992**, 188, 405.

(2) Schurko, R. W., *Acc. Chem. Res.* **2013**, 46, 1985.

(3) Goncharova-Zapata, O.; Chatterjee, P. B.; Hou, G.; Quinn, L. L.; Li, M.; Yehl, J.; Crans, D. C.; Polenova, T., *CrystEngComm* **2013**, 15, 8776.