Supporting Information For:

Vanadyl as a Stable Structural Mimic of Reactive Ferryl Intermediates in Mononuclear Non-heme-iron Enzymes

Ryan J. Martinie[a], Christopher J. Pollock[a], Megan L. Matthews[c], J. Martin Bollinger, Jr.[a,b]* Carsten Krebs [a,b]*, and Alexey Silakov [a]*

[a]Department of Chemistry and [b]Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, Pennsylvania 16802, United States

[c]Department of Chemical Physiology and The Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037, United States

Supplementary Figures

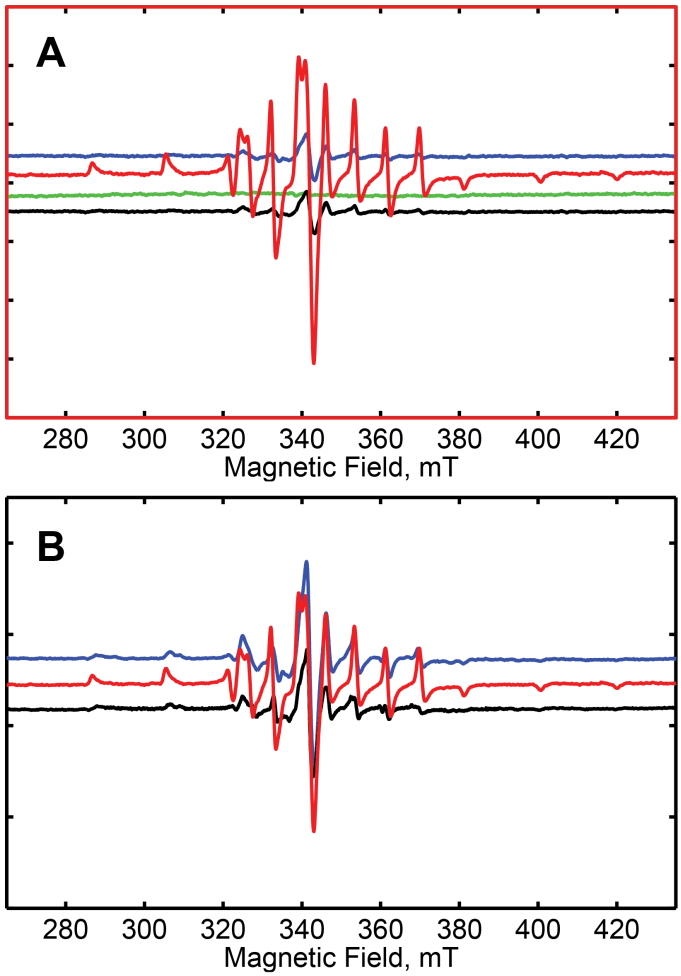

Figure S1. Comparison of continuous-wave (CW) EPR signals for solutions containing vanadyl. (**A**) Comparison of taurine only (green); succinate and taurine (blue); 2OG and taurine (black); or TauD, succinate, and taurine (red). Spectra are shifted vertically for clarity. (**B**) Comparison of TauD•vanadyl in the presence of succinate only (blue), 2OG only (black), or succinate and taurine (red). Concentration of species (if present): vanadyl sulfate (1.0 mM), succinate (3.0 mM), 2-oxo-glutarate (3.0 mM), TauD (1.6 mM) in 50 mM sodium HEPES pH 7.6. Spectra were recorded at 80 K with microwave frequency 9.625 GHz, microwave power 200 μW, 10 G modulation amplitude, and 40 ms time constant and conversion time.

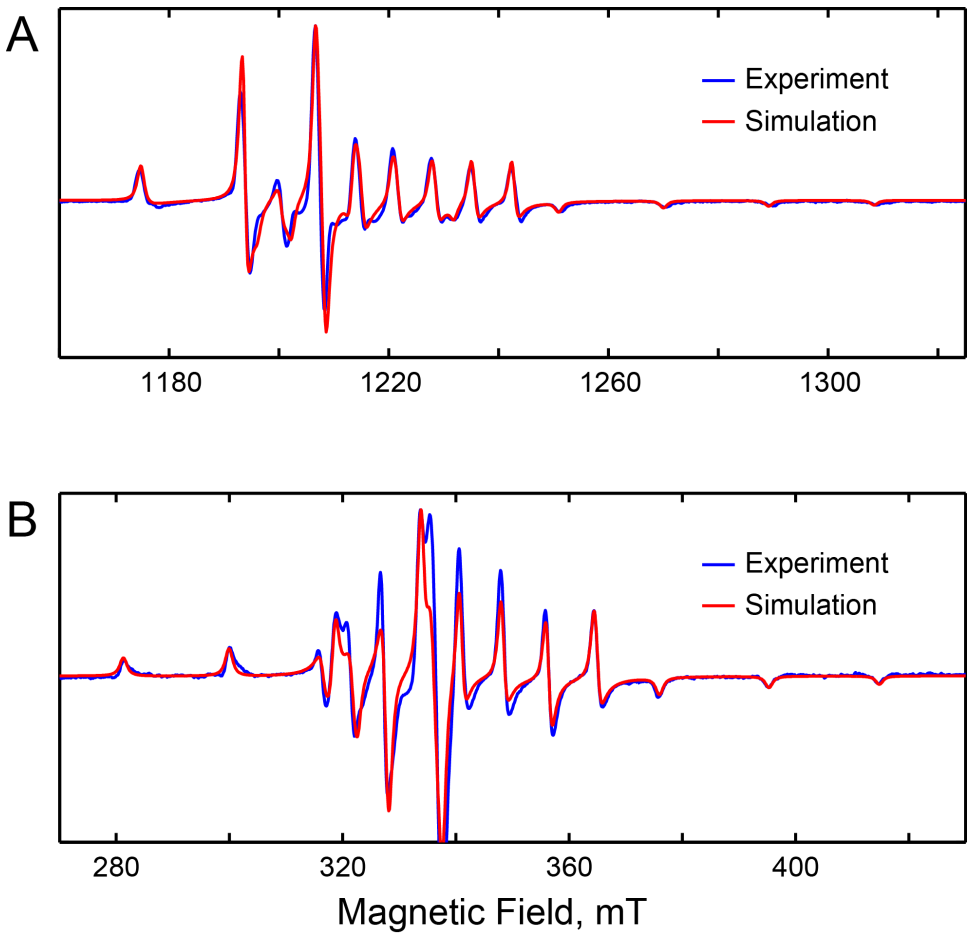

Figure S2. Experimental (blue) and simulated (red) electron paramagnetic resonance spectra of the TauD•($V^{IV}O$)•taurine•succinate complex, collected at Q-band (**A**) and X-band (**B**). The Q-band spectrum was collected as a two-pulse Hahn echo experiment with τ = 200 ns and 250 µs shot repetition time at 40 K, with the pseudo-modulated spectrum shown. X-band spectrum was acquired using continuous-wave excitation at 80 K. X- and Q-band spectra were acquired at 9.478 & 33.788 GHz, respectively. Sample composition: TauD (1.5 mM), vanadyl sulfate (1.0 mM), succinate (5.0 mM), and taurine (5.0 mM). Simulation parameters: g = [1.944, 1.979, 1.981] ± 0.001 and A_V = [519, 185, 192] ± 3 MHz.

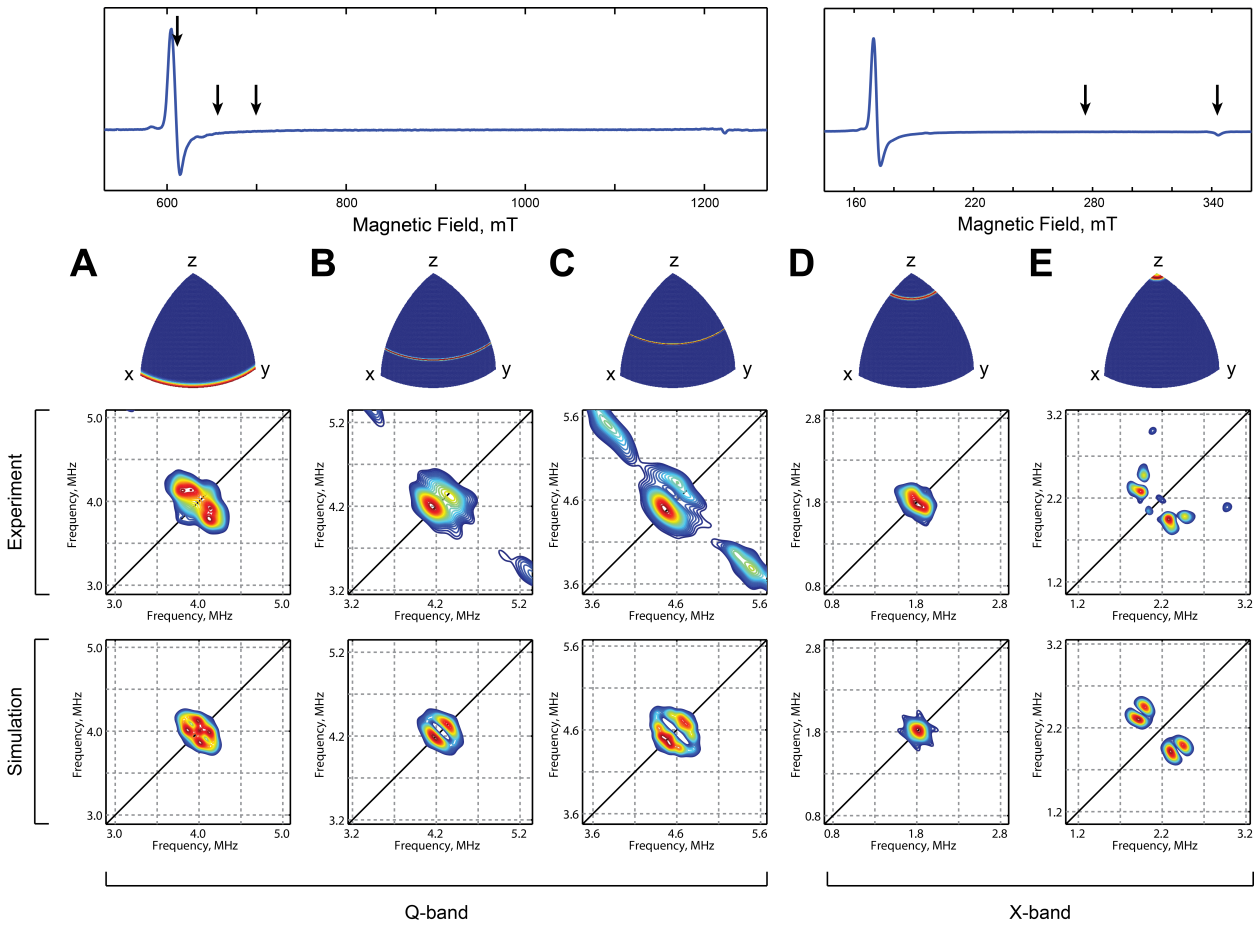

Figure S3. Field-dependent ^{2}H-HYSCORE spectra of the {Fe-NO}^{7} form of TauD in the presence of d_4-taurine and 2OG collected at Q-band (**A**-**C**) and X-band (**D**-**E**). Orientation selectivities probed by each magnetic field position (shown above the spectra) are color coded using an RGB color scheme (red - fully excited; blue, not excited). Magnetic field positions of 610 (**A**), 650 (**B**), 700 (**C**), 275 (**D**), and 329 (**E**) mT are indicated by black arrows on the one-dimensional EPR spectra (top). Spectra were collected with microwave frequency 34.276 (Q-band) and 9.308 GHz (X-band), temperature 4.0 K, $\pi/2$ pulse length of 8 ns, and τ of 140, 140, 140, 220, and 200 ns, respectively. Simulation parameters: hyperfine coupling [0.26, 0.44, -0.70] ± 0.05 MHz with Euler angles of [0, 30, 93] ± 10° and deuterium nuclear quadrupole coupling [-0.04, -0.04, 0.08] ± 0.03 MHz with Euler angles [0, 40, 25] ± 10°. Sample preparation is described in the Materials and Methods.

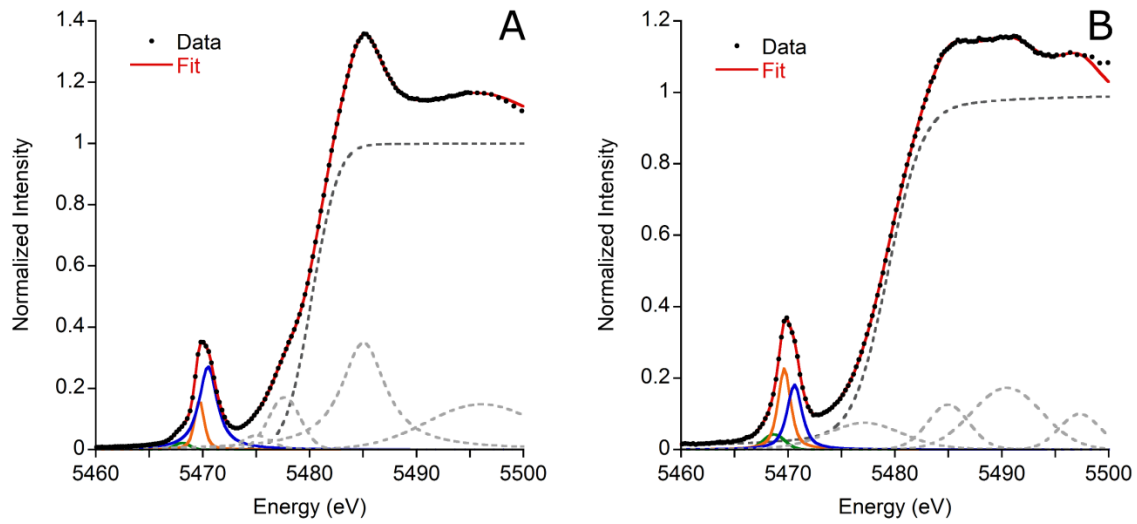

Figure S4. Representative fits of the XANES data (points) for $VO(H_2O)_5^{2+}$ (**A**) and the TauD•($V^{IV}O$)•taurine•succinate complex (**B**). Solid lines represent components of the pre-edge, whereas gray, dashed lines are background components.

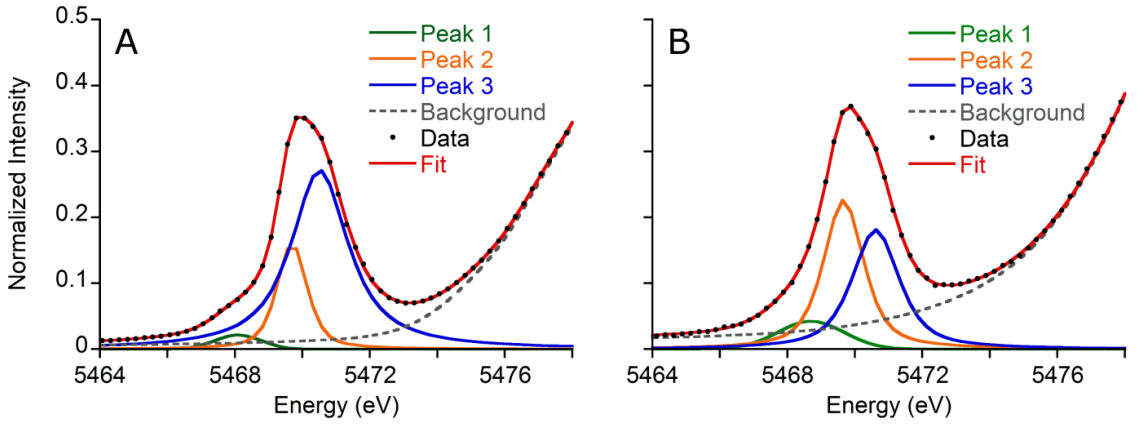

Figure S5. Representative fits of the pre-edge features in the XANES data for $VO(H_2O)_5^{2+}$ (**A**) and the TauD•($V^{IV}O$)•taurine•succinate complex (**B**). Solid, colored lines represent components that were included in calculations of the pre-edge areas.

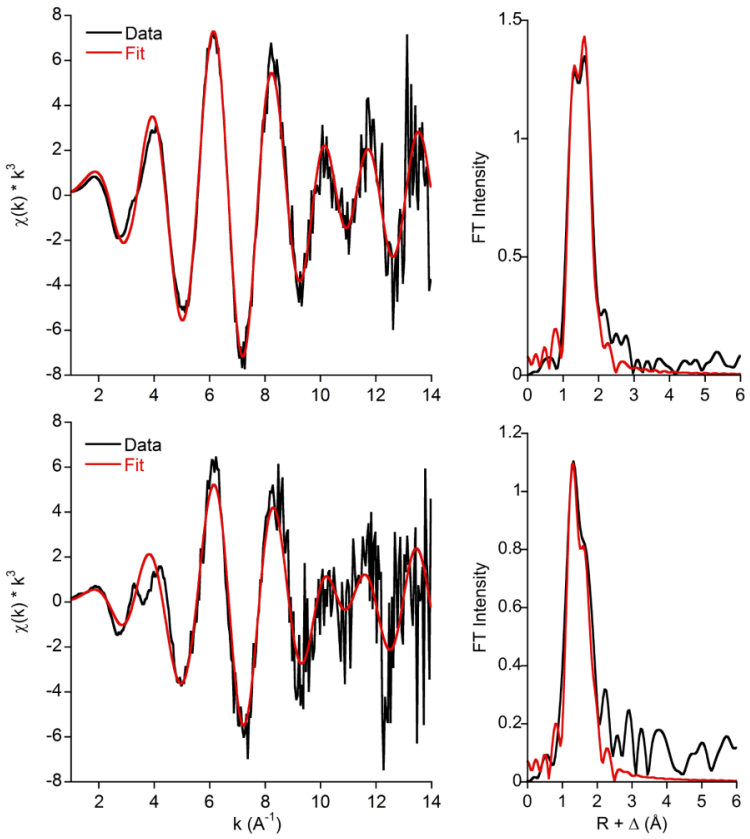

Figure S6. Fits to the k^3-weighted EXAFS data and Fourier transforms for $VO(H_2O)_5^{2+}$ (**top**) and the TauD•($V^{IV}O$)•taurine•succinate complex (**bottom**).

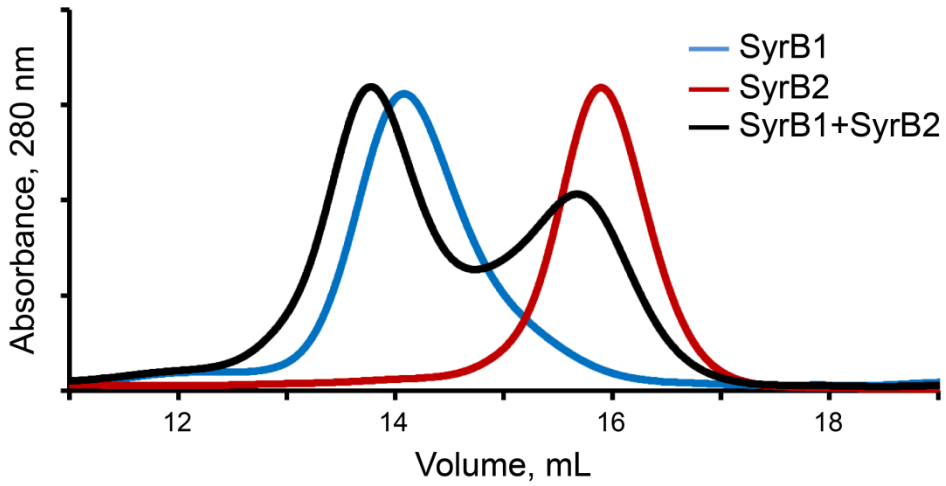

Figure S7. Elution chromatograms from size-exclusion chromatographic analysis of samples containing SyrB1 (**blue**), SyrB2 (**red**) or equi-molar quantities of both proteins (**black**). The shift of the mixed proteins relative to SyrB1 and SyrB2 alone is attributed to dynamic association during chromatography. SyrB2 (0.15 mM) and L-cyclopropylglycinyl-*S*-SyrB1 (0.15 mM) were applied to a GE Healthcare Superdex 200 10/300 GL column at 4 °C using a 100 µL sample loop and eluted at 0.7 mL/min with 1.2 column volumes of 50 mM sodium HEPES pH 7.6, 150 mM NaCl.

Supplementary Tables

Table S1: EXAFS fit parameters for $VO(H_2O)_5^{2+}$ (top) and the TauD•($V^{IV}O$)•taurine•succinate complex

$VO(H_2O)_5^{2+}$				TauD-VO			
Scatterer	**N**	**R (Å)**	**σ^2 (Å^2)**	**Scatterer**	**N**	**R (Å)**	**σ^2 (Å^2)**
V-N/O	1	1.59	0.00157	V-N/O	1	1.60	0.00136
V-N/O	4	2.02	0.00284	V-N/O	3	2.05	0.00246
F		0.3314		F		0.5716	
E_0		-0.14 eV		E_0		4.04 eV	

Table S2: EXAFS fitting results for $VO(H_2O)_5^{2+}$

			V=O			V-N/O			V-N/O		
χ^2	**F**	**E_0**	**N**	**R**	**σ^2**	**N**	**R**	**σ^2**	**N**	**R**	**σ^2**
7.4718	0.8803	3.97134	1	1.55	0.0058						
4.6009	0.6907	3.97131	2	1.58	0.00301						
9.2426	0.979	2.81078	3	1.57	0.01124						
5.1255	0.7291	-1.6467	1	2.02	-0.0027						
3.6942	0.619	-0.5603	2	2.03	-0.0003						
3.0418	0.5616	-3.0165	3	2.02	0.00146						
2.8068	0.5395	-4.091	4	2.01	0.00292						
2.8515	0.5438	-5.8326	5	2.00	0.00424						
1.6161	0.4079	-3.6211	1	1.59	0.00205	6	2.01	0.00573			
1.2644	0.3608	-1.9091	1	1.59	0.00179	5	2.02	0.00429			
1.0671	0.3314	-0.141	1	1.59	0.00157	4	2.02	0.00284			
1.13	0.3411	1.56566	1	1.60	0.00142	3	2.03	0.0013			
1.0402	0.326	0.11454	1	1.59	0.00157	4	2.02	0.00283	1	2.45	0.02339
1.181	0.3474	-1.518	1	1.59	0.00177	5	2.02	0.00426	1	2.38	0.01299

Table S3: EXAFS fitting results for the TauD•($V^{IV}O$)•taurine•succinate complex

			V=O			V-N/O			V-N/O		
χ^2	F	E_0	N	R	σ^2	N	R	σ^2	N	R	σ^2
5.4733	0.8579	3.51693	1	1.59	0.00179						
5.8301	0.8854	4.03515	2	1.60	0.00556						
6.7101	0.9499	-16.408	3	1.54	0.00882						
5.6113	0.8686	4.03522	1	2.05	0.00109						
4.635	0.7895	2.31054	2	2.05	0.00087						
4.2853	0.7591	0.03089	3	2.04	0.00266						
4.1533	0.7473	-2.9326	4	2.02	0.00417						
4.1585	0.7478	-5.6757	5	2.01	0.00548						
4.2502	0.7560	-9.2176	6	1.99	0.00664						
2.6946	0.5997	4.0352	1	1.60	0.00121	2	2.04	0.00064			
2.4478	0.5716	4.03512	1	1.60	0.00136	3	2.05	0.00246			
2.4883	0.5763	1.45792	1	1.60	0.00150	4	2.03	0.00415			
2.6389	0.5935	-0.3822	1	1.59	0.00170	5	2.03	0.00574			
2.8534	0.6171	-2.4176	1	1.59	0.00175	6	2.02	0.00734			
2.8588	0.6177	4.03522	2	1.61	0.00629	3	2.04	0.0029			
2.709	0.6013	4.03518	2	1.61	0.00610	4	2.03	0.00519			
2.7346	0.6041	2.79102	2	1.61	0.00624	5	2.03	0.0069			
2.4007	0.5639	3.55141	1	1.60	0.00137	3	2.04	0.00248	1	4.10	-0.0016
2.4951	0.5633	4.03517	1	1.60	0.00152	3	2.05	0.00254	2	2.56	0.01735
2.2906	0.5509	4.03489	1	1.60	0.00139	3	2.04	0.00254	1	2.61	-0.0009
2.4549	0.5703	4.03511	1	1.60	0.00150	3	2.04	0.00259	2	2.61	0.00371
2.4176	0.5659	4.03504	1	1.60	0.00156	3	2.05	0.00254	3	2.56	0.02223
2.3226	0.5547	2.24498	1	1.60	0.00144	4	2.04	0.0042	1	2.61	-0.0007
2.3775	0.5612	3.21365	1	1.60	0.00151	4	2.04	0.00417	2	2.50	0.02194
2.4408	0.5686	-4.875	1	1.58	0.00162	5	2.01	0.00627	1	2.34	0