

Supporting Information

Vibrational Probes of Molybdenum Cofactor–Protein Interactions in Xanthine Dehydrogenase.

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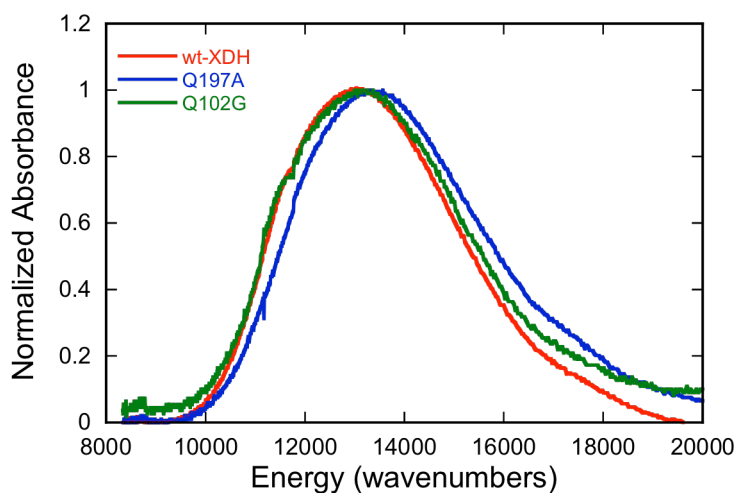


Figure S1. Normalized electronic absorption spectra detailing the NIR MLCT band for $w_{\text{red-2,4-TV}}$ (red), $Q197A_{\text{red-2,4-TV}}$ (blue), and $Q102G_{\text{red-2,4-thioviolatperin}}$ ($Q102G_{\text{red-4-TV}}$) (green).

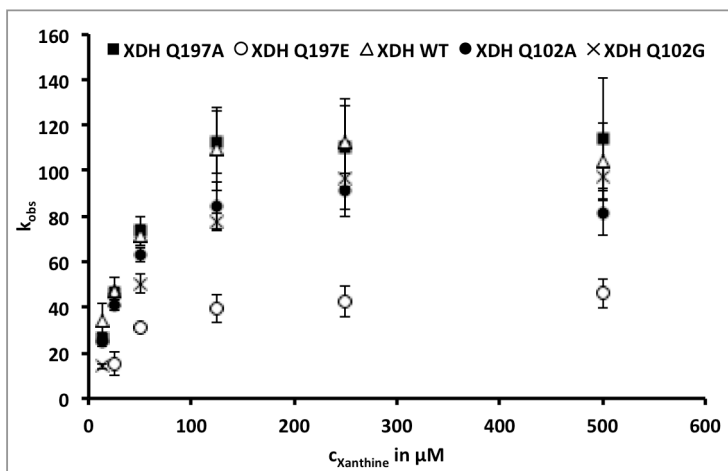


Figure S2. Kinetic data for obtaining k_{red} , and K_{D} .

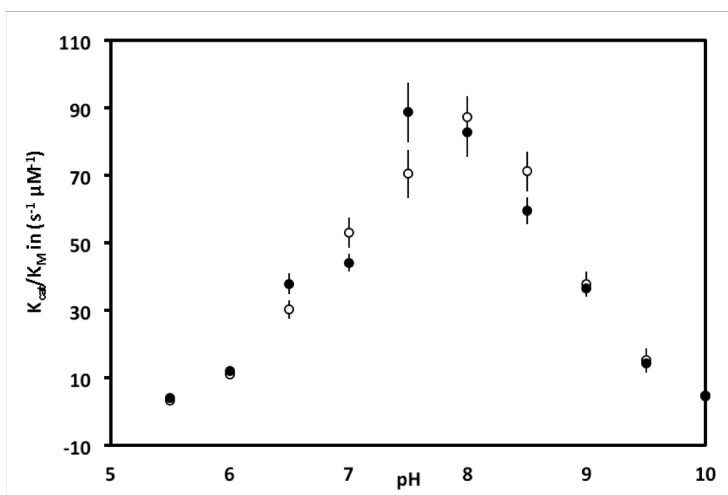


Figure S3. **pH dependent comparison of $k_{\text{cat}}/K_{\text{M}}$ for wild type XDH (open circles) and XDH Q197A (closed circles).** Assays were conducted at 25°C in 1 ml cuvettes in 100 mM CAPS, 100 mM KCl (pH 9-10); 50 mM Tris, 100 mM NaCl (pH 7-9) and 100 mM MES, 100 mM KCl (pH 5.5-6.5) using 500 μM NAD^+ and varying concentrations of xanthine (25, 50, 75, 100, 200, 500 μM). The reaction was followed 1 min at 340 nm with a Shimadzu UV-2401PC UV-VIS recording spectrophotometer.

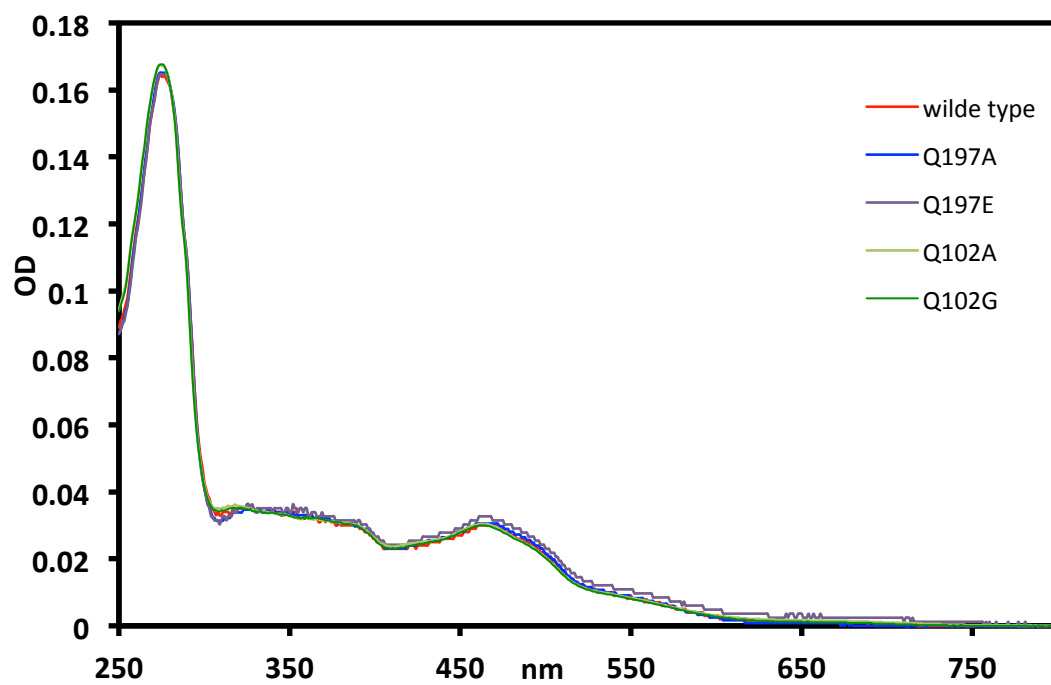
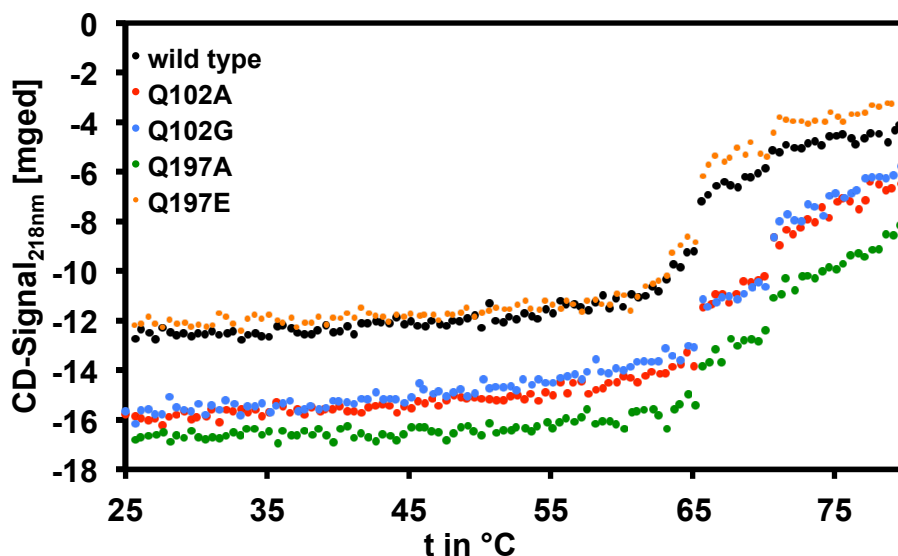


Figure S4: **UV-Vis spectra of wild type XDH and variants.** Spectra of wild type (red), Q197A (blue), Q197E (purple), Q102A (light green) and Q102G (green) were recorded in the range of 250-800 nm and normalized to wild type at 280 nm.

Figure S5. CD-Spectroscopy:



S5. CD-Spectroscopy: Thermal denaturation curves of XDH wild type (black), Q102A (red), Q102G (blue), Q197A (green) and Q197E (orange) were recorded at 218 nm at temperatures ranging from 25°C to 80°C. Signals were recorded using a 0.1 cm cuvette at 1°C/min and a data integration time of 8 sec using a Jasco (J-815) CD-Spectropolarimeter. Protein concentration was adjusted to 1 μM ($\epsilon_{465} = 31600 \text{ M}^{-1}\text{cm}^{-1}$).

Table S1. Metal content analysis for *wt*, Q102 variants, and Q197 variants.

Protein	Mo	Fe
	Saturation in %	Saturation in %
<i>wt</i>	68.28 \pm 7.15	93.04 \pm 1.84
Q197A	72.14 \pm 6.72	99.62 \pm 2.70
Q197E	49.82 \pm 7.21	90.53 \pm 4.03
Q102A	67.76 \pm 2.93	105.84 \pm 1.98
Q102G	69.77 \pm 2.33	95.95 \pm 1.71

Table S1. **Metal content analysis.** Metal content was analyzed using an Optima 2100DV inductively coupled plasma-optical emission spectrometer. Protein samples were incubated overnight in a 1:1 mixture with 65% nitric acid at 100 °C. This mixture was diluted with a 4-fold volume of ultrapure water prior to ICP-OES analysis. The multielement standard solution XVI (Merck) was used as a reference (1). The percent saturation is related to a 100% saturation with molybdenum and two [2Fe2S] clusters (4 iron atoms in total).

Cartesian (x,y,z) Coordinates for Mo(IV)-4-TV

N	7.13039500	0.30806500	1.28096500
C	8.33056900	-0.36085400	1.12224000
N	8.29954600	-1.35416300	0.14931000
C	7.20731500	-1.74717700	-0.67033600
C	6.00203600	-1.00705100	-0.44579400
C	3.76433100	-0.53177100	-0.89814100
N	4.83602100	-1.26305500	-1.15952500
C	3.73999900	0.53945000	0.10159500
C	5.96466700	0.03736500	0.54116300
N	4.90084900	0.79013400	0.82290100
C	-2.63742900	-1.58476900	2.80598400
C	-2.27979700	-1.76604000	1.33798000
O	-3.58598000	-2.16620200	0.71764100
C	-1.61201000	-0.59367300	0.64512500
S	0.13659700	-0.29618300	1.11087800
C	-2.21626100	0.14041900	-0.31843900
S	-1.36804100	1.50683500	-1.19283100
N	-7.48782000	-1.88259100	-0.72499000
C	-8.51687100	-1.13151700	-0.38729000
N	-9.82078900	-1.63549500	-0.48055700
N	-8.36211900	0.17315800	0.03398300
C	-7.09396400	0.82597300	0.14557400
O	-7.05123600	2.02624700	0.56065700
N	-4.68528200	0.49833600	-0.20310400
C	-3.58424900	-0.23243900	-0.85249400
C	-3.80384700	-1.75823600	-0.67236900
N	-5.15710400	-2.12862300	-1.02954400
C	-5.97825000	0.00288200	-0.24665100
C	-6.22358200	-1.31537100	-0.66046000
O	2.68057600	1.23480600	0.33508000
O	0.47695000	3.13400300	1.09375100
Mo	0.75364100	1.82852400	0.01195400
S	1.62639400	2.64526000	-2.16881100
C	-2.28452000	-0.56169700	3.60685400
H	-1.66212600	0.25297300	3.24804100
H	-2.60312300	-0.53429500	4.64868800
H	-3.25446500	-2.39997200	3.19219700
H	-4.50714400	1.40633400	0.20641500
H	-5.37196500	-3.10992200	-1.15924500
H	-9.16009200	0.76778700	0.23431800
H	-9.87794400	-2.59384800	-0.79797800
H	-10.53307800	-1.31183400	0.16018600
H	7.07686400	1.04799100	1.97292400
H	9.16435800	-1.86505300	0.00575800
H	2.98245100	2.66951800	-1.88593800
H	2.85442800	-0.73180100	-1.45631600
H	-3.09830700	-2.29073300	-1.32695000
H	-3.59038400	-0.03387100	-1.93793700
H	-1.61325700	-2.64260500	1.24941800
S	7.49276500	-3.02781200	-1.78902200
O	9.36628100	-0.09780700	1.79632200

Cartesian (x,y,z) Coordinates for Mo(IV)-2,4-TV

N	6.90452200	0.27719500	1.08462800
C	8.07935500	-0.40228000	0.90115400
N	8.02719400	-1.38359600	-0.05917700
C	6.91564000	-1.76986400	-0.86493800
C	5.72275400	-1.01907400	-0.61838400
C	3.48293200	-0.51819800	-1.02290500
N	4.54028800	-1.26238100	-1.30866200
C	3.49100300	0.55269800	-0.02287800
C	5.71634400	0.02471700	0.36905900
N	4.67044300	0.79052200	0.67481300
C	-2.81588000	-1.55864200	2.81929500
C	-2.47977400	-1.74748000	1.34722300
O	-3.79188600	-2.16105000	0.74922500
C	-1.83106400	-0.57508300	0.63664400
S	-0.07628500	-0.26590200	1.06836500
C	-2.45560300	0.15006200	-0.32033900
S	-1.62849700	1.51266700	-1.21988900
N	-7.72115700	-1.90479500	-0.62409000
C	-8.74825500	-1.15884100	-0.26929300
N	-10.05055800	-1.66992600	-0.33918000
N	-8.59332400	0.14727100	0.14766800
C	-7.32710700	0.80720900	0.23568600
O	-7.28304000	2.00801400	0.64881400
N	-4.92362100	0.49359000	-0.15808600
C	-3.83128800	-0.23202600	-0.82697600
C	-4.03779900	-1.75889100	-0.63821600
N	-5.39479800	-2.13822100	-0.96954800
C	-6.21424300	-0.01012100	-0.17602700
C	-6.45927100	-1.33037700	-0.58282900
O	2.44424500	1.25927700	0.23240000
O	0.25822400	3.16421400	1.01636100
Mo	0.51489400	1.84991800	-0.05913800
S	1.34401600	2.64628400	-2.26262100
C	-2.46212900	-0.52511000	3.60619800
H	-1.85491700	0.29377000	3.23132900
H	-2.76418800	-0.49282300	4.65273000
H	-3.41727800	-2.37754800	3.22178000
H	-4.74292300	1.40148000	0.25044500
H	-5.60617100	-3.12095200	-1.09400500
H	-9.39100900	0.73758600	0.36149300
H	-10.10865200	-2.62860100	-0.65540800
H	-10.75411400	-1.34865600	0.31228000
H	6.87150700	1.01701500	1.77864000
H	8.88186400	-1.90666100	-0.22157900
H	2.70582500	2.65701000	-2.00886400
H	2.55921100	-0.70780100	-1.56171600
H	-3.34097400	-2.28926100	-1.30372200
H	-3.85967200	-0.03691700	-1.91264100
H	-1.80817800	-2.61962100	1.25422500
S	7.17764300	-3.05161700	-1.98524100
S	9.51419700	-0.05756100	1.80132300