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**Supporting information for article:**

# Cholesterol oxidase: ultrahigh-resolution crystal structure and multipolar atom model-based analysis

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# Supplementary Materials.

**Cholesterol oxidase: ultra high resolution crystal structure and multipolar atom model based analysis.**

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**Table S1.** Topological properties at the CPs of all the interactions between the protein and FAD ligand. $d_{12}$ is the distance between the two atoms and $d_{1CP}$ and $d_{2CP}$ are the distances from the atoms to the critical point, $\rho(r_b)$ is the electron density and $\nabla^2\rho(r_b)$ is the Laplacian at the bond CP, $\lambda_1$, $\lambda_2$ and $\lambda_3$ are the Hessian eigenvalues and $\varepsilon$ is the bond ellipticity. $G^{CP}$, $V^{CP}$ and $E^{CP}$ are the kinetic, potential and total electronic energies (Abramov, 1997) at critical point, respectively; $D_e$ is the dissociation energy. The strongest hydrogen bonds ($D_e \geq 20$ kJ/mol) are highlighted in bold.

| Residue # | Protein Atom | FAD Atom | $d_{12}$ | $d_{1CP}$ (Å) | $d_{2CP}$ | $\rho(\mathbf{r})$ (e·Å$^{-3}$) | $\nabla^2\rho(\mathbf{r})$ (e·Å$^{-5}$) | $\lambda_1$ | $\lambda_3$ (e·Å$^{-5}$) | $\lambda_3$ | $\varepsilon$ | $G^{CP}$ (kJ | $V^{CP}$ / mol/ | $E^{CP}$ bohr$^{-3}$) | $D_e$ (kJ/mol) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 19 | HA3 | O5B | 2.6567 | 1.1261 | 1.5468 | 0.0429 | 0.53 | -0.15 | -0.09 | 0.77 | 0.41 | 11.2 | -18.4 | -7.1 | 9.2 |
| 114 | HA3 | O5B | 2.927 | 1.274 | 1.6536 | 0.0219 | 0.30 | -0.07 | -0.06 | 0.42 | 0.20 | 6.0 | -9.8 | -3.9 | 4.9 |
| 20 | HD1 | O5' | 2.6016 | 1.1019 | 1.5007 | 0.0472 | 0.59 | -0.16 | -0.16 | 0.9 | 0.00 | 12.6 | -20.7 | -8.1 | 10.4 |
| 289 | HA | O4B | 2.4995 | 1.0457 | 1.4548 | 0.0582 | 0.73 | -0.22 | -0.21 | 1.17 | 0.04 | 15.9 | -26.3 | -10.3 | 13.2 |
| 17 | CA | O4B | 3.3475 | 1.7875 | 1.5645 | 0.0316 | 0.56 | -0.10 | -0.04 | 0.70 | 0.56 | 11.1 | -19.2 | -8.1 | 9.6 |
| 20 | HE1 | O4' | 2.7406 | 1.1749 | 1.5671 | 0.0330 | 0.56 | -0.11 | -0.07 | 0.74 | 0.32 | 11.2 | -19.3 | -8.0 | 9.6 |
| 122 | H | O4 | 2.1005 | 0.8261 | 1.2756 | 0.1419 | 1.36 | -0.68 | -0.67 | 2.71 | 0.02 | 36.8 | -59.7 | -23.0 | 29.9 |
| 122 | HB2 | O4 | 2.5469 | 1.0784 | 1.4688 | 0.0528 | 0.83 | -0.17 | -0.13 | 1.13 | 0.25 | 17.4 | -29.7 | -12.3 | 14.8 |
| 114 | HA2 | O3B | 2.3377 | 0.9663 | 1.3735 | 0.0818 | 1.33 | -0.32 | -0.25 | 1.9 | 0.20 | 28.9 | -50.0 | -21.0 | 25.0 |
| 111 | O | O3B | 2.9943 | 1.504 | 1.4906 | 0.0402 | 0.70 | -0.11 | -0.11 | 0.92 | 0.03 | 14.2 | -24.5 | -10.3 | 12.2 |
| 111 | H | O3B | 2.8356 | 1.2482 | 1.5915 | 0.0312 | 0.37 | -0.09 | -0.09 | 0.55 | 0.03 | 7.7 | -12.3 | -4.7 | 6.2 |
| 486 | HG2 | O3' | 2.5254 | 1.0685 | 1.4572 | 0.0542 | 0.83 | -0.2 | -0.16 | 1.19 | 0.22 | 17.5 | -29.7 | -12.2 | 14.9 |
| 475 | H | O2P | 1.9967 | 0.7717 | 1.2257 | 0.1777 | 1.51 | -0.91 | -0.9 | 3.31 | 0.01 | 45.0 | -72.7 | -27.7 | 36.3 |
| 474 | HB2 | O2P | 2.3726 | 0.9716 | 1.4013 | 0.0849 | 0.98 | -0.33 | -0.28 | 1.58 | 0.15 | 22.9 | -37.6 | -14.7 | 18.8 |
| 290 | HA2 | O2P | 2.634 | 1.1231 | 1.5119 | 0.0442 | 0.50 | -0.16 | -0.15 | 0.81 | 0.05 | 10.8 | -17.3 | -6.5 | 8.7 |
| 490 | HD13 | O2P | 3.1559 | 1.4054 | 1.7625 | 0.0167 | 0.19 | -0.04 | -0.03 | 0.26 | 0.21 | 3.8 | -6.0 | -2.2 | 3.0 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 110 | HD3 | O2B | 2.5926 | 1.0897 | 1.5039 | 0.0461 | 0.66 | -0.16 | -0.15 | 0.96 | 0.09 | 13.8 | -23.2 | -9.4 | 11.6 |
| 110 | HA | O2B | 2.6821 | 1.1448 | 1.539 | 0.0408 | 0.47 | -0.14 | -0.14 | 0.76 | 0.03 | 10.0 | -16.1 | -6.1 | 8.1 |
| 41 | HG2 | O2B | 2.7041 | 1.1564 | 1.5483 | 0.0383 | 0.61 | -0.12 | -0.10 | 0.82 | 0.15 | 12.4 | -21.2 | -8.7 | 10.6 |
| 110 | HH11 | O2B | 2.8352 | 1.2084 | 1.6319 | 0.0309 | 0.36 | -0.09 | -0.05 | 0.50 | 0.49 | 7.5 | -12.0 | -4.5 | 6.0 |
| 119 | HD22 | O2' | 2.2021 | 0.8597 | 1.3429 | 0.1083 | 1.06 | -0.49 | -0.49 | 2.05 | 0.01 | 26.9 | -43.4 | -16.4 | 21.7 |
| 119 | HB3 | O2' | 2.8083 | 1.2144 | 1.5958 | 0.0292 | 0.47 | -0.08 | -0.06 | 0.61 | 0.31 | 9.4 | -16.0 | -6.6 | 8.0 |
| 487 | H | O2 | 1.9015 | 0.7071 | 1.1947 | 0.2255 | 1.84 | -1.26 | -1.25 | 4.34 | 0 | 59.5 | -97.2 | -37.6 | 48.6 |
| 485 | HB3b | O2 | 2.3422 | 0.9261 | 1.4261 | 0.0731 | 1.01 | -0.26 | -0.18 | 1.44 | 0.31 | 22.3 | -37.6 | -15.2 | 18.8 |
| 21 | H | O1P | 2.3254 | 0.9621 | 1.3654 | 0.0866 | 0.86 | -0.37 | -0.36 | 1.6 | 0.03 | 20.9 | -33.4 | -12.5 | 16.7 |
| 20 | H | O1P | 2.5212 | 1.1571 | 1.4214 | 0.0602 | 1.01 | -0.2 | -0.13 | 1.35 | 0.35 | 21.2 | -36.6 | -15.4 | 18.3 |
| 115 | H | O1A | 1.9918 | 0.7648 | 1.2284 | 0.1837 | 1.54 | -0.94 | -0.92 | 3.4 | 0.02 | 46.5 | -75.2 | -28.7 | 37.6 |
| 40 | OE1 | HO3A | 1.8408 | 1.1855 | 0.6577 | 0.2501 | 1.27 | -1.56 | -1.52 | 4.36 | 0.03 | 54.1 | -83.9 | -29.8 | 42.0 |
| 40 | OE2 | HO2A | 1.7604 | 1.1537 | 0.6076 | 0.3002 | 1.21 | -2.03 | -2.02 | 5.26 | 0 | 64.1 | -98.9 | -34.9 | 49.5 |
| 122 | O | HN3 | 1.9363 | 1.2283 | 0.711 | 0.1787 | 1.96 | -0.99 | -0.81 | 3.76 | 0.18 | 53.3 | -89.3 | -35.9 | 44.6 |
| 107 | Ob | HM83 | 2.4184 | 1.3772 | 1.0455 | 0.0542 | 0.92 | -0.2 | -0.12 | 1.24 | 0.37 | 19.1 | -33.0 | -13.9 | 16.5 |
| 118 | O | HM71 | 3.1128 | 1.7457 | 1.3775 | 0.0133 | 0.19 | -0.04 | -0.02 | 0.25 | 0.43 | 3.7 | -6.1 | -2.4 | 3.0 |
| 446 | O | H9 | 2.373 | 1.4032 | 0.9707 | 0.0655 | 1.09 | -0.24 | -0.18 | 1.51 | 0.26 | 23.1 | -39.9 | -16.8 | 19.9 |
| 289 | O | H8A | 2.5868 | 1.486 | 1.1358 | 0.0445 | 0.75 | -0.16 | -0.10 | 1.01 | 0.36 | 15.4 | -26.4 | -11.0 | 13.2 |
| 250 | O | H61A | 2.0592 | 1.2953 | 0.7662 | 0.126 | 1.42 | -0.64 | -0.56 | 2.63 | 0.12 | 35.7 | -59.1 | -23.4 | 29.6 |
| 288 | O | H52A | 2.3955 | 1.4072 | 0.9911 | 0.0693 | 0.88 | -0.29 | -0.26 | 1.43 | 0.09 | 19.6 | -32.5 | -12.9 | 16.3 |
| 109 | O | H3B | 2.7331 | 1.558 | 1.1756 | 0.0297 | 0.48 | -0.1 | -0.07 | 0.65 | 0.3 | 9.6 | -16.3 | -6.7 | 8.2 |
| 39 | O | H2A | 2.6493 | 1.5264 | 1.1297 | 0.0372 | 0.64 | -0.13 | -0.07 | 0.84 | 0.42 | 12.9 | -22.2 | -9.3 | 11.1 |
| 248 | O | H2A | 2.6571 | 1.5371 | 1.1231 | 0.0339 | 0.57 | -0.12 | -0.08 | 0.76 | 0.36 | 11.5 | -19.6 | -8.2 | 9.8 |
| 446 | O | H1'2 | 2.3922 | 1.4113 | 0.9832 | 0.0649 | 0.87 | -0.25 | -0.22 | 1.35 | 0.11 | 19.1 | -31.8 | -12.8 | 15.9 |
| 40 | OE2 | H1B | 2.4429 | 1.4415 | 1.0041 | 0.0659 | 0.87 | -0.25 | -0.21 | 1.33 | 0.16 | 19.2 | -31.9 | -12.8 | 16.0 |
| 289 | HB1 | N7A | 2.4965 | 1.0156 | 1.4833 | 0.0718 | 0.81 | -0.22 | -0.21 | 1.24 | 0.02 | 18.6 | -30.2 | -11.6 | 15.1 |
| 297 | HD11 | N6A | 3.0914 | 1.3247 | 1.7754 | 0.0202 | 0.24 | -0.06 | -0.05 | 0.35 | 0.09 | 4.8 | -7.7 | -2.9 | 3.8 |
| 119 | HB2 | N5 | 2.3854 | 0.9574 | 1.4364 | 0.0993 | 1.08 | -0.3 | -0.15 | 1.53 | 0.51 | 26.3 | -42.9 | -16.6 | 21.4 |
| 41 | H | N3A | 2.4543 | 0.9873 | 1.4712 | 0.0744 | 0.81 | -0.29 | -0.25 | 1.34 | 0.14 | 18.8 | -30.4 | -11.6 | 15.2 |
| 40 | HA | N3A | 3.0029 | 1.2734 | 1.7343 | 0.023 | 0.31 | -0.07 | -0.04 | 0.42 | 0.36 | 6.2 | -10.2 | -4.0 | 5.1 |
| 250 | H | N1A | 2.245 | 0.8612 | 1.3857 | 0.1254 | 1.13 | -0.6 | -0.54 | 2.26 | 0.1 | 30.3 | -48.5 | -18.2 | 24.3 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 249 | HA | N1A | 2.72 | 1.1258 | 1.6007 | 0.0436 | 0.52 | -0.15 | -0.09 | 0.76 | 0.38 | 11.1 | -18.0 | -6.9 | 9.0 |
| 16 | HG23 | N1A | 3.1157 | 1.594 | 1.8103 | 0.0216 | 0.34 | -0.04 | -0.03 | 0.41 | 0.12 | 6.7 | -11.3 | -4.6 | 5.7 |
| 447 | HD2 | N10 | 2.9061 | 1.2106 | 1.7077 | 0.0319 | 0.48 | -0.06 | -0.04 | 0.58 | 0.33 | 9.7 | -16.3 | -6.6 | 8.2 |
| 486 | HD2 | N1 | 2.6333 | 1.0812 | 1.555 | 0.0506 | 0.6 | -0.16 | -0.15 | 0.92 | 0.08 | 13.1 | -21.2 | -8.1 | 10.6 |
| 19 | N | H4B | 2.9116 | 1.7231 | 1.2054 | 0.0342 | 0.41 | -0.08 | -0.08 | 0.57 | 0.04 | 8.6 | -13.8 | -5.3 | 6.9 |
| 490 | HD13 | H5'1 | 2.3894 | 1.2756 | 1.1344 | 0.0338 | 0.56 | -0.1 | -0.09 | 0.75 | 0.13 | 11.3 | -19.3 | -8.0 | 9.6 |
| 18 | H | H4B | 2.1199 | 1.0714 | 1.0519 | 0.0593 | 0.48 | -0.25 | -0.23 | 0.96 | 0.1 | 11.5 | -17.3 | -5.8 | 8.7 |
| 487 | HB3 | H2' | 2.5571 | 1.2858 | 1.2747 | 0.0266 | 0.21 | -0.09 | -0.09 | 0.39 | 0.07 | 4.5 | -6.5 | -2.0 | 3.3 |
| 486 | HG2 | H1'1 | 2.5145 | 1.3263 | 1.2122 | 0.0256 | 0.34 | -0.07 | -0.06 | 0.48 | 0.16 | 6.9 | -11.3 | -4.4 | 5.6 |
| 446 | CE2 | HM72 | 2.8035 | 1.6823 | 1.1242 | 0.042 | 0.45 | -0.11 | -0.07 | 0.64 | 0.4 | 9.8 | -15.4 | -5.7 | 7.7 |
| 119 | HA | C6 | 2.7208 | 1.1066 | 1.6152 | 0.0491 | 0.52 | -0.14 | -0.09 | 0.75 | 0.32 | 11.5 | -18.2 | -6.7 | 9.1 |
| 41 | HB3 | C2A | 3.0248 | 1.2827 | 1.7496 | 0.0252 | 0.29 | -0.05 | -0.04 | 0.37 | 0.13 | 5.9 | -9.4 | -3.5 | 4.7 |
| 446 | HB3 | C9 | 2.912 | 1.1567 | 1.8171 | 0.0451 | 0.44 | -0.12 | -0.06 | 0.62 | 0.52 | 9.8 | -15.2 | -5.4 | 7.6 |
| 110 | NH1 | C8A | 3.4899 | 1.6851 | 1.8464 | 0.0244 | 0.41 | -0.06 | -0.04 | 0.5 | 0.32 | 8.1 | -13.8 | -5.7 | 6.9 |
| 41 | SD | C5A | 3.4467 | 1.8086 | 1.6557 | 0.0487 | 0.5 | -0.08 | -0.07 | 0.64 | 0.15 | 11.1 | -17.5 | -6.4 | 8.7 |
| Residue # | Protein Atom | FAD Atom | $d_{12}$ | $d_{1CP}$ (Å) | $d_{2CP}$ | $\rho(\mathbf{r})$ (e·Å$^{-3}$) | $\nabla^2\rho(\mathbf{r})$ (e·Å$^{-5}$) | $\lambda_1$ | $\lambda_3$ (e·Å$^{-5}$) | $\lambda_3$ | ε | $G_{CP}$ (kJ/ | $V_{CP}$ mol/ | $E_{CP}$ bohr$^3$) | $De$ (kJ/mol) |

**Table S2**

| Resolution | Compl | Nwork | Nfree | R_work | <Fobs> | <Fmodel> |
|---|---|---|---|---|---|---|
| 44.477-12.721 | 80.65 | 93 | 7 | 0.3288 | 13.517 | 12.515 |
| 12.667-10.195 | 86.21 | 96 | 4 | 0.2517 | 14.142 | 13.485 |
| 10.194-8.204 | 94.20 | 196 | 15 | 0.2242 | 14.143 | 13.583 |
| 8.201-6.603 | 94.70 | 376 | 17 | 0.2218 | 11.587 | 11.264 |
| 6.596-5.313 | 94.28 | 703 | 39 | 0.1817 | 12.067 | 11.767 |
| 5.312-4.276 | 95.95 | 1376 | 69 | 0.1312 | 15.415 | 15.222 |
| 4.275-3.441 | 97.70 | 2664 | 137 | 0.1151 | 16.401 | 16.221 |
| 3.441-2.769 | 99.05 | 5177 | 270 | 0.1183 | 12.022 | 11.913 |
| 2.769-2.228 | 99.98 | 9888 | 571 | 0.1074 | 8.488 | 8.390 |
| 2.228-1.793 | 99.71 | 19032 | 958 | 0.1004 | 6.153 | 6.105 |
| 1.793-1.443 | 99.74 | 36330 | 1907 | 0.0978 | 3.477 | 3.447 |
| 1.443-1.161 | 99.23 | 69189 | 3649 | 0.0935 | 2.191 | 2.179 |
| 1.161-0.935 | 99.98 | 133428 | 7163 | 0.1032 | 1.109 | 1.104 |
| 0.935-0.740 | 58.04 | 163390 | 8585 | 0.1959 | 0.399 | 0.383 |

**Table S3 |**

| Resolution range | Comple-teness | No. Refl. work | No. Refl. test | R-factors work | R-factors test |
|---|---|---|---|---|---|
| 44.5578 - 2.3007 | 0.98 | 18679 | 1022 | 0.1238 | 0.1348 |
| 2.3007 - 1.8261 | 1.00 | 18802 | 959 | 0.1004 | 0.1101 |
| 1.8261 - 1.5953 | 1.00 | 18742 | 978 | 0.1007 | 0.1106 |
| 1.5953 - 1.4494 | 1.00 | 18747 | 976 | 0.0950 | 0.1061 |
| 1.4494 - 1.3455 | 0.99 | 18589 | 957 | 0.0957 | 0.1113 |
| 1.3455 - 1.2662 | 0.99 | 18520 | 1035 | 0.0952 | 0.1080 |
| 1.2662 - 1.2028 | 0.99 | 18524 | 938 | 0.0944 | 0.1031 |
| 1.2028 - 1.1504 | 1.00 | 18654 | 1023 | 0.0856 | 0.0921 |
| 1.1504 - 1.1061 | 1.00 | 18710 | 991 | 0.0882 | 0.0906 |
| 1.1061 - 1.0679 | 1.00 | 18667 | 1004 | 0.0910 | 0.1034 |
| 1.0679 - 1.0346 | 1.00 | 18697 | 984 | 0.1002 | 0.1156 |
| 1.0346 - 1.0050 | 1.00 | 18685 | 984 | 0.1096 | 0.1179 |
| 1.0050 - 0.9785 | 1.00 | 18684 | 1018 | 0.1145 | 0.1175 |
| 0.9785 - 0.9546 | 1.00 | 18606 | 1024 | 0.1253 | 0.1386 |
| 0.9546 - 0.9329 | 1.00 | 18639 | 1005 | 0.1397 | 0.1485 |
| 0.9329 - 0.9131 | 1.00 | 18686 | 982 | 0.1490 | 0.1561 |
| 0.9131 - 0.8948 | 1.00 | 18731 | 931 | 0.1556 | 0.1582 |
| 0.8948 - 0.8779 | 0.99 | 18506 | 962 | 0.1699 | 0.1734 |
| 0.8779 - 0.8623 | 0.99 | 18519 | 953 | 0.1820 | 0.1929 |
| 0.8623 - 0.8476 | 0.94 | 17474 | 974 | 0.2034 | 0.2289 |
| 0.8476 - 0.8340 | 0.85 | 15803 | 827 | 0.2186 | 0.2275 |
| 0.8340 - 0.8211 | 0.75 | 14005 | 701 | 0.2320 | 0.2433 |
| 0.8211 - 0.8091 | 0.61 | 11235 | 626 | 0.2510 | 0.2667 |
| 0.8091 - 0.7977 | 0.47 | 8758 | 513 | 0.2627 | 0.2777 |
| 0.7977 - 0.7869 | 0.36 | 6695 | 360 | 0.2762 | 0.3094 |
| 0.7869 - 0.7767 | 0.27 | 5036 | 252 | 0.2800 | 0.2883 |
| 0.7767 - 0.7669 | 0.15 | 2841 | 140 | 0.3173 | 0.3186 |
| 0.7669 - 0.7577 | 0.14 | 2650 | 132 | 0.3241 | 0.3145 |
| 0.7577 - 0.7489 | 0.12 | 2162 | 96 | 0.3727 | 0.3562 |
| 0.7489 - 0.7405 | 0.05 | 892 | 44 | 0.4900 | 0.5356 |

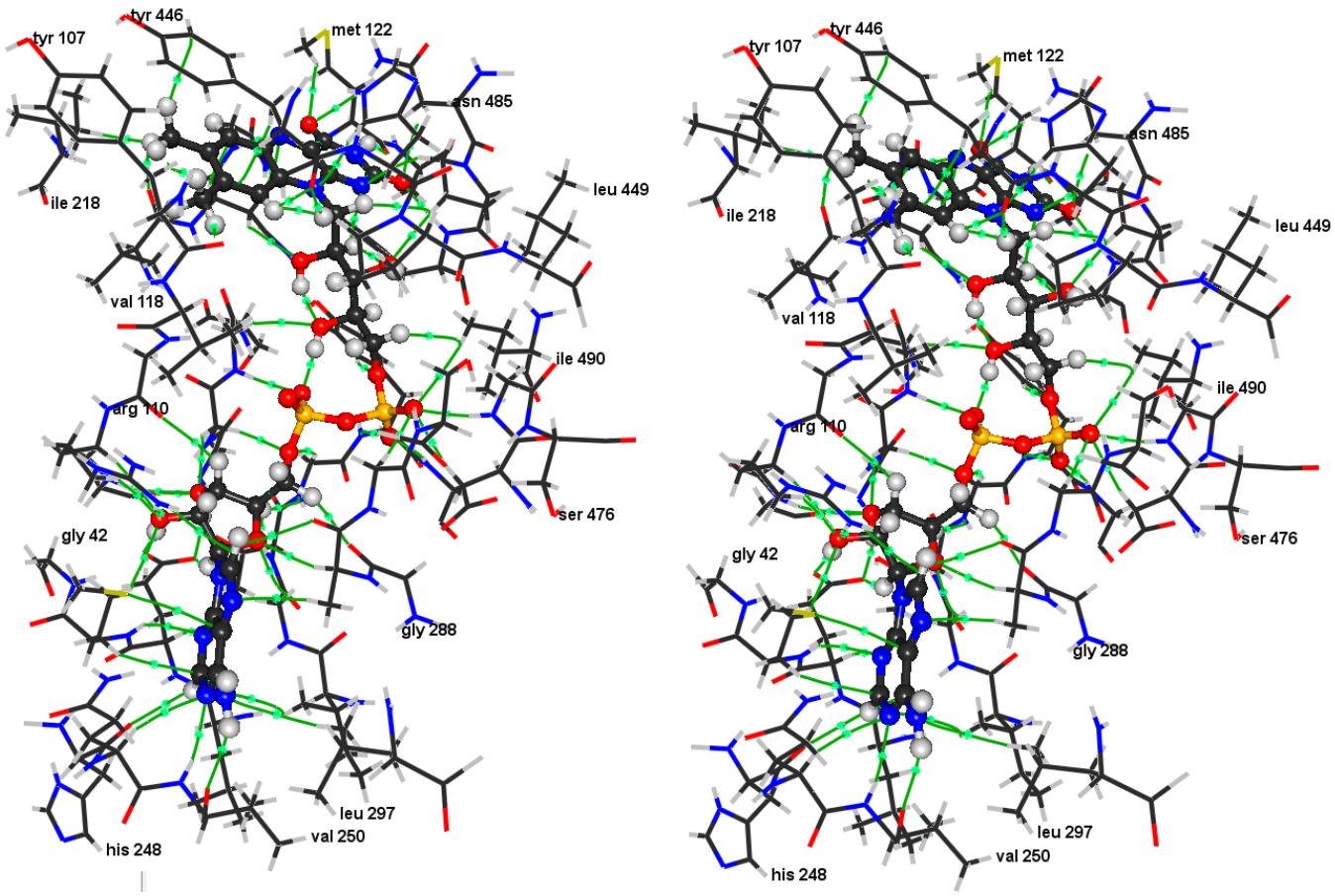


**Figure S1**: Stereo view (wall eyes) showing protein residues forming intermolecular interactions with the FAD cofactor. The interactions are shown in the form of (3, -1) critical points and bond paths (green lines). All the interacting protein residues are shown as bond lines whereas the cofactor is highlighted in ball and stick model