The complete catalytic mechanism of Xanthine Oxidase: a computational study

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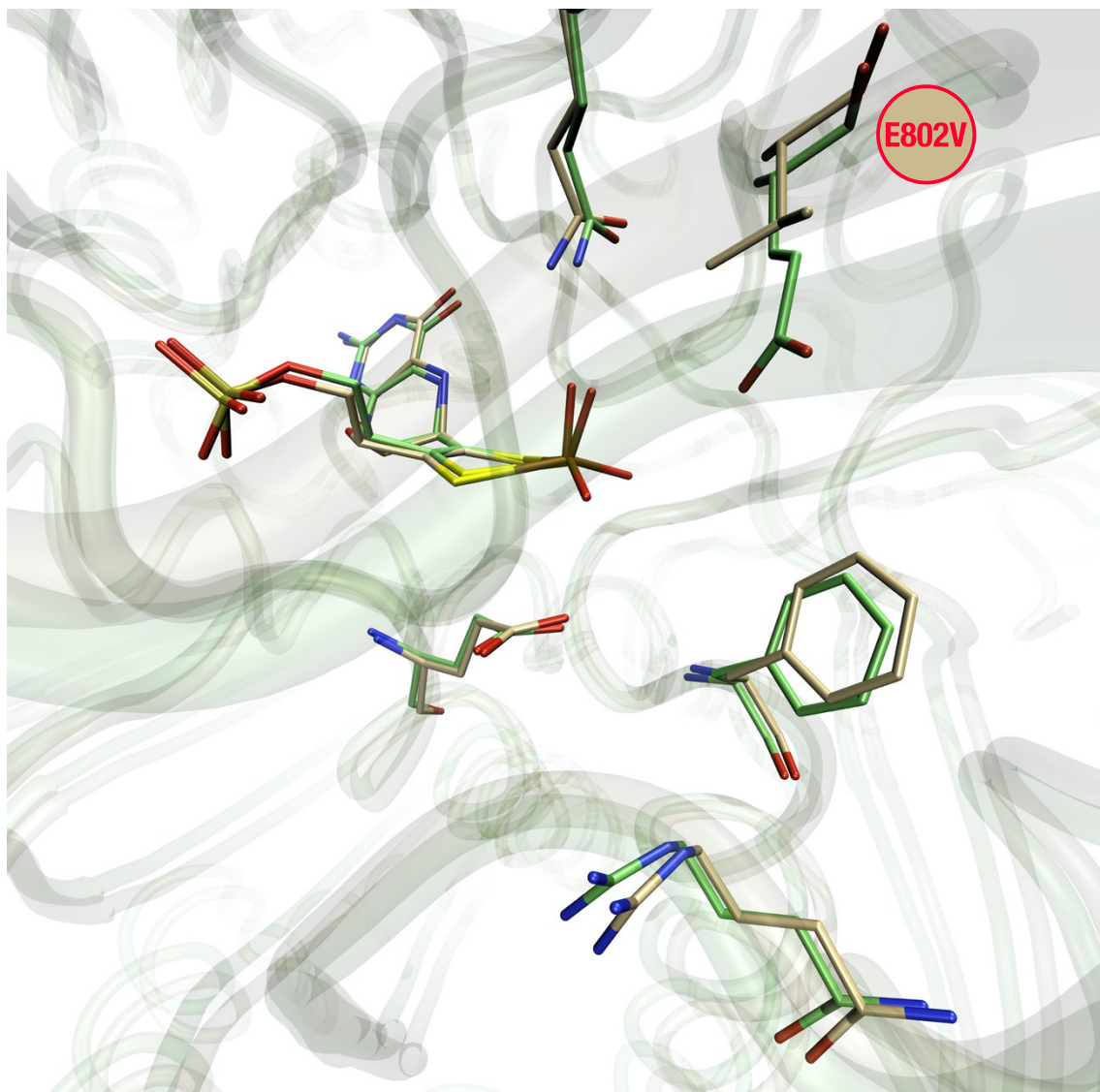
Supporting Information

Figure S 1. Overlapping of the structures of the active site residues of bovine and human forms of xanthine oxidase.
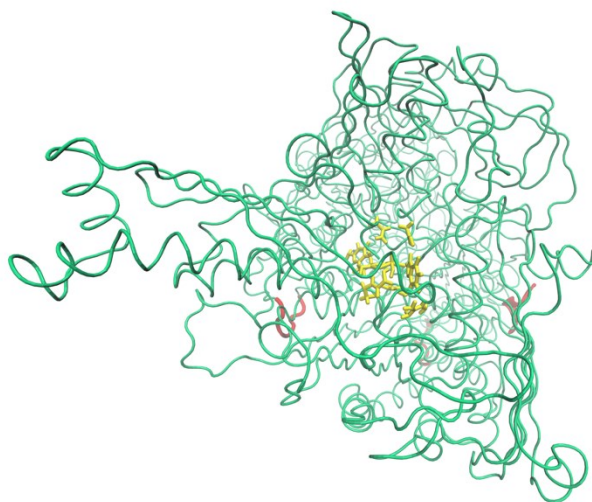
Figure S 2. Representation of the XO structure with the three regions modeled by homology highlighted in red. The active site residues and pterin cofactor are represented in licorice colored by yellow.
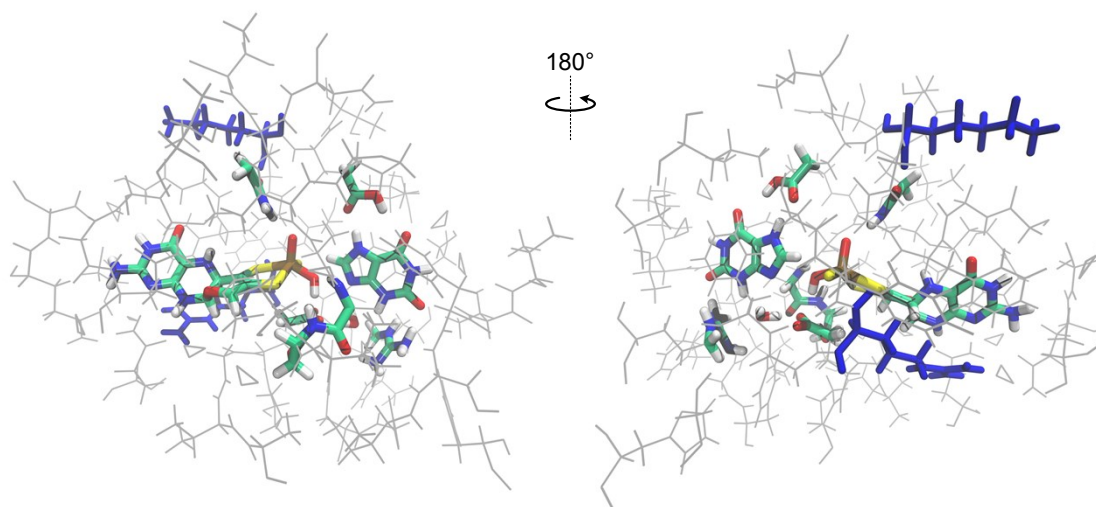
Figure S 3. Representation of all the atoms included in the high-level (HL) layer treated using quantum mechanics (licorice colored by element). The residues that are up to 5 Å from the HL layer were represented in grey lines, and the charged residues within that shell are highlighted in blue. A 180 ° rotation across the z-axis was applied from the left to the right images.

Methodology: QM/MM Calculations (Detailed Version)

The initial structure for the QM/MM calculations was retrieved as a representative structure of the most populated cluster from the MD simulation[1]. The model was built using molUP[2] plugin for Visual Molecular Dynamics (VMD)[3] software. The QM/MM model includes the full protein, the substrate (XAN), the Moco, and a 4.0 Å coat of water molecules around the full protein. The coat of water molecules mimics the solvent effect (water) around the enzyme, through intermolecular interactions and limiting the free movements of the solvent-exposed parts of the enzyme. The total system is composed of about 24,500 atoms that were treated using two different theoretical methods following a 2-layers subtractive ONIOM QM/MM approach[4-6]: the high-level (HL) layer, which was calculated with density functional theory (DFT), and the low-level (LL) layer, which was calculated with molecular mechanics (MM). The HL layer included the Moco, a water molecule (two in the last step), the XAN (except for the last step) molecule, and the Ala1043, Ser1044, Arg844, Glu1225, Glu766, and Gln731 amino acid residues, accounting for 84 to 97 atoms (**Figure S3**). The remaining atoms of the system were included in the LL layer. Hydrogen atoms were used as link atoms to complete the valence of the bonds crossing between the two layers.

The geometry optimization of the HL layer was performed using the B3LYP[7] functional (DFT), based on the very good results in the study of biological systems. The 6-31G(d)[8-12] basis set and LanL2dz[13] pseudo-potential (Mo atom) were employed for the geometry optimizations as available in Gaussian 09[14]. During the catalytic mechanism, the oxidation state of Mo atom changes from +6 to +4, and back to +6 at the enzymatic turnover. The +6 state corresponds to a Mo atom with no valence electrons (Mo(VI): [Kr] $4d^0$). In the +4 state, Mo atom assumes the Mo(IV): [Kr] $4d^2$ configuration, where two electrons are paired. Therefore, the spin multiplicity was set 1 for all the calculations.

The reactional space was explored through linear transit scans along with the reaction coordinates implicated in each step of the mechanism. Then, the transition states (TS)

were optimized, using the structures of higher energy obtained with the previous scans. Internal Reaction Coordinate (IRC) calculations were conducted to obtain the reactant and product for each step. The TSs were confirmed by vibrational frequency calculations, resulting in a single imaginary frequency with the correct transition vectors assigned. The vibrational frequency calculations were also conducted for the minima, confirming no imaginary frequencies. The zero-point energy (ZPE), thermal, and entropic energy corrections were estimated at 310.15 K and 1.0 bar during the frequency calculation of TS and related minima structures. The activation and reaction Gibbs free energies, for each step, were calculated through the difference between the computed Gibbs free energies of TS or product and the reactant, respectively.

The final energies were refined through single-point energy calculations using a more complete basis set: 6-311++G(3df,3pd)[11, 15-19].

All the calculations were accomplished using Gaussian 09 software[14], whereas the preparation of input files and analysis of results were made using molUP[2, 20] plugin for VMD[3].

This general protocol has been used with success in the study of the catalytic mechanism of several different enzymes[21-25], including other important Mo dependent systems[26-28].

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