Supplemental Data

"The first mammalian aldehyde oxidase crystal structure: insights into substrate specificity" MS ID: JBC/2012/390419

MOLECULAR DYNAMICS AND DOCKING STUDIES

Experimental Procedure

Molecular Dynamics: For the mAOX3 modeling, construction of the missing loops was carried out with program Modeller9 version 8 (2-5). The missing parameters were determined using a $Fe_2S_2(SCH_3)_4$ model for the FeS cluster and a model of the entire Moco cofactor (molybdenum pentacoordinated to molybdopterin , sulfido, oxo, and hydroxo ligands). The Moco and FeS model structures were geometry optimized using DFT (6), with the exchange correlation functional B3LYP and basis set 6-311G++(2d, 2p) for all atoms except molybdenum, for which the LanL2DZ pseudopotential was employed. Frequency calculations were performed to assure the existence of true local minima and to obtain the cartesian Hessian matrices. An in-house developed program was applied to calculate the force constants for the bond and angles parameters according to the Seminario *et al* method (7). The vdw parameters for molybdenum were taken from experimental data. The atomic charges necessary to perform the MD simulations were calculated further resorting to the RESP method (8). MD simulations were performed with Amber10, a package of molecular simulation programs (9), using the ff99SB parameter set (10) for the protein and cofactors. The parameter set used for the ligands was the generalized amber force field (GAFF) (11). Accordingly, the initial structures of the substrates /inhibitors were taken from the PubChem compound library, and geometry optimized using the Hartree-Fock method and a 6-31G* basis set (6). The atomic charges were evaluated with the RESP method. An initial energy minimization was performed followed by an equilibration of 100 ps to slowly heat the system from 0 to 300 K. The equilibration was performed in an NVT ensemble using the Langevin dynamics with small restraints on the protein. Production simulations were carried out at 300 K in the NPT ensemble using Langevin dynamics with a collision frequency of 1.0 ps^{-1}. Constant pressure periodic boundary conditions were imposed with an average

pressure of 1 atm. Isotropic position scaling was used to maintain pressure with a relaxation time of 2 ps. The time step was set to 2 fs. Shake constraints were applied to all bonds involving hydrogen atoms. The particle mesh Ewald (PME) method was used to calculate electrostatic interactions with a cut-off distance of 8Å. The total time of each simulation was 10 ns.

Molecular Docking: Known mAOX3 substrates (benzaldehyde, 2-OH-pyrimidine, phthalazine and retinal,) and inhibitors (menadione, norharmane, benzamidine and raloxifene) (12) were docked onto the mAOX3 active site using the software AutoDock 4.0 (13,14) with the Lamarckian genetic algorithm. The grid map was centered at the Moco and remaining active site. 150 docks were carried out per run of substrate/inhibitor. The best solutions were used as the starting structures for the MD simulations of the complexes.

Results and Discussion

Molecular Docking with Substrates: Molecular docking studies performed with benzaldehyde, retinal, 2-OH-pyrimidine and phthalazine indicate that substrate binding is largely modulated by aromatic stacking interactions with Phe919 (Fig.2 SI). Glu1266 and Lys889 are also very important for the placement of the substrate relative to Moco, with Glu1266 close to the OH ligand of Mo. In the case of aldehydes as substrates (benzaldehyde and retinal), both adopt similar positions at the substrate-binding pocket (Fig.2A, 2B SI). Due to the polar nature of the aldehyde functionality, its carbonyl group is oriented towards the side chain of Lys889 establishing a dipole-ion interaction with it. Additionally, Lys889 approaches Glu1266 establishing a salt bridge (Nz…Oe ~2.7 - 2.8Å). These two interactions place the carbon atom of the aldehyde group close to the OH Mo ligand (~4.5Å) (Fig.2 SI). In the case of benzaldehyde, the distance between the carbonyl carbon atom and the oxygen atom of the OH Moco ligand varies between 3-5Å during the simulation (Fig.2A SI). As to the N-heterocycle 2-OH-pyrimidine, the hydroxyl group of the substrate interacts with Glu1266 (distance O…O 3.4Å) (Fig.2C SI). We expect the enzyme to proceed via a nucleophilic attack to one of the carbons that is connected to an electronegative group, which is likely of the carbon atoms adjacent to the nitrogen atoms of the symmetrical pyrimidine ring. The distance of one of those

carbons to the OH ligand of Mo is around 4.1Å. The results of molecular docking with phthalazine show that it adopts a similar position as the other substrates. Phthalazine engages again in an aromatic p-p interaction with Phe919. It also establishes a hydrogen bond with Lys889 (N…HN_{Lys889}) (Fig.2D SI). In all the substrate-bound forms analyzed, Lys889 adopts a position highly similar to that of Arg880 in XOR allowing the correct positioning of the substrate in relation to the Mo center. The mutation of the positively charged Lys889 to a histidine (as in XOR – His884) changed the active site significantly (Fig.3 SI). Docking and molecular dynamics simulations with benzaldehyde show that this substrate interacts simultaneously with the aromatic imidazole and phenol rings of His889 and Tyr885, respectively, and displaces the substrate from the Moco group (Fig.2A SI).

Molecular Docking with Inhibitors: Molecular docking studies of mAOX3 with the inhibitors menadione, norharmane, benzamidine and raloxifene reveal that Phe919 interacts with the phenyl rings of inhibitors and substrates in a similar fashion (Fig.4 SI and Fig.8) (15,16). The large size of norharmane and menadione prevents them from entering deeply into the active site pocket and interacting with Lys889 and/or Glu1266 (Fig.4A, 3B SI). On the other hand, benzamidine adjusts to the active site pocket and its NH_2 group interacts with Glu1266, leaving only the phenyl ring accessible to the OH ligand of Mo (Fig.4C SI). The results with raloxifene are reported in the manuscript body (Fig.8).

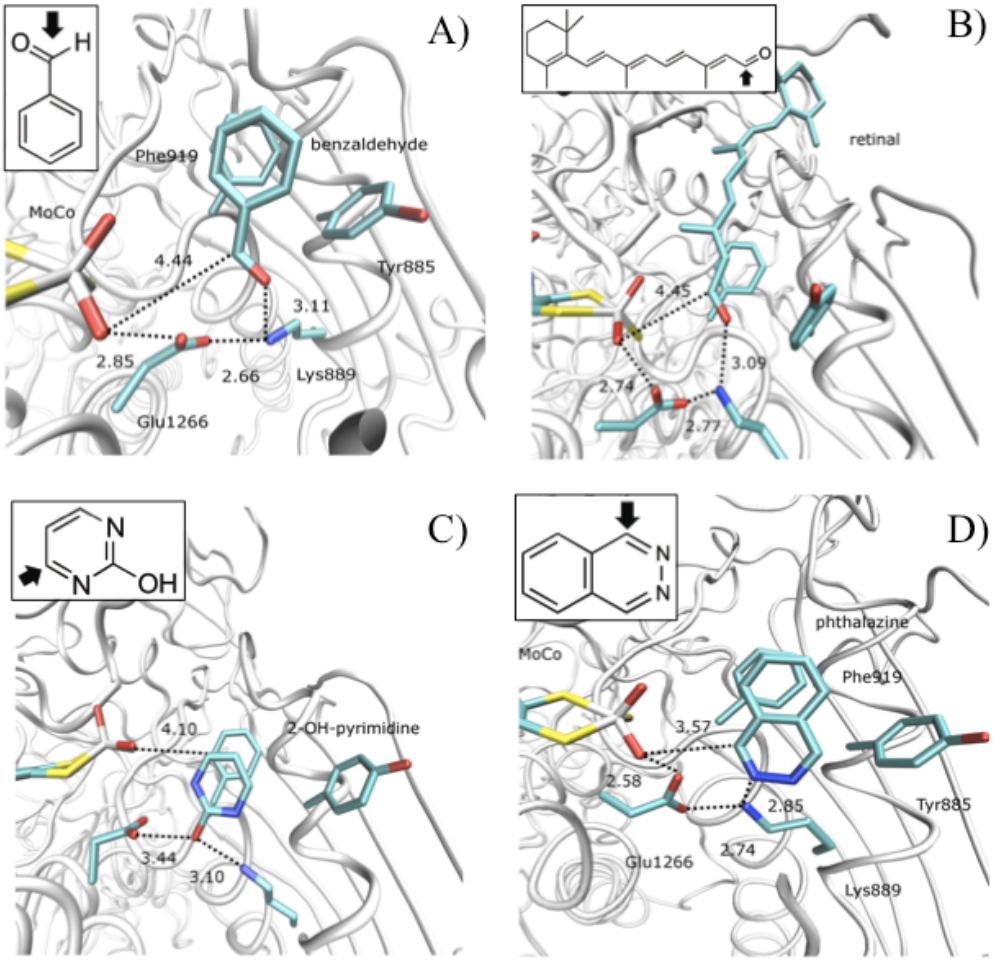

FIGURE 1 SI - Molecular docking with the substrates: (A) benzaldehyde; (b) retinal; (C) 2-OH pyrimidine and (D) phthalazine.

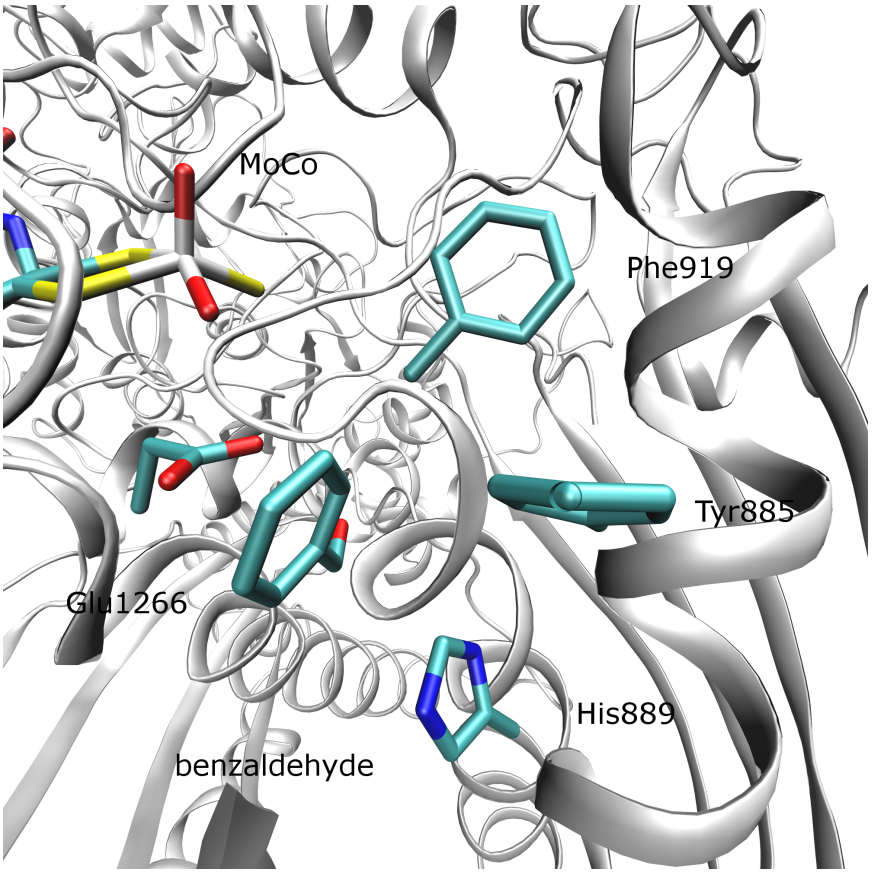

FIGURE 2 SI - Docking with benzaldehyde and K889H variant. The mutation of the positively charged Lys889 to a histidine changed the active site significantly. Docking and molecular dynamics simulations with benzaldehyde show that this substrate interacts simultaneously with the aromatic imidazole and phenol rings of His889 and Tyr885, respectively. This displaces the substrate from the Moco group.

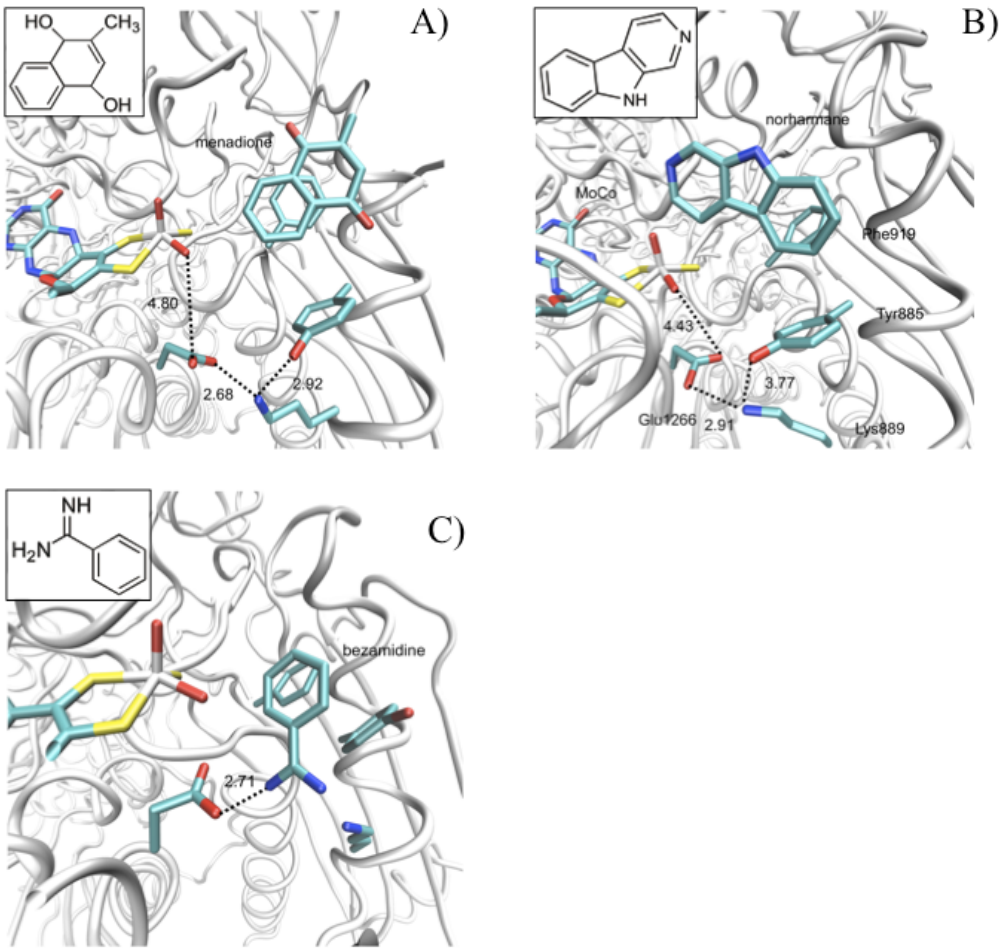

FIGURE 3 SI - Molecular docking with the inhibitors: (A) menadione; (B) norharmane and (C) benzamidine.

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