Supplementary information

Different inhibitory potency of febuxostat towards mammalian and bacterial xanthine oxidoreductases: insight from molecular dynamics

AUTHORS:

Hiroto Kikuchi[1], Hiroshi Fujisaki[1,3], Tadaomi Furuta[2,3], Ken Okamoto[4], Silke Leimkühler[5], Takeshi Nishino[4,6, 7*]

[1.] Department of Physics, Nippon Medical School, 2-297-2 Musasikosugi, Nakahara-Ku, Kawasaki 211-0063, Japan

[2.] Center for Biological Resources and Informatics, Tokyo Institute of Technology, B-62 4259 Nagatsuta-cho, Midori-ku, Yokohama 226-8501, Japan

[3.] Computational Science Research Program, RIKEN, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan

[4.] Department of Biochemistry, Nippon Medical School, 1-1-5 Sendagi, Bunkyo-ku, Tokyo 113-8602, Japan

[5.] Department of Molecular Enzymology, Institute for Biochemistry and Biology, University of Potsdam, Karl-Liebknecht Str. 24-25, 14476 Potsdam, Germany

[6.] Department of Biochemistry, University of California, 1463 Boyce Hall, Riverside, CA92521-0122, USA

[7.] Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, University of Tokyo, 1-1-1 Yayoi, Bunkyo-Ku, Tokyo, 113-8657, Japan

*Correspondence and requests for materials should be addressed to T.N. (nishino@nms.ac.jp)

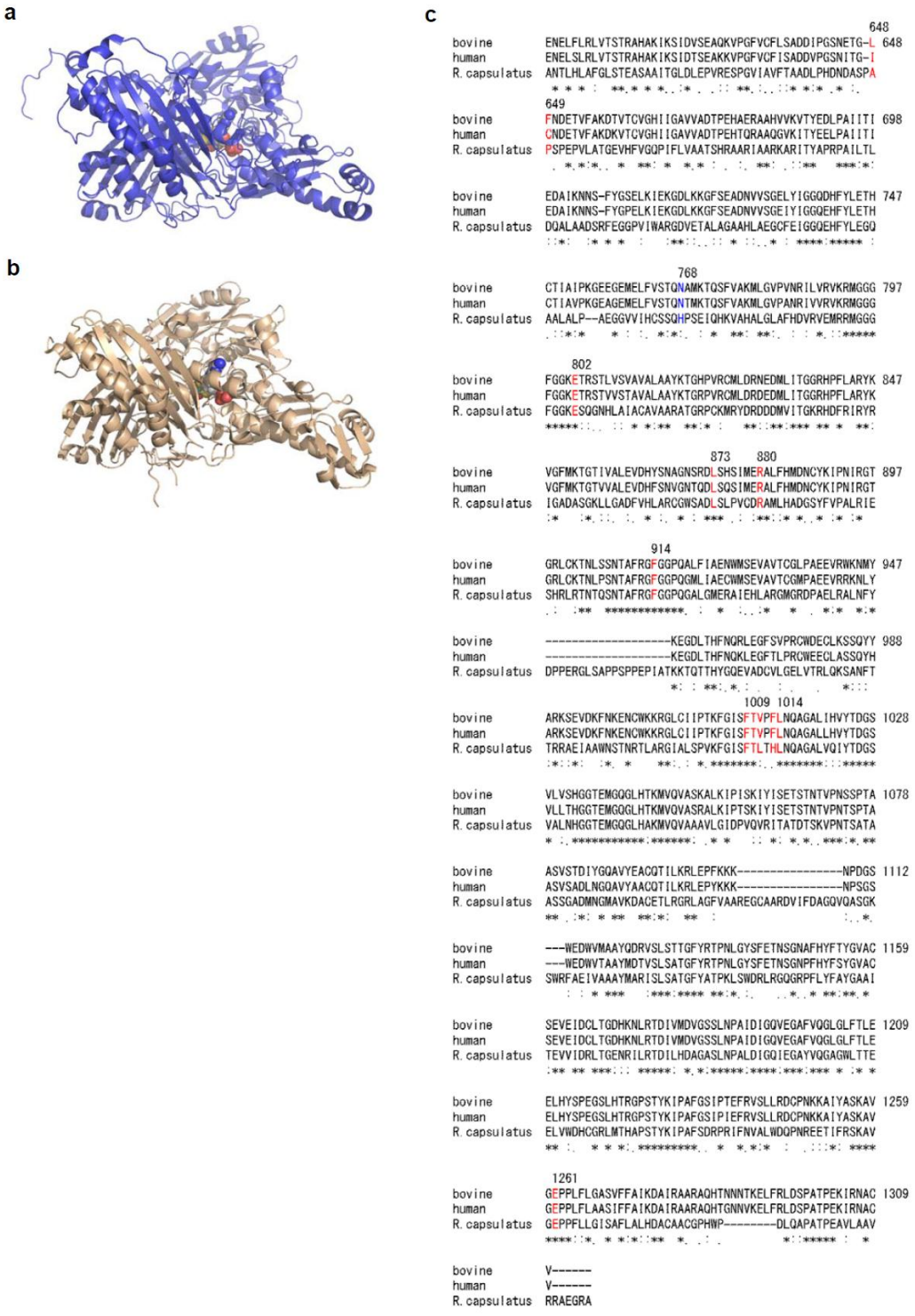


Figure S1. Structure/sequence comparison of human, bovine, and bacterial XORs. (a)-(b) Residues 600-1310 for bovine XOR (bXOR) and residues 30-377 & 402-777 for *Rhodobacter capsulatus* XOR (*Rc*XOR), which were selected for the MD simulation, are shown. Molybdenum cofactors are shown in van der Waals presentation. (c) Alignment of bovine, human, and *R. capsulatus* amino acid sequences is shown. Residues that interact with febuxostat in bXOR and corresponding residues in human and *Rc*XOR are shown in red. Asp 768 and corresponding residues are shown in blue. Conserved residues are marked with *.

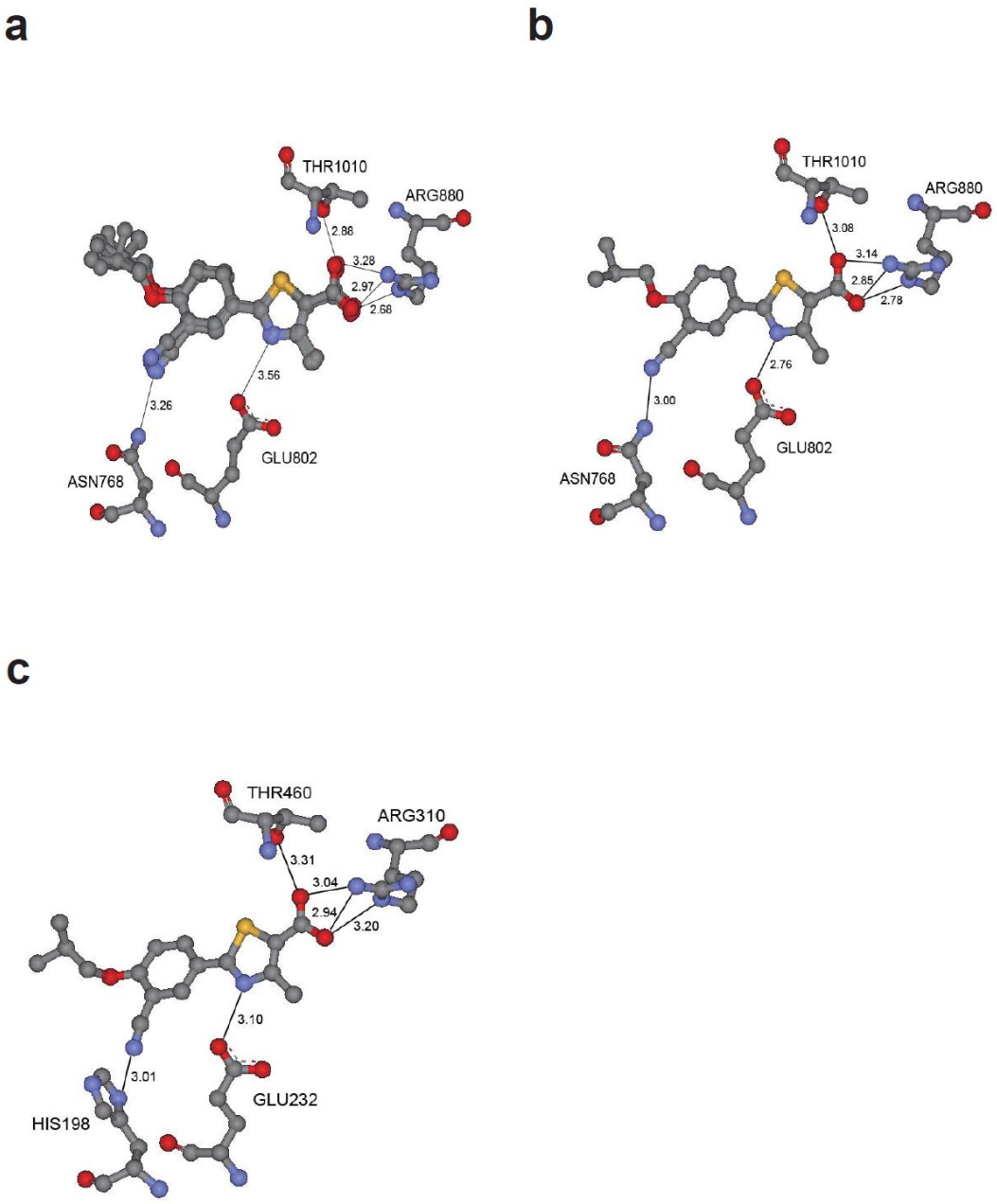
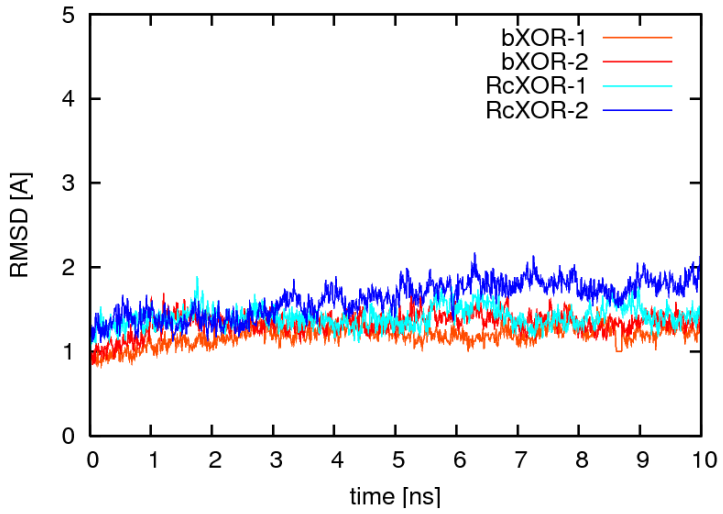


Figure S2. Initial positions of febuxostat in the cavity of bXOR or *Rc*XOR for MD calculations. (a) The spatial relationship between several residues in the cavity of bXOR based on PDB code 1FO4 and ten febuxostat poses obtained by using a protein-ligand docking algorithm. The ten poses almost overlap each other except for the four carbon atoms of the tail: C15, C16, C17, and C18. One of them was used as the initial position of febuxostat in the MD simulations, and the information on lengths was calculated using the initial position. The RMSD of all heavy atoms in febuxostat between the initial position and the position of febuxostat in 1N5X, which is the crystal structure of febuxostat-bound bXOR in S2 (b), is 0.672 Å. The RMSD for the case of the heavy atoms without the four carbon atoms of the tail is 0.295 Å. This means that the initial position of febuxostat obtained by the docking calculations is almost identical with the position of febuxostat in 1N5X. (b) The spatial relationship between febuxostat and several residues in the cavity of bXOR based on PDB code 1N5X. (c) The spatial relationship between febuxostat and several residues in the cavity of *Rc*XOR based on PDB code 1JRO. The position of febuxostat is also one of the docking poses obtained by the docking calculations, S2 (a).



	avg	sd	min	max	num	Final RMSD(10 ns)
bXOR-1	1.190	0.120	0.836	1.562	1000	1.379
bXOR-2	1.333	0.126	0.843	1.693	1000	1.354
RcXOR-1	1.405	0.123	1.023	1.892	1000	1.303
RcXOR-2	1.624	0.214	1.103	2.174	1000	1.907

So the average 1FO4 RMSD (1.379+1.354)/2=1.3665 ~ **1.4**

and the average 1JRO RMSD (1.303+1.907)/2=1.605 ~ **1.6**

Figure S3. Backbone RMSD. The time courses of the backbone fluctuations in terms of RMSD are shown for two characteristic runs for each species. The RMSD increase in *Rc*XOR-2 after ~4 ns might be caused by the movement of febuxostat.

1FO4(Blue) (Residue: Y947, K948, E949, G950, D951, L952(vdW, Purple, 4/6 res.))

1JRO(Red) (Residue: Y377, T402(vdW, Red), 378-401(dashed loop, Orange, **24** res.))

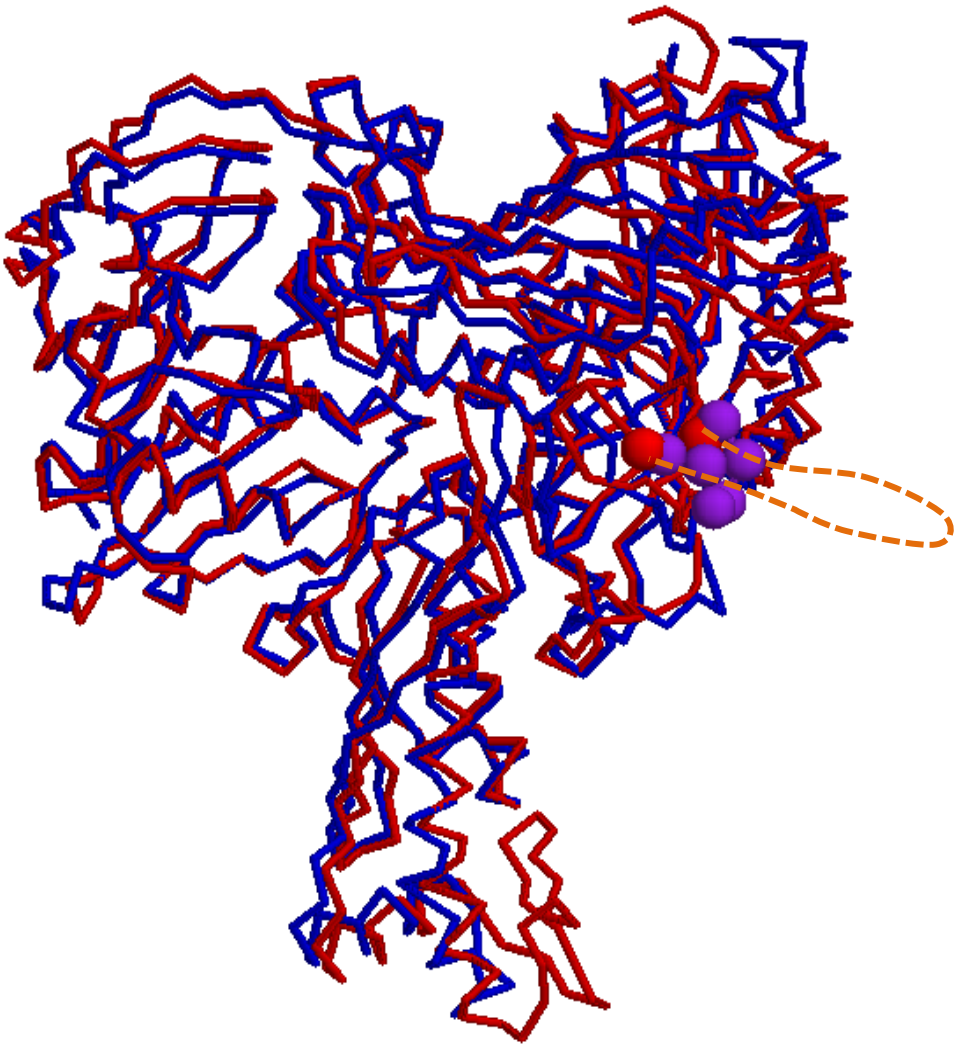


Figure S4. Truncated (disordered) region. In the PDB structure of bacterial XOR (1JRO), there is a structurally missing region from the 378th to 401st amino acids as depicted by the orange dashed curve here. In the MD simulations conducted in this work, the missing (possibly disordered) region was removed. See the methods section for detail.

Febuxostat(Red), Molybdopterin(Blue), Cap[NME, ACE](Green)

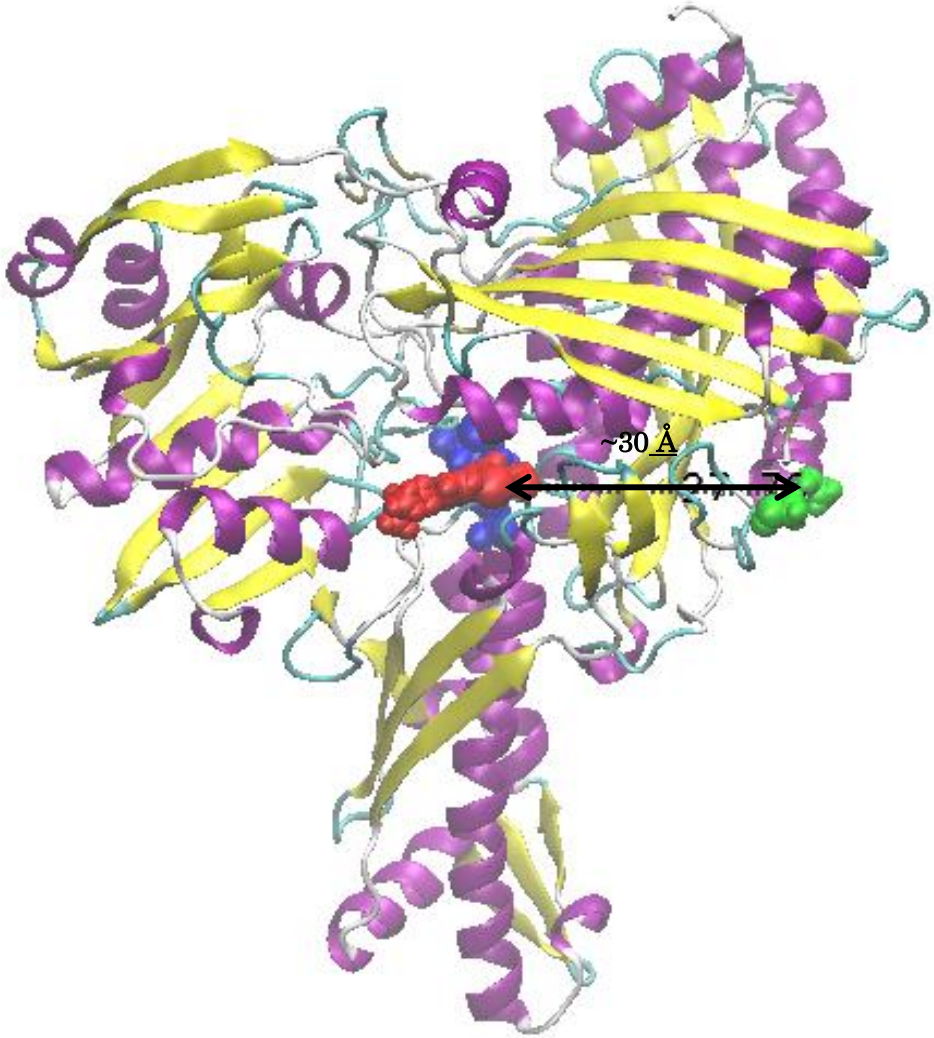


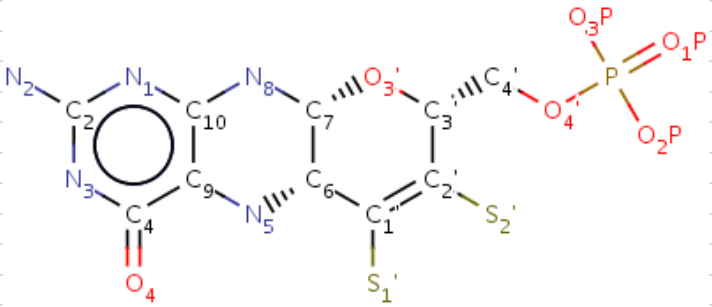
Figure S5. Distance between febuxostat and the disordered region. The bound febuxostat lies far (~30 Angstroms) from the capped part of the disordered region of bacterial XOR, as illustrated here.

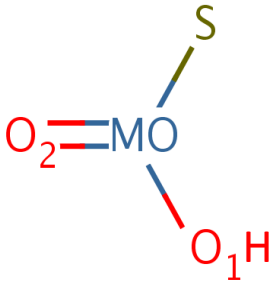
Partial charges for MTE part

MTE	(PHOSPHONIC ACIDMONO-(2-AMINO-5,6-DIMERCAPTO-4-OXO-3,7,8A,9,10,10A-HEXAHYDRO-4H-8-OXA-1,3,9,10-TETRAAZA-ANTHRACEN-7-YLMETHYL)ESTER)

Other non-electrostatic (GAFF) parameters were automatically assigned for this pyranopterin part using the antechamber module.

atom	charge
C1'	-0.110262
S1'	-0.409631
C2'	-0.089339
S2'	-0.508988
C3'	0.188266
C4'	0.461128
O3'	-0.356157
O4'	-0.573016
P	1.288178
O1P	-0.790039
O2P	-0.791697
O3P	-0.780348
N1	-0.805805
C2	0.843612
N2	-0.985533
N3	-0.657014
C4	0.518219
O4	-0.615023
N5	-0.431553
C6	0.150907
C7	0.334505
N8	-0.706836
C9	-0.220222
C10	0.628876
HC3'	0.018031
HC4'1	0.004078
HC4'2	-0.015572
HN21	0.396006
HN22	0.349318
HN3	0.353928
HN5	0.338535
HC6	0.010644
HC7	0.019511
HN8	0.377365





Force Field parameters for MOS part

MOS (DIOXOTHIOMOLYBDENUM(VI) ION)

atom	charge	
MO	1.096299	(mass: 95.94)
O1	-0.689388	
O2	-0.487081	
S	-0.688175	
HO1	0.334271	

Hydrogen bond:

MOS(HO1)-Glu1261(OE2) in mammalian

MOS(HO1)-Glu730(OE2) in bacteria

Bond length including MO (in MTE-MOS complex)

bond	length [Å]
S1'-MO	2.552
S2'-MO	2.431
S-MO	2.023
O1-MO	1.950
O2-MO	1.716

bond	angle [deg]
S-MO-O1	100.029
S-MO-O2	100.031
O1-MO-O2	100.010

Strong force coefficients were assigned for bond lengths / bond angles: 5000 kcal/mol Å^2 / 500 kcal/mol deg^2.
(Standard values were assigned for dihedral etc.)

Figure S6. Parameters for MTE and MOS (MTS). For the parameterization of molybdenum cofactor (called MTS here) for molecular dynamics simulations, the molecule was first decomposed into two fragments, MTE and MOS. The partial charges for each fragment are shown here with non-electrostatic parameters for the Amber force field.