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Supporting information for article:

Crystal structure of rofecoxib bound to human cyclooxygenase-2

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Table S1 List of contacts made between Vioxx and residues in monomer A of the huCOX-2:Vioxx crystal structure. Hydrophilic contacts were defined with a cutoff distance of 3.6Å and are colored in red. Hydrophobic interactions were defined with a cutoff of 4Å.

COX Residue	COX Atom	Vioxx Atom	Distance (Å)
His-90	ND1	O21	3.56
	NE2	O21	3.58
Val-349	CG1	C1	3.9
	CG1	C3	3.86
	CG1	C4	3.46
Leu-352	CD1	C11	3.96
	C	C14	3.66
	CB	C14	3.68
	C	C15	3.44
	CB	C15	3.99
Ser-353	CA	C13	3.7
	CA	C14	3.68
	CA	C15	3.73
	CA	C16	3.8
	CA	C17	3.81
	CA	C18	3.76
Tyr-355	CZ	C18	3.98
Trp-387	CH2	C10	3.76
	CZ2	C10	3.68
Arg-513	NH1	O21	3.24
Ala-516	CB	C22	3.66
Ile-517	CG2	C22	3.88
Phe-518	CD2	C15	3.87
	CA	C22	3.5
	C	C22	3.89
	CB	C22	3.13
	CG	C22	3.73
	CD2	C22	3.74
Val-523	CA	C8	3.99
	CG1	C13	3.66
	CG2	C15	3.96
	CG2	C16	3.49
	CG1	C17	3.3
	CG2	C17	3.57
	CG1	C18	3.07
	CG2	C22	3.95
Gly-526	CA	C9	3.89
Ala-527	CA	C1	3.9
	CB	C1	3.71
	CA	C4	3.61
	CB	C4	3.37
	CA	C7	3.89

Table S2 Comparison of unit cell parameters from X-ray crystal structures of mouse and human COX-2 obtained in polyacrylic acid 5100 (PAA 5100) versus polyethylene glycol 400 (PEG 400) as the primary precipitant. Crystals obtained from both conditions belong to the orthorhombic space group I222. Crystals obtained in PEG 400 conditions have an approximately 20% larger unit cell volume.

Species	**Ligand**	**Precipitant**	**a (Å)**	**b (Å)**	**c (Å)**	**Volume (Å^3)**	**Ref.**
mouse	AA	PAA 5100	119.98	132.55	180.52	2870873	(1)
mouse	1-AG	PAA 5100	118.96	131.77	179.86	2819370	(2)
mouse	NS-398	PAA 5100	120.43	131.21	179.57	2837497	(3)
mouse	ibuprofen	PAA 5100	120.94	132.23	180.46	2885898	(4)
human	aspirin	PAA 5100	114.20	130.13	178.03	2645676	(5)
human	Vioxx	PEG 400	126.99	149.42	185.06	3511485	this study

Table S3 Comparison of atomic displacement (B) factors for residues around the cyclooxygenase active site channel.

Residue B-factor	**Monomer A (Å^2)**	**Monomer B (Å^2)**
His-90	43.3	67.3
Val-349	28.7	39.1
Leu-352	33.4	41.6
Ser-353	32.7	49.3
Arg-513	42.4	54.2
Phe-518	38.0	47.2
Val-523	32.6	45.3
Ala-527	30.2	45.4

Figure S1 Crystals of human COX-2 derived from polyethylene glycol 400. Rod shaped crystals grew to dimensions of 50μm x 50μm x 300μm in two weeks and were subsequently harvested for data collection.

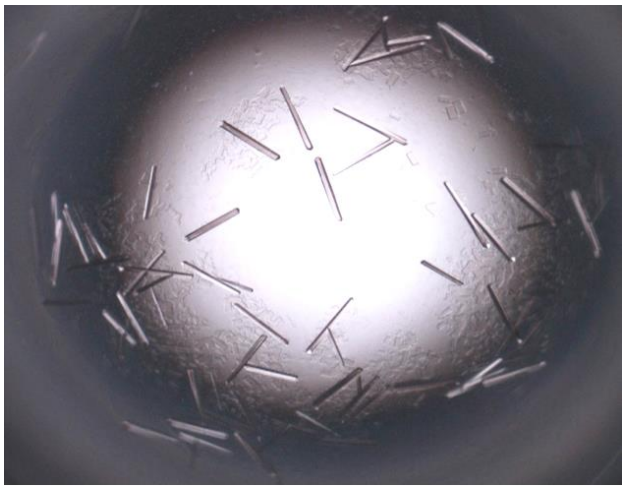


Figure S2 Comparison of crystal packing orientations in X-ray crystal structures of COX-2 obtained from polyacrylic acid 5100 and polyethylene glycol 400 conditions. Crystal packing diagrams for structures obtained using crystals from (A and C) Polyacrylic acid 5100 or (B and D) Polyethylene glycol 400 crystallization conditions. The membrane-binding domain helices are colored red. (E) Alignment of structures obtained from Polyacrylic acid 5100 (red, PDB id 5IKQ) and Polyethylene glycol 400 (grey, human COX-2 in complex with Vioxx). The root mean square deviation between structures is 0.22Å for 984 Cα pairs.

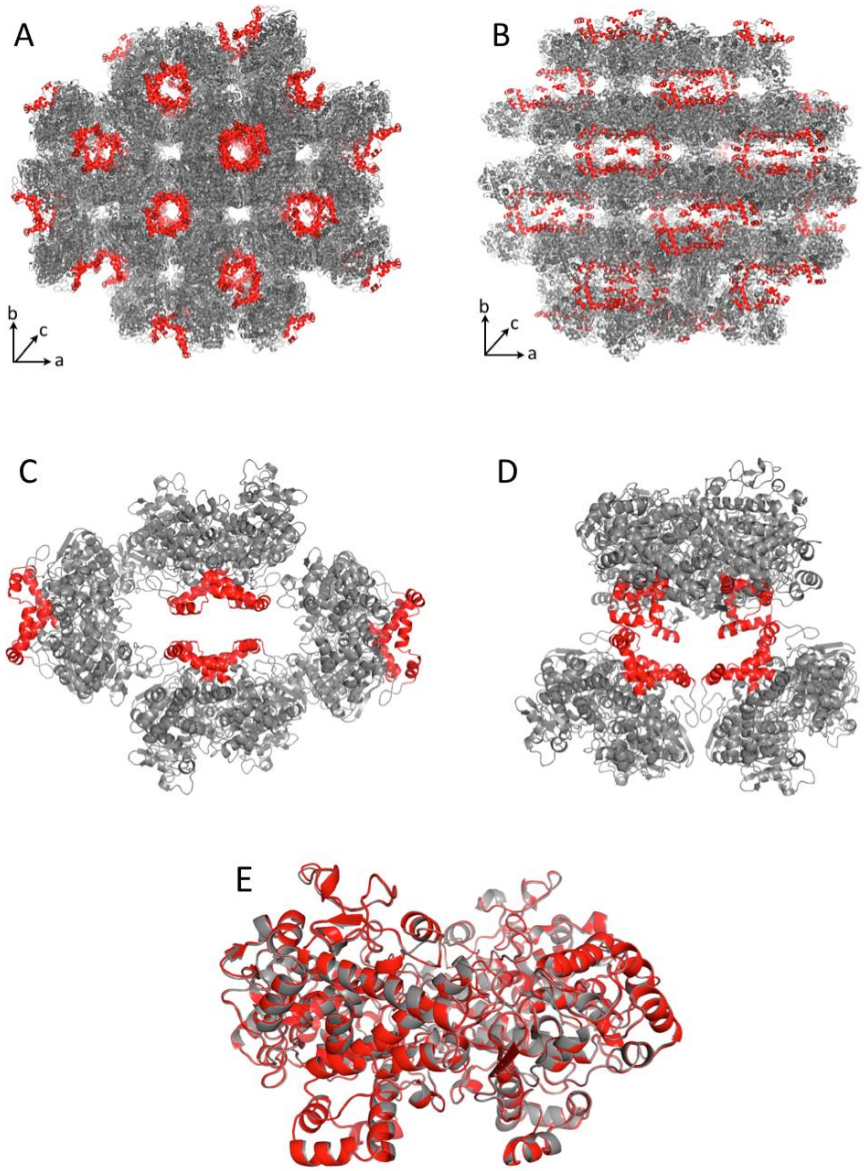


Figure S3 Comparison of crystal packing and B-factor for monomer A and monomer B in the huCOX-2 crystal structure in complex with Vioxx. A) Shown on the left is a crystal-packing diagram for the huCOX-2:Vioxx crystal structure. One layer from the packing diagram was extracted and rotated 90° to show the relative solvent exposure of monomer A (green) and monomer B (cyan). Monomer B is significantly more exposed to solvent channels than monomer A. B) B-factor putty diagram showing atomic displacement factors for the two monomers. Monomer B has higher B-factors in protein regions surrounding the Vioxx (magenta spheres) binding site. The B-factor magnitude increases from blue (skinny cartoon tube) to orange (thicker cartoon tube).

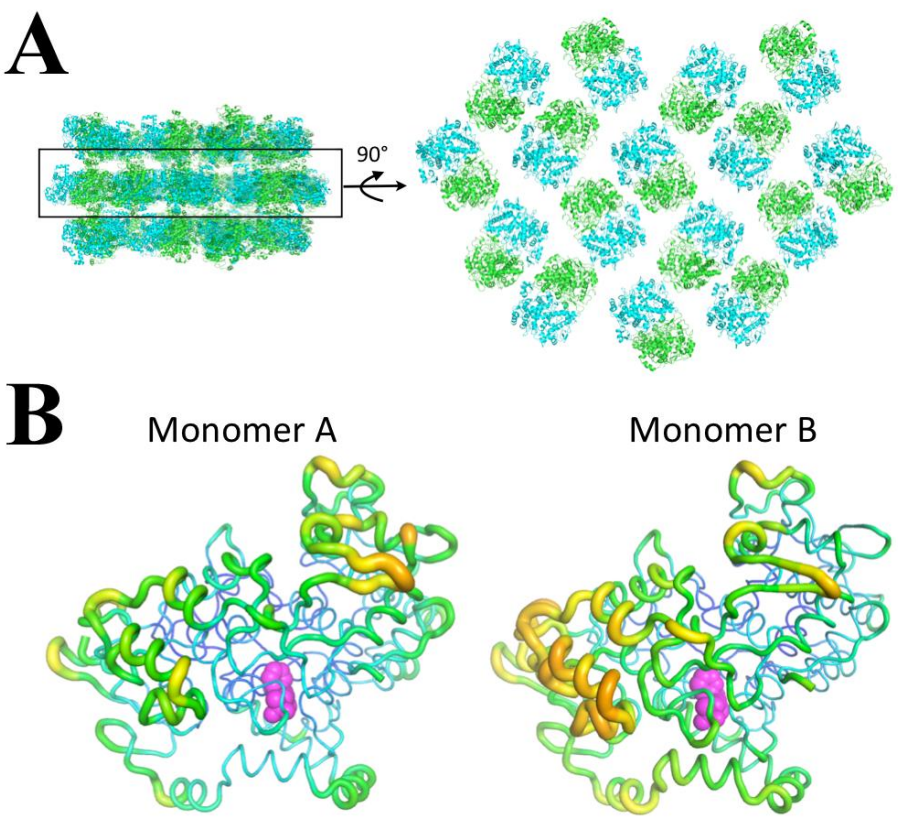
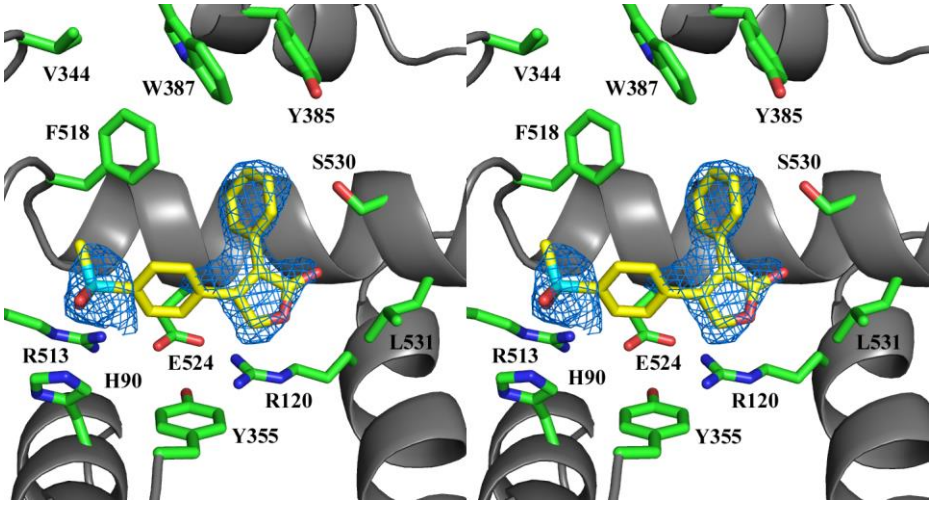


Figure S4 Vioxx Bound within the Cyclooxygenase Channel of Monomer B of Human COX-2. Stereo view of Vioxx bound within the cyclooxygenase channel of monomer B of the huCOX-2:Vioxx crystal structure. F_O-F_C simulated annealing omit electron density (blue), contoured at 3σ, is shown with the final refined model of Vioxx (yellow). Residues lining the cyclooxygenase channel are labeled accordingly. Carbon atoms of residues lining the channel are colored green, while, nitrogen, oxygen, and sulfur atoms are colored blue, red, and cyan, respectively.



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