Supporting Information

Li *et al.* 10.1073/pnas.0804099105

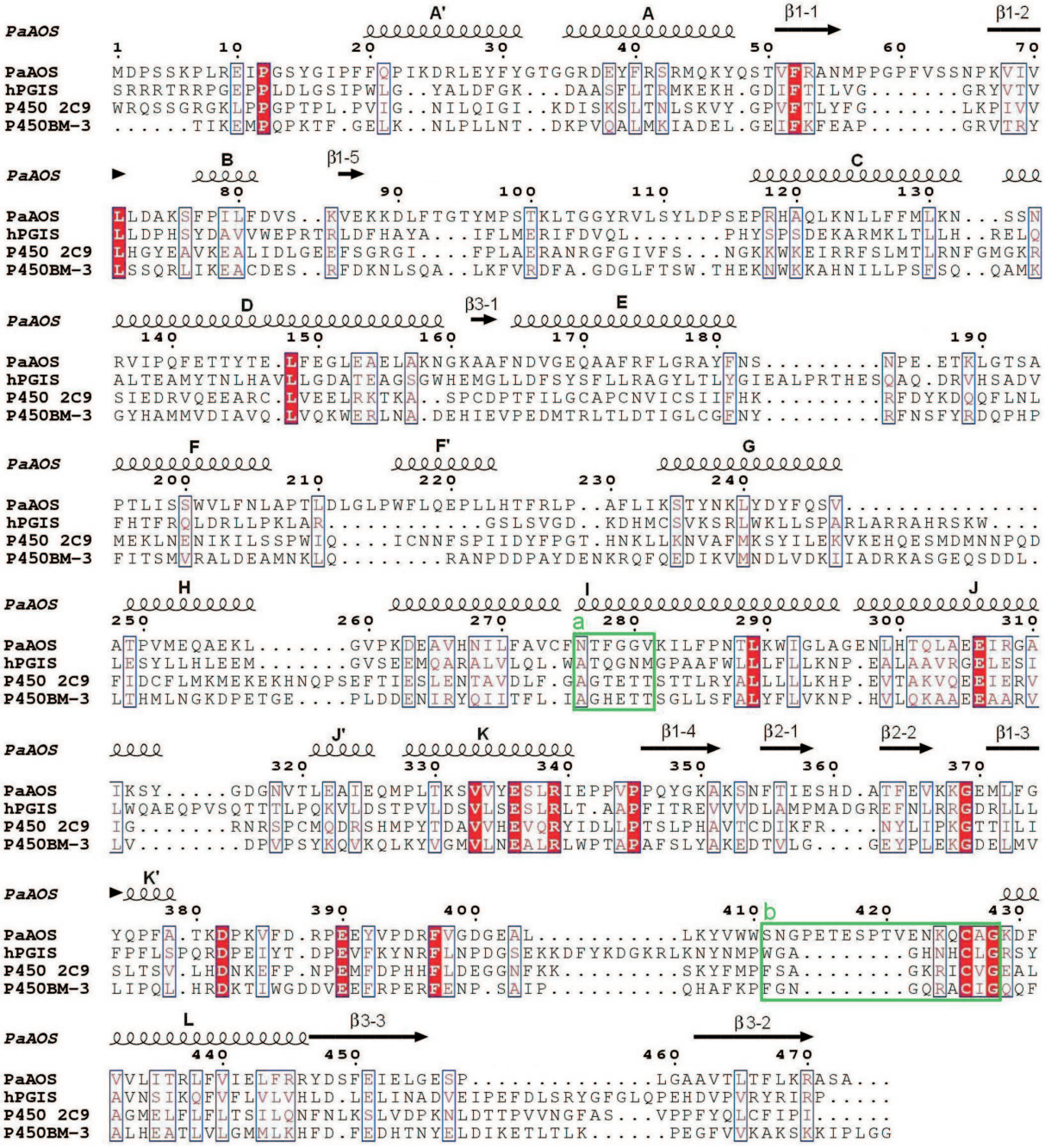

Fig. S1. Structure-based sequence alignment of allene oxide synthase (AOS) and human prostacyclin synthase (PGIS), human P450 2C9, and bacterial P450BM-3. The secondary structure elements observed in the AOS structure are shown above the alignment. The P450 signature sequence and heme-binding motif are enclosed in green boxes a and b, respectively. Conserved residues are highlighted. This figure was produced with ENDscript [Gouet P, Courcelle E (2002) ENDscript: A workflow with web interface to display sequence and structure information. *Bioinformatics* 18:767–768].

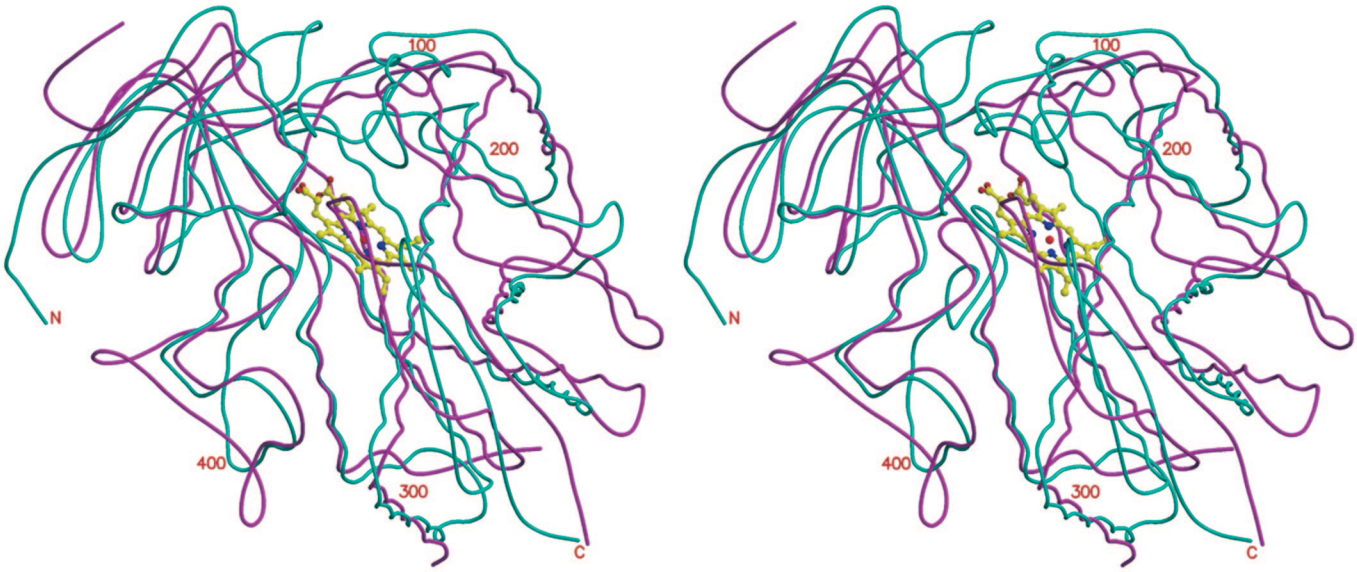

Fig. S2. Stereo diagram showing the superimposition of the structures of AOS (cyan) and human PGIS (magenta; PDB ID: 2IAG).

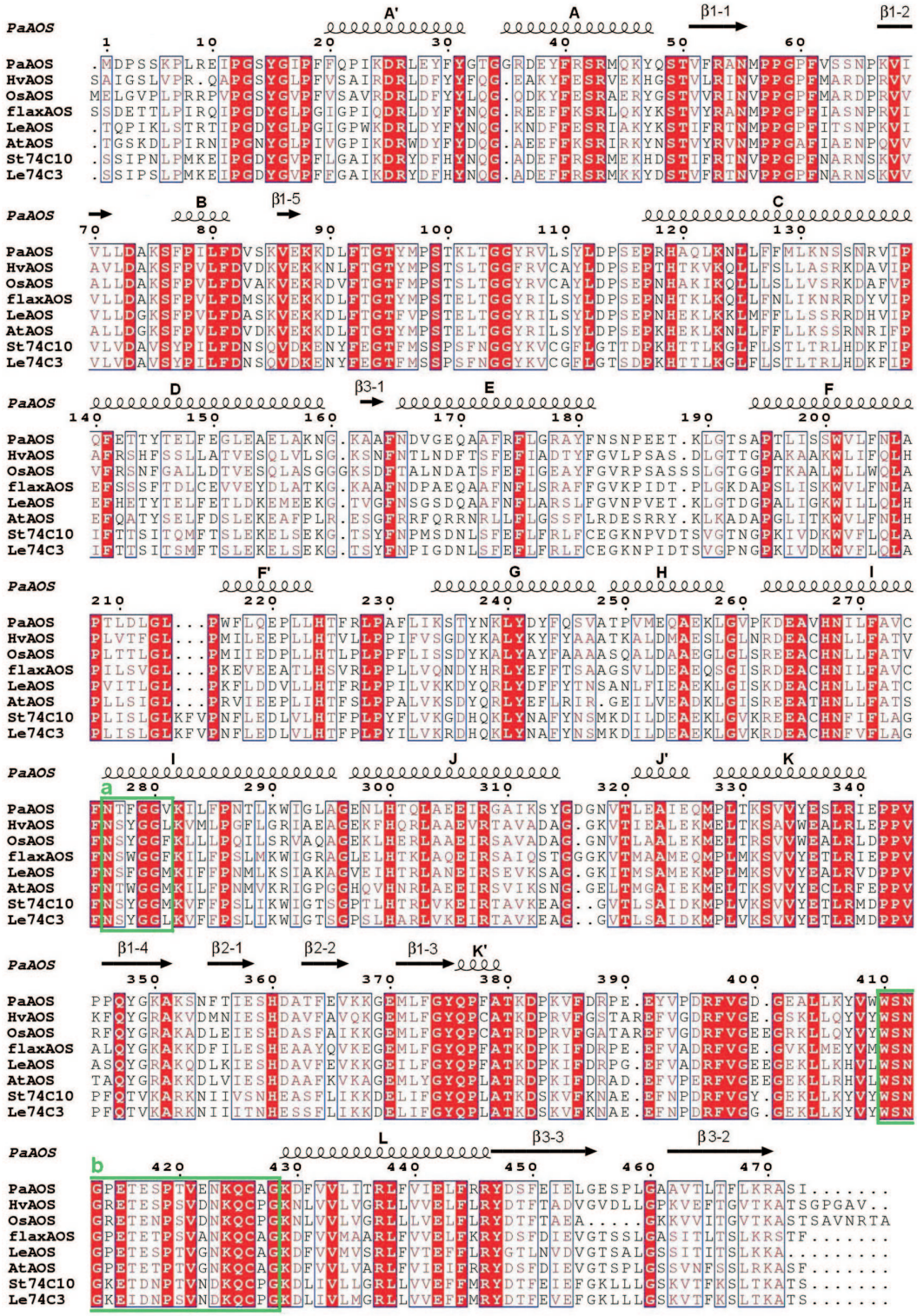

Fig. S3. Sequence alignment of AOSs from guayule (*Parthenium argentatum*), barley (*Hordeum vulgare*), rice (*Oryza sativa*), flaxseed (*Linum usitatissimum*), tomato (*Lycopersicon esculentum*), and *Arabidopsis thaliana*; CYP74C10 from potato (*Solanum tuberosum*) and CYP74C3 from *L. esculentum*. The N-terminal transit sequences were omitted for flax AOS, *Le*AOS, *At*AOS, *St*CYP74C10, and *Le*CYP74C3. The P450 signature sequence and heme-binding motif are enclosed in green boxes a and b. Conserved residues are highlighted.

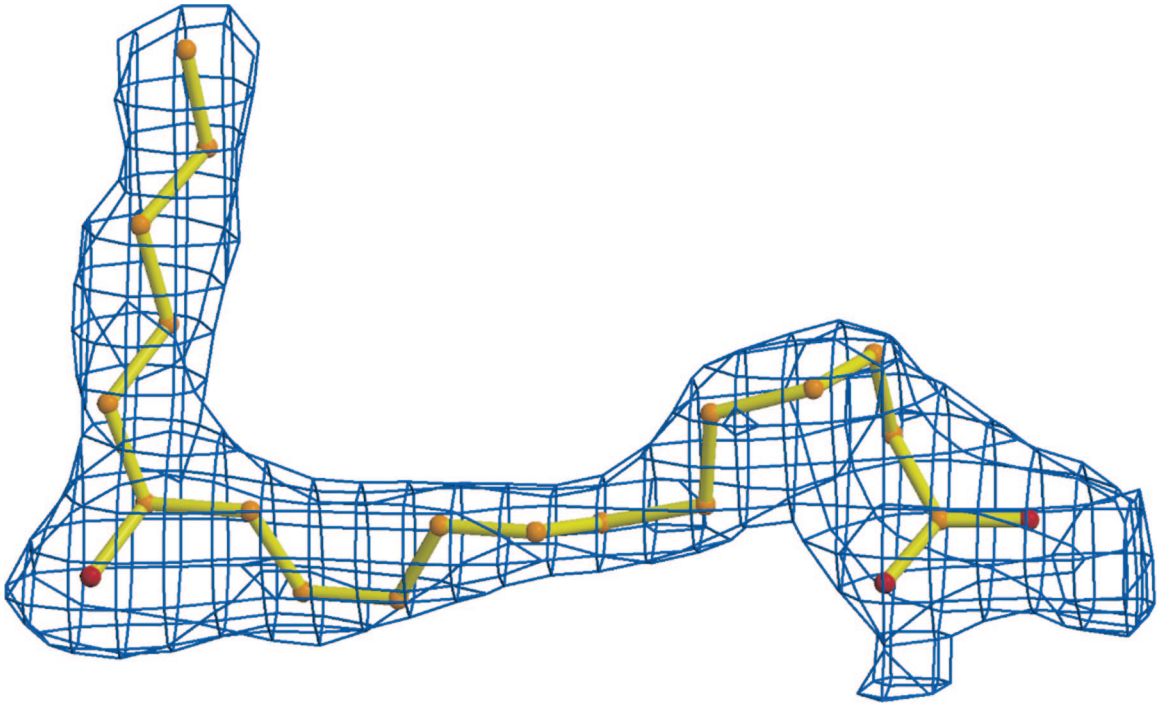

Fig. S4. $2F_o - F_c$ electron density omit map of substrate analog 13(*S*)-HODE contoured at 1.0 σ.

Table S1. Data collection and phasing statistics

Parameter	Native 1	Se	Fe	Native 2	13(*S*)-HODE
Space group	*I*422	*I*422	*I*422	I422	*I*422
Cell, Å	$a = b = 126.5$	$a = b = 126.0$	$a = b = 125.7$	$a = b =113.5$	$a = b = 128.3$
	$c = 163.9$	$c = 167.1$	$c = 166.3$	$c = 163.8$	$c = 160.0$
Resolution, Å	28.4–2.4 (2.49–2.4)	40.7–2.8 (2.9–2.8)	39.8–2.6 (2.69–2.6)	28.4–1.8 (1.86–1.8)	49.2–2.6 (2.69–2.6)
Wavelength, Å	1.5418	0.972	1.742	1.001	1.5418
Total reflections	127,004	181,074	199,076	407,286	107,397
Unique reflections	26,120	16,692	20,068	48,912	20,482
Completeness, %	98.6 (100)	99.0 (92.4)	96.0 (96.4)	98.1 (99.2)	98.0 (96.0)
R_{sym}[†], %	8.0 (59.4)	10.6 (58.8)	7.8 (55.9)	6.0 (45.5)	11.3 (33.3)
I/σ, I	15.2 (2.4)	17.9 (2.1)	24.0 (3.7)	29.5 (4.0)	10.5 (3.2)
Figurer of merit	0.22				

Numbers in parentheses are for the highest resolution shell.

[†]$R_{sym} = \Sigma_{hkl}|I - \langle I\rangle|/\Sigma I$, where I is the observed intensity and $\langle I\rangle$ is the average intensity from observations of symmetry-related reflections. A subset of the data (10%) was excluded from the refinement and used to calculate the free R value (R_{free}). R factor $= \Sigma||F_o| - |F_c||/\Sigma|F_o|$.

Table S2. Refinement statistics

Parameter	Native 1	Native 2	w. 13(*S*)-HODE
R factor, %	19.8	18.0	21.9
R_{free}, %	24.3	20.8	27.0
No. of protein atoms	3,742	3,782	3,742
No. of solvent atoms	206	378	147
No. of ligand atoms	43	43	64
Average *B* factors, Å^2			
All atoms	42.9	20.9	27.2
Protein	43.2	19.4	27.4
Solvent	40.7	36.0	24.4
Ligands	34.3	14.8	22.9
Rmsd from ideal values			
Bond length, Å	0.006	0.005	0.009
Bond angle, °	1.40	1.30	1.40