Table S1. Basic kinetic parameters for AA and AA-PE oxygenation by 15LO1, 15LO2 and LoxA in the absence and in the presence of PEBP1, Related to Figure 6.

Enzyme	Sample ID	V_{max} Pmole/min	V_{max} p value vs "no PEBP1"	K_M mM	K_M p value vs "no PEBP1"	$K_{cat}*10^{-2}$ s^{-1}	K_{cat}/K_M*10^{-3} $(mM\ S)^{-1}$	N
15LO1	Free AA no PEBP1	49.5 ± 9.1		150.9 ± 41.3		41.2	2.7	3
15LO1	Free AA plus PEBP1	33.4 + 5.3	0.224	65.7 ± 20.4	0.178	27.8	4.2	3
15LO1	AA-PE no PEBP1	1.9 ± 0.5		41.3 ± 17.2		0.08	0.019	6
15LO1	AA-PE plus PEBP1	2.7 ± 0.7	0.011	41.4 ± 11.4	0.986	0.11	0.027	6
15LO2	Free AA no PEBP1	228.2 ± 35.1		37.3 ± 7.2		47.5	12.7	3
15LO2	Free AA plus PEBP1	285.3 ± 61.8	0.236	62.4 ± 12.0	0.036	59.4	9.5	3
15LO2	AA-PE no PEBP1	14.5 ± 7.9		13.2 ± 5.5		0.6	0.5	8
15LO2	AA-PE plus PEBP1	33.0 ± 13.3	0.003	7.7 ± 3.8	0.038	1.4	1.8	7
LoxA	Free AA no PEBP1	78.4 ± 26.4		362.5 ± 124.9		65.3	1.8	4
LoxA	Free AA plus PEBP1	126.6 ± 19.6	0.066	442.1 ± 84.5	0.265	105.5	2.4	3
LoxA	AA-PE no PEBP1	1.8 ± 0.7		109.9 ± 65.4		0.08	0.007	3
LoxA	AA-PEBP plus PEBP1	1.1 ± 0.3	0.176	80.6 ± 37.2	0.575	0.02	0.006	3

Data are mean ± SD

Table S2. "Multiple docking" calculations of arachidonic acid (AA) to PEBP1 mutants, Related to Figure 6.

	wt PEBP1 (human PEBP1, 1beh)	Truncated C-terminal helix (175-186)	P112E mutant PEBP1
ENERGY (ΔG, kcal/mol)**	-5.7 ± 0.4	-5.4 ± 0.4	-5.2 ± 0.4
	-5.5 ± 0.1	-5.4 ± 0.2	-5.2 ± 0.3
	-5.1 ± 0.5	-4.4 ± 0.2	-4.8 ± 0.3
	-4.4 ± 0.3	-4.7 ± 0.2	-4.8 ± 0.0
	-4.3 ± 0.2	-4.7 ± 0.1	-4.9 ± 0.2
	-4.1 ± 0.3	--	-4.1 ± 0.1
	-3.7 ± 0.4	--	-4.1 ± 0.4[#]
	-3.5 ± 0.2	--	-4.2 ± 0.6
	-3.5 ± 0.3	-4.5 ± 0.2	-4.3 ± 0.4
Total energy (kcal/mol)	-39.8 ± 2.7	-29.1 ± 1.3	-37.5 ± 2.7
ΔΔG (a)(kcal/mol)	**0**	**10.7**	**2.3**
AA interaction with PEBP1†	76	35	38
Number of interactions present in wt*	76	30	32

(a) ΔΔG = change in binding free energy with respect to that of wt PEBP1 (i.e. ΔG (mutant) – ΔG (wt). A positive value indicates an increase in binding energy, which is unfavorable.

* Number of residues located with 3.5 Å from all nine AA ligands and present in a wt PEBP1.

† Excluding AAs (energy suffixed with[#]) which are interacting only with AA (lack of residues within 3 Å).

** Avarage value with standard deviation of 5 runs per value.

Table S3. Results on AA-binding (to PEBP1 (case 1) or Y176X (case 2)) energetics(a), Related to Figure 6.

Protein/ Mutant and substrate	Run1	Run2	Run3	Run4	Run5	Avg change(c) in binding energy compared to wt PEBP1-AA (kcal/mol)
Case 1 wtPEBP1 + 9AA molecules **	-5.7 ± 0.5	-6.2 ± 0.3	-5.7 ± 0.4	-5.7 ± 0.5	-5.4 ± 0.4	
	-5.3 ± 0.2	-5.6 ± 0.4	-5.5 ± 0.3	-5.2 ± 0.1	-5.4 ± 0.3	
	-5.3 ± 0.1	-5.2 ± 0.1	-5.3 ± 0.3	-5.6 ± 0.3	-5.3 ± 0.2	
	-4.8 ± 0.1†	-4.8 ± 0.2	-5.2 ± 0.3	-4.3 ± 0.4†	-4.8 ± 0.2	
	-4.5 ± 0.4	-4.5 ± 0.1	-4.8 ± 0.3	-4.4 ± 0.4	-4.4 ± 0.3	
	-4.5 ± 0.2†	-4.5 ± 0.3	-4.2 ± 0.2	-4.1 ± 0.4	-4.5 ± 0.4	
	-4.6 ± 0.2†	-4.3 ± 0.2	-4.3 ± 0.3#	-4.3 ± 0.3	-4.2 ± 0.2#	
	-4.4 ± 0.3	-4.6 ± 0.4#	-4.2 ± 0.2	-4.3 ± 0.3#	-4.5 ± 0.3	
	-4.1 ± 0.3	-4.4 ± 0.3	-4.5 ± 0.3	-4.2 ± 0.3	-4.3 ± 0.2	
TotalΔG(b) (kcal/mol)	**-29.5 ± 2.3**	**-39.5 ± 2.3**	**-39.4 ± 2.6**	**-33.5 ± 3.0**	**-38.6 ± 2.5**	**ΔΔG (kcal/mol) 0**
Case 2 Y176X + 9AA molecules **	-5.4 ± 0.4	-5.4 ± 0.1	-5.3 ± 0.4	-5.1 ± 0.1	-5.2 ± 0.1	
	-5.4 ± 0.2	-5.3 ± 0.3	-5.4 ± 0.3	-5.4 ± 0.3	-5.8 ± 0.4	
	-4.4 ± 0.2	-4.8 ± 0.1	-4.3 ± 0.1	-4.5 ± 0.1	-4.5 ± 0.3†	
	-4.7 ± 0.2	-5.1 ± 0.2†	-4.7 ± 0.3	-5.0 ± 0.4	-5.0 ± 0.3†	
	-4.7 ± 0.1	-4.6 ± 0.3	-5.0 ± 0.1	-4.8 ± 0.2†	-5.1 ± 0.1†	
	-5.0 ± 0.5	-4.5 ± 0.1#	-4.8 ± 0.5	-4.5 ± 0.1†	-5.0 ± 0.2	
	-5.0 ± 0.4†	-4.1 ± 0.3	-4.7 ± 0.4#	-4.2 ± 0.2†	-4.5 ± 0.3	
	-4.8 ± 0.4†	-4.3 ± 0.4	-4.1 ± 0.3#	-4.5 ± 0.4†	-4.4 ± 0.5#	
	-4.5 ± 0.2†	-4.4 ± 0.5	-4.4 ± 0.4#	-4.7 ± 0.2#	-4.6 ± 0.4	
Total ΔG (kcal/mol) (b)	**29.1 ± 2.6**	**-32.9 ± 2.3**	**-29.5 ± 2.8**	**-20.0 ± 2.0**	**-25.1 ± 2.6**	**ΔΔG (kcal/mol) 9.76**

(a) The entries in the table show the interaction energy between wt PEBP1 or Y176X mutant PEBP1 and AA molecules, upon binding one small molecule at a time, up to 9 molecules binding. #refer to interactions of AA molecules with previously bound AA molecules; †refers the same, except for the involvement of 1 PEBP1 residues.

(b) Total ΔG is obtained by summing up all the black entries in the corresponding run.

(c) Average change in free energy, relative to that obtained for the wt PEBP1 + 9 AA molecules.

** Avarage value with standard deviation of 5 runs per value.