Supporting Information Appendix

Binding site for coenzyme A revealed in the structure of pyruvate:ferredoxin oxidoreducatase from *Moorella thermoacetica*

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SI Appendix – Materials and Methods

SI Appendix – Figures S1-S11

SI Appendix – Tables S1-S4

SI Appendix – References

SI Appendix – Materials and Methods

Protein purification and activity. *Moorella thermoacetica* growth, enzyme isolation and protein quantification were performed as described previously (1). However, in the purification protocol, Tris-HCl buffer at pH 7.6 was used instead of MOPS. Enzyme quality was assessed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and enzyme activity assays. Enzyme assays were conducted at 20 ºC in 50 mM Tris-HCl buffer at pH 7.6 including 2 mM DTT, 1 mM TPP, 1 mM $MgCl_2$, 1 mM Coenzyme A hydrate, 10 mM sodium pyruvate, and 10 mM oxidized methyl viologen. The assay was started by adding PFOR and the absorbance at 578 nm of reduced methyl viologen was followed as a function of time. Activities of the different preparations of enzyme used in this study appeared homogeneous by SDS-PAGE analyses and exhibited pyruvate oxidoreductase activities of 8.3, 12.0, and 13.8 µmol pyruvate oxidized min^{-1} mg^{-1}.

Crystallization of native *Mt*PFOR and pyruvate soaking experiment. *Mt*PFOR was crystallized by sitting drop crystallization method in a Coy anaerobic chamber with an Ar/H_2 environment at room temperature. Brown plate crystals grew within 4-7 days of mixing 1.5 µL of 6.2 mg/mL *Mt*PFOR in storage buffer (50 mM Tris pH 7.5, 1 mM TPP (Sigma-Aldrich), and 1 mM $MgCl_2$) with 0.5 µL well solution (15-16% (w/v) PEG 4000, 0.21 M $(NH_4)_2SO_4$, and 0.10 M MES pH 6.0) to make a 2.0 µL sitting drop in a sealed well with 500 µL well solution. The crystal used to determine the native structure was transferred to cryoprotectant (20% (v/v) glycerol, 20% (w/v) PEG 4000, 0.07 M $(NH_4)_2SO_4$, and 0.10 M MES pH 6.0) briefly and flash-cooled in liquid nitrogen. The crystals used in pyruvate soaking experiments were transferred into cryoprotectant (20% (v/v) glycerol, 20% (w/v) PEG 4000, 0.18 M $(NH_4)_2SO_4$, 0.10 M MES pH 6.0, and 50 mM pyruvate (Sigma-Aldrich)) for 15 mins or 12 hrs and flash-cooled in liquid nitrogen. All the chemicals, unless stated otherwise, are from Hampton Research. The native *Mt*PFOR and pyruvate-soaked PFOR crystals formed in the space group *C2* with unit cell constants a = 340 Å, b = 108 Å, and c = 240 Å, β = 109°.

Cocrystallization of *Mt*PFOR with coenzyme A. *Mt*PFOR was crystallized with CoA in a Coy anaerobic chamber with an Ar/H_2 environment at room temperature using the sitting drop crystallization method. Brown plate crystals grew within 7 days of mixing 1.5 µL of 5.2 mg/mL *Mt*PFOR in storage buffer with coenzyme A (50 mM Tris pH 7.5, 1 mM TPP (Sigma-Aldrich), 1 mM $MgCl_2$, and 10 mM CoA (Sigma-Aldrich)) with 0.5 µL

well solution (13% (w/v) PEG 6000 and 0.06 M sodium cacodylate pH 6.5) to make a 2.0 μL sitting drop in a

sealed well with 500 μL of well solution. The crystal used to determine the CoA cocrystal structure was briefly

soaked into a cryoprotectant (10 mM CoA (Sigma-Aldrich), 15% (w/v) PEG 6000, and 20% (v/v) glycerol) and

flash-cooled in liquid nitrogen. All the chemicals, unless stated otherwise, are from Hampton Research.

Crystals formed in space group *C2* with unit cell constants a = 338 Å, b = 107 Å, and c = 120 Å, β = 110°.

Data collection and processing. Data were collected at the Advanced Photon Source on Northeastern

Collaborative Access Team beamline 24-ID-C on a Pilatus 6MF detector. Data were indexed and scaled in

HKL2000(2). Resolution cutoffs were chosen as CC1/2 ~ 0.8, which agrees with decrement of signal-to-noise

ratio and Rsym. Data for the native crystal, the pyruvate soaked crystals and the CoA cocrystal extended to

2.60-Å, 3.00-Å, 3.20-Å, and 3.30-Å resolution, respectively.

Structure determination and refinement. The structure of native *Mt*PFOR was determined to 2.60-Å

resolution by molecular replacement (MR) using Phaser(3) implemented in Phenix(4). The *Da*PFOR model

(PDB ID: 2C42(5)), which shares more than 60% sequence identity with *Mt*PFOR, was trimmed with the

Sculptor(6) algorithm implemented in Phenix(4) as the MR search model. The occupancy of TPP and [4Fe-4S]

clusters were set to zero for MR search. The MR result gave a single solution of three copies of PFOR

homodimer per asymmetric unit (ASU) with a log-likelihood gain (LLG) value of 18300 and a z-score of 114.

One round of rigid-body refinement and simulated annealing was performed after MR, with initial R_{work}/R_{free} =

35%/39%. The atomic coordinates and B-factors was iteratively refined in Phenix Refine(4) with model building

and manual adjustment of model in Coot(7). Water molecules were added manually throughout real space

refinements using Fo-Fc electron density contoured to 3.0σ as criteria. Non-crystallographic symmetry (NCS)

restraints were used throughout refinement. Restraints for [4Fe-4S] clusters were based on *M. thermoacetica*

carbon monoxide dehydrogenase/acetyl-CoA synthase (PDB ID: 3I01(8)). Restraints for TPP were based on

the crystal structure of *Saccharomyces cerevisiae* pyruvate decarboxylase (PDB ID: 2VK8(9)). Final cycles of

refinements include TLS parameterizations with three TLS groups per monomer, domain I-II, domain III, and

domain IV-VI. The division of TLS groups is assigned to capture the flexible nature of domain III. The final

model of the native structure contains residues listed in Table S2, one TPP per chain, and three [4Fe-4S]

clusters per chain.

Two structures of pyruvate-soaked *Mt*PFOR and one of *Mt*PFOR co-crystallized with CoA were determined to 3.00-Å, 3.20-Å, and 3.30-Å resolution by MR, respectively. The structure of native *Mt*PFOR was used as the MR search model in all cases. The MR results gave three dimers of PFOR per ASU in pyruvate-soaked *Mt*PFOR and three monomers of PFOR per ASU in CoA-cocrystallized *Mt*PFOR. In the latter case, two of the three monomers form a homodimer within the ASU, and the other monomer forms a homodimer with a monomer in a neighboring ASU across a crystallographic symmetry axis. The refinement protocol used was the same as for the native structure described above. However, additional restraints for CoA and the TPP adduct observed were required. In particular, restraints for CoA were based on the crystal structure of *Escherichia coli* acetyltransferase MccE (PDB ID: 3R9F(10)). Restraints for acetyl-TPP adduct and lactyl group of lactyl-TPP intermediate were generated from geometry optimizations followed by frequency calculations of 2-acetyl-3,4,5-trimethylthiazol-3-ium and 2-hydroxyl-2-methylpropanoate using B3LYP/6-311++G(11-13) with Gaussian 03(14). The calculated bond distances and angles for lactyl-TPP intermediate and acetyl-TPP adduct were used to update TPP restraints. The C2 of pyruvate in lactyl-TPP intermediate are restrained to be coplanar with the thiazolium ring. The carboxylic acid group and C2 of pyruvate in lactyl-TPP adduct are restrained to be planar. The acetyl group in acetyl-TPP adduct was restrained to be coplanar with the thiazolium ring. The final models contain three [4Fe-4S] clusters and one TPP or TPP adducts in each monomer. The model of PFOR cocrystallized with CoA contains one CoA molecule in each monomer. The detailed residue and TPP/TPP adduct composition is in Table S1 and S2.

Composite-omit electron density maps were calculated using Phenix(4) and used to verify all three models. All structure figures and solvent accessible surface area of proximal [4Fe-4S] clusters were rendered in PyMOL. Software used to process crystallography datasets was provided by SBGrid(15).

Activity Assays with PFOR crystals. Native PFOR crystals were grown to ~100x20x5 µm³ as described above, washed sequentially in two 2-µL of the crystallization well solution, and added to 2-µL of assay solution. Assay solution consisted of 50 mM HEPES 8.0, 10 mM oxidized methyl viologen, and a combination of reagents as follows: Drop 1 of **Figure S6**– 5 mM pyruvate and 5 mM CoA (no crystal); Drop 2 – 5 mM CoA and

one crystal (no pyruvate), Drop 3 – 5 mM pyruvate and one crystal (no pyruvate); Drop 4 – 5 mM pyruvate, 5

mM CoA, and one crystal. Drops were sealed and reagents were allowed to incubate. Photos of drops were

taken after 15 min and 12 hrs. After 12 hr, 1 µL of 5 mM CoA was added to each drop and incubated for

another 15 mins before the third photo was taken.

Multisequence alignment. Selection of representative sequences was described previously (16). Sequences

of OORs, which do not bind CoA, were not included and sequences of *St*OFORs, which were not considered

previously (16), were added. The alignment was performed using PROMAL3D(17). For sequences in which

"domain III" is not the first domain of a protein chain, residues prior to domain III were manually removed

before alignment to increase alignment quality. The full alignment is shown in Table S3. The UniProt IDs of

sequences chosen and alignment results are shown in Table S4.

SI Appendix – Figures

a Glycolysis

$CoAS^- +$ [pyruvate structure] $\longrightarrow CO_2 + 2e^- +$ [acetyl-SCoA structure]

Pyruvate:ferredoxin Oxidoreductase

$CO_2 \xrightarrow{+ 6e^-} \left[CH_3^+\right]$

Corrinoid Iron-Sulfur Protein

$CO_2 \xrightarrow[\text{Carbon Monoxide Dehydrogenase}]{+ 2e^-} CO \xrightarrow[\text{Acetyl-CoA Synthase}]{}$ [SCoA structure]

The Wood-Ljungdahl Pathway

ATP Synthesis
Biomass Synthesis

b

[oxalate structure] $\longrightarrow 2CO_2 + 2e^-$

Oxalate Oxidoreductase

$CO_2 \xrightarrow{+ 6e^-} \left[CH_3^+\right]$

Corrinoid Iron-Sulfur Protein

$CO_2 \xrightarrow[\text{Carbon Monoxide Dehydrogenase}]{+ 2e^-} CO \xrightarrow{}$ [SCoA structure]

The Wood-Ljungdahl Pathway

ATP Synthesis
Biomass Synthesis

$CO_2 + 2e^- +$ [acetyl-SCoA structure] $\longrightarrow CoAS^- +$ [pyruvate structure]

Pyruvate:ferredoxin Oxidoreductase

Biomass Synthesis

Figure S1. Relationships of PFOR and OOR to the Wood-Ljungdahl pathway. (a) Pyruvate from glycolysis is cleaved by PFOR, and the products can feed into the Wood-Ljungdahl pathway under certain cellular conditions. (b) OOR cleaves oxalate, generating both carbon dioxide and electrons for the Wood-Ljungdahl pathway. PFOR can convert the acetyl-CoA that is produced by the Wood-Ljungdahl pathway into pyruvate.
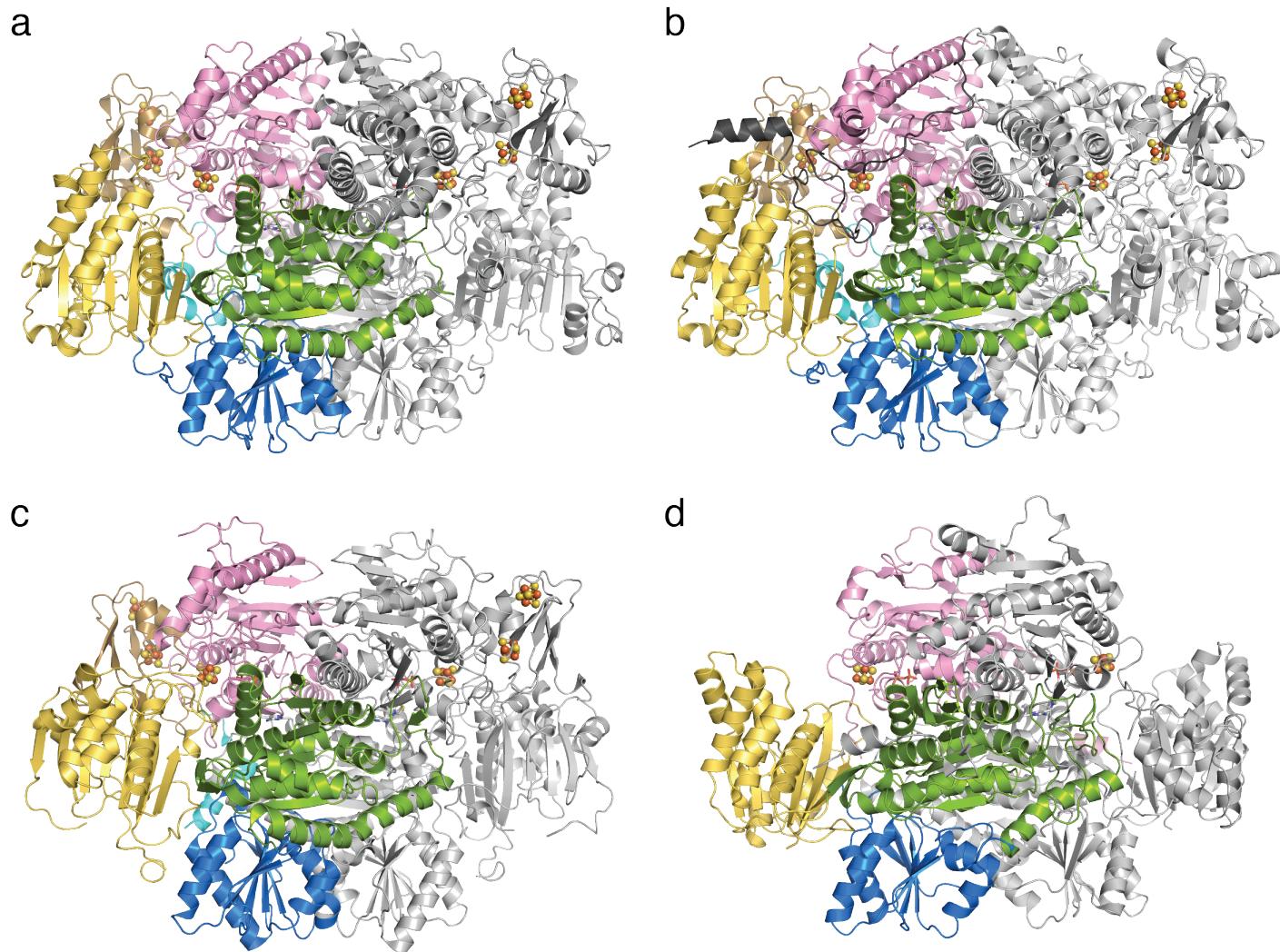
Figure S2. Overall OFOR structures. (a) *Mt*PFOR (b) *Da*PFOR (PDB ID: 2C3M(5)) (c) *Mt*OOR (PDB ID: 5C4I(18)) (d) *St*OFOR2 (PDB ID: 5B46(19)) Both *Mt*PFOR and *Da*PFOR are α_2 homodimers and are very similar in terms of overall structure. One difference is that domain VII is only found in OFORs from the *Desulfovibrio* genus. *Mt*OOR is a $(\alpha\gamma\beta)_2$ dimer of heterotrimers. Both structurally characterized *St*OFORs are dimers of heterodimers $(\alpha\beta)_2$ in which chain α contains domains I, II, and III, but the order of the domains with respect to the primary sequence is domain III-I-II from N-terminus to C-terminus. Color schemes and domain arrangements are in **Figure 2A**; domain VII of DaPFOR is shown as black ribbons.
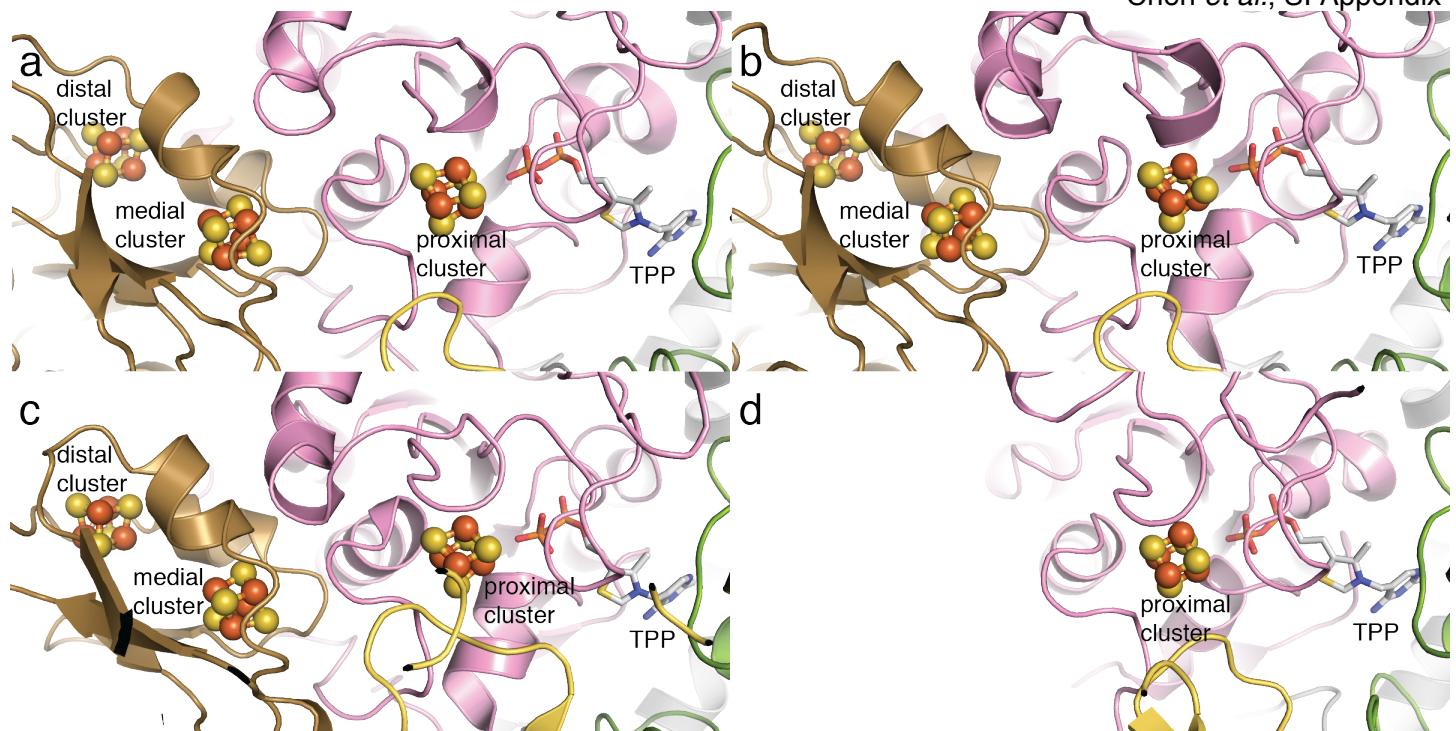
Figure S3. The redox active cofactors are oriented similarly. (a) *Mt*PFOR (b) *Da*PFOR (PDB ID: 2C3M(5)) (c) *Mt*OOR (PDB ID: 5C4I(18)) (d) *St*OFOR2 (PDB ID: 5B46(19)). Despite the differences in functions and oligomeric states, each catalytic unit adapts a similar fold that binds one [4Fe-4S] cluster and one TPP through domain VI and two [4Fe-4S] clusters in domain V. *St*OFORs lack domain V, but TPP and the only enzyme-bound [4Fe-4S] cluster are arranged in a similar orientation with respect to the other OFORs. TPP molecules are drawn in sticks. [4Fe-4S] clusters are drawn in ball-and-stick representations. Domain I, III, V and VI of each protein) are shown as ribbons in the same color scheme as **Figure 2a**.
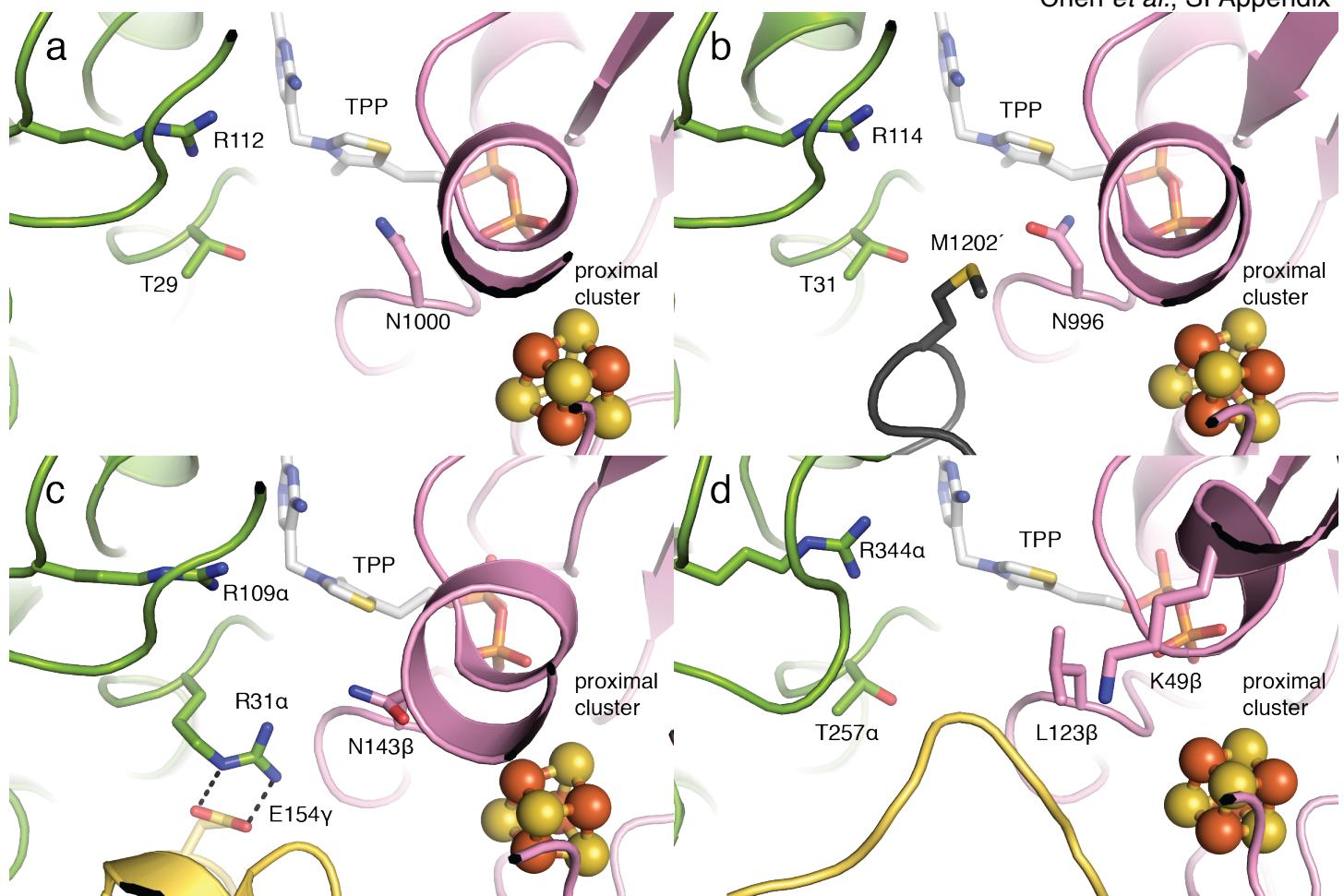
Figure S4. Active site residues in OFOR enzymes. (a) *Mt*PFOR (b) *Da*PFOR (PDB ID: 2C3M(5)) (c) *Mt*OOR (PDB ID: 5C4I(18)) (d) *St*OFOR2 (PDB ID: 5B46(19)). The active site residues are conserved between *Mt*PFOR and *Da*PFOR except for Met1202´, which belongs to *Da*PFOR's domain VII. Met1202´ plugs the active site, and thus pyruvate-binding residues are not solvent accessible in the crystal structure of *Da*PFOR. Active site residues in *Mt*OOR are similar to what is found in PFORs except Arg31α replaces Thr29 (*Mt*PFOR numbering). The difference affords a more electrostatic positive active site to bind oxalate, a dicarboxylic acid. The active site of *St*OFOR is larger than the other structurally characterized OFORs and contains two positively charged residues, Arg334α and Lys49β. The figures are in the same orientation as **Figure 4E**. Domain I, III and VI of each protein are shown as ribbons in the same color scheme as **Figure 2A**; domain VII of *Da*PFOR is shown as black ribbons. Active site residues and TPP molecules are drawn in sticks. [4Fe-4S] clusters are drawn in ball-and-stick representations.
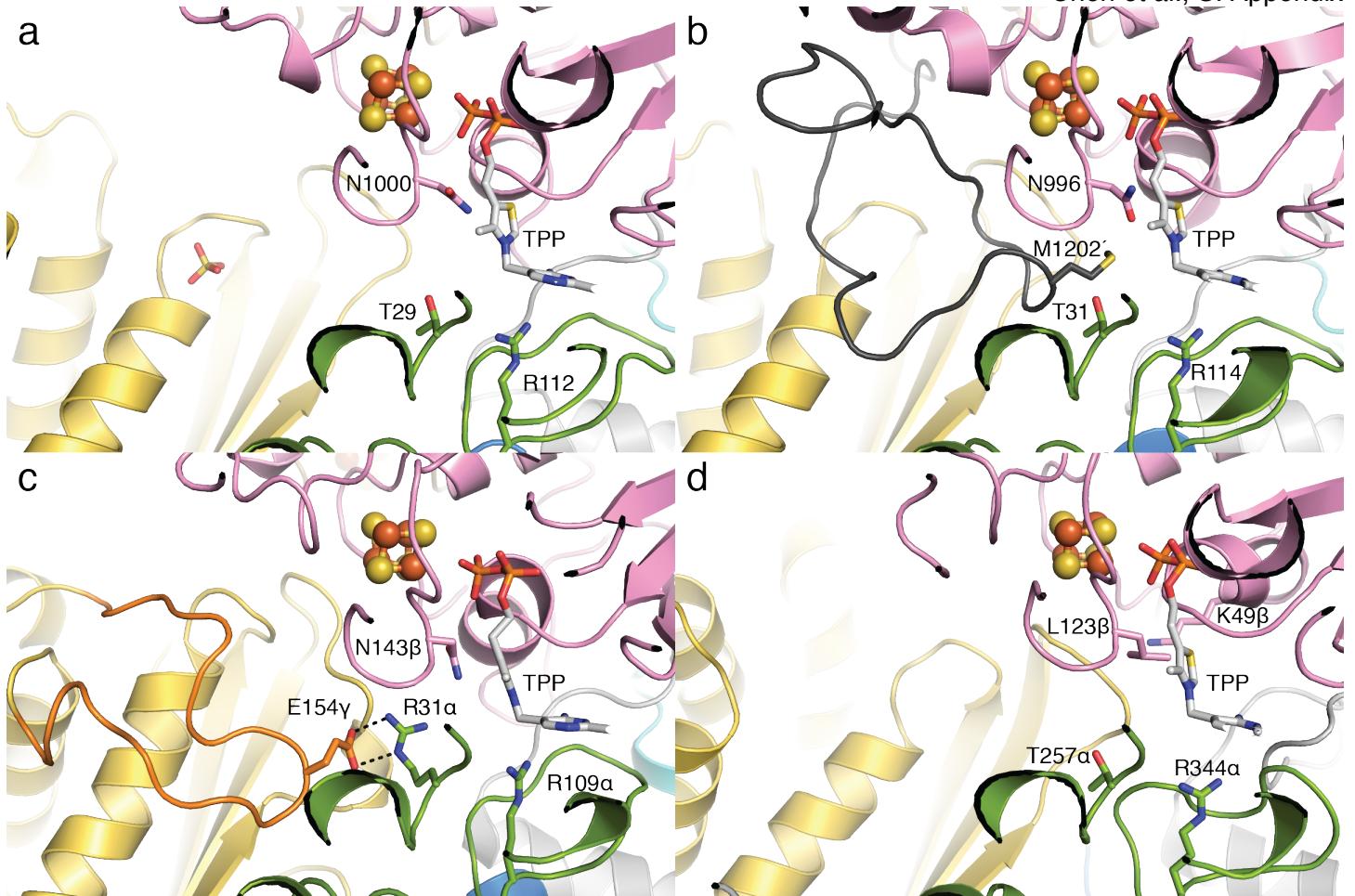
Figure S5. Differences in active site accessibility in OFORs. (a) The active site of *Mt*PFOR is open. Thr29 and Asn1000, which were identified as pyruvate-binding residues are solvent accessible. (b) The active site of *Da*PFOR (PDB ID: 2C3M(5)) is blocked by domain VII (black) from the other monomeric subunit of the homodimer in the crystal structure. (c) The active site of *Mt*OOR in the resting state (PDB ID: 5C4I(18)) is blocked by an extension from domain III referred to as the plug loop (orange). (d) The active site of *St*OFOR2 (PDB ID: 5B46(19)) is open. The figures are in the same orientation as **Figure 4A.** Domain I, III and VI of each protein are shown as ribbons in the same color scheme as **Figure 2A**. Active site residues and TPP molecules are drawn in sticks. [4Fe-4S] clusters are drawn in ball-and-stick representations.
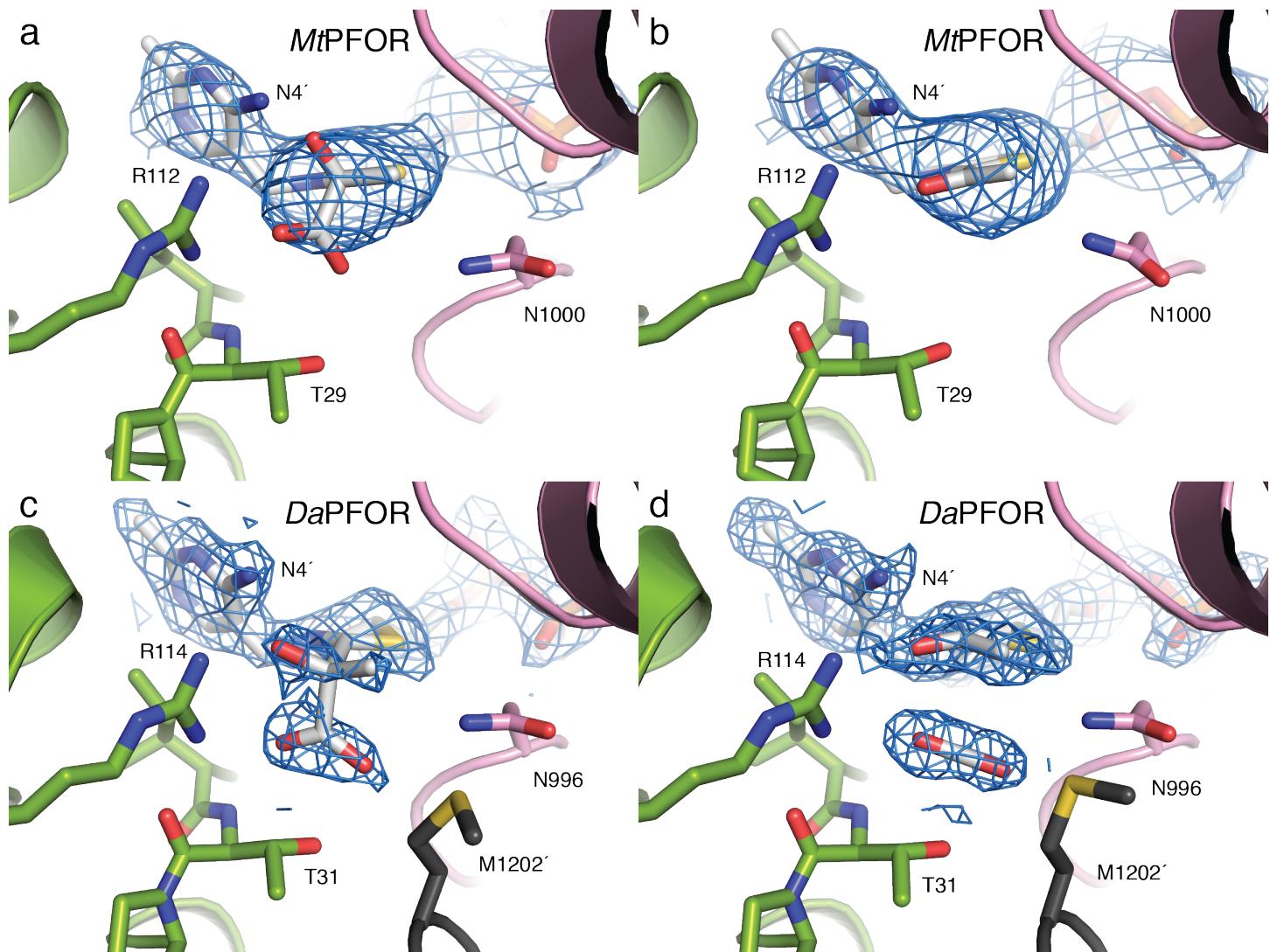
**Figure S6. The electron density of lactyl-TPP intermediates and acetyl-TPP adduct observed in *Mt*PFOR and *Da*PFOR.** (a) Composite-omit electron density map contoured to 1.0 σ in blue mesh for *Mt*PFOR soaked with pyruvate for 15-min. Lactyl-TPP intermediate state is modeled in the electron density. Four of six active sites in the asymmetric unit (ASU) show omit map density indicative of adduct formation. (b) Composite-omit electron density map contoured to 1.0 σ in blue mesh for *Mt*PFOR soaked with pyruvate for 12-hr. An acetyl-TPP adduct is modeled in the electron density. Density for an acetyl-TPP adduct is present in all six active sites in ASU. (c) 2Fo-Fc electron density map contoured to 1.0 σ is shown for lactyl-TPP intermediate state in *Da*PFOR (PDB ID: 2C3P(5)). The carbon-carbon bond between C2 carbon of TPP and the lactyl moiety is long at 1.9-Å. (d) 2Fo-Fc electron density map contoured to 1.0 σ for acetyl-TPP intermediate in *Da*PFOR (PDB ID: 2C3Y(5)). Carbon dioxide are refined into density near the acetyl-TPP adduct.

Figure S7. Crystallized PFOR retains pyruvate oxidation activity. Each 2- µL drop contains 50 mM HEPES pH 8.0, 10 mM oxidized methylviologen (MV) and a combination of substrates as follows: Drop 1 – negative control drop (no crystal) containing 5 mM CoA and 5 mM pyruvate; Drop 2 – negative control drop (no pyruvate) containing one crystal and 5 mM CoA; Drop 3 – negative control drop (no CoA) containing one crystal and 5 mM pyruvate; Drop 4 – one crystal, 5 mM CoA and 5 mM pyruvate. Crystals were looped and washed, before being added into each drop. (a) Results of 15 min incubation. MV is reduced in drop 4 turning the solution purple, but not in the negative control drops (1-3) (b) Results of 12 hr incubation. No MV reduction is observed in control drops (1-3). (c) Following a 12 hr incubation, 1 µL of 5 mM CoA was added into each drop and allowed to incubate for 15 min. With the addition of CoA to all drops, Drop 3 (the no CoA control) now turns purple.
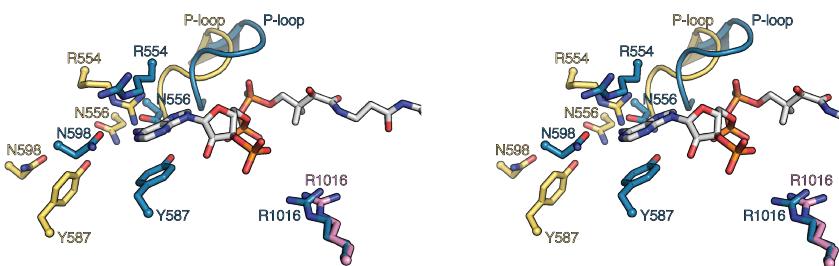
Figure S8. Stereo view showing that residues that contact CoA are not pre-organized for CoA binding. Adenine binding residues (sticks) and P-loop (ribbons) adopt different conformations with CoA (teal) and without (yellow). Movement of domain III also brings 3´-phospho group of CoA toward Arg1016 (without CoA – pink; with CoA – teal). Cαs are labeled as spheres.
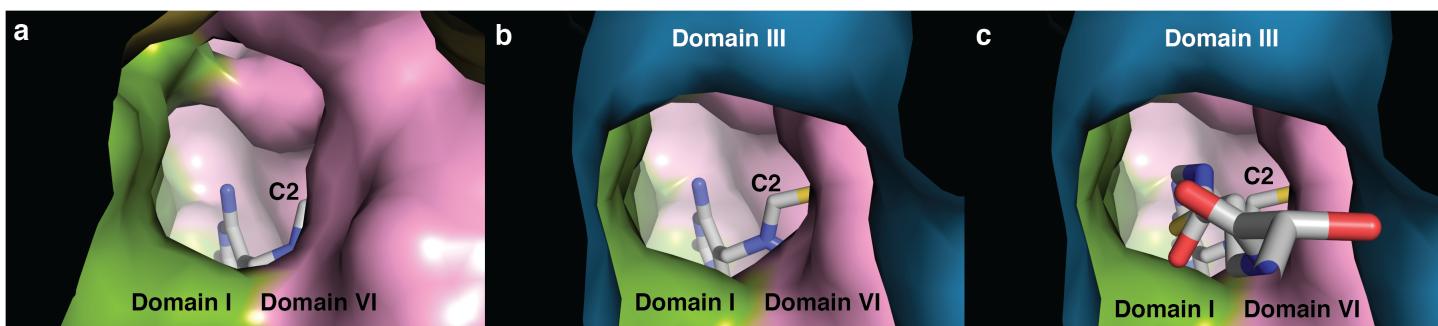
Figure S9. Surface representations of the *Mt*PFOR active site in different liganded states. (a) Channel to TPP is open in the CoA-free structure of *Mt*PFOR. (b) Channel to TPP is narrower in the CoA-bound structure of *Mt*PFOR due to movement of domain III (teal surface). CoA is omitted for clarity. (c) CoA-bound *Mt*PFOR structure showing CoA filling the narrow channel to TPP, occluding the TPP from solvent. CoA and TPP are shown as sticks. Position C2 of TPP, which initiates the nucleophilic attack on pyruvate, is labeled. Domain I surface is in green, domain III in teal, and domain VI in pink.
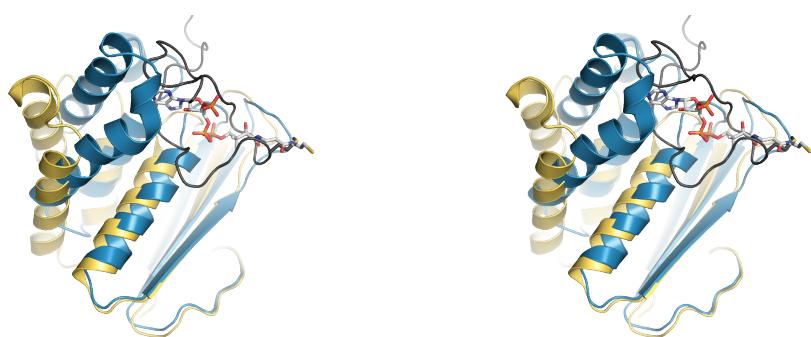
Figure S10. Stereo view of domain III and VII of *Da*PFOR (PDB ID: 2C3M(5)) **overlaid with domain III of *Mt*PFOR from CoA-bound structure.** Domain VII of *Da*PFOR (black) occupies the space where CoA binds in *Mt*PFOR and clashes with CoA-bound position of *Mt*PFOR's domain III. Domain III of *Mt*PFOR with CoA bound is colored teal; domain III of *Da*PFOR is colored yellow.
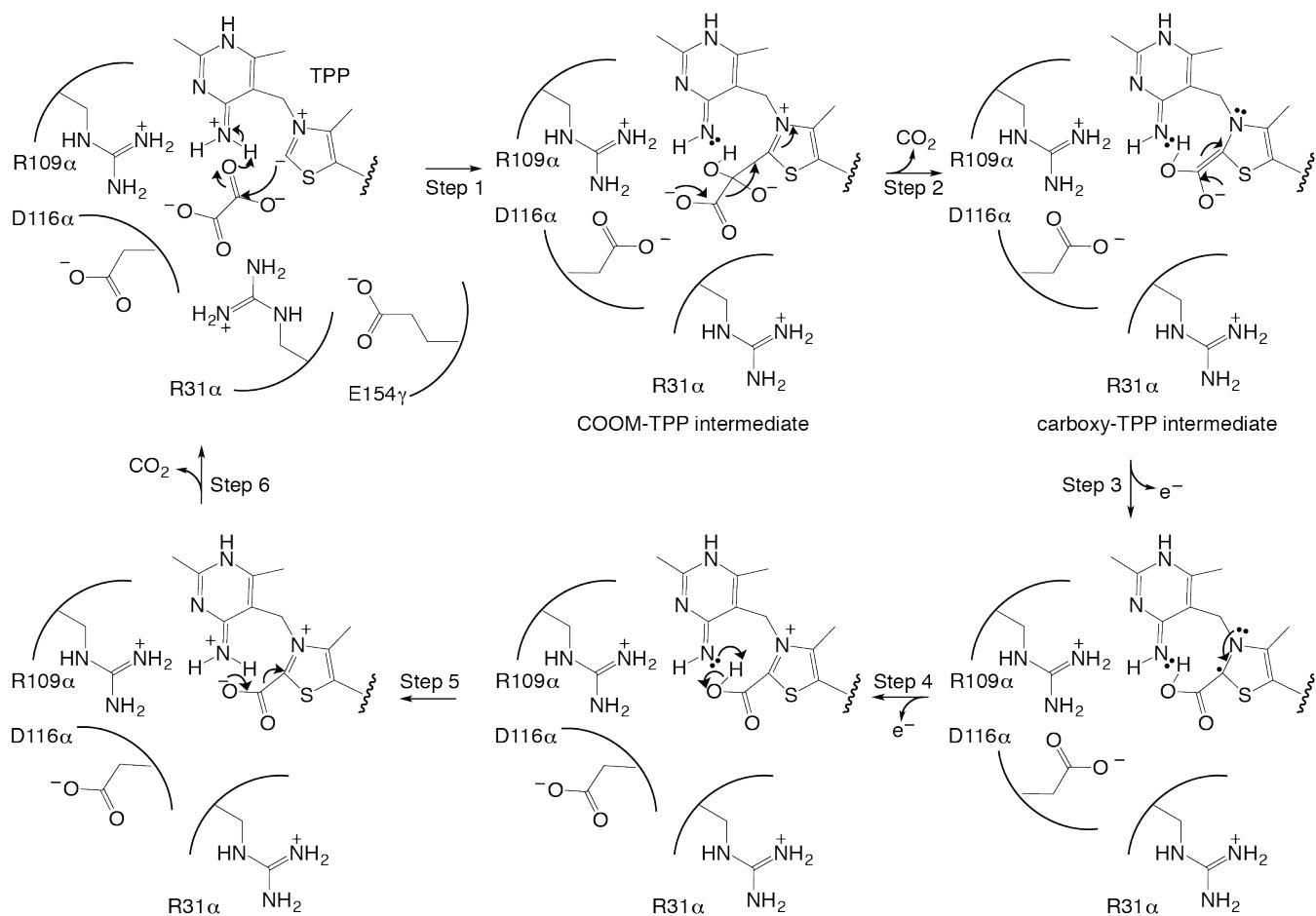
Figure S11. A bait-and-switch mechanism proposed for OOR. This mechanism for *Mt*OOR has been proposed based on structural snapshots of *Mt*OOR(18, 20) and computational studies of reaction intermediates (21-24). Active site residues Arg31α and Arg109α are proposed to activate oxalate, a dicarboxylic acid, for nucleophilic attack by the TPP, and protonation of the carboxy-di-oxido-methyl-TPP (COOM-TPP) is proposed to occur with the N4′ of TPP acting as the catalytic acid (**step 1**). Asp116α, which is part of a loop called the 'switch loop', is observed by crystallography to flip into the active site and point directly toward the COOM-TPP adduct, which is expected to facilitate decarboxylation through charge-charge repulsion (**step 2**). CO_2 release further requires movement of Arg31α out of the active site, which additionally alters the active site electrostatic environment to be less positively charged. Arg31α is not free to move, however, without the accompanying movement of domain III and the so-called plug loop that houses Glu154γ. The plug loop is named for the fact that it 'plugs' the active site burying the COOM-TPP adduct. The 'plugging' is secured by interaction between Glu154γ and Arg31α. Following decarboxylation, the first electron is transferred to a [4Fe-4S] cluster (**step 3**) and then the second electron is transferred (**step 4**). It has been proposed that the presence of negatively charged Asp116α in the active site facilitates these oxidations through charge repulsion (20). In the last step before release of the second CO_2 (**step 5**), Asp116α has been proposed to flip back, away from the active site, consistent with one crystal structure of a carboxyl-TPP intermediate in *Mt*OOR. This loop rearrangement removes the negative charge next to the carboxyl-TPP, thus facilitating deprotonation of the carboxylate and CO_2 release (**step 6**).

SI Appendix – Tables
Table S1. Data collection and model refinement statistics of *Mt*PFOR

	Native	Pyruvate soaked (15 mins)	Pyruvate soaked (12 hrs)	CoA cocrystallization (10 mM)
PDB ID	6CIN	6CIO	6CIP	6CIQ
Beamline	APS 24-ID-C	APS 24-ID-C	APS 24-ID-C	APS 24-ID-C
Space group	C2	C2	C2	C2
Cell dimensions (Å)	a=340.61, b=106.63, c=239.08, β=109.31°	a=340.39, b=107.10, c=239.56, β=109.67°	a=342.01, b=108.26, c=240.44, β=109.32°	a=337.71, b=106.99, c=120.47, β=109.85°
Wavelength (Å)	0.9791	0.9791	0.9789	0.9789
Resolution (Å)	100.-2.60 (2.64-2.60)	50.-3.00 (3.11-3.00)	100.-3.20 (3.26-3.20)	100.-3.30 (3.36-3.30)
# unique reflections	247841	157684	133390	58817
Completeness (%)	98.5 (95.7)	97.4 (92.6)	97.1 (84.9)	97.3 (83.0)
Redundancy	3.3 (3.0)	4.9 (4.1)	3.3 (2.7)	3.5 (2.7)
$<I/\sigma I>$	9.4 (2.1)	7.2 (1.7)	7.4 (1.5)	6.1 (2.1)
R_{sym}	0.075 (0.582)	0.160 (0.793)	0.092 (0.580)	0.159 (0.454)
$CC_{1/2}$	(0.796)	(0.781)	(0.836)	(0.798)
Resolution (Å)	89.0 - 2.60	48.5 - 3.00	97.5. - 3.20	80.3 - 3.30
# unique reflections	244551	157125	132694	58800
R_{work} (%) / R_{free} (%)	19.5/22.7	20.7/24.1	18.7/22.5	18.4/22.3
RMS bond lengths (Å)	0.003	0.003	0.003	0.003
RMS bond angles (°)	0.561	0.554	0.550	0.566
Number of Atoms/Molecules				
Protein atoms	53090	53163	52606	26589
TPP	6	2	0	3
Lactyl-TPP	0	4	0	0
Acetyl-TPP	0	0	6	0
[4Fe-4S] clusters	18	18	18	9
CoA molecules	0	0	0	3
Water molecules	439	54	27	29
Average B-factor (Å²)	77.5	75.3	103.4	95.0
Protein chains	77.7	75.5	103.5	95.0
TPP	68.9	69.0	n/a	66.7
Lactyl-TPP	n/a	61.1	n/a	n/a
Acetyl-TPP	n/a	n/a	89.9	n/a
[4Fe-4S] clusters	88.4	86.5	112.1	95.3
CoA molecules	n/a	n/a	n/a	127.1
Water molecules	51.3	36.5	57.1	40.1
Ramachandran plot				
Favored (%)	98.45	98.40	98.31	97.93
Allowed (%)	1.55	1.60	1.69	2.07
Outliers (%)	0.00	0.00	0.00	0.00
Rotamer outliers (%)	0.37	0.42	0.41	0.40

Table S2. Residues and cofactors modeled in each chain of all four structures.

	Chain					
	A	B	C	D	E	F
Native	2-1140, 1147-1170, 3 [4Fe-4S], 1 TPP	2-1170, 3 [4Fe-4S], 1 TPP	2-623, 630-1170, 3 [4Fe-4S], 1 TPP	3-586, 592-622, 629-1170, 3 [4Fe-4S], 1 TPP	2-625, 630-1170, 3 [4Fe-4S], 1 TPP	3-586, 597-1170, 3 [4Fe-4S], 1 TPP
Pyruvate soaking (15 mins)	2-1140, 1146-1170, 3 [4Fe-4S], 1 lactyl-TPP	2-1140, 1146-1170, 3 [4Fe-4S], 1 TPP	2-623, 632-1170, 3 [4Fe-4S], 1 lactyl-TPP	2-587, 594-624, 629-1170, 3 [4Fe-4S], 1 lactyl-TPP	2-622, 632-1142, 1146-1170, 3 [4Fe-4S], 1 TPP	2-587, 594-622, 631-1170, 3 [4Fe-4S], 1 lactyl-TPP
Pyruvate soaking (12 hrs)	2-1140, 1145-1170, 3 [4Fe-4S], 1 acetyl-TPP	2-1140, 1145-1170, 3 [4Fe-4S], 1 acetyl-TPP	2-623, 630-1170, 3 [4Fe-4S], 1 acetyl-TPP	2-624, 630-1170, 3 [4Fe-4S], 1 acetyl-TPP	2-624, 630-1170, 3 [4Fe-4S], 1 acetyl-TPP	2-1170, 3 [4Fe-4S], 1 acetyl-TPP
CoA cocrystallization	2-1170, 3 [4Fe-4S], 1 TPP, 1 CoA	2-624, 628-1170, 3 [4Fe-4S], 1 TPP, 1 CoA	2-622, 631-1170, 3 [4Fe-4S], 1 TPP, 1 CoA	N/A	N/A	N/A

Table S3. Sequence alignments of domain IIIs of CoA-dependent OFORs. The sequences are arranged in the same order as Table S4.

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Conservation:                                                  8 66 9
sp_O05650_PORC_THEMA_Pyru    1  --------------------MPVAKKYFEIRWHGRAGQGAKSASQMLAEAA-LEAGKYVQAFPEYGAER    48
tr_M3NSB1_M3NSB1_HELPX_Py    1  ------------------------MFQIRWHARAGQGAITGAKGLADVI-SKTGKEVQAFASYGSAK    42
tr_Q3Z8I7_Q3Z8I7_DEHM1_Py    1  ----------------------MKHFIEIRWHGRGGQGAVTSAELIAQAA-IGKGKYAQAFPSFGPER    45
sp_P80523_PORC_METBF_Pyru    1  -----------------------MKEIRIHGRGGQGSVTAAEMLSVAA-FEDGKFSQAFPAFGVER    42
sp_P80902_PORC_METTM_Pyru    1  -----------------------MIEIRFHGRGGQGAVTAAEILAKAA-FEDGKYSQAFPFFGVER    42
tr_W8CQB1_W8CQB1_9EURY_Py    1  -----------------------MIEIRFHGRGGQGAVTAANILAEAA-FLEGKYVQAFPFFGVER    42
tr_Q4KY23_Q4KY23_TRIVA_Py  411  ------------------------PEGTKQCMFWGLGSDGTVGANKQAIKLIVSNTKLYGQAYFAYDAHK   456
sp_P94692_POR_DESAF_Pyruv  414  ------------------------PKGTIQCQFWGLGADGTVGANKQAIKIIGDNTDLFAQGYFSYDSKK   459
tr_Q2RMD6_Q2RMD6_MOOTA_Py  411  ------------------------PKGTFRCKFFGLGSDGTVGANKNSIKIIGDHTDMYAQGYFVYDSKK   456
tr_C4LTX6_C4LTX6_ENTHI_Py  409  ------------------------PEGTTQCMFWGLGSDGTVGANHDAIRIIGQNTDMYVQGYFSYDAHK   454
sp_Q968X7_PNO_CRYPV_Pyruv  417  ------------------------TSETKQCLFWGLGSDGTVSANKNAIKIIGESTDLQVQGYFAYDAKK   462
sp_Q94IN5_PNO_EUGGR_Pyruv  480  ------------------------PEGTRQCVFWGIGSDGTVGANRSAVRIIGDNSDLMVQAYFQFDAFK   525
tr_L8B958_L8B958_CHLRE_Py  522  ------------------------PKGTFECLFWGMGSDGTVGANKEAIKIIASSAGMSAQAYFSYDAHK   567
sp_Q53046_NIFJ_RHORT_Pyru  422  ------------------------SADIKRSVFFGLGADGTVGANKNSIKIIISDSPTIHGQGYFVYDSKK   467
tr_B5XPH3_B5XPH3_KLEP3_Py  414  ------------------------HAGITACKFWGMGSDGTVGANKSAIKIIGDNTPLYAQAYFSYDSKK   459
tr_Q24982_Q24982_GIAIN_Py  437  ------------------------PEGTTECILWGLGSDGTIGACRNAMKILSDRVGCECQANFEFDGKK   482
tr_I2K9Y6_I2K9Y6_9PROT_Py    1  ------------------MAEQKKKERYNIRISGLGGQGVVTTAHILGSTM-DNAGKYASLVPFFGSEK    50
tr_O67231_O67231_AQUAE_Fe    1  ---------------------M--KRYNIRIAGVGGQGVVTSAHIIGNAM-AAAGKYASLVPFFGSEK    44
tr_I0IRW0_I0IRW0_LEPFC_Fe    1  --------------------MNKERYNIRMAGIGGQGVVTASHILSNGM-VIMGGESTLVPFFGSEK    46
tr_J9ZD37_J9ZD37_LEPFM_Py    1  --------------------MSAMKRINIRMSGLGGQGVVTSAHIMAMAA-SKEDKFSISNPFFGAEK    48
tr_D3DJJ8_D3DJJ8_HYDTT_Py    1  --------------------MKRRRVNIRMPALGGQGAVTAAHIIATAA-DYEGYYAVSNPFFGAEK    46
sp_P84820_PORC_THELN_Pyru    1  ------------------------MIEIRFHGRGGQGAVTAANILAEAA-FLEGKYVQAFPFFGVER    42
sp_P80907_VORA_METTM_Keto  290  --------------------DDPEFREVRVKIAGFGGQGVLSMGLTLAQAA-CSEGRHTSWYPAYGPEQ   337
tr_A1HTT9_A1HTT9_9FIRM_Py    1  ------------------------MTHEIIMAGFGGQGVMLMGQLVTYAG-MIEGKQVSWIPSYGPEM    43
tr_B0R3G0_B0R3G0_HALS3_Ox    1  ---------------------MHDDLNWAIGGEAGDGIASTGKIFAQAL-SRAGRHVFTSKDFASRI    45
tr_H3ZPH3_H3ZPH3_THELN_2-    1  -----------------------MQIRLAGIGGQGVVLAGIILGEAA-AIEGLNVIQTQDYGSQS    41
tr_Q1IQP1_Q1IQP1_KORVE_2-    1  ---------------------MQRLQLTEIRIAGFGGQGVILSAIVIGKAASILEGGFATMTQSFGPEA    48
tr_E8RJ92_E8RJ92_DESPD_2-    1  ----------------MSKNAPSSQTEIIVTGFGGQGIILAGRILGMAASLGDKKESTLVQAYGPES    51
sp_P80906_KORC_METTM_2-ox    1  ---------------------MRKEIRIAGFGGQGVILAGIVLGKAASLYDGLYAVQTQSYGPEA    44
tr_O68230_O68230_HELPX_Oo    1  ---------------------MEAQLRFTGVGGQGVLLAGEILAEAK-IASGGYGTKTSTYTSQV    43
sp_O53182_KORA_MYCTU_2-ox    1  MDPNGSGAGPESHDAAFHAAPDRQRLENVVIRFAGDSGDGMQLTGDRFTSEA-ALFGNDLATQPNYPAEI    69
tr_H0DIR4_H0DIR4_9STAP_2-    1  ----------------------MRNQISWKVGGQQGEGIESTGEIFATAM-NRKGYYLYGYRHFSSRI    45
tr_O87870_O87870_THAAR_2-    1  ---------------------MTARSVSITFAGSGGAGVMTAGSMLLDAA-GHAGWYAYMTRSSGAQI    46
tr_B0R4X6_B0R4X6_HALS3_Py    1  ----------------------MTDDELIWRIAGGSGDGIDSTSQNFAKAL-MRSGLDVFTHRHYPSRI    46
tr_D3DI99_D3DI99_HYDTT_2-    1  ----------------------MAFDLTIKIGGEGGEGVISAGDFLTESA-ARAGYYVVNFKSFPAEI    45
sp_O07836_IORB_THEKO_Indo    1  -----------------------MKEYNIVITGVGGQGILTAANLLGWAA-LRAGYKVRVGEVHGMSQ    44
tr_A1RYA4_A1RYA4_THEPD_In    1  ----------------------MAGKSIVIAGVGGQGLITIGTVVAQAL-IRKGYSVRVGEVHGLSQ    44
tr_K4MBD5_K4MBD5_9EURY_In    1  ------------------MSAPGISQFDVIIAGVGGQGAILASDIIGKAA-VKENLSVRAAETHGMAQ    49
sp_P80911_IORB_METTM_Indo    1  -----------------------MSYNIYVCGVGGQGIIKTSVIGEAA-MNEGMNVVMSEIHGMAQ    43
tr_P72578_P72578_SULSP_2-    1  ----------------------MRLSWVIGGAQGTGIDTAANIFGNAV-ASAGYYIYGNREYYSNI    43
tr_Q96Y66_Q96Y66_SULTO_2-    1  ----------------------MRLSWVIGGAQGTGIDTAANIFGNAV-ASAGYYIYGNREYYSNI    43
tr_Q96XT2_Q96XT2_SULTO_2-    1  ----------------------MTRIVWMIGGAQGLGVDTSANIFGNAV-AKAGYYLFGNREYYSNI    44
Consensus_aa:                   ............................ph.h.G.ttpGh..hsp.h.phh....s..s......@ssp.
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Yellow – P-loop
Red – Positively changed residue predicted to be within hydrogen bond distance with the proximal [4Fe-4S] cluster

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Conservation:                        6          6                    6
sp_O05650_PORC_THEMA_Pyru     49  TG------APMRAFNRIGDEYIRVRS-AVENPDVVVVIDETLLSP----------------AIVEGLSED    95
tr_M3NSB1_M3NSB1_HELPX_Py     43  RG------AAMMAYNRVDDEPILNHE-RFMQPDYVLVIDPGLVFIE--------------NIFANEKED    90
tr_Q3Z8I7_Q3Z8I7_DEHM1_Py     46  RG------APVQSFNRISDDKPIRERSGISEPDIVVVLDPSLVIIG--------------NVISGLKEG    94
sp_P80523_PORC_METBF_Pyru     43  RG------APVQAFTRINNNPIRLRS-QVYTPDYVIVQDATLLETV--------------DVASGVKDD    90
sp_P80902_PORC_METTM_Pyru     43  RG------APVMAFTRINDEPIRRRY-QVYNPDYVVVLDEGLVQVN--------------DVFSGLKAD    90
tr_W8CQB1_W8CQB1_9EURY_Py     43  RG------APVTAFTRIDEKPIRIKT-QIYEPDIVVVLDPSLLDTV--------------DVTAGLKDG    90
tr_Q4KY23_Q4KY23_TRIVA_Py    457  SG------GVTTPHLRFGAKPINAPY-YVQNADYIACHNPSYLHKF--------------DMTKQLKKG   504
sp_P94692_POR_DESAF_Pyruv    460  SG------GITISHLRFGEKPIQSTY-LVNRADYVACHNPAYVGIY--------------DILEGIKDG   507
tr_Q2RMD6_Q2RMD6_MOOTA_Py    457  SG------GVTISHLRFGKQPIQSAY-LIDQADLIACHNPSYVGRY--------------NLLEGIKPG   504
tr_C4LTX6_C4LTX6_ENTHI_Py    455  SG------GVTVSHLRFGQKPIKSQY-LIQNADYTACHFPNYVKKY--------------KLLDAAKPN   502
sp_Q968X7_PNO_CRYPV_Pyruv    463  AG------GATMSHLRFGPKPIKSAY-LLQRCDYVAVHHPSYVHKF--------------DVLENIKQG   510
sp_Q94IN5_PNO_EUGGR_Pyruv    526  SG------GVTSSHLRFGPKPITAQY-LVTNADYIACHFQEYVKRF--------------DMLDAIREG   573
tr_L8B958_L8B958_CHLRE_Py    568  SG------GVTVSHLRFGPSPIDSPY-LVQQADYLAVNHQSYMAKY--------------DTLASLKPG   615
sp_Q53046_NIFJ_RHORT_Pyru    468  SG------AITISHLRFGPRPIRAPY-LIDEADFIACHHFSFLDKV--------------DVLETAAVG   515
tr_B5XPH3_B5XPH3_KLEP3_Py    460  SG------GITVSHLRFGDRPITSPY-LIHRADFIACSQQSYVDRY--------------DLLEGLKPG   507
tr_Q24982_Q24982_GIAIN_Py    483  SG------GTTVSHLRFGPKKIRAQY-NIEEAGYVACHAQSYVSKF--------------NVLHGIKED   530
tr_I2K9Y6_I2K9Y6_9PROT_Py     51  RM------APVEAYVRASSEPIYEVG-EVVYPDIIMIYHSQVVTHGKS----------YTMPFYTGLKPN  103
tr_O67231_O67231_AQUAE_Fe     45  RM------APVEAYVRASDQPIYEVG-EVVYPNVIMIYHPQVITHGKS----------YTMPFYSGLKEN   97
tr_I0IRW0_I0IRW0_LEPFC_Fe     47  RL------APVESYVRIANGKIYEIG-EIIYPNLIMIFHPQVITHGKS----------YTMPFYSGLKPN   99
tr_J9ZD37_J9ZD37_LEPFM_Py     49  RM------APAESYVRIGPEKIYDRG-ELVYPDVVMVFHPQVITMGKS----------YTMPFYSGIKKN  101
tr_D3DJJ8_D3DJJ8_HYDTT_Py     47  RM------APAESYARIGIEPIYDRG-EVVYPDVIMVFHPQVITMGKS----------YTMPFYSGIKKN   99
sp_P84820_PORC_THELN_Pyru     43  RG------APVTAFTRIDDKPIRIKT-QIYEPDVVVVLDPSLLDTV--------------DVTAGLKEG    90
sp_P80907_VORA_METTM_Keto    338  RG------GTSSCGVVISGERVGSP--AVDTPDVLVAFNQPSLDE----------------FAGDVREG   382
tr_A1HTT9_A1HTT9_9FIRM_Py     44  RG------GTANCSVIVSDEAIGAP--IVTEPTAVVAMNLPSLDK----------------FESALLPG    88
tr_B0R3G0_B0R3G0_HALS3_Ox     46  RG------GYTAYKVRTSVDQVQSV---VDRLDILIALTERTVDE----------------NLDELHAD    89
tr_H3ZPH3_H3ZPH3_THELN_2-     42  RG------GHSIADLIISKEPIYDL--MVTKADILVALAQLGYNS----------------TKNSLREG    86
tr_Q1IQP1_Q1IQP1_KORVE_2-     49  RG------GACSAQVVIDSKPVLYP--YVTNPDILIVMSQEAYTK----------------FGPELKPG    93
tr_E8RJ92_E8RJ92_DESPD_2-     52  RG------GACNAQVIISDVPIHYP--YVNTPKILVAMSQAGYDK----------------FAPALVPE    96
sp_P80906_KORC_METTM_2-ox     45  RG------GASRAEVVISDEEIDYP--KVQSPDILVAMSHQALLT----------------YMDDLKAG    89
tr_O68230_O68230_HELPX_Oo     44  RG------GPTKVDLSLDRNEIIFPYGKGEGEIDFMLSVAQISYNQ----------------FKSDIKQG    90
sp_O53182_KORA_MYCTU_2-ox     70  RAPAGTLPGVSSFQIQIADYDILTA---GDRPDVLVAMNPAALKA----------------NIGDLPLG   119
tr_H0DIR4_H0DIR4_9STAP_2-     46  KG------GHTNNKIRVSTSPVHAV---SDNLDILVAFDQETIEV----------------NHHEMRAD    89
tr_O87870_O87870_THAAR_2-     47  RG------GEAAAMLRLSTTPVQSH---DDHFDMLVAIDWQNVGRF--------------AAEVPMTAD    92
tr_B0R4X6_B0R4X6_HALS3_Py     47  RG------GHTYVEIRARDGTVTSR---GDGYNFLLALGDSFARNPSEEAVYGDEEVKPLTENLDDLRAG  107
tr_D3DI99_D3DI99_HYDTT_2-     46  KG------GYAQSTIRVSNKKLYTT---GDGFDILCCFNGEAYEF----------------NRKHLRPG    89
sp_O07836_IORB_THEKO_Indo     45  RF------GSVIAYVRFGEDVYGAMV-PEGKADVILSFEPVEALR----------------YINYLKKG    90
tr_A1RYA4_A1RYA4_THEPD_In     45  RG------GSVVVFLKYGQGPLSPIV-DQGEADVLLGLELIETLR----------------RVPLLSKE    90
tr_K4MBD5_K4MBD5_9EURY_In     50  RG------GSVVNHIRIGCTLGSMI--SLGGADVLLALEPSEALR----------------YLDYLAED    94
sp_P80911_IORB_METTM_Indo     44  RG------GAVSTEIRFGDVRGSII--PQGEADLVIAFEPLEALR----------------ALPKMSED    88
tr_P72578_P72578_SULSP_2-     44  KG------GHSYFSLTISDKRVRSN---TQKIDILVSFDAETVFQ----------------HFYDVKDI    87
tr_Q96Y66_Q96Y66_SULTO_2-     44  KG------RHSYFSLTISDKRVRSN---TQKIDILVSFDAETVFQ----------------HFYDVKDI    87
tr_Q96XT2_Q96XT2_SULTO_2-     45  KG------RHSYFEVVISEKPIRSL---SSYVNILASFDAETVFQ----------------HFTETKEY    88
Consensus_aa:                     pt......t.s.s.h.hs...l..........shlhsh....h.....................hp.s
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Conservation:
sp_O05650_PORC_THEMA_Pyru    96  -----GILLVNTV--KDFE----------------------FVRK--KTGFNGKICVVDATDIALQEI  132
tr_M3NSB1_M3NSB1_HELPX_Py    91  -----TTYIITSY--LNKE----------------------ELFEKKPELKTRKVFLVDCLKISMETL  129
tr_Q3Z8I7_Q3Z8I7_DEHM1_Py    95  -----GTLIINTT--KPLD----------------------YFVS--EYGDRWKIATVDATAIAKELL  131
sp_P80523_PORC_METBF_Pyru    91  -----GIIIVNTT--ENPESL--------------------KLNTKARVMTVDATKVAMDII        125
sp_P80902_PORC_METTM_Pyru    91  -----GVVLLNTA--GTFT----------------------SENAKIHTIDATGIALENL         121
tr_W8CQB1_W8CQB1_9EURY_Py    91  -----GMVIINTE--KSKE----------------------EVLEK--LKKKPAKLALVDATTIALEIL  128
tr_Q4KY23_Q4KY23_TRIVA_Py   505  -----GVFVINFP--GSAD--LNK-----------------DLPGSFRKA--IAEKDAKLYTIDATQIAIDLK  549
sp_P94692_POR_DESAF_Pyruv   508  -----GTFVLNSP--WSSLEDMDK-----------------HLPSGIKRT--IANKKLKFYNIDAVKIATDVG  554
tr_Q2RMD6_Q2RMD6_MOOTA_Py   505  -----GIFLLNST--WSA-EEMDS-----------------RLPADMKRT--IATKKLKFYNIDAVKIAQEIG  550
tr_C4LTX6_C4LTX6_ENTHI_Py   503  -----SVFVLNCP--WTGA-ELEA-----------------QLPGSLKRV--IAEKQIKFYTIDAIKIGQEVK  548
sp_Q968X7_PNO_CRYPV_Pyruv   511  -----GCFVLNCP--WSTLEELNH-----------------ELPSKIKHQ--IASRDVKFYVIDAQRIAQESN  557
sp_Q94IN5_PNO_EUGGR_Pyruv   574  -----GTFVLNSR--WTT-EDMEK-----------------EIPADFRRN--VAQKKVRFYNVDARKICDSFG  619
tr_L8B958_L8B958_CHLRE_Py   616  -----GVLVLNTV--FTSPDSLGK-----------------YLPDKVKKQ--IAALKPQLYVIDAQSVAKASG  662
sp_Q53046_NIFJ_RHORT_Pyru   516  -----ATLLLNSP--HDKD-TVWD-----------------ALPRPVQQT--IIDRDLKLFVIDANKVAQETG  561
tr_B5XPH3_B5XPH3_KLEP3_Py   508  -----GTFLLNCS--WSEAE-LEQ-----------------HLPVSVRRY--LAQEKIDFYTLNAVDIARELG  553
tr_Q24982_Q24982_GIAIN_Py   531  -----GFFVLNTE--HDTVETLEK-----------------YLPAEMKRE--IARKNIRVYAVNANKVAQSVG  577
tr_I2K9Y6_I2K9Y6_9PROT_Py   104  -----SLIIINTD--FDVL----------------------NEDDIKV--LEDLNATVVQFDATKLALDIA   143
tr_O67231_O67231_AQUAE_Fe    98  -----GMIIINSD--VDII----------------------PDEDKKI--LEELNAKIYYIPATQIARDIA   137
tr_I0IRW0_I0IRW0_LEPFC_Fe   100  -----GVVLINSETPINLV------------------------ADEEREL--MER-NARVYYLPATQLSREIA  140
tr_J9ZD37_J9ZD37_LEPFM_Py   102  -----GIIIINDD--IELL----------------------TDSEKEE--LDQMGVLVYYVPATKMALDIA   141
tr_D3DJJ8_D3DJJ8_HYDTT_Py   100  -----GLIIINSE--EDLL----------------------TDEDKEF--LESLNVKVLNFSATKFAIDIA   139
sp_P84820_PORC_THELN_Pyru    91  -----GMVIVNTE--KTKE----------------------EVLEK--LKKKPAKLALVDATTIALEIL    128
sp_P80907_VORA_METTM_Keto   383  -----GIVLYDTA--TADF----------------------SKKENLRAIGVPALEIAKEHG         415
tr_A1HTT9_A1HTT9_9FIRM_Py    89  -----GVLIINSS--LIERS---------------------SKRDDITVYRVPANDIAAELG         122
tr_B0R3G0_B0R3G0_HALS3_Ox    90  -----SIIIYDGD--RTE-----------------------FADF--ESPAEVTGLDIPLKDLAEDAG    125
tr_H3ZPH3_H3ZPH3_THELN_2-    87  -----GLLVIDTD----------------------------LVKPDREYIGAPFTRLAEEKT         115
tr_Q1IQP1_Q1IQP1_KORVE_2-    94  -----GVLIVEQD--LVKIT---------------------GMSQAGRVYSAPATRLAEELG         127
tr_E8RJ92_E8RJ92_DESPD_2-    97  -----SVLLVDQD--LVN-----------------------PENAPCDHFAIAATRMAENLG         128
sp_P80906_KORC_METTM_2-ox    90  -----GTLIVDPD--MVIE----------------------NEIQDF--VEERNISYFRAPATRTAEEKV   128
tr_O68230_O68230_HELPX_Oo    91  -----GIVVMDPN--LVTPTK--------------------EDEEKYQLYKIPIISIAKDEV         125
sp_O53182_KORA_MYCTU_2-ox   120  -----GMVIVNSD--EFTKRNLTK------------VGYVTNPLES--GELSDYVVHTVAMTTLTLGAV    167
tr_H0DIR4_H0DIR4_9STAP_2-    90  -----SVIIADSK--AKPDK---------------------PEDCRAQMIDLPFTKTAKELG         123
tr_O87870_O87870_THAAR_2-    93  -----GLVLGDPD--GGE-------------------FPEQ--ILAKGTRRGDIPFKKIAKEID       128
tr_B0R4X6_B0R4X6_HALS3_Py   108  -----GVIIYDEG--LLDDEDV---------------GDLEQQ--ADANDWHLYPLDLRGLAKEHG      149
tr_D3DI99_D3DI99_HYDTT_2-    90  -----TVLVYDSS--DFEP----------------------EEHEGVVMYPVPLSHLAKDIM         122
sp_O07836_IORB_THEKO_Indo    91  -----GLVFTNAR--PIPPVQVSMGLA--------TYPTLDEMKKIV--EEDFGGKFMAFDAEKLAMEAG   143
tr_A1RYA4_A1RYA4_THEPD_In    91  -----GVVLANNF--FLPPPAAKS------------PSRSAVLNA--LKGLGARVVLLEADELALKAG    137
tr_K4MBD5_K4MBD5_9EURY_In    95  -----GVVIVNTE--PILPVTVTSGL----------CSYPDVGEIMA--SLQGKRKVVGFNATQLAVEAG   145
sp_P80911_IORB_METTM_Indo    89  -----ACVIVNTS--KIPPFNLIKSP---------HPYPPLEEIIKT--LEENAGRVRSFNGEKIAVEAG   140
tr_P72578_P72578_SULSP_2-    88  LIYNKAVETTKID--AVQSMEPELAERIKDFLTKQGYETTVKGALEY--ASKNNVTLIPVNYDEIAKKVA   153
tr_Q96Y66_Q96Y66_SULTO_2-    88  LIYNKAVETTKID--AVQSMEPELAERIKDFLTKQGYETTVKGALEY--ASKNNVTLIPVNYDEIAKKVA   153
tr_Q96XT2_Q96XT2_SULTO_2-    89  LIYNVEYENTTVD--LVKSMEPEMAEQVKEALSKERLGFTIKDVLEY--LKRRGVKVIGFNYTELIKKIA   154
Consensus_aa:                    .....thhlhss..............................p.........phh.hsh.plt.p..
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Conservation:                                      8                                  7
sp_O05650_PORC_THEMA_Pyru  133 K---------RGIPNTPMLGALVRV-----TGI-VPLEAIEKRIEKMFG---KKFPQEVIDANKRALRRG 184
tr_M3NSB1_M3NSB1_HELPX_Py  130 K---------RPIPNTPMLGALMKV-----SGM-LEIEAFKEAFKKVLG---KKLTQEVIDANMLAIQRA 181
tr_Q3Z8I7_Q3Z8I7_DEHM1_Py  132 G---------VNIVNTTMLGALIKA-----TGL-AGIEDFEEPLKHRFG--------KLAAKNMAAMKKA 178
sp_P80523_PORC_METBF_Pyru  126 G---------VPIVNTVLLGAFAGA-----TGE-INVESIQHAIRARFS-------GKVGEKNANAIQKA 173
sp_P80902_PORC_METTM_Pyru  122 R---------RPIVNTVMLGAFAGV-----TGL-VSIDSLIKIIKETFP-------GKIGDKNAEAARIA 169
tr_W8CQB1_W8CQB1_9EURY_Py  129 G---------LPITNTSILGAVAKA-----TGI-VKIESVEKAIKETFS-------GELGEKNAKAAREA 176
tr_Q4KY23_Q4KY23_TRIVA_Py  550 L---------PGRINMLMQTVFFGL-----ANI-IPAEECIALLKKSIAKQYARKGKEVIQKNWDMVDHA 604
sp_P94692_POR_DESAF_Pyruv   554 L---------GGRIMMIMQTAFFKL-----AGV-LPFEKAVDLLKKSIHKAYGKKGEKIVKMNTDAVDQA 609
tr_Q2RMD6_Q2RMD6_MOOTA_Py  551 L---------GSRINVIMQTAFFKI-----ANV-IPVDEAIKYIKDSIVKTYGKKGDKILNMNFAAVDRA 605
tr_C4LTX6_C4LTX6_ENTHI_Py  549 L---------GRRINMIMQTVFFKL-----ANV-IPFEKAIVLLKEAVQKTYGAKGPAIVKMNHDAIDKA 603
sp_Q968X7_PNO_CRYPV_Pyruv   558 L---------GRRINNILMVVFFSL-----TNI-IPLDLAIKLVKEAIKKTYGKKGDAVVNSNWKAVDLT 612
sp_Q94IN5_PNO_EUGGR_Pyruv   630 L---------GKRINMLMQACFFKL-----SGV-LPLAEAQRLLNESIVHEYGKKGGKVVEMNQAVVNAV 674
tr_L8B958_L8B958_CHLRE_Py  663 L---------GKHVNMVMQTVFFNL-----SGV-LPMEKALALLKKSITKAYERKGPEVVAKNHSAVDMA 717
sp_Q53046_NIFJ_RHORT_Pyru   562 M---------GQRINTIMQTCFFAL-----SGV-MPRDEAIEEIKKAISKTYARKSQKVIDANFAAVDQT 616
tr_B5XPH3_B5XPH3_KLEP3_Py  554 L---------GGRFNMLMQAAFFKL-----TAI-IDPQTAADYLKQAVEKSYGSKGASVIEMNQRAIELG 608
tr_Q24982_Q24982_GIAIN_Py  578 L---------GGRINTIMILFFLKLGLSKLLDFDVACEDMKAAARITYA----KQKAEVIEANVKAIDVA 634
tr_I2K9Y6_I2K9Y6_9PROT_Py  144 GT--------ELATNMAMMGMLLGL-----TKL-VTTENIEAAVKERFL-----GTSFVSSGGTAMLDSA 194
tr_O67231_O67231_AQUAE_Fe  138 GT--------ELATNMAMVGTFFGI-----TRL-VTLEHIEKALIERFL-----GGTFVASGGTTALDSA 188
tr_I0IRW0_I0IRW0_LEPFC_Fe  141 DT--------DLATNMAMVGAVSAI-----MGI-PDLPSLEQSVKERFL-----GKGFVVSGGTAALDNV 191
tr_J9ZD37_J9ZD37_LEPFM_Py  142 GT--------ELSTNMAMIGSVSGL-----TDV-IGMEALDLALQDRFG------KKYVASGGTATLDEA 191
tr_D3DJJ8_D3DJJ8_HYDTT_Py  140 GT--------ELSTNMAMIGALFGA-----VGC-VGLEAIEHGIKSRFL------KKFVASGGTASLDSA 189
sp_P84820_PORC_THELN_Pyru   129 G---------LPITNTSILGAVAKA-----TGI-VKIESVEEAIKDTFS-------GELGKKNAKAAREA 176
sp_P80907_VORA_METTM_Keto   416 T---------GRAANTAMLGVMMAL----GITG-LDEESFRDAIRFTFS-----GKDKIIDINLKILEAG 466
tr_A1HTT9_A1HTT9_9FIRM_Py  123 N---------SKVANMVVLGALIAA-----TGA-VATTSVLKAFQKMFA-----KKPELLAINEQAIHRG 172
tr_B0R3G0_B0R3G0_HALS3_Ox  126 G---------AIMRNIVALGAVCAV-----ADF--PIENLDESLEKRFS-----GKGEQIIENNKQAARLG 175
tr_H3ZPH3_H3ZPH3_THELN_2-  116 GL--------ALTVNMVALGYLVAK-----INI-VKKESVEKAIRRRVP-------KGTEEINLKAFRIG 164
tr_Q1IQP1_Q1IQP1_KORVE_2-  128 K---------RMILNIVMVGFTAAV-----TNI-LQKESLREAVASSVP-------PSFRELNLKAFDRG 175
tr_E8RJ92_E8RJ92_DESPD_2-  129 N---------KMMANIIMLGFCTAI-----TKA-VSSEAAQATIRQSVP-------KGTEERNIEAFTKG 176
sp_P80906_KORC_METTM_2-ox   129 GI--------TIVANMVMIGALTEA-----TGV-VSVRAAEEAIKNSVP-------PGTEEKNIMAFQAG 177
tr_O68230_O68230_HELPX_Oo  126 GN--------IITQSVVALAITAEF-----TKC-VEENIALDTMLKKVP-------AKVADTNKKAFEIG 174
sp_O53182_KORA_MYCTU_2-ox   168 EAIGASKKDGQRAKNMFALGLLSWM-----YGR--ELEHSEAFIREKFA-----RKPEIAEANVLALKAG 225
tr_H0DIR4_H0DIR4_9STAP_2-  124 T---------TLMKNMVAVGATCAL-----MDL--ETETFESLITAMFE----KKGDKVVEMNIQALHEG 173
tr_O87870_O87870_THAAR_2-  129 G---------GRPNMIALGTVAAL-----VGL--PEDAVLKVIKDSLA----KKGPAALAASEASVRAG 177
tr_B0R4X6_B0R4X6_HALS3_Py  150 R---------EVMRNTAGVGATAAL-----IDM--DLDHIEDLMSDAMG-------GDILEQNLTVLRDA 196
tr_D3DI99_D3DI99_HYDTT_2-  123 KA--------YITKNVIALGVLCGL-----FDI--PVQSIKDSIKAKFL----RKGQEIIELNYKALETG 173
sp_O07836_IORB_THEKO_Indo   144 N---------IVTTNVVLIGALSQT-----PGFPLSEEQIKEVIRISVP-------PKTIDVNMRAFELG 192
tr_A1RYA4_A1RYA4_THEPD_In  138 S---------PITVNMVMLGALIGT----GRID-LTLEDAADVLKSRFK-------GKVLEMNLEALKLG 186
tr_K4MBD5_K4MBD5_9EURY_In  146 N---------AQAMNVIMVGAISNY-----LPL--SPDIMLDCVRELVP-------PKTVDINVKAFELG 192
sp_P80911_IORB_METTM_Indo   141 H---------ILSLNMVMLGAAAAT-----TGFPLGEETLIESMKNNLP-------PKLMEVNLRAFHEG 189
tr_P72578_P72578_SULSP_2-  154 DEMKVPLSVTERVKNIVGITISYKL-----LGL--DVNYLIEAINSTFK------QDLYRKMNELAVKDS 210
tr_Q96Y66_Q96Y66_SULTO_2-  154 DEMKVPLSVTERVKNIVGITISYKL-----LGL--DVNYLIEAINSTFK------QDLYRKMNELAVKDS 210
tr_Q96XT2_Q96XT2_SULTO_2-  155 DTFKVPMSVVERAKNMIAVGASYGL-----LGL--KFDYLKDAISSTFK------NELFIKFNTMAAELG 211
Consensus_aa:                  ..............Nhhh.shhh.h......sh.l..p.h...hpp.h..........h.p.N..thc.t
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Cyan – Adenine binding asparagine
Green – Aromatic residues that can possibly form pi-pi interaction with adenine moiety of CoA
Magenta – Cationic residues that can possibly form cation-pi interactions with adenine moiety of CoA

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Conservation:
sp_O05650_PORC_THEMA_Pyru   185 YEE--VKCSE---------------------------------------------------------- 192
tr_M3NSB1_M3NSB1_HELPX_Py   182 YEE--VQ------------------------------------------------------------- 186
tr_Q3Z8I7_Q3Z8I7_DEHM1_Py   179 LEE--TAVKEL-KVG---------------------------------------------------- 190
sp_P80523_PORC_METBF_Pyru   174 YKL--IRGEEA-------------------------------------------------------- 182
sp_P80902_PORC_METTM_Pyru   170 YEK---MKHSG-------------------------------------------------------- 177
tr_W8CQB1_W8CQB1_9EURY_Py   177 FEK--TVVYEL-------------------------------------------------------- 185
tr_Q4KY23_Q4KY23_TRIVA_Py   605 LQG--LKEFKY-NKAEWLN--AP----------VEPRPKHE----------------GIR--HIIDMSI 640
sp_P94692_POR_DESAF_Pyruv   610 VTS--LQEFKY-PDSWKDA--PAET--------KAEPMTNE----------------FFK--NVVKPIL 647
tr_Q2RMD6_Q2RMD6_MOOTA_Py   606 LEA--LEEIKY-PASWADA--VDEA--------AATVTEEP----------------EFIQ-KVLRPIN 644
tr_C4LTX6_C4LTX6_ENTHI_Py   604 LDG--LVEVKV-PAEWANA--PLET--------VTKIEAPE----------------FVT--DVLMPQL 641
sp_Q968X7_PNO_CRYPV_Pyruv   613 LES--LIQISYDKSQWISK--DKCGEKS-LPATAVETGNKDQEITKSTVLKQKPEHDVNQFVKDILGPVN 677
sp_Q94IN5_PNO_EUGGR_Pyruv   675 FAGDLPQEVQV-PAAWANA--VDTS--------TRTPTGIE----------------FVD--KIMRPLM 714
tr_L8B958_L8B958_CHLRE_Py   718 VAA--LKKLDI-PASWSSL--PTHVVNP-NPPAEGNTSRWE----------------FIE--TVAKPML 762
sp_Q53046_NIFJ_RHORT_Pyru   617 LSR--LQSVTI-PGVLTGH--ALPP--------LVSAGAPD----------------FVR--NVTAVML 654
tr_B5XPH3_B5XPH3_KLEP3_Py   609 MAA--LHRVTV-PAHWATL--EAPA-------PQASALMPD----------------FIR--DILQPMN 647
tr_Q24982_Q24982_GIAIN_Py   635 RSI--IEQCHI-EYDKARW--VNADPSESTANQTYGPEPDK----------------YVK--DIILPAV 680
tr_I2K9Y6_I2K9Y6_9PROT_Py   195 IEK--KFKKKE-ELLAKNM--EVIN----------------------------------------- 214
tr_O67231_O67231_AQUAE_Fe   189 IEK--KFKKKQ-ELLAKNM--EVIK----------------------------------------- 208
tr_I0IRW0_I0IRW0_LEPFC_Fe   192 IER--KFAKKE-QLLKKNM--EVIV----------------------------------------- 211
tr_J9ZD37_J9ZD37_LEPFM_Py   192 IKK--KFAKKE-MLLQKNL--DTIK----------------------------------------- 211
tr_D3DJJ8_D3DJJ8_HYDTT_Py   190 LER--KFKKKL-ELIEKNL--STAK----------------------------------------- 209
sp_P84820_PORC_THELN_Pyru   177 FEK--TVVYEL-------------------------------------------------------- 185
sp_P80907_VORA_METTM_Keto   467 ADW--ARKNLE-GEF---------------------------------------------------- 478
tr_A1HTT9_A1HTT9_9FIRM_Py   173 AEC--IKK----------------------------------------------------------- 178
tr_B0R3G0_B0R3G0_HALS3_Ox   176 AEY--VAEEFE-DVTLPYE--LETT---------DEDYV--------------------LLNGDEAIGMGA 212
tr_H3ZPH3_H3ZPH3_THELN_2-   165 YEE--GLR----------------------------------------------------------- 170
tr_Q1IQP1_Q1IQP1_KORVE_2-   176 YEY--GVQALQ-TTPETGV--DENT--------VKVY----------------------------- 199
tr_E8RJ92_E8RJ92_DESPD_2-   177 FDY--GLSTLK-GREKRAA--GQTG--------AKAQ----------------------------- 200
sp_P80906_KORC_METTM_2-ox   178 REL--IMEGQK-------------------------------------------------------- 186
tr_O68230_O68230_HELPX_Oo   175 KKH--ALEALK-K------------------------------------------------------ 184
sp_O53182_KORA_MYCTU_2-ox   226 WNY--GETTEA-FGTTYEIPPATLP--------PGEYR--------------------QISGNTALAYGI 264
tr_H0DIR4_H0DIR4_9STAP_2-   174 YRL--MQEQLE-TVEGDFQ--LTASQ-------QDPHL--------------------YMIGNDAIGLGA 211
tr_O87870_O87870_THAAR_2-   178 MAF--AASLPP-SKKLAAA--AGGE--------RQRLW--------------------SITGNEAAGLGA 214
tr_B0R4X6_B0R4X6_HALS3_Py   197 YEQ--VSEMEH-THDLSVP--TGSH--------DEPQV--------------------LMSGSHAIAYGA 233
tr_D3DI99_D3DI99_HYDTT_2-   174 INY--VRENIK-KLDGYLF-PPAKE--------PKDVV--------------------IMEGNQAIAKGA 211
sp_O07836_IORB_THEKO_Indo   193 VKA--AKEMLG-L------------------------------------------------------ 202
tr_A1RYA4_A1RYA4_THEPD_In   187 YTA--AQEQLE-GQRL--------------------------------------------------- 199
tr_K4MBD5_K4MBD5_9EURY_In   193 RAQ--THV----------------------------------------------------------- 198
sp_P80911_IORB_METTM_Indo   190 FET--VNCD---------------------------------------------------------- 196
tr_P72578_P72578_SULSP_2-   211 YDI--VESRYN-LKPSSK-------------ERRRF-------------------WLDGNTAVAIGK 242
tr_Q96Y66_Q96Y66_SULTO_2-   211 YDI--VESRYN-LKPSSK-------------ERRRF-------------------WLDGNTAVAIGK 242
tr_Q96XT2_Q96XT2_SULTO_2-   212 YNS--VPNVYK-LQEYKI-------------EKQRI-------------------QVDGNTISAMGK 243
Consensus_aa:                   hp............................................................
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Table S4. Sequences of domain III used in sequence alignment

Number	Organism	UniProt ID
1	*Thermotoga maritime*	O05650
2	*Helicobacter pylori*	M3NSB1
3	*Dehalococcoides mccartyi*	Q3Z8I7
4	*Methanosarcina barkeri*	P80523
5	*Methanothermobacter marburgensis*	P80902
6	*Thermococcus guaymasensis*	W8CQB1
7	*Trichomonas vaginalis*	Q4KY23
8	***Desulfovibrio africanus***	P94692
9	***Moorella thermoacetica***	Q2RMD6
10	*Entamoeba histolytica*	C4LTX6
11	*Cryptosporidium parvum*	Q968X7
12	*Euglena gracilis*	Q94IN5
13	*Chlamydomonas reinhardtii*	L8B958
14	*Rhodospirillum rubrum*	Q53046
15	*Klebsiella pneumoniae*	B5XPH3
16	*Giardia intestinalis*	Q24982
29	*Sulfurovum sp. AR*	I2K9Y6
30	*Aquifex aeolicus*	O67231
31	*Leptospirillum ferrooxidans*	I0IRW0
32	*Leptospirillum ferriphilum*	J9ZD37
33	*Hydrogenobacter thermophiles*	D3DJJ8
34	*Thermococcus litoralis*	P84820
35	*Methanothermobacter marburgensis*	P80907
36	*Thermosinus carboxydivorans*	A1HTT9
37	*Halobacterium salinarum*	B0R3G0
38	*Thermococcus litoralis*	H3ZPH3
39	*Koribacter versatilis*	Q1IQP1
40	*Desulfobulbus propionicus*	E8RJ92
41	*Methanothermobacter marburgensis*	P80906
42	*Helicobacter pylori*	O68230
43	*Mycobacterium tuberculosis*	O53182
44	*Staphylococcus pettenkoferi*	H0DIR4
45	*Thauera aromatica*	O87870
46	*Halobacterium salinarum*	B0R4X6
47	*Hydrogenobacter thermophilus*	D3DI99
48	*Thermococcus kodakaraensis*	O07836
49	*Thermofilum pendens*	A1RYA4
50	*Methanolobus psychrophilus*	K4MBD5
51	*Methanothermobacter marburgensis*	P80911
52	*Sulfolobus sp.*	P72578
53	***Sulfolobus tokodaii***	Q96Y66
54	***Sulfolobus tokodaii***	Q96XT2

Cyan: "Group 3" PFOR/VOR; Purple: "Group 4" PFOR; Blue: "Group 5 PFOR/OGOR; Yellow: "Group 6" IOR; Red: "Group 7" VOR; Orange : "Group 8" OGOR.

The numbers 1-16 and 29-52 are the same as the numbers used in our previous phylogenetic analysis. Two OFORs from *Sulfolobus tokodaii* are numbered 53 and 54. Based on sequence and domain composition, two *St*OFORs would be classified as Group 8 OGOR.

SI Appendix – References

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