Supporting Information

Crystal structures of fumarate hydratases from *Leishmania major* in a complex with inhibitor 2-thiomalate

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Table S1. Data collection and refinement statistics of LmFH isoforms

Data name	**LmFH-1-thio**	**LmFH-2-thio**	**LmFH-2-thio-S-peak**[a,b]
Co-crystallization	*RS*-2-thiomalate	*RS*-2-thiomalate	*RS*-2-thiomalate
Ligand in the structure	*S*-2-thiomalate	*S*-2-thiomalate	*S*-2-thiomalate
Data collection			
Space group	$P2_1$	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions			
a, b, c (Å)	78.74, 138.44, 138.07	65.73, 84.68, 239.99	65.97, 84.82, 240.96
α, β, γ (°)	90, 90, 90	90, 90, 90	90, 90, 90
Wavelength (Å)	0.9792	0.9792	1.7969
Resolution (Å)	50 – 2.05 (2.09 – 2.05)[c]	50 – 1.6 (1.63 – 1.60)[c]	50 – 2.03 (2.07 – 2.03)[c]
No. of unique reflections	180,831	178,641	166,783
R_{sym}	0.150 (0.605)[c]	0.135 (0.805)[c]	0.163 (0.804)[c]
I/σ(I)	9.75 (1.33)[c]	10.16 (1.11)[c]	25.56 (1.80)[c]
CC1/2	(0.706)[c]	(0.642)[c]	(0.687)[c]
Completeness (%)	98.6 (94.7)[c]	99.8 (99.5)[c]	99.5 (96.7)[c]
Redundancy	6.3 (4.5)[c]	5.4 (4.8)[c]	10.0 (5.0)[c]
Refinement			
No. of reflections used	180,807 (16,857)[c]	178,536 (16,623)[c]	
R_{work} / R_{free}[d]	0.1594 / 0.2045	0.1530 / 0.1699	
No. atoms			
Protein	16,437	8,647	
[4Fe-4S] cluster	32	16	
S-2-thiomalate	54	36	
Glycerol	48	-	
Pentaethylene glycol	16	-	
Tetraethylene glycol	26	-	
Triethylene glycol	50	-	
Di(hydroxyethyl)ether	14	-	
Water	870	821	
Average B-factors (Å^2)			
Protein	28.5	17.8	
[4Fe-4S] cluster	25.2	12.1	
S-2-thiomalate	16.5	6.8	
Glycerol	30.7	-	
Pentaethylene glycol	32.0	-	
Tetraethylene glycol	20.6	-	
Triethylene glycol	32.5	-	
Di(hydroxyethyl)ether	30.0	-	
Water	25.5	28.1	
R.m.s deviations			
Bond lenghts (Å)	0.008	0.004	
Bond angles (°)	1.021	0.717	
Rotamer outliers (%)	1.03	0.57	
Ramachandran analysis[e] (%)			
Favored	96.4	97.3	
Allowed	3.5	2.7	
Outliers	0.1	0	

[a] Bijvoet pairs were not merged during data processing.
[b] Structure was not refined to completion.
[c] Highest resolution shell is shown in parenthesis.
[d] R_{free} was calculated with 5% of the data.
[e] Distribution of dihedral angles in Ramachandran diagram were calculated with *MolProbity* program.[1]

Table S2. Hydrogen bonds formed between residues of chains A and B from LmFH-1

	Chain A				Chain B				
Hydrogen bond no.	Atom name	Residue name	Residue number	Chain	Atom name	Residue name	Residue number	Chain	Distance
1	O	ALA	10	A	OH	TYR	145	B	2.12
2	OD1	ASP	116	A	NE2	HIS	316	B	2.77
3	NH2	ARG	126	A	O	SER	182	B	2.94
4	O	VAL	132	A	NZ	LYS	183	B	2.94
5	O	GLY	134	A	NZ	LYS	183	B	2.84
6	OD1	ASP	136	A	NZ	LYS	183	B	2.83
7	OH	TYR	145	A	O	ALA	10	B	2.34
8	O	SER	156	A	N	GLY	317	B	2.94
9	N	ASN	158	A	O	ARG	315	B	2.87
10	O	ASN	158	A	N	ARG	315	B	3.07
11	O	MET	163	A	NE	ARG	312	B	3.00
12	O	MET	163	A	NH2	ARG	312	B	2.86
13	OE1	GLU	166	A	NH2	ARG	315	B	3.03
14	OE2	GLU	166	A	NH2	ARG	312	B	3.18
15	OE2	GLU	166	A	NE	ARG	315	B	2.66
16	OE1	GLN	176	A	OG1	THR	245	B	2.87
17	OE1	GLN	176	A	OG	SER	246	B	2.52
18	OD1	ASP	178	A	NZ	LYS	252	B	2.72
19	O	LEU	179	A	N	ALA	181	B	2.86
20	N	ALA	181	A	O	LEU	179	B	2.79
21	O	SER	182	A	NH2	ARG	126	B	2.88
22	NZ	LYS	183	A	O	VAL	132	B	3.15
23	NZ	LYS	183	A	O	GLY	134	B	2.65
24	NZ	LYS	183	A	OD1	ASP	136	B	2.77
25	O	ALA	199	A	NE2	GLN	206	B	3.29
26	OD1	ASN	200	A	OG	SER	319	B	2.87
27	ND2	ASN	200	A	O	ALA	318	B	2.87
28	O	SER	202	A	NE2	GLN	206	B	2.92
29	NE2	GLN	206	A	O	ALA	199	B	3.19
30	NE2	GLN	206	A	O	SER	202	B	2.93
31	O	SER	210	A	NH2	ARG	412	B	2.73
32	OG1	THR	245	A	NE2	GLN	176	B	2.82
33	OG	SER	246	A	NE2	GLN	176	B	2.72
34	OE2	GLU	248	A	NZ	LYS	255	B	2.42
35	NZ	LYS	252	A	OD1	ASP	178	B	2.85
36	NZ	LYS	255	A	OE2	GLU	248	B	3.11
37	NE	ARG	312	A	O	MET	163	B	3.04
38	NH2	ARG	312	A	O	MET	163	B	2.86
39	NH2	ARG	312	A	OE2	GLU	166	B	3.03
40	N	ARG	315	A	O	ASN	158	B	3.07
41	O	ARG	315	A	N	ASN	158	B	2.93
42	NE	ARG	315	A	OE2	GLU	166	B	2.61
43	NH2	ARG	315	A	OE1	GLU	166	B	3.14
44	NE2	HIS	316	A	OD1	ASP	116	B	2.6
45	N	GLY	317	A	O	SER	156	B	2.86
46	O	ALA	318	A	ND2	ASN	200	B	2.77
47	OG	SER	319	A	OD1	ASN	200	B	2.88
48	NH2	ARG	412	A	O	SER	210	B	2.95

Table S3. Tacsimate composition and its variation* in the presence of 2-thiomalate.

	100% Tacsimate pH 5	**100% Tacsimate* pH 5 with 2-thiomalate**
Ammonium citrate tribasic	0.25 M	0.25 M
Sodium acetate trihydrate	0.40 M	0.40 M
Sodium formate	0.50 M	0.50 M
Ammonium tartrate dibasic	0.16 M	0.16 M
RS-Malic acid	0.30 M	-
Malonic acid	1.83 M	-
Succinic acid	0.12 M	-
RS-2-thiomalate	-	0.30 M

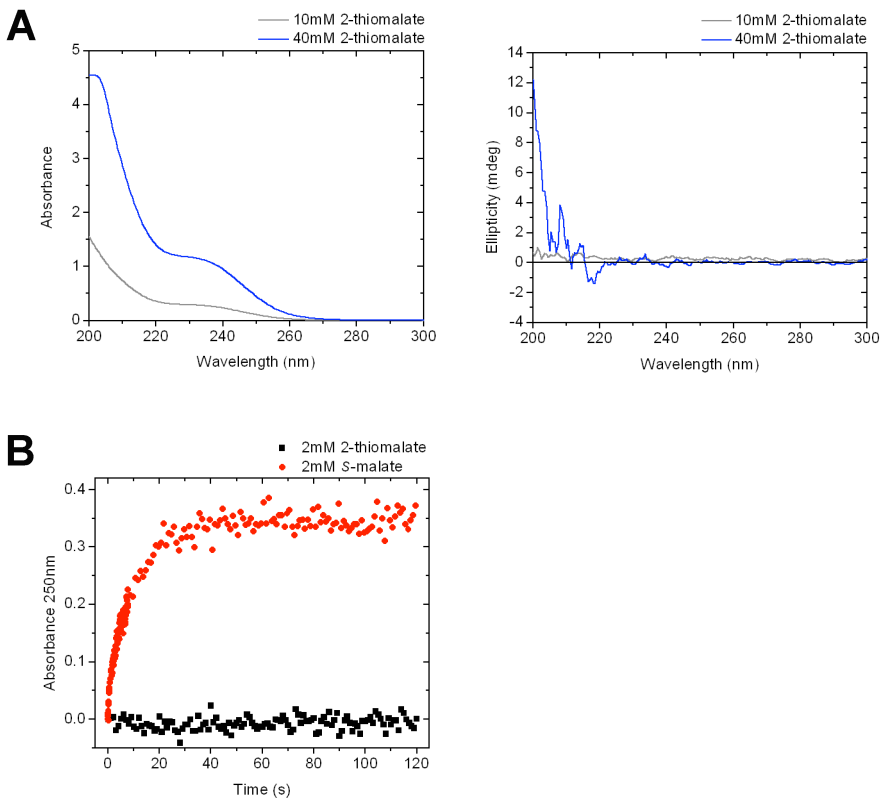

Figure S1. *RS*-thiomalate characterization and use. (A) Circular dichroism (CD) of *RS*-2-thiomalate. The *Left* and *Right* panels show absorption and CD spectra of *RS*-2-thiomalate (10 and 40 mM), respectively. No chiroptical signals were observed in the CD spectra (~240 nm) indicating that *RS*-2-thiomalate is a 50:50 racemic mixture. **(B) Activity of LmFH-2 with *S*-malate and *RS*-2-thiomalate as substrate.** The production of fumarate was measured spectrophotometrically at 250 nm. No production of fumarate was observed with *RS*-2-thiomalate.

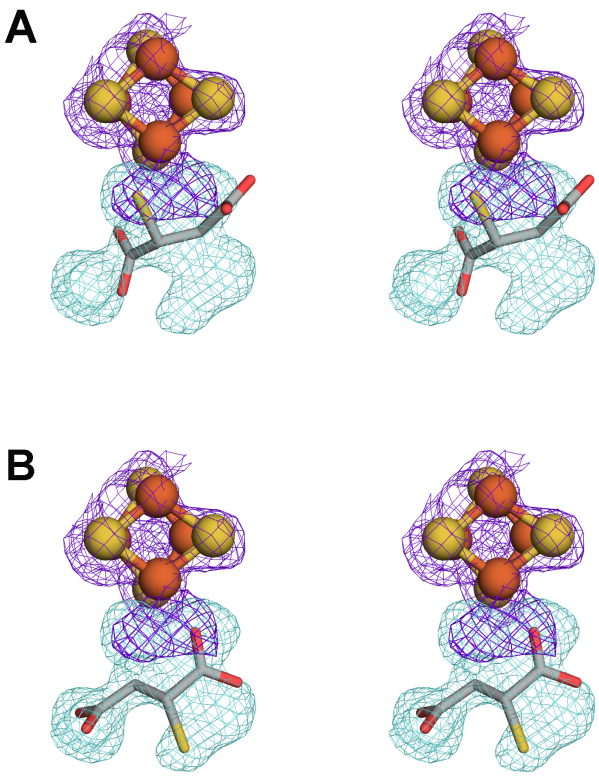

Figure S2. Stereo view of *R*-2-thiomalate modeled in the active site of LmFH-2. *R*-2-thiomalate (grey) was modeled in two possible orientations (**A** and **B**) into F_o–F_c difference electron density map that was contoured at 3.0 rmsd (green mesh). Neither orientation of *R*-2-thiomalate is a good fit to the density. A sulfur anomalous difference electron density map contoured at 3.0 rmsd (purple mesh) indicates that the sulfur atom of 2-thiomalate must be coordinated to the unique Fe of the [4Fe-4S] cluster (orange (Fe) and yellow (S) spheres). (**A**) When the C2-thiol group of *R*-2-thiomalate is placed into the sulfur anomalous difference density, the rest of the molecule is a poor fit to the F_o–F_c difference electron density map. This orientation of *R*-2-thiomalate also clashes with amino acids in the active site (not shown). (**B**) When *R*-2-thiomalate is positioned such that its C2-thiol group is pointed away from the cluster, the overall fit to the F_o–F_c difference electron density map is better, but there is no sulfur anomalous signal to support this orientation of the *R*-enantiomer.

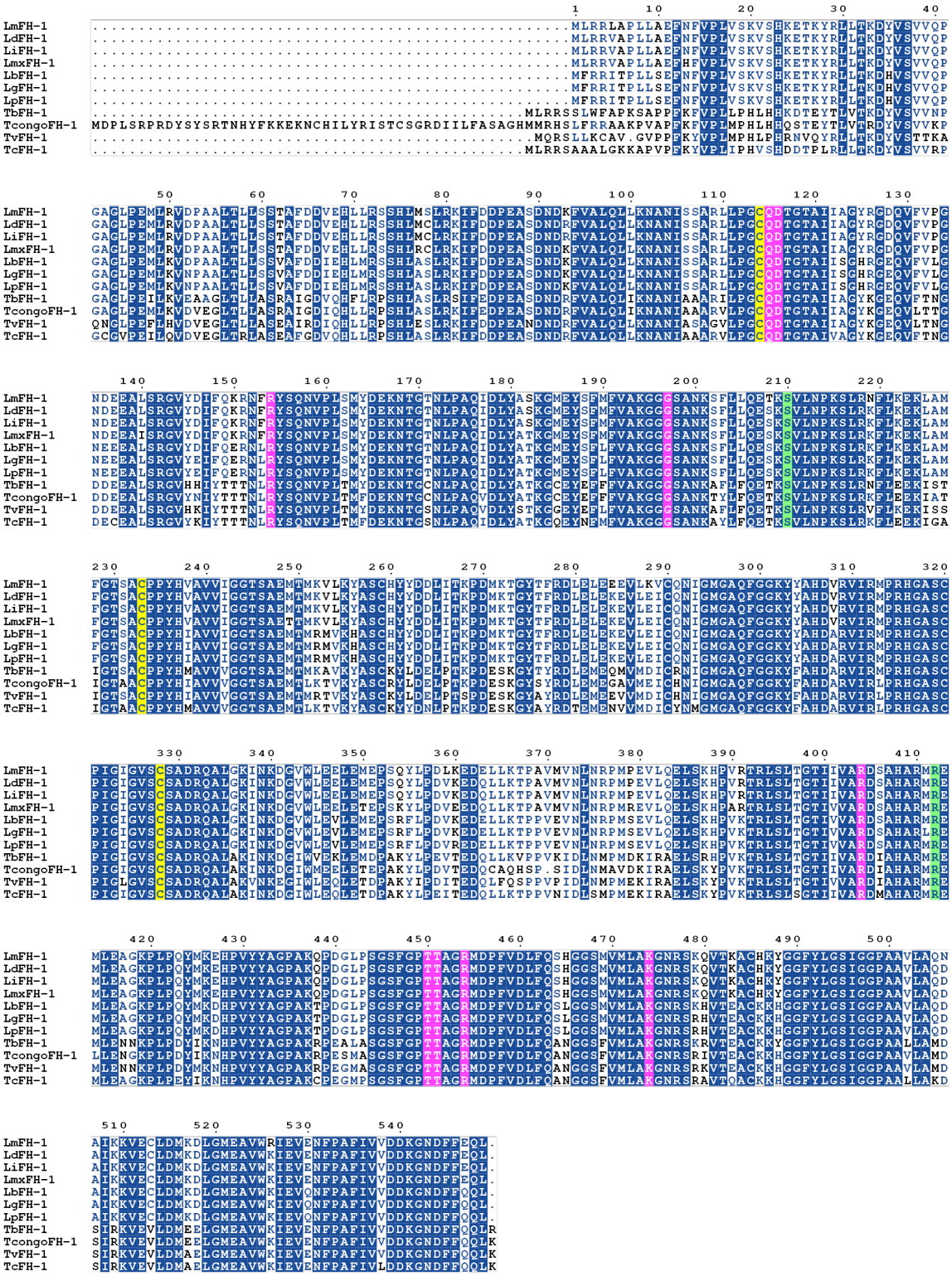

Figure S3. Sequence alignment of mitochondrial class I FH isoforms. LmFH-1 (*Leishmania major*), LdFH-1 (*Leishmania donovani*), LiFH-1 (*Leishmania infantum*), LmxFH-1 (*Leishmania mexicana*), LbFH-1 (*Leishmania braziliensis*), LgFH-1 (*Leishmania guyanensis*), LpFH-1 (*Leishmania panamensis*), TbFH-1 (*Trypanosoma brucei*), TcongoFH-1 (*Trypanosoma congolense*), TvFH-1 (*Trypanosoma vivax*), TcFH-1 (*Trypanosoma cryzi*). Conserved residues are in blue boxes; similar residues are in blue text; active site residues that bind the inhibitor *S*-2-thiomalate are in pink boxes; cysteine residues that coordinate the [4Fe-4S] cluster are in yellow boxes; dimer interface residues Ser-210 and Arg-412 are in green boxes. The alignment was performed using MULTALIN[2] and graphically displayed using ESPript.[3]

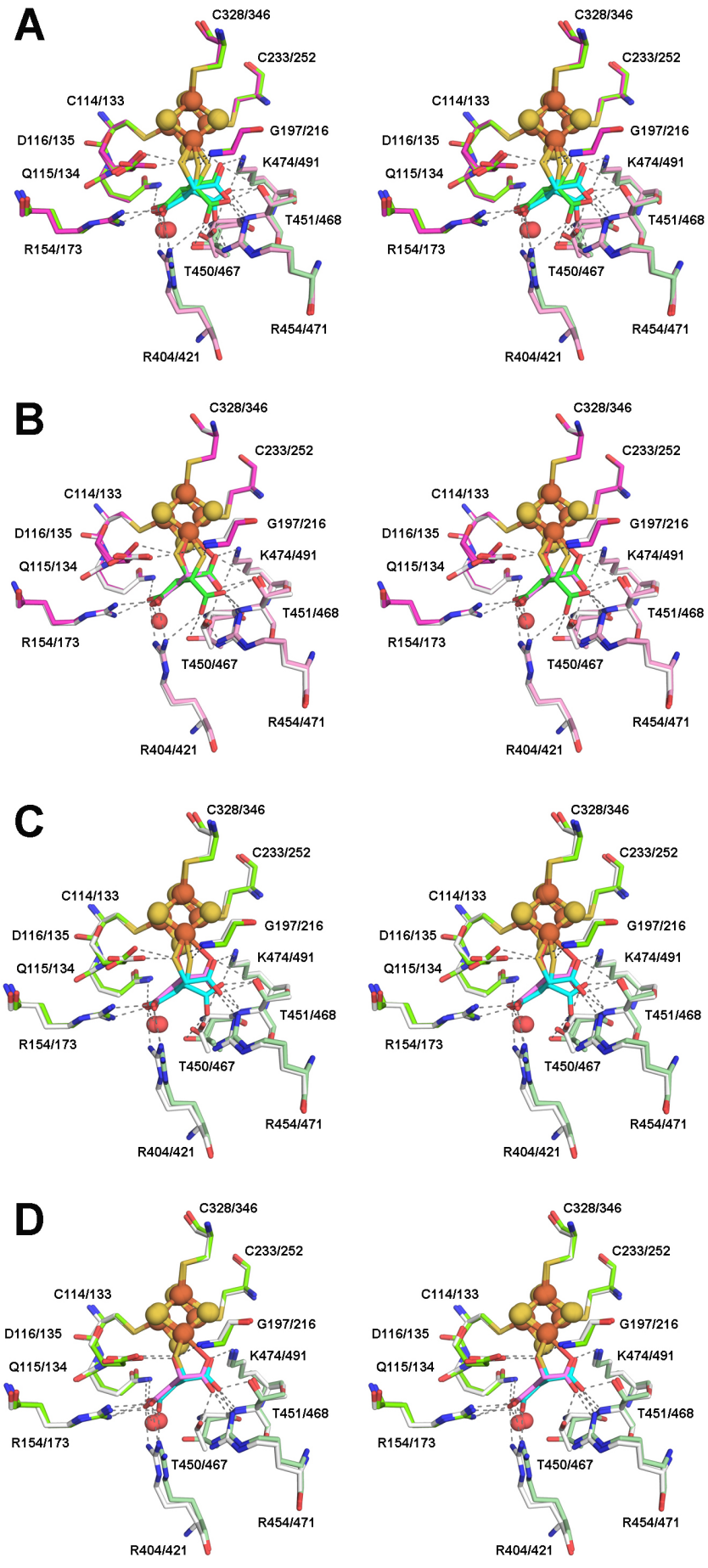

Figure S4. Stereo view of LmFHs active sites in a complex with *S*-2-thiomalate and *S*-malate. (A) Superposition of LmFH-1 (green) and LmFH-2 (pink) active sites in a complex with *S*-2-thiomalate (green and cyan, double conformation). **(B)** Superposition of LmFH-2 (pink) in a complex with *S*-2-thiomalate (green, double conformation) and LmFH-2 (white) in a complex with *S*-malate (violet). **(C)** Superposition of LmFH-1 (green) in a complex with *S*-2-thiomalate (cyan, double conformation) and LmFH-2 (white) in a complex with *S*-malate (violet). **(D)** Superposition of LmFH-1 (green) in a complex with *S*-2-thiomalate (cyan, canonical conformation) and LmFH-2 (white) in a complex with *S*-malate (violet). The [4Fe-4S] cluster is shown in orange (Fe) and yellow (S) spheres. The water molecule is shown in red sphere. The residues numbers separated by a *slash* are from LmFH-1/LmFH-2. The PDB code of LmFH-2 in a complex with *S*-malate is 5L2R.

References

(1) Chen, V. B., Arendall, W. B., III, Headd, J. J., Keedy, D. A., Immormino, R. M., Kapral, G. J., Murray, L. W., Richardson, J. S., and Richardson, D. C. (2010) MolProbity: all-atom structure validation for macromolecular crystallography, *Acta Crystallogr. D Biol. Crystallogr. 66*, 12–21.

(2) Corpet, F. (1988) Multiple sequence alignment with hierarchical-clustering, *Nucleic Acids Res. 16*, 10881–10890.

(3) Gouet, P., Courcelle, E., Stuart, D. I., and Metoz, F. (1999) ESPript: analysis of multiple sequence alignments in PostScript, *Bioinformatics 15*, 305–308.