

Supplemental Table 1: Data collection and refinement statistics of Ma-Flr-1.

^a Data collection		Refinement	
Wavelength (Å)	1.5414	Resolution (Å)	20-2.05
Resolution (Å)	30-2.05	R _{work} /R _{free} (%)	20.84/26.50
No. of Reflections (tot./uni.)	339,679/22,357	B-factors:	
Completeness (%)	98.3 (84.9)*	Protein	20.796
$I/\sigma(I)$	31.3 (3.7)*	Ligand	15.776
R _{sym} ^b (%)	9.5 (44.0)*	Water	21.865
Wilson B factor (Å ²)	35.2	R.m.s deviations:	
		Bond lengths (Å)	0.011
		Bond angles (°)	1.361

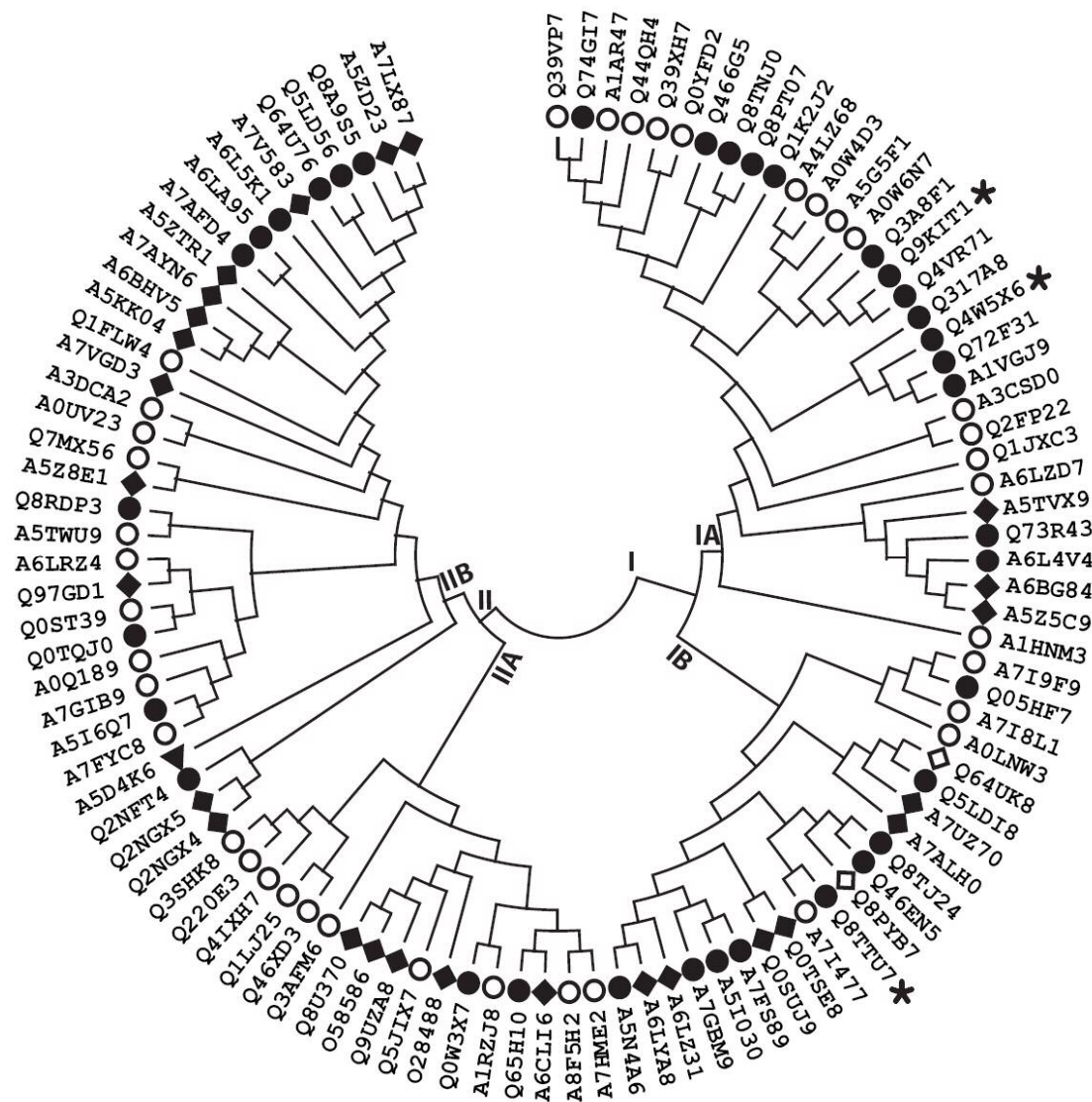
^aData set was collected on a Rigaku RU-200 rotating anode generator equipped with Osmic confocal “blue” optics, and diffraction intensities were recorded on an R-axis IV image plate system; ^bR_{sym} = $\sum |I - \langle I \rangle| / \sum I$, where I is observed intensity and $\langle I \rangle$ is average intensity obtained from multiple observations of symmetry related reflections; *highest resolution shell (2.12-2.05 Å) is shown in parenthesis.

Supplemental Figure S1. Sequence alignments of representative flavodoxin homologs shown in Fig. S2. The grey-shadowed residues are proposed to contribute in FMN binding and the black-shadowed glycine for accommodating space necessary for FMN binding. The numbers in parentheses indicate percent identity to Ma-Flr-1 (Q8TTU7) and the clades shown in Figure S2.

Q8TTU7 (100/IB)	<i>M. acetivorans</i>	-----MAEKIKINNNVFIYPMPVTLGANKV-G	27
A7I477 (55/IB)	<i>M. boonei</i>	-----MEKIEIP-KNFFIPMPVVLVGTVMN-G	
Q8TJ24 (51/IB)	<i>M. acetivorans</i>	-----METGEKKAIGAKNFLYPMPVTLVGADVN-G	
Q8PYB7 (48/IB)	<i>M. mazei</i>	MKRATKNLDFCLYNFVEDLRVELGEKKTIGSRNFLYPMPVTLVGADVN-G	
Q46EN5 (48/IIA)	<i>M. barkeri</i>	-----MPTTLVGACVN-G	
Q4W5X6 (31/IA)	<i>D. vulgaris</i>	-----MKKSLGARTLAYPTPLFLVGTYDRDS	
Q2NGX4 (28/IIB)	<i>M. stadtmanae</i>	-----MKIDVDSNWIRPMPNAIISCRSKDG	
O28488 (36/IIA)	<i>A. fulgidus</i>	-----MRSEVVMLEYLYPLRITYLIVSGVE--	
Q64U76 (26/IIB)	<i>B. fragilis</i>	-----MKQDWKPGTLIYPLPAVLVSCGSDDES	
Q65H10 (28/IIA)	<i>B. licheniformis</i>	-----MRAPIDEPIWYAYPGMVAVVTAQYEG	
Q9KIT1 (26/IA)	<i>D. gigas</i>	-----MKRSLGAKPLLFPPTVLVVGTYDDQG	
Q8TTU7 (100/IB)	<i>M. acetivorans</i>	KANLMALGWVSRVNNANPPMLGVGVNKSHTYPEGIAENGFSVNFPSYSGMV	77
A7I477 (55/IB)	<i>M. boonei</i>	RANFMAVGWCARVNGNPPMIACGISNSHATPGGITATKNFSVNIPSSALL	
Q8TJ24 (51/IB)	<i>M. acetivorans</i>	KPNYLTVAFCGIVQASPPMIAVTLGKMHYTNEGIRENNCFSVNIPSRMV	
Q8PYB7 (48/IB)	<i>M. mazei</i>	KPNYLTVAFCGIVQAGPPMIAVTLGKMHYTNEGIRENKCFSVNIPSRHML	
Q46EN5 (48/IIA)	<i>M. barkeri</i>	KPNYLTVAFCGVVQASPPMIAVTLGKIHTNEGIRENQCFSVNIPSRMV	
Q4W5X6 (31/IA)	<i>D. vulgaris</i>	RPNIMAAAWAGICCSQPPSIAVSLRKATYTYRSITERGAFITISIPSRAYV	
Q2NGX4 (28/IIB)	<i>M. stadtmanae</i>	HDNALAIGFISNIATDPLMIMVAIRPSRYSHEIIKETKNFVNLPTKDFK	
O28488 (36/IIA)	<i>A. fulgidus</i>	KPNVMTADWVPLSFSPQLLGVAIGHRSRYTHSLIKECGEFVAVPTIELL	
Q64U76 (26/IIB)	<i>B. fragilis</i>	EYNILTVAWTGTICTNPPMCYISVRPERHSYPIIKKNMEFVINLTTRDMA	
Q65H10 (28/IIA)	<i>B. licheniformis</i>	EKNAMASGWHYIIGSSPGMYGISLNNETFTYKLIKSGVFGVNFPLPGRCS	
Q9KIT1 (26/IA)	<i>D. gigas</i>	RPNAMTAAGGICCSKPPCVTVSLRKATYTYASLMARKAYTLHVTDEPHL	
Q8TTU7 (100/IB)	<i>M. acetivorans</i>	KKTDYCGLVSGEKVDKSG--LFEVFGELKTAPMIKECTNLNECRVETL	125
A7I477 (55/IB)	<i>M. boonei</i>	EKTDYCGMVGSKTADKSG--VFDVFGTLKTAPMIRECPVTECQLVQVV	
Q8TJ24 (51/IB)	<i>M. acetivorans</i>	EVTDYCGIVSGKKADKSG--IFKTFYKLEKAPMIRECPVNECRLADTL	
Q8PYB7 (48/IB)	<i>M. mazei</i>	EVTDYCGIVSGKKADKSD--IFRSFYGKLGKAPMIECPVNECRLVDTL	
Q46EN5 (48/IIA)	<i>M. barkeri</i>	ESTDYCGIVSGKRIDKSE--IFETFYGKLERAPMIRECPVNECKLIDIL	
Q4W5X6 (31/IA)	<i>D. vulgaris</i>	RHADYAGIYSGENEDKFASLGLTPVPGEHVDAPYVGEFPMALIELKLIHQI	
Q2NGX4 (28/IIB)	<i>M. stadtmanae</i>	REFDYLGISVSGYDEDKLTSTINTT--DADVINAFLTDCPINYECSLIDVY	
O28488 (36/IIA)	<i>A. fulgidus</i>	KDVWKAGTSLGAKENKMEKLSLTLVESKKVKVPSIKECQANLECRVVKEV	
Q64U76 (26/IIB)	<i>B. fragilis</i>	FATDWCGVRSGKDYHKKFEEMKLTGPKCSVVNAPLIEESPLCIECRVKEIV	
Q65H10 (28/IIA)	<i>B. licheniformis</i>	ELIQALGTHSGRDKINKFEAFHIQYEEGLKAEVPILTDAYFAYECKVHSIS	
Q9KIT1 (26/IA)	<i>D. gigas</i>	TASDFLGMASSGRDGDGLGLTTLTVRSELVDAPIIQEYPLVLECKIVHVV	
Q8TTU7 (100/IB)	<i>M. acetivorans</i>	EFP-TNYFFVGEIIAAYSEEQYLIQ-GKPDIKKMDPLLLTMPDNSYWTVG	273
A7I477 (55/IB)	<i>M. boonei</i>	ALP-THTLFIGEIIAGAYADSRVIKD-GKPDFPAIDPLFLTMPDNRYWALG	
Q8TJ24 (51/IB)	<i>M. acetivorans</i>	DFGGTNEVFGEIIVESYAEDRYLCN-GIPDIEKIEPIVFSMYDNNYWGIG	
Q8PYB7 (48/IB)	<i>M. mazei</i>	DFGGGSEVFGEIIMESYAEEKYLCN-GIPDVEKIEPIVFSMYDNNYWGIG	
Q46EN5 (48/IIA)	<i>M. barkeri</i>	DFGGASEVFGEIIVESYAEEERYLCN-EIPDIEKIEPIVFSMYDNNYWGIG	
Q4W5X6 (31/IA)	<i>D. vulgaris</i>	EIG-LHTQFIGEIMDVKVDDECLRDDGLPDINKVDPVIFAPVSREYYAVG	
Q2NGX4 (28/IIB)	<i>M. stadtmanae</i>	SPG-SHDLFIGKVEKVHCDDKYLTKKE-----	
O28488 (36/IIA)	<i>A. fulgidus</i>	ETG-DHTLFVGEILHVTHGDAFKDG-----KPDINYKFVMH	
Q64U76 (26/IIB)	<i>B. fragilis</i>	SLG-SHDMFIADVNNIRADDRHLNRETGKLELAEANPLVYVHGYYNLGE	
Q65H10 (28/IIA)	<i>B. licheniformis</i>	TLG-DQEWIAGEVLQRYQDKELFLKKGMPNLEKLDVPLHIGGSSYRILNS	
Q9KIT1 (26/IA)	<i>D. gigas</i>	DLG-LHTMFGVEVQDIKADARVLDEKGHLLLDALKPLGFMPEVRTYHGMG	
Q8TTU7 (100/IB)	<i>M. acetivorans</i>	DYAGAALKTGKSLMEKR	190
A7I477 (55/IB)	<i>M. boonei</i>	TYAGDAWSAGKHLKSSTG	
Q8TJ24 (51/IB)	<i>M. acetivorans</i>	EHLGRAWCVGKKFGENINDNRNENNS	
Q8PYB7 (48/IB)	<i>M. mazei</i>	EHLGKAWSGKKFQSNINEDRSENMM	
Q46EN5 (48/IIA)	<i>M. barkeri</i>	EHLGKAWSIGKNFAEGGNEKKSWKR	
Q4W5X6 (31/IA)	<i>D. vulgaris</i>	EFLAKAFSAGKGLRS	
Q2NGX4 (28/IIB)	<i>M. stadtmanae</i>	---SVNWEKIDLL	
O28488 (36/IIA)	<i>A. fulgidus</i>	ASFGKNFTTNSSERFEP	
Q64U76 (26/IIB)	<i>B. fragilis</i>	KIGKFGWSVEKKTK	
Q65H10 (28/IIA)	<i>B. licheniformis</i>	QAEHQHAFNSEAD	
Q9KIT1 (26/IA)	<i>D. gigas</i>	PALGRAFDAGKLHMPKAE	

Supplemental Figure S2. Phylogenetic tree of flavoredoxin homologs. The primary accession numbers and organism codes (in parentheses) were derived from the Swiss-prot/TrEMBL data bank. The symbols denote primary annotations: Flr (●); flavin reductase or flavin reductase-like protein FMN binding domain (○); uncharacterized protein (◆); NADPH:flavin oxidoreductase (◇); conserved hypothetical protein (▲). The Swiss-Prot/TrEMBL primary accession numbers (clockwise) and corresponding species names: Q39VP7, *Geobacter metallireducens* ; Q74GI7, *Geobacter sulfurreducens* ; A1AR47, *Pelobacter propionicus* ; Q44QH4, *Chlorobium limicola* ; Q39XH7, *Geobacter metallireducens* ; Q0YFD2, *Geobacter* sp. FRC-32 ; Q466G5, *Methanosarcina barkeri* ; Q8TNJ0, *Methanosarcina acetivorans* ; Q8PT07, *Methanosarcina mazei* ; Q1K2J2, *Desulfuromonas acetoxidans* ; A4LZ68, *Geobacter bemidjensis Bem* ; A0W4D3, *Geobacter lovleyi* SZ ; A5G5F1, *Geobacter uraniumreducens Rf4* ; A0W6N7, *Geobacter lovleyi* SZ ; Q3A8F1, *Pelobacter carbinolicus* ; Q9KIT1, *Desulfovibrio gigas* ; Q4VR71, *Desulfovibrio gigas* ; Q317A8, *Desulfovibrio desulfuricans* ; Q4W5X6, *Desulfovibrio vulgaris* ; Q72F31, *Desulfovibrio vulgaris* (strain Hildenborough) ; A1VGJ9, *Desulfovibrio vulgaris* subsp. *vulgaris* (strain DP4) ; A3CSD0, *Methanoculleus marisnigri* ; Q2FP22, *Methanospirillum hungatei* ; Q1JXC3, *Desulfuromonas acetoxidans* ; A6LZD7, *Clostridium beijerinckii* ; A5TVX9, *Fusobacterium nucleatum* subsp. *Polymorphum* ; Q73R43, *Treponema denticola* ; A6L4V4, *Bacteroides vulgatus* ; A6BG84, *Dorea longicatena* ; A5Z5C9, *Eubacterium ventriosum* ; A1HNM3, *Thermosinus carboxydivorans* ; A7I9F9, *Methanoregula boonei* ; Q05HF7, Uncultured methanogenic archaeon ; A7I8L1, *Methanoregula boonei* ; A0LNW3, *Syntrophobacter fumaroxidans* ; Q64UK8, *Bacteroides fragilis* ; Q5LDI8, *Bacteroides fragilis* ; A7UZ70, *Bacteroides uniformis* ; A7ALH0, *Parabacteroides merdae* ; Q8TJ24, *Methanosarcina acetivorans* ; Q46EN5, *Methanosarcina barkeri* ; Q8PYB7, *Methanosarcina mazei* ; Q8TTU7, *Methanosarcina acetivorans* ; A7I477, *Methanoregula boonei* ; Q0TSE8, *Clostridium perfringens* ; Q0SUJ9, *Clostridium perfringens* ; A7FS89, *Clostridium botulinum* ; A5I030, *Clostridium botulinum* ; A7GBM9, *Clostridium botulinum* ; A6LZ31, *Clostridium beijerinckii* ; A6LYA8, *Clostridium beijerinckii* ; A5N4A6, *Clostridium kluyveri* ; A7HME2, *Fervidobacterium nodosum* ; A8F5H2, *Thermotoga lettingae* ; A6CLI6, *Bacillus* sp. SG-1 ; Q65H10, *Bacillus licheniformis* ; A1RZJ8, *Thermophilum pendens* ; Q0W3X7, Uncultured methanogenic archaeon ; O28488, *Archaeoglobus fulgidus* ; Q5JIX7, *Pyrococcus kodakaraensis* ; Q9UZA8,

Pyrococcus abyssi ; O58586, *Pyrococcus horikoshii* ; Q8U370, *Pyrococcus furiosus* ; Q3AFM6,
Carboxydotherrmus hydrogenoformans ; Q46XD3, *Ralstonia eutropha* ; Q1LJ25, *Ralstonia metallidurans* ;
Q4IXH7, *Azotobacter vinelandii* ; Q220E3, *Rhodoferrax ferrireducens* ; Q3SHK8, *Thiobacillus denitrificans* ;
Q2NGX4, *Methanosphaera stadtmanae* ; Q2NGX5, *Methanosphaera stadtmanae* ; Q2NFT4, *Methanosphaera*
stadtmanae ; A5D4K6, *Pelotomaculum thermopropionicum* ; A7FYC8, *Clostridium botulinum* ; A5I6Q7,
Clostridium botulinum ; A7GIB9, *Clostridium botulinum* ; A0Q189; *Clostridium novyi* ; |Q0TQJ0, *Clostridium*
perfringens ; Q0ST39, *Clostridium perfringens* ; Q97GD1, *Clostridium acetobutylicum* ; A6LRZ4, *Clostridium*
beijerinckii ; A5TWU9, *Fusobacterium nucleatum* subsp. *polymorphum* ; Q8RDP3, *Fusobacterium nucleatum*
subsp. *nucleatum* ; A5Z8E1, *Eubacterium ventriosum* ; Q7MX56, *Porphyromonas gingivalis* ; A0UV23,
Clostridium cellulolyticum H10 ; A3DCA2, *Clostridium thermocellum* ; A7VGD3, *Clostridium* sp.L2-50 ;
Q1FLW4, *Clostridium phytofermentans* ISDg ; A5KK04, *Ruminococcus torques* ; A6BHV5, *Dorea longicatena*
; A7AYN6, *Ruminococcus gnavus* ; A5ZTR1, *Ruminococcus obeum* ; A7AFD4, *Parabacteroides merdae* ;
A6LA95, *Parabacteroides distasonis* ; A6L5K1, *Bacteroides vulgatus* ; A7V583, *Bacteroides uniformis* ;
Q64U76, *Bacteroides fragilis* ; Q5LD56, *Bacteroides fragilis* ; Q8A9S5, *Bacteroides thetaiotaomicron* ;
A5ZD23, *Bacteroides caccae* ; A7LX87, *Bacteroides ovatus*.



Supplemental Figure S3. Electrostatic potential maps. Clock wise: Ma-Flr1 (this work), flavoredoxin from *D. vulgaris* (PDB code: 2D5M), flavin reductase from *B. thermoglucosidasius* (PDB code: 1RZ1), and ferric reductase from *A. fulgidus* (PDB code: 1IOS).

