Supplementary Materials
The Magnetosome Protein, Mms6, is a Lipid-Activated Ferric Reductase

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Table S1. List of protein sequences used in this work

protein name	Sequence
Mms6	VGGTIWTGKGLGLGLGLGLGAWGPIILGVVGAGAVYAYMKSRDIESAQSDEEVELRDALA
m1Mms6	VGGTIWTGKGLGLGLGLGLGAWGPIILGVVGAGAVYAYADSEDIESAQSRRKVELEDALA
m2Mms6	VGGTIWTGKGLGLGLGLGLGAWGPIILGVVGAGAVYAYAKDRSIDEAQESDSVELREALA
m3Mms6	VGGTIWTGKGLGLGLGLGLGAWGPIILGVVGAGAVYAYAQSLERAEDEDADISAVEKLSR
C21Mms6	KSRDIESAQSDEEVELRDALA
GLtoGA	VGGTIWTGKGAGAGAGAGAGAWGPIILGVVGAGAVYAYMKSRDIESAQSDEEVELRDALA
W103A	VGGTIATGKGLGLGLGLGLGAWGPIILGVVGAGAVYAYMKSRDIESAQSDEEVELRDALA
W119A	VGGTIWTGKGLGLGLGLGLGAAGPIILGVVGAGAVYAYMKSRDIESAQSDEEVELRDALA
S138A	VGGTIWTGKGLGLGLGLGLGAWGPIILGVVGAGAVYAYMKARDIESAQSDEEVELRDALA
D140A	VGGTIWTGKGLGLGLGLGLGAWGPIILGVVGAGAVYAYMKSRAIESAQSDEEVELRDALA
S146A	VGGTIWTGKGLGLGLGLGLGAWGPIILGVVGAGAVYAYMKSRDIESAQADEEVELRDALA
D147A	VGGTIWTGKGLGLGLGLGLGAWGPIILGVVGAGAVYAYMKSRDIESAQSAEEVELRDALA
E148A	VGGTIWTGKGLGLGLGLGLGAWGPIILGVVGAGAVYAYMKSRDIESAQSDAEVELRDALA
E149A	VGGTIWTGKGLGLGLGLGLGAWGPIILGVVGAGAVYAYMKSRDIESAQSDEAVELRDALA
D154A	VGGTIWTGKGLGLGLGLGLGAWGPIILGVVGAGAVYAYMKSRDIESAQSDEEVELRAALA
S146E148A	VGGTIWTGKGLGLGLGLGLGAWGPIILGVVGAGAVYAYMKSRDIESAQADAEVELRDALA
Histidine tag:	MGGSHHHHHHGMASMTGGQQMGRDLYDDDDKDPTLGGHM

Legend: All proteins, except for C21Mms6, which was chemically synthesized, were produced as fusion proteins with the N-terminal Histidine tag.

Table S2. Summary of kcat determinations

Operator	Conditions		Kcat	Individual preparations							
Individual	prep	date	/sec	1178	1628	1629	1633	1634	1639	14611	14619
1	1628	8/2013	0.10		0.10						
2	1633	12/2013	0.14				0.14				
2	1634	12/2013	0.18					0.18			
2	1629	12/2013	0.19			0.19					
2	1629	12/2013	0.16			0.16					
2	1633	12/2013	0.19				0.19				
2	1634	12/2013	0.27					0.27			
2	1629	1/2014	0.14			0.14					
2	1633	1/2014	0.051				0.051				
2	1178	2/2014	0.21	0.21							
2	1639	6/2014	0.025						0.025		
3	14611	10/2020	0.073							0.073	
3	14611	10/2020	0.058							0.058	
3	14619	6/2022	0.66								0.66
3	14619	6/2022	0.61								0.61
3	14611	6/2022	140							140	
3	14611	6/2022	140							140	
3	14611	6/2022	0.24							0.24	
3	14611	6/2022	0.44							0.44	
3	14611	7/2022	0.56							0.56	
3	14611	7/2022	0.39							0.39	
3	14611	7/2022	0.025							0.025	
3	14611	7/2022	0.018							0.018	

	averages									
	All values	minus highest and lowest values	Individual preparations							
Average	12	0.24	0.21	0.10	0.16	0.13	0.23	0.025	25	0.64
standard deviation	40	0.2			0.025	0.070	0.064		59	0.035
coefficient of variation	325%	85%			15%	55%	28%		209%	6%
Median	0.19	0.18			0.16	0.14	0.23		0.32	0.64
Minimum	0.018	0.025			0.14	0.051	0.18		0.018	0.61
Maximum	140	0.66			0.19	0.19	0.27		140	0.66
N	23	20	1	1	3	3	2	1	10	2

LEGEND: Kcat results for a range of Mms6 preparations by three different people over a period of 9 years.

Table S3. Homologs identified by TM-Align

PDB	Homologs identified by TM-Align	Microbial source
3V2H	D-beta-hydroxybutyrate dehydrogenase	*Sinorhizobium meliloti*
3GVI	Lactate/malate dehydrogenase in complex with ADP	*Brucella melitensis*
3FG2	Ferredoxin Reductase for the CYP199A2 System	*Rhodopseudomonas palustris*
7DNM	AgCarB2-C2 complex	*Arthrobacter globiformis*
5LC5	Mammalian respiratory complex I, class2	*Bos taurus*
6AGS	L310R/Q401C mutant of malic enzyme	*Escherichia coli*
2VCF	Cytosolic ascorbate peroxidase with isoniazid (INH)	*Glycine max*
6T15	III2-IV(5B)1 respiratory supercomplex	Saccharomyces cerevisiae
1d4c	Flavocytochrome c fumarate reductase, uncomplexed	Shewanella putrefaciens strain mr-2
3EF6	Toluene 2,3-dioxygenase reductase	*Pseudomonas putida*
1YCG	Nitric oxide reductase	*Moorella thermoacetica FprA*
3KD9	Pyridine nucleotide disulfide oxidoreductase	*Pyrococcus horikoshii*

LEGEND: Enzymes identified by TM-Align as structural homologs of Mms6. The top three structures were used by I-TASSER as the basis of structural prediction.

Table S4. Summary of reported kinetic parameters

Bacterium	MW	pH opt	electron donor	cofactor	electron acceptor (Kd)		Kcat (nmole/min/mg)	Reference
			NADH or NADPH	FMN vs. FAD				
Mms6, M. magnetotacticum			NADH preferred	FAD		Fe-Citrate with FAD, NADH (76)	Fe-citrate (5.7)	this work
Mms6, M. magnetotacticum in bicelles							Fe-citrate (20)	this work
Archaeoglobus fulgidus	18	7	both	both	Fe-EDTA with NADH (61), with NADPH (80); flavin not specified	Fe-citrate (20.6)		1
Azotobacter vinelandii	44.6 and 69	7.5	preferred (16)	FMN preferred	Fe-Citrate with NADH, FMN (10)	Fe-citrate (21)	Fe-citrate (197)	2
Bacillus subtilis	N/R		NADPH	FMN	Fe-HBA with NADPH, FMN (55)	Fe-citrate (197)	Fe-DCIP (3.8)	3
Bacillus subtilis	13		NADPH (78) with oxygen as acceptor	FMN (12.5) with oxygen as acceptor	Fe-DCIP (385)	Fe-DCIP (3.8)		4
E. coli	26		both equal	both but FMN preferred				5
E. coli	26		NADH (9), NADPH (30)	FMN (1.5), FAD (1.9)				5
L. pneumophila	38		NADH (11), NADPH (9)				Fe-citrate (7)	6
M. magnetotacticum	36	7	NADH (1.3) NADPH (119)	FMN (0.035)	Fe-Citrate with FMN, NADH (15)	Fe-citrate (3111)		7
R. sphaeroides	32	7.1	NADH (18) not NADPH	FMN (3.2), FAD (14.1)	Fe-Citrate with FMN, NADH (8.3)			8
R. sphaeroides	41		NADH (20) not NADPH		Fe-Citrate with FMN, NADH (4.2)	Fe-citrate (1300), Fe-EDTA (13100)		8
Acidithiobacillus ferrooxidans	28	6.5	NADPH (76) not NADH	FMN	Fe-EDTA with NADPH, FMN (30)	Fe-citrate (1300), ferric-EDTA (13000)		9
Paracoccus denitrificans	19		NADH (5.5) not NADPH	FMN (20)	Fe-NLA with FMN, NADH (17)	Fe-EDTA (0.005); Fe-NLA (7.6)		10
Paracoccus denitrificans	18		NADH (2.6)	FAD		Fe-NLA (3.1), Fe-EDTA (0.16), Fe-citrate (0.08)	Fe-NLA (4.8)	10
Pseudomonas putida	29.5		NADPH (6.3)	both	Fe-EDTA with NADH, FAD (19), with NADH, FMN (8.7), with NADPH, FMN or FAD (~7); Fe-citrate with NADH, FAD (12), with FAD, NADPH (6.4)			11
Pseudomonas putida	29.4		NADH (2.4)	both	Fe-EDTA with NADH, FAD (13), with NADH, FMN (6.5), with NADPH, FMN or FAD (~15): Fe-citrate with NADH, FAD (6.2), with FAD, NADPH (12)			11
Magnetospirillum gryphiswaldense	16	6.5	NADH not NADPH	FMN (0.24)	Fe-citrate with FAD, NADPH (45)			12
G. sulfurreducens	78, 87	5.5	NADPH (25) not NADH	none added, a flavin is associated with the enzyme	Fe-NLA with NADPH (1000)	Fe-NLA with NADPH (65000)	Fe-NLA (65000)	13

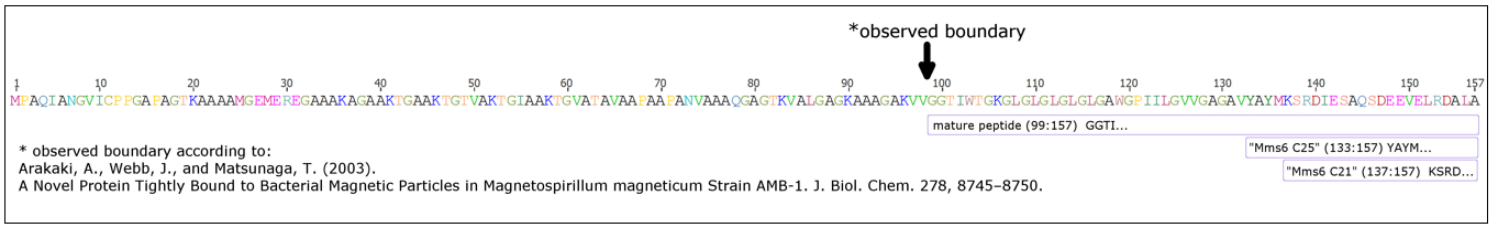
Summary of values

Bacterium	MW (Kda)	pH opt	electron donor (Kd)	Cofactor (Kd)	Fe(III)-EDTA (Km)	Fe(III)-citrate (Km)	Kcat (nmole/min/mg)	
Average	38	7	27	8.3	27	13	1775	
STD	21	1	35	8.3	26	11	4103	
Cv	56%	10%	1.3	1.0	1.0	0.84	231%	
Median	31	7	16	7.9	15	12	14	
N	18	7	15	6.0	9.0	12	10	
Minimum	13	5.5	1.3	0.035	6.5	4.2	5E-03	
Maximum	87	7.5	119	20	80	45	13100	

LEGEND: Summary of kinetic parameters for other reductases reported in the literature. The numbers in parentheses are the reported Kd or Km values and these are summarized in the lower part of the table where the average, media, maximum and minimum values are reported.

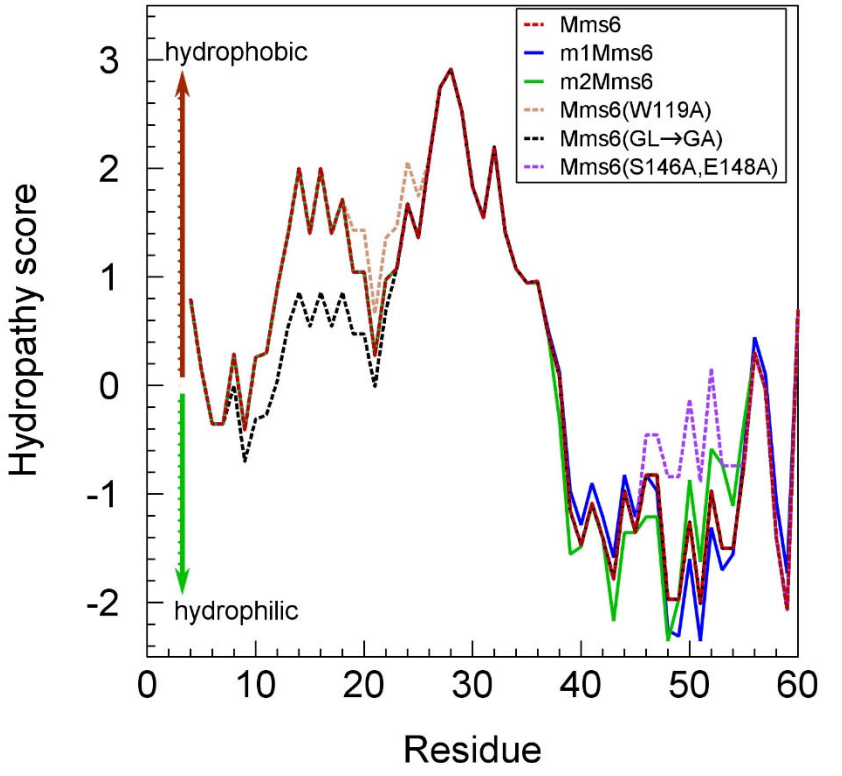
FIGURES

Figure S1. Residue numbering for Mms6 and mutants



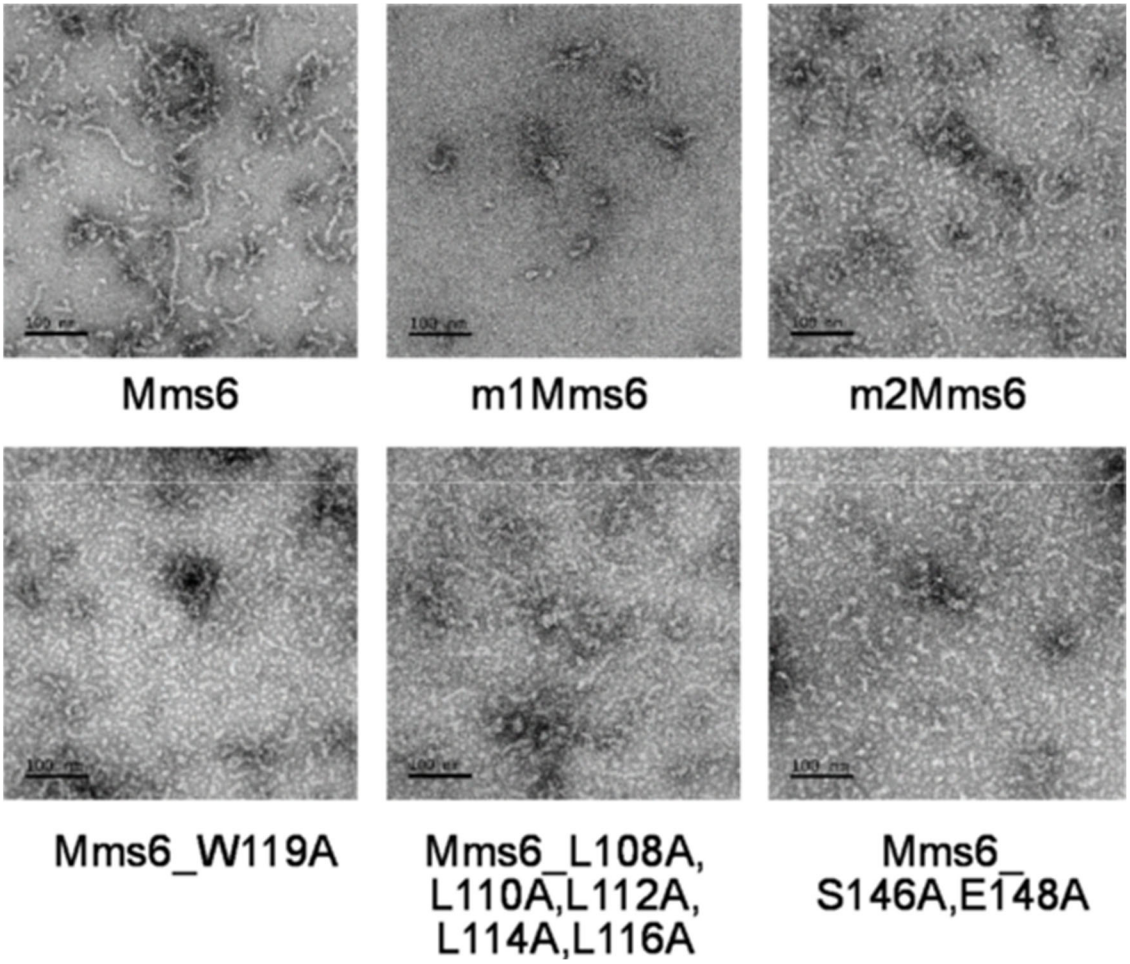
LEGEND: The numbering scheme for Mms6 residues relative to the translation of the complete gene sequence.

Figure S2. Hydropathy plots of Mms6 and its sequence variants used in this work.



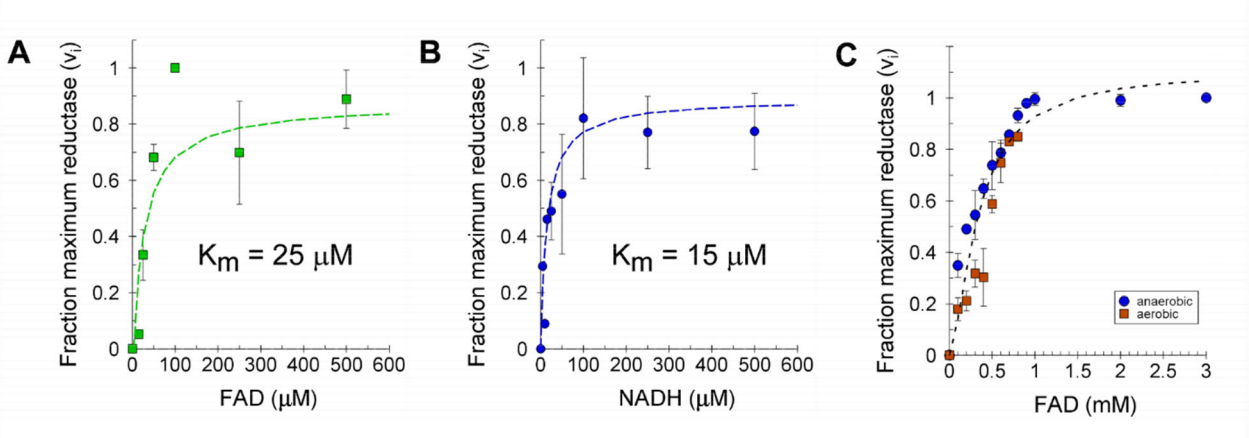
LEGEND: Hydropathy plots were obtained using the Web tool: ProtScale (on the ExPASy Server) http://expasy.org/tools/protscale.html. Parameter settings were: window size = 7, Relative weight of the window edges = 100%, Choices in modeling were: Weight variation model = linear, and normalized scale = no.

Figure S3. The effects of C-terminal sequence scrambles on the ability of Mms6 to form micelles.



LEGEND: Morphologies of Mms6 mutants observed in TEM images.

Figure S4. Km estimates for FAD and NADH and a comparison between aerobic and anaerobic conditions.



LEGEND: Reductase activity (initial velocity) was determined under aerobic conditions over a range of (A) FAD concentrations and (B) NADH concentrations. Calculated Km values are shown. These are considered rough estimates as the signal was low and the errors are large. (C) In a separate set of experiments, the reductase activity at various concentrations of FAD was compared under aerobic and anerobic conditions

Figure S5. Effect of hemin on Mms6 reductase activity.

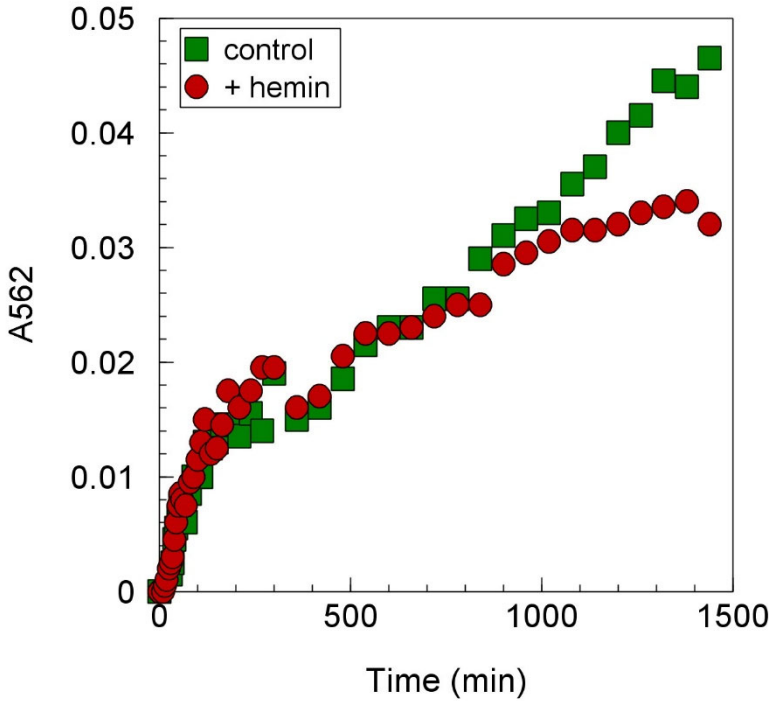
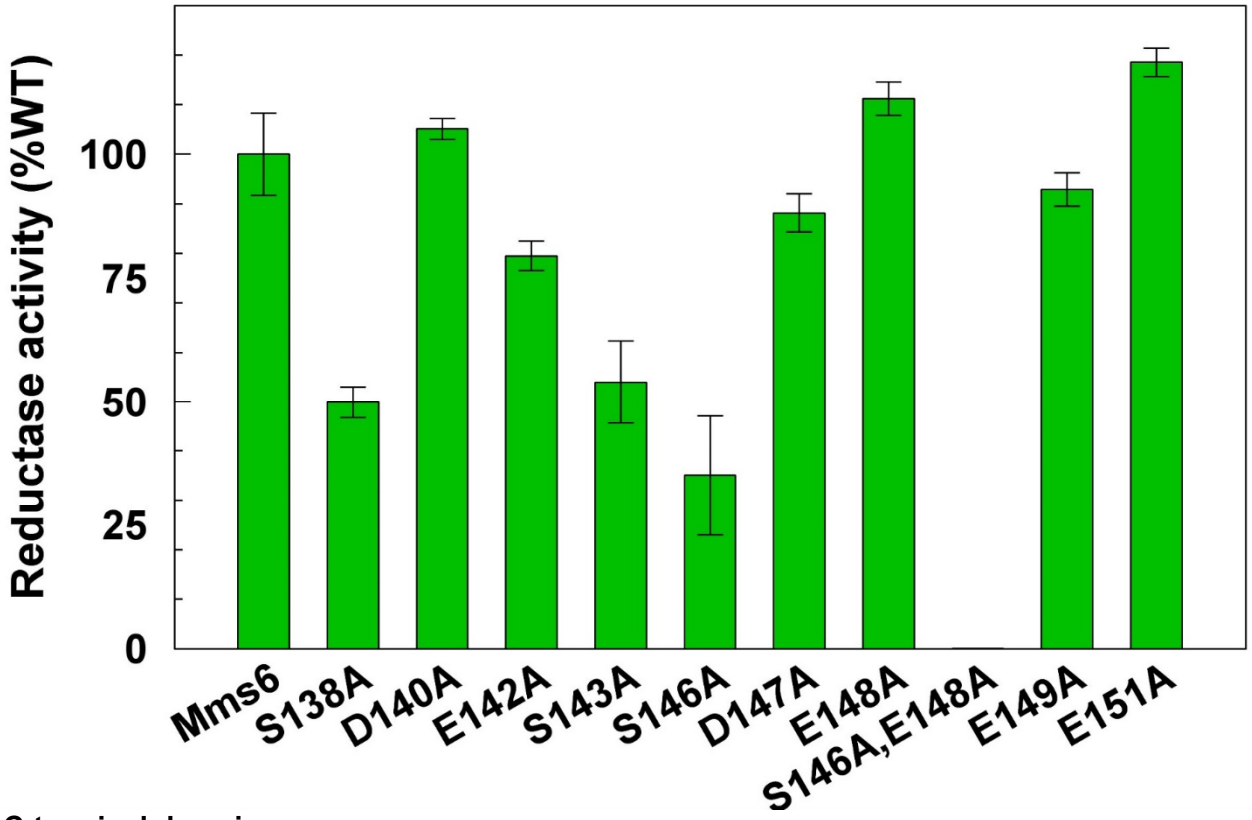
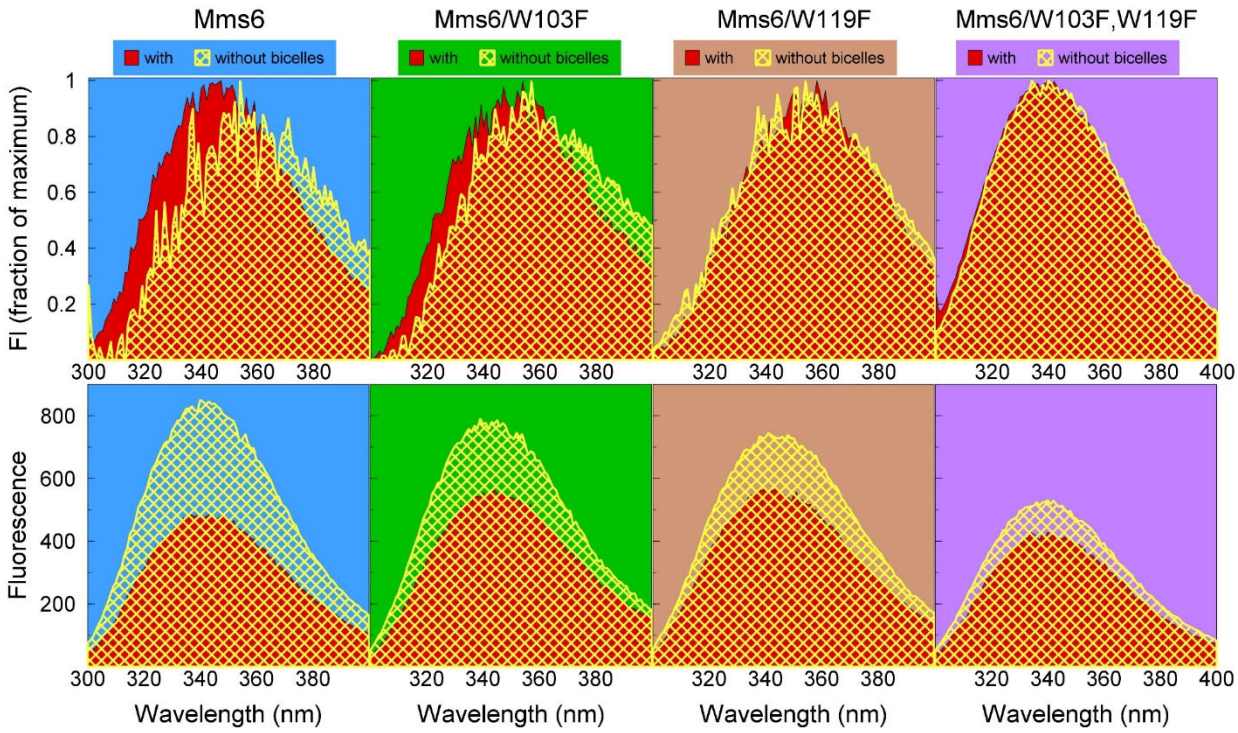


Figure S6. Ferric reductase activities of Mms6 and mutants with Ala-substitutions in the C-terminal domain.



LEGEND: The ferric reductase activity of each recombinant protein was determined as described in Materials and Methods with 20 μM protein and 100 μM ferric-citrate.

Figure S7. Identification of the Trp that changes in its hydrophobic environment when Mms6 associates with bicelles.



LEGEND: The fluorescence shift lost with W119F but not with W103F.

Citations

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