Wang et al. Supplemental Information

Materials and Methods

Cloning and express of recombinant mature Mms6 protein

Genomic DNA was obtained from *Magnetospirillum magneticum* strain AMB-1. The DNA was amplified using primers that are complementary to internal sites on the Mms6 gene coding region. The genomic DNA was amplified using primers that are complementary to internal sites on the Mms6 gene coding region. The Mms6 was expressed as recombinant protein with a poly-His tag attached to the N-terminus and purified as described previously.[1] The sequence of the recombinant Mms6 is: MGGSHHHHHHGMASMTGGQQMGRDLYDDDDKDPTLGGHM**VGGTIWTGKGLGLGLGL GLGAWGPIILGVVGAGAVYAYMKSRDIESAQSDEEVELRDALA** (Bold: the sequence of the mature Mms6 protein cloned).

Verification of Mms6 monomeric mass and sequence

The purified protein was analyzed by mass spectrometry with the result that the expected mass (10.2 kDa) was obtained. In addition, the protein was excised from the gel and subjected to N-terminal protein sequencing to verify the presence of the histidine tag. It should be noted that the protein runs anomalously through an SDS-PAGE gel with an apparent molecular weight larger than expected by its sequence or measured by mass spectrometry. The basis of this discrepancy is unknown.

Materials:

Peptides were provided by Genscript as powders that were solubilized in deionized, distilled water for further use.

Expression vectors for m2Mms6 and m3Mms6 were prepared using annealed complementary oligonucleotides encoding the appropriate mutant sequence to replace the previously cloned wild-type sequence. CLC protein workbench software (CLC bio) was used to identify the sequences for m2Mms6 and m3Mms6 that possessed similar hydropathy plots to that of Mms6.

Column chromatography:

C21Mms6 does not contain an aromatic side chain therefore in all chromatographic studies of C21Mms6, it was quantified fluorescently by using o-phtalaldehyde[2] (OPA, Thermo Scientific) by adding 200 µL of OPA to 20 µL of column fraction and measuring fluorescence (Ex: 350 nm, Em: 450 nm). C25Mms6 (YAYMKSRDIESAQSDEEVELRDALA) was used as the standard (its concentration was determined by A280 using extinction coefficient of 2980 M^{-1} cm^{-1}).

C21Mms6 was also found not to adsorb efficiently to nitrocellulose filters, the C21Mms6-bound and unbound Fe^{3+} were separated by size exclusion chromatography. The ferrozine assay was used to determine the concentration of iron in each column fraction and OPA was used to quantify the peptide.

Liposome preparation:

1-palmitoyl-2-oleoyl-*sn*-glycero-3-choline (POPC) and 1,2-dioleoyl-*sn*-glycero-3-phospho-L-serine (DOPS) in chloroform at a molar ratio of 85:15, were dried by vacuum and resuspended in 20 mM Tris-HCl, 100 mM KCl, pH 7.5. Liposomes were prepared by extrusion through polycarbonate filters[3]. All reagents were from Avanti Polar Lipids.

Proteolytic digestion in the presence and absence of liposome pre-incubation:

Two conditions were used for proteolytic digestion of Mms6. In the first case, 1 mg/mL Mms6 was digested with 0.1 mg/mL proteinase K (Sigma) in 20 mM Tris-HCl, 100 mM KCl, pH 7.5 and incubated at 25℃ for up to 24h. In the second case, 1 mg/mL Mms6 was first incubated with 10 mM (total lipid concentration) liposome in 20 mM Tris-HCl, 100 mM KCl, pH 7.5 with and without 0.5% Triton X-100 and incubated at 25℃ or 4℃ for 1h with constant inversion. Samples that included Triton X-100 were then dialyzed against 20 mM Tris-HCl, 100 mM KCl, pH 7.5 with three changes in the presence of Bio-Beads SM-2 detergent adsorbent beads (Bio-Rad) to remove the detergent.[3] Mms6 (0.8 mg/mL) with liposomes was digested with 0.2 mg/mL proteinase K in 20 mM Tris-HCl, 100 mM KCl, pH7.5 at 25℃ for up to 24h. All reactions were stopped with 9 mM phenylmethylsulfonyl fluoride (Sigma) then 95℃ for 5 min. The samples were resolved by 15% SDS-PAGE.

$^{55}Fe^{3+}$ binding assays:

Mms6 (100 nM or 1 μM) in 100 mM KCl was incubated with ^{55}Fe (PerkinElmer) as ferric chloride (pH 3) or ferric citrate, 20 mM Tris-HCl (pH 7.5) at various concentrations of iron. Unless otherwise noted, the incubations were 2h at 25℃. The incubations with ferric citrate were performed in brown test tubes to prevent possible auto-reduction of ferric citrate. The samples were filtered through 0.45 μm prewashed nitrocellulose membranes (Millipore) followed by two consecutive 5 mL volumes of buffer at the same temperature as during the incubation. The radioactivity was counted using Scintiverse BD LSC Cocktail scintillation solution (Fisher) and liquid scintillation analyzer (Packard, model 1600 TR).

Magnetite nanoparticle formation

In a typical synthesis, 100 μL of 25 wt% Pluronic F127 solution and 100 μL protein solutions containing 20 μg Mms6, m2Mms6 or m3Mms6 were added to a 5 mL degassed pear-shape flask. The flask was then degassed, filled with argon and incubated at 4°C for 30 min for the protein and Pluronic to mix. Then, the flask was degassed, added 50 μL of 0.5 M $FeCl_3$ and 50 μL of 0.25M $FeCl_2$, and filled with argon. The flask was then incubated at 4°C again for mixing. The resulting solution was then brought to room temperature, and titrated slowly with the room-temperature 1400 μL of 0.1 M NaOH solution under constant argon flow. As the titration progressed, there was a black particle layer formed on the top of the solution. Nanoparticles were allowed to grow and precipitate at room temperature in the argon purged and sealed flask for 5 days, after which the precipitated nanoparticles were washed with degassed water three times before characterization.

Supplemental Data:

Figure S1. CD spectra of Mms6 at pH 3 and 7. All CD measurements were performed on a Jasco J-710 spectropolarimeter in a 0.1 cm path-length quartz cell at 25 ℃. CD data were collected from 190 to 250 nm with a scanning speed of 50 nm/min, resolution of 0.2 nm, bandwidth of 1.0 nm, sensitivity of 20 millidegree, time response of 8 s and average of 2 scans. CD spectra were expressed as the mean residue ellipticity, [θ] (deg*cm^2/dmole). CD data were analyzed by the program JFIT. Shown are the CD spectrum of 50μM Mms6 in 20 mM Tris, 100 mM KCl, pH 7.5 (●) or 50 mM sodium formate, 100 mM KCl, pH 3 (○).

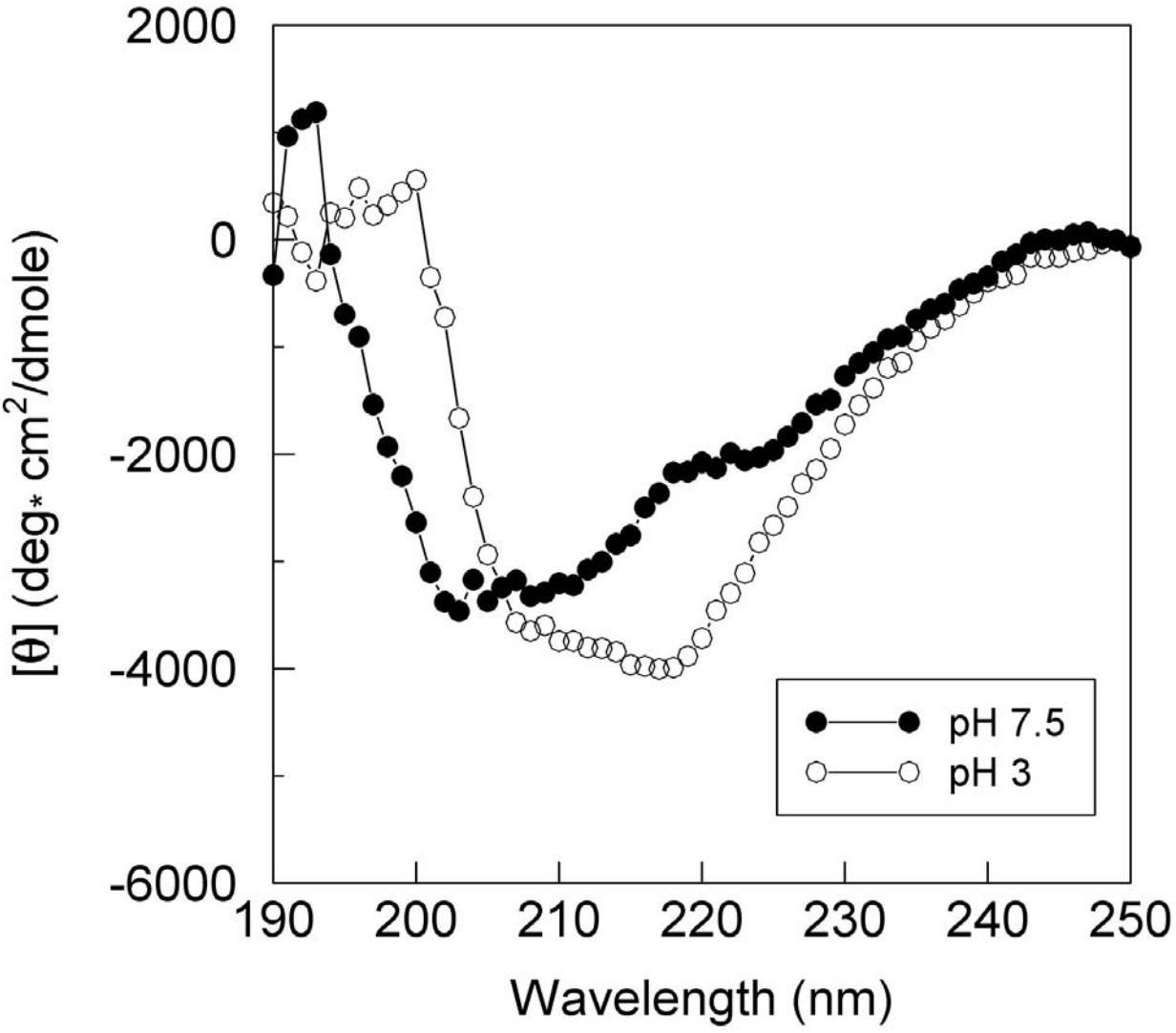
Figure S2. Standard curve for Superdex G75 column chromatography. Blue dextran was used to determine the void column volume (Vo) of the column. The elution volumes (Ve) of bovine serum album (MM 66 kDa), ovalbumin (MM 44.3 kDa), carbonic anhydrase (MM 29 kDa), myoglobin (MM 17.6 kDa), aprotinin (MM 6.5 kDa) and B12 (MM 1355 Da) (all from Sigma) obtained by Superdex G75 10/300GL were used to generate the standard curve for the apparent molecular mass estimations of Mms6, m2Mms6 and m3Mms6.

Figure S3. Isothermal titration calorimetry. Isothermal titration calorimetry (ITC) experiments were performed using a Nano ITC isothermal titration calorimeter (TA Instruments, New Castle, DE). In each experiment, 300 μM $FeCl_3$ was titrated using the computer-controlled syringe into the sample cell (0.95 mL) containing 15 μM Mms6 at 25 °C. Both Mms6 and $FeCl_3$ were in 50 mM sodium formate, 100 mM KCl, pH 3. The syringe was set at a stirring speed of 250 rpm. After a 300 s initial delay, each titration involved an initial 1 μL injection followed by 24 serial injections of 10 μL each at intervals of 300 s. The raw data obtained in each experiment were corrected for the effect of titrating $FeCl_3$ from the syringe into the sample cell containing 50 mM sodium formate, 100 mM KCl, pH 3 but no Mms6. The thermodynamic parameters were calculated using an independent binding model in the software (Nanoanalyze software, version 2.2.0) provided by TA Instruments (New Castle, DE).

References:

1. Prozorov, T., Mallapragada, S. M., Narasimhan, B., Wang, L., Palo, P., Nilsen-Hamilton, M., Williams, T. J., Bazylinski, D. A., Prozorov, R., and Canfield, P. C. (2007) *Adv. Funct. Mater.* **17**, 951-957
2. Drescher, D. G., and Lee, K. S. (1978) *Anal. Biochem.* **84**, 559-569
3. Lu, X., Zhang, Y., and Shin, Y.-K. (2008) *Nat. Struct. Mol. Biol.* **15**, 700-706

Figure S1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0
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-4000
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[θ] (deg* cm2/dmole)
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200
210
220
230
240
250
Wavelength (nm)
pH 7.5
pH 3

Figure S2

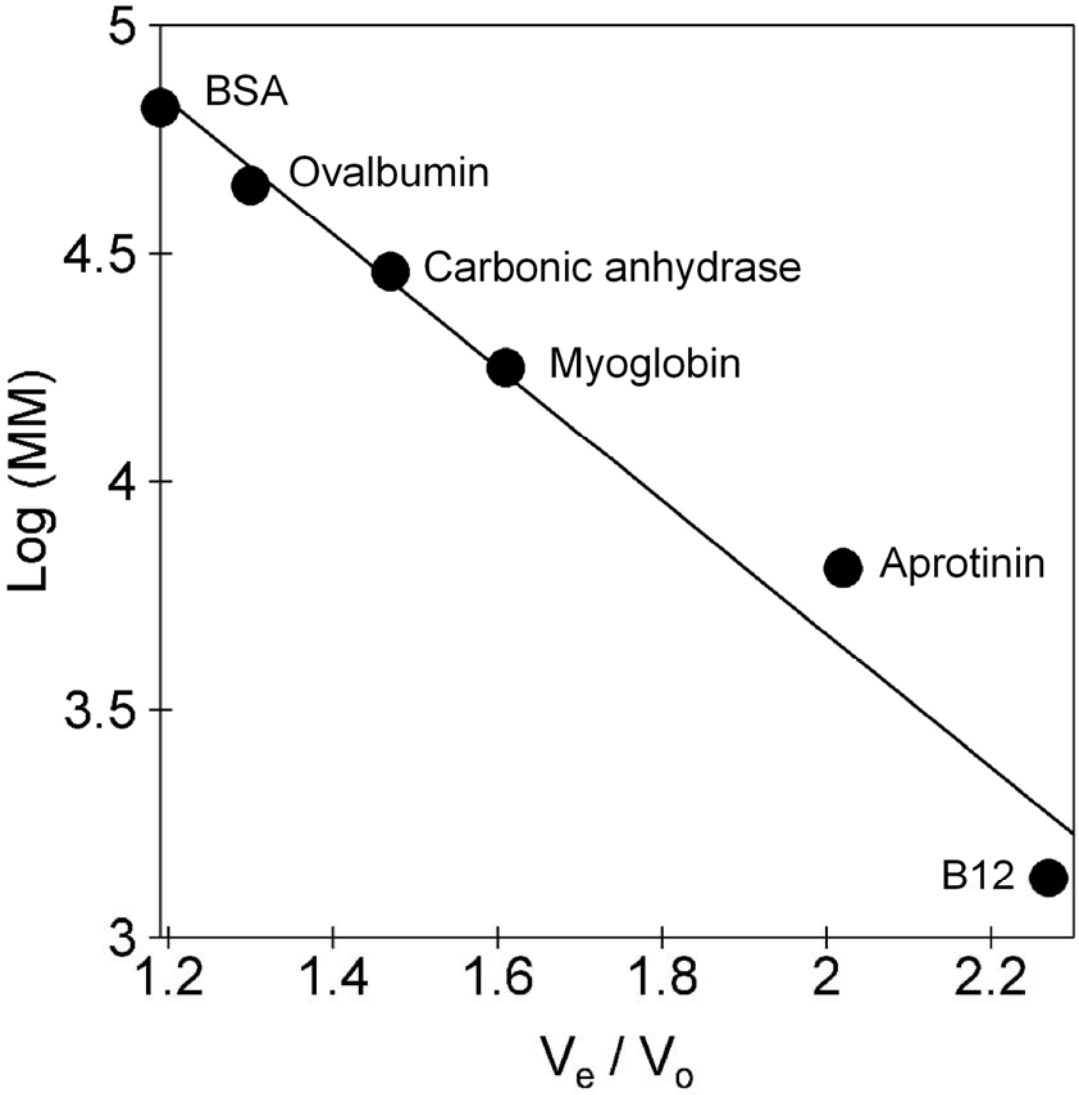
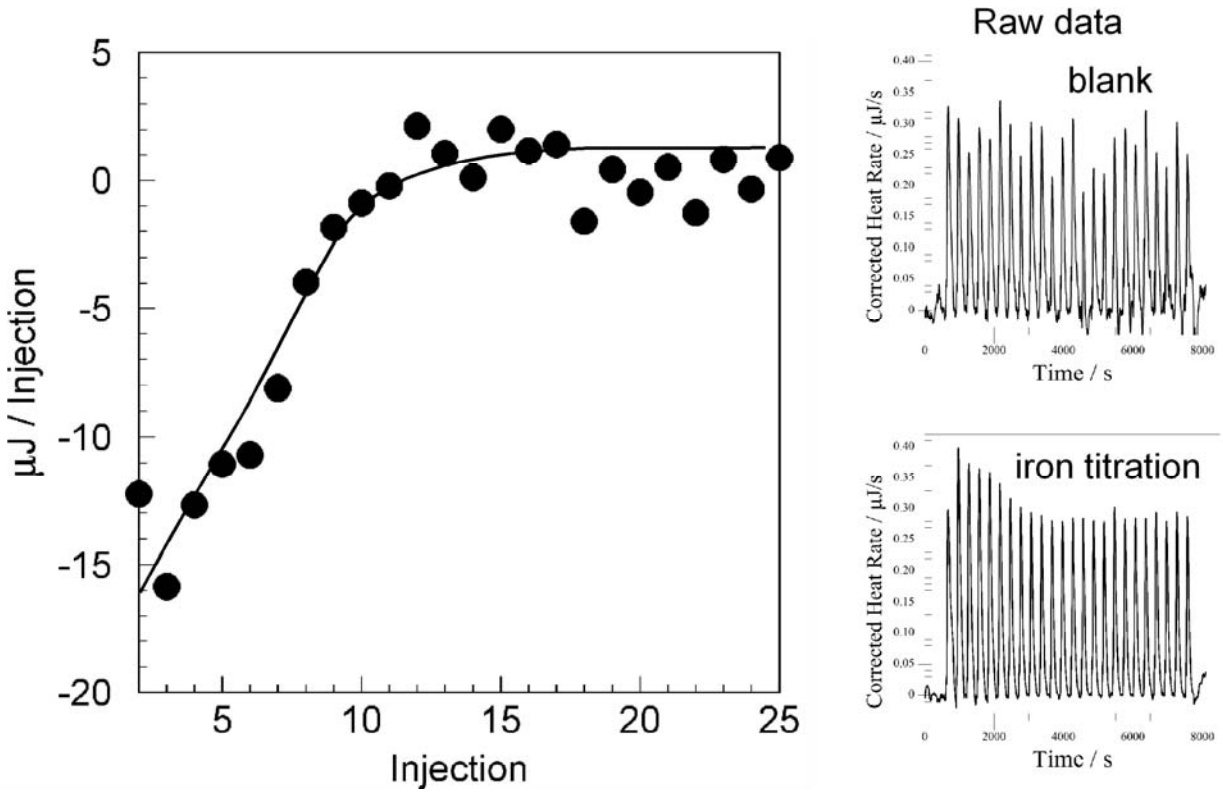

5
4.5
4
3.5
3
Log (MM)
BSA
Ovalbumin
Carbonic anhydrase
Myoglobin
Aprotinin
B12
1.2
1.4
1.6
1.8
2
2.2
V_e / V_o

Figure S3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Injection
Raw data
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iron titration
Corrected Heat Rate / µJ/s
Time / s