

# UC Berkeley

## UC Berkeley Electronic Theses and Dissertations

**Title**

Strategies of Organelle Organization in Magnetotactic Bacteria

**Permalink**

<https://escholarship.org/uc/item/0g2863pg>

**ISBN**

9798189271533

**Author**

Ra, Yein

**Publication Date**

2026-05-01

Peer reviewed|Thesis/dissertation

Strategies of Organelle Organization in Magnetotactic Bacteria

By

Yein Ra

A dissertation submitted in partial satisfaction of the

requirements for the degree of

Doctor of Philosophy

in

Microbiology

in the

Graduate Division

of the

University of California, Berkeley

Committee in charge:

Professor Arash Komeili, Chair

Professor Michi E. Taga,

Professor Karine Gibbs,

Professor Matthew D. Welch

Spring 2026



## Abstract

### Strategies of Organelle Organization in Magnetotactic Bacteria

by

Yein Ra

Doctor of Philosophy in Microbiology

University of California, Berkeley

Professor Arash Komeili, Chair

Of the three domains of life, bacteria are by far the most ancient and most diverse [1]. Bacteria thus have evolved creative and varied solutions to different biochemical, structural, and cellular problems. One representation of this diversity is the catalog of different intracellular compartments that bacterial species produce to store excess nutrients, make biochemical reactions more efficient, and/or coordinate cellular processes [2]. The number of bacterial organelles identified has greatly expanded in the last few decades, owing to the development of high-resolution imaging technology. However, many questions regarding the cell biology of these organelles still remain unanswered. How does the bacterial cell regulate the production and function of their organelles? What mechanisms are in place to control the spatial distribution of organelles, and are there any similarities to those in eukaryotic systems? In species that produce multiple types of compartments, is there any coordination in their activity? To address these unknowns, this dissertation features new research that advances our knowledge of how magnetotactic bacteria (MTB) organize their organelles.

I start with a literature review of known organelle positioning systems in Chapter 1. The best-studied models of bacterial organelle localization - featuring the carboxysome and the magnetosome - are discussed in detail. Additionally, the current knowledge on the organization of polyhydroxybutyrate and polyphosphate granules is discussed. This chapter also explores the future outlook on the bacterial organelle biology field.

Chapter 2 features original research conducted to understand how magnetosomes, lipid membrane-bound compartments where a magnetite crystal is biomineralized [3], are organized in MTB. I focus on how two proteins, McaA and McaB, create subchains of magnetite in the model MTB *Magnetospirillum magneticum* AMB-1. Using biochemical and microscopy methods, we discovered that McaA and McaB control the dynamics of a bacterial actin-like protein MamK to arrange magnetosomes into a complex pattern.

Chapter 3 contains the preliminary findings of how different organelles may be co-regulated in bacteria. In eukaryotic cells, many organelles undergo interorganelle communication to coordinate their activity and physical position in the cell [4–6]. To uncover whether bacteria also have interorganelle communication, we tested whether the absence of any magnetosome genes impacts a second organelle, the polyhydroxybutyrate granule (PHBG). We discovered that MamA regulates both the total number of magnetite crystals in a cell and PHBG size. We use biochemical and genetic experiments to characterize the PHBG and its relationship with MamA. This work provides the foundation for further studies on how magnetosomes and PHBGs are coordinated by a single protein in MTB.

Finally, Chapter 4 summarizes the work and states the major conclusions of this thesis. I finish with how this work contributes to the field of bacterial cell biology and directions for future studies in this area.

This dissertation is dedicated to my 9th grade biology teacher, Mr. Nayif Abdullah, the first person to show me the fascinating world of molecular biology and whose dedication for teaching created a passion for science that still motivates me today.

## **Table of Contents**

### **Chapter 1**

Organizing organelles: a review of bacterial organelle positioning systems

### **Chapter 2**

Magnetosome organelles are organized through interactions between McaA and McaB that alter the dynamics of the bacterial actin-like protein MamK

### **Chapter 3**

Magnetosome protein MamA regulates size of another organelle, the PHB granule, in *Magnetospirillum magneticum* AMB-1

### **Chapter 4**

Conclusions and Future Directions

## **Chapter 1**

Organizing organelles: a review of bacterial organelle positioning systems

Yein Ra and Arash Komeili

Department of Plant and Microbial Biology, University of California, Berkeley, CA, USA

Adapted from a version published in Current Opinion in Microbiology [7].  
[doi.org/10.1016/j.mib.2025.102614](https://doi.org/10.1016/j.mib.2025.102614)

## ABSTRACT

Bacteria contain multiple subcellular compartments that enable a variety of biochemical activities and behaviors. In many cases, the organization of these organelles is not random and is directly linked to their function. In the last decade, mechanistic studies have uncovered the machinery responsible for organelle positioning in some bacterial systems. In this chapter, I review currently known positioning systems with an emphasis on two arrangement patterns and the molecular mechanisms used to achieve them. We start with carboxysomes as an illustration of how a ParA/MinD ATPase system is used to equally distribute organelles in a cell. We follow with an example of an actin-like cytoskeletal system that links lipid-bounded magnetosome organelles into a continuous chain. We finally explore emerging models of bacterial organelle positioning and conclude with an outlook on the future opportunities in the study of bacterial organelle cell biology.

## INTRODUCTION

Having organelles is no longer considered a strictly eukaryotic trait; we now know that a vast repertoire of organelles exists in bacteria [2]. These subcellular compartments, spanning from lipid-bounded ferrosomes to nanoscale encapsulins, are characterized by a distinct boundary with a specific internal composition that often enables the execution of otherwise inefficient or toxic biochemical reactions [8,9]. Functions of organelles include concentration of substrates with their enzymes, containment of toxic metabolites, and storage of energy-rich compounds [2]. Careful observations of organelle distributions reveal that many are not randomly positioned within the cell. Indeed, bacterial species have evolved dedicated positioning systems to localize organelles. One common motivation is to ensure the proper segregation of organelles during cell division, which is typically achieved by equally distributing organelles along the cell length [10,11]. Other times, an organelle's position and organization is directly linked to its cellular function [12].

This review aims to summarize different strategies and the underlying molecular mechanisms of organelle positioning in bacteria. We first detail the current knowledge on the two best-studied examples of bacterial organelle positioning: the ParA/MinD family ATPase-based system of carboxysome organization and the cytoskeleton-based system of magnetosome chain formation. We next describe two emerging models of bacterial organelle positioning: the polyhydroxybutyrate (PHB) granule and the polyphosphate granule. We conclude with a generalized model of bacterial organelle organization and an outlook on future research endeavors in this area.

## DISCUSSION

### Carboxysomes – Spacing through McdA gradients

Organisms that fix carbon through the Calvin-Benson-Bassham cycle face an issue with ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO): the carbon-fixing enzyme also reacts with oxygen, leading to unproductive photorespiration. Cyanobacteria and other carbon-fixing proteobacteria have solved this problem by concentrating carbon dioxide in the same compartment that houses RuBisCO. This bacterial organelle – the carboxysome – has a proteinaceous shell and is the best studied bacterial microcompartment (BMC) [13].

Carboxysome positioning has been primarily studied in  $\beta$ -cyanobacterium *Synechococcus elongatus* PCC 7942 and chemoautotrophic proteobacterium

*Halothiobacillus neapolitanus* [14,15]. In both organisms, carboxysomes are linearly distributed uniformly along the cell length by the action of two interacting proteins, McdA and McdB [10,14]. The importance of the McdA and McdB can be clearly seen via genetic analyses since deletion of *mcdA* and/or *mcdB* results in mislocalized and aggregated carboxysomes [10,14]. A series of elegant biochemical experiments show that McdA and McdB work similarly to the well-described ParAB DNA segregation system [14,16]. McdA is a ParA-type ATPase [10,14]. When ATP-bound, it dimerizes and nonspecifically binds to the nucleoid [14,17,18]. McdB localizes to carboxysomes and triggers the ATPase activity of McdA [14]. This causes McdA to be released from DNA, creating zones of differing McdA concentrations along the nucleoid. Because McdB-bound carboxysomes migrate towards higher concentrations of McdA while also clearing nearby DNA-bound McdA, carboxysomes in close proximity are more likely to move away from one another. The overall result of the McdAB system is uniformly distributed carboxysomes along the nucleoid (Figure 1) [14].

Deeper investigation of the molecular workings of McdB has revealed a connection between carboxysome positioning and phase separation (PS), a process in which a molecule solution is separated into a dilute and concentrated phase [19]. Sequence analysis of over 500 predicted McdBs show that the protein varies widely in both sequence and length [15,20]. The few features shared among all McdB homologs, such as an intrinsic disordered domain, indicate that the ability to perform PS is conserved [15,20]. In *S. elongatus*, McdB PS is pH-dependent [20,21] and partially driven by its positively charged N-terminal intrinsic disordered region [21]. Replacing the positively charged residues with glutamine drastically reduces McdB condensate formation and its ability to position carboxysomes [21]. The positively charged residues might also be required for interactions with McdA, therefore whether the mispositioning of carboxysomes is due to lack of PS or loss of McdA interaction remains unresolved. Another conserved feature across all McdB proteins is an invariant tryptophan at the C-terminal end. The tryptophan is necessary for McdB localization to carboxysomes and also affects its ability to form condensates [22]. Replacing the tryptophan with other aromatic residues partially restores McdB localization and PS ability to varying degrees. Greater condensate formation *in vitro* is correlated with higher frequency of McdB carboxysome localization, providing further evidence that PS plays a role in carboxysome targeting [22].

The conservation of *mcdAB* highlights the significance of correct carboxysome positioning [14,15,20]. Indeed, the equidistant distribution ensures equal division of carboxysomes between the two daughter cells after cell division [10]. Cells that inherit carboxysomes have shorter doubling times and greater carbon fixation rates compared to cells that synthesize all carboxysomes *de novo* [10]. Furthermore, *S. elongatus* strains with mispositioned carboxysomes have increased occurrence of elongated cells, asymmetrical cell division, slower growth rates, and higher RuBisCO levels when grown with high CO<sub>2</sub> [23]. Given the importance of correct carboxysome positioning, it is unsurprising that *mcdAB* is present in the majority of genomes of carboxysome-producing organisms analyzed [15,20]. An unexpected exception are  $\alpha$ -cyanobacteria, for which no *mcdAB* system could be identified [20]. Whether  $\alpha$ -cyanobacteria have a dedicated carboxysome positioning system is still an open question.

Though most *mcdAB* operons are encoded near carboxysome genes, occasionally *mcdAB*-like genes are detected near operons of other BMCs, such as ethanolamine utilization microcompartments or 1,2-propanediol utilization microcompartments (PDU) [15]. This suggests that McdAB-like proteins position BMCs other than carboxysomes in selected organisms. It also appears that McdAB-like proteins are not the only BMC positioning system. For example, the minor shell protein PduK in *Salmonella enterica* serovar Typhimurium prevents aggregation of PDU at the cell poles [24]. More directed studies on the spatial organization of non-carboxysome BMCs will undoubtedly uncover novel organelle positioning strategies.

### **Magnetosomes – Linking through cytoskeletal filaments**

Many microbes face the challenge of navigating towards desired nutrients or stimuli. To improve the efficiency of this process, magnetotactic bacteria (MTB) have evolved to align to and navigate along the geomagnetic field using magnetosomes, membrane-bounded compartments in which a magnetic crystal is biomineralized [3]. This behavior is thought to simplify the organism's search for the oxic-anoxic transition zone of the water column, a process termed magnetoaerotaxis. A prerequisite to magnetoaerotaxis is the formation of a linear chain of magnetosomes, allowing individual magnetic crystals to collectively act as a single compass needle to orient the cell [3]. MTB strains with aggregated or dispersed magnetosomes have severely impaired magnetoaerotaxis [12,25], exemplifying how an organelle's position can directly affect its function.

Magnetosome chain assembly and positioning have been best studied in two model MTB, *Magnetospirillum gryphiswaldense* MSR-1 and *Magnetospirillum magneticum* AMB-1 (referred to as MSR-1 and AMB-1, respectively). Both species employ a similar cohort of proteins to assemble their magnetosome chain (Figure 2) [3]. The system's three key components—MamK, MamJ, and MamY—work in concert to form a cohesive magnetosome chain centered along the positive curvature of the inner cell membrane [12,26,27].

At the core of building a magnetosome chain is MamK, which forms a distinct branch of the bacterial actin-like family of proteins [26]. MamK polymerizes into a collection of parallel filaments, spanning from cell pole to pole, on which magnetosomes attach [26,28]. Similar to other actins, MamK monomers polymerize into double-stranded filaments when bound to ATP [29]. While incorporated in the filament, MamK hydrolyzes its ATP to ADP, which favors monomer disassociation [29]. The ATP-dependent polymerization and depolymerization gives MamK its dynamic behavior. In MSR-1, MamK polymerization occurs at the cell poles, which pushes the connected magnetosomes towards the midcell to establish a single, continuous chain (Figure 2B) [30]. The centered magnetosome chain that becomes polarly localized due to cell division also relies on MamK to migrate to the new midcell [30]. Removing MamK's ATPase activity or deleting *mamK* causes large gaps between magnetosomes, reduction in the cell's ability to align with a magnetic field, and unequal magnetosome segregation during cell division [26,28,30–32]. Thus, MamK governs the spacing between magnetosomes in the cell.

MamK's function in this system requires it to interact with magnetosomes. This role is filled by the acidic protein MamJ [12]. In the absence of *mamJ*, MamK filaments are no longer associated with magnetosomes and magnetosomes form an aggregate in MSR-1

[33]. MamJ's magnetosome localization requires MmsF or MmxF, two distant Tic20 protein transporter homologs [25]. Further studies are required to identify how MmsF and MmxF recognize MamJ and other client proteins as a cargo for magnetosome targeting.

A particular challenge for species like MSR-1 and AMB-1 is positioning a straight chain in their spirillum-shaped cell, which has no obvious linear landmarks. This task is achieved by relying on the distinct cell membrane curvatures of these species: because of their helical cell shape, AMB-1 and MSR-1 have areas of positive curvature, where the inner cell membrane is convex [27]. Similar to a fireman's pole running down the center of a spiral staircase, the magnetosomes follow the positive curvature of the cell body to maintain a linear chain. MamY is a membrane protein that localizes to the positive curvature and, like MamK, relies on MamJ to connect to magnetosomes. MamY recognition of the positive cell curvature is driven by self-interaction and appears to be an intrinsic property of the protein, since it localizes similarly in a heterologous host [27]. In the absence of *mamY*, the magnetosome chain is localized to the incorrect curvature and the cell is less efficient at orienting with a magnetic field [27,34]. Interestingly, MamK and MamY appear to act as two independent systems where the former pushes magnetosomes into a single chain and the latter localizes the chain to the positive curvature.

Although MSR-1 and AMB-1 are closely related MTB, there are a number of differences in their magnetosome chain assembly. For example, while MSR-1 has only one copy of *mamK*, AMB-1 has a second copy dubbed *mamK-like* [35]. MamK-like interacts with MamK, contributes to magnetosome chain formation, and increases MamK filament turnover [36]. Similarly, AMB-1 has multiple homologs of MamJ, two of which—MamJ and LimJ—appear to play redundant roles [32,34]. Perhaps the most visible difference between MSR-1 and AMB-1 is the organization of the magnetosome chain. In MSR-1, older magnetosomes are found at the chain center and newer magnetosomes, many of which have not initiated biomineralization, are at the chain's edge (Figure 2B). In contrast, AMB-1's magnetosome chain features subchains of either older or newer magnetosomes that join end-to-end (Figure 2C) [34]. The distinct AMB-1 organization is produced by the action of McaA and McaB. McaA localizes to the positive cell curvature and McaB localizes to magnetosomes that have started biomineralization. Analysis of *mcaA* and *mcaB* deletions demonstrates that the differences in chain organization are correlated with alterations in the directionality of MamK dynamics. One model suggests that McaA and McaB direct MamK to group older, crystal-containing magnetosomes into shorter subchains by promoting more local turnover of MamK filaments. This allows new magnetosomes to form in the space between these subchains. Thus, modulating MamK dynamics leads to a change in global organelle organization [34].

The mechanisms of magnetosome positioning described so far are based on work done on *Alphaproteobacteria* MTB. However, MTB are diverse and include species throughout the *Proteobacteria*, *Nitrospirae*, and several other phyla [37,38], many of which have complex chain architectures. For example, *Magnetobacterium bavaricum* fashions its hundreds of magnetosomes into an elaborate rosette-like arrangement organized around tube-like filamentous structures [33]. Fortunately, *Desulfovibrio magneticus* RS-1 (RS-1 for simplicity) has emerged as a *Deltaproteobacteria* model to reveal conserved and divergent aspects of magnetosome cell biology. RS-1 has a linear magnetosome chain localized to the positive cell curvature [39], but unlike AMB-1 and

MSR-1 magnetosomes which are lipid-bound [28,40], no membranes have been observed surrounding RS-1 magnetosomes [8]. Recent work shows that RS-1 uses a unique combination of actin-like proteins and coiled-coil proteins to position its membrane-less organelles [41]. In place of a lipid membrane, it seems possible that Mad10, a coiled-coil protein that has been described to bind magnetite, creates a proteinaceous casing around the magnetosomes on which other positioning proteins can bind and act upon [41,42]. All coiled-coil proteins involved in RS-1 magnetosome positioning are from a set of magnetosome proteins, named the Mad proteins, that are unique to *Deltaproteobacteria* and other phylogenetically distant MTB [37,41]. Thus, it is hypothesized that the coiled-coil protein system evolved as a distinct magnetosome positioning system in deep-branching MTB [41]. Exploring the diversity of MTB will expand our knowledge of both the MamK-MamJ-MamY system and novel magnetosome positioning mechanisms.

### **Polyhydroxybutyrate and Polyphosphate Granules – Tethered to the nucleoid**

When growing in an abundance of carbon, some bacterial species will store the excess carbon in the form of a PHB granule [43]. PHB granules, also known as carbonosomes [44], are composed of a hydrophobic PHB core surrounded by a layer of proteins that synthesize, depolymerize, and regulate the number, size, and localization of the granule [43]. *pha* genes required for forming PHB granules are found in a wide range of bacteria and archaea, highlighting their potentially broad relevance to cellular health and survival [45,46]. PHB granules have also attracted wide ranging interest in applied settings since the carbon stored within them is a bioplastic [43].

Though the arrangement can vary from species to species, PHB granules are typically positioned along the cell length to prevent aggregation and ensure equal segregation [47–50]. PHB granules are also large compartments that can take up a substantial volume of the cell, thus proper PHB granule placement might be necessary to reduce interruptions to other cellular processes [51]. *Cupriavidus necator* (also known as *Ralstonia eutropha*), *Pseudomonas putida*, and *Caulobacter crescentus* equally distribute their PHB granules along the nucleoid, but use different proteins to achieve that positioning: PhaM, PhaF, and PhaH, respectively (Figure 3A) [11,48,51,52]. All three proteins use their N-terminal end to bind to PHB granules and their C-terminal end to bind DNA nonspecifically [11,48,52]. Although the nonspecific DNA binding aspect is shared between PHB granule and carboxysome positioning, the three Pha proteins have no homology to ParA. PhaM and PhaF use a histone-like domain to bind DNA while PhaH relies on a helix-hairpin-helix domain [11,48,52]. Random positioning through nonspecific attachment to the nucleoid could be sufficient to prevent aggregation of PHB granules, however it is also possible that additional undiscovered elements are involved. For example, perhaps the localization of the PhaM, PhaF, and PhaH is influenced by another protein, and the spacing between granules is achieved through a system analogous to that of carboxysome positioning. Another alternative mechanism is that PHB granules are actively repelled from one another to regulate the distance between them.

In *C. necator*, PHB granule position is closely tied with granule number and size. While WT *C. necator* has up to six PHB granules, *C. necator*  $\Delta$ *phaM* instead has one or two oversized PHB granules [11,47]. Furthermore, when *phaM* is overexpressed, cells contain many, smaller PHB granules that aggregate [11,47]. PhaM is known to activate

the PHB-synthesizing enzyme PhaC, therefore the increased number of PHB granules in *phaM* overexpression strains is likely due to elevated PhaC activity [53,54]. PhaM complexes with PhaC, and together they are recruited to PHB granules during the early stages of granule formation and could be restricting the granule localization thereafter [50]. *C. necator* PhaM is sufficient to suppress polar localization of heterologously produced PHB granules in *Escherichia coli* [55]. Despite these advances, the biochemical mode of PhaM action in PHB granule organization remains unclear.

In *Pseudomonas putida*, a *phaF* deletion also results in aggregated PHB granules, although granule size does not change [48]. PhaF contribution to PHB granule position could be related to its interaction with PHB granule-associated Phal or transcription regulator PhaD [56,57]. The DNA-binding domain of PhaF shares a high degree of homology with that of another Pseudomonad protein AlgP [58]. Interestingly, AlgP is responsible for the equal spatial distribution of a second intracellular structure, the polyphosphate (polyP) granule (Figure 3B) [58]. Removing the DNA-binding C-terminal domain of AlgP or deleting its gene completely in *Pseudomonas aeruginosa* causes the formation of fewer, enlarged polyP granules [58,59]. This phenotype is speculated to arise from increased polyP granule fusion that occurs when granule position is no longer regulated [58]. The parallels of PhaF and AlgP demonstrate a possible case where a strategy is recycled to position different compartments.

Given the large number of bacterial PHB and polyP granule producers, there is variation in how these compartments are organized across species. For example, in *C. crescentus*, polyP positioning is reliant on the cell cycle and chromosome segregation [60]. In early stages of cellular growth, a single polyP granule is found at the midcell. Before cell division, a second polyP granule forms and the two granules are repositioned to the future middle of each daughter cell. This remarkable polyP granule localization is lost when chromosome replication or segregation is disrupted, suggesting that chromosome segregation is driving the granule repositioning. The dependence on proper chromosome segregation strongly implies that polyP granules in *C. crescentus* also require association with the nucleoid, but this is not yet confirmed and the proteins responsible for this positioning have not been identified [60]. Other polyP granule organizations include polar localization, as in *Agrobacterium tumefaciens* and *Corynebacterium glutamicum* [61,62]. Further investigation of PHB and polyP granules can reveal the diverse evolutionary strategies used to position functionally and structurally similar compartments in species with different morphologies or physiologies.

## CONCLUSION

Examination of the examples above reveals a trend of the typical components found within bacterial organelle positioning systems (summarized in Table 1). In all cases, an element of the cell, such as the nucleoid or the cell membrane, serves as the scaffold to guide organelle organization. The scaffold is marked by a specific protein that works in concert with a second component, an organelle-bound protein. In some cases, a single protein performs both functions, as seen with the Pha proteins and AlgP [11,48,52,58]. A third component determines the spacing between organelles. For instance, McdA's dynamic distribution across the nucleoid results in evenly spaced carboxysomes [14]. On the other hand, magnetosomes are pushed together to center the chain by MamK [30]. The nonspecific DNA-binding activity of PhaM, PhaF, PhaH, and AlgP may be sufficient

to generate the uniform distribution of PHB and polyP granules, or there may be additional proteins involved that have not been uncovered yet [47,48,52,58]. Interestingly, MamK and McdA use ATP hydrolysis to drive organelle positioning [14,29]. It remains to be seen if similar expenditure of energy is needed for PHB and polyP granule localization.

Given the diversity of mechanisms discussed here, it is almost certain that there are strategies of bacterial organelle positioning left to be discovered. For example, encapsulins are well-described in their predicted function and applicative utility, but their cell biology is underexplored [9]. Innovative methods are essential for establishing and expanding on organelle organization model systems. One such experimental technique is the use of an unbiased, imaged-based screen to find changes in protein or organelle localization. These microscopy screens take advantage of high-throughput fluorescence imaging, which generally involves imaging strains in a multi-well format with automated well-to-well stage movement and auto-focusing software [63–65]. Additionally, bacterial organelle research can be further enhanced by increasing accessibility to high-resolution microscopy techniques, such as cryo-electron tomography and super-resolution fluorescence microscopy, allowing cell biologists to overcome the miniscule size of bacterial compartments [9]. An upcoming solution is the development of expansion microscopy, in which an organism is isotropically enlarged in a hydrogel to increase spatial resolution [66]. Though methods are still being adapted for bacterial samples [67,68], maturation of this technique and others will expand the boundaries of bacterial cell biology.

Defining pathways of organelle assembly and positioning can also be critical in engineering and scaling up production of organelles in applied settings. For example, there are currently efforts to heterologously express carboxysomes in both bacterial and eukaryotic hosts for purposes such as optimizing photosynthesis or redesigning the carboxysome to hold novel cargo [69]. Knowledge about carboxysome positioning and segregation is vital for ensuring that these structures are retained in the hosts for many cell divisions. In another case, the magnetic properties of magnetosomes have formed the basis for targeted drug delivery through payload-attached MTB that are guided towards specific tissue with a magnetic field [70]. Alignment and response of MTB cells to the magnetic field—driven by magnetosome organization—is a critical component for the success of these applications. Indeed, the cell biology of bacterial organelles cannot be ignored in many applicative contexts. Thus, the study of organelle positioning not only provides a context to examine broader aspects of bacterial cell biology, but will also make biotechnological advances more attainable. With increasing efforts and improving technology, we look forward to the future discoveries in bacterial organelle organization.

| Organelle     | Organism(s)                                                    | Scaffold      | Scaffold Binding | Organelle Binding | Organelle Spacing | ATP-dependent? | Ref.       |
|---------------|----------------------------------------------------------------|---------------|------------------|-------------------|-------------------|----------------|------------|
| Carboxysome   | <i>S. elongatus</i> PCC7942,<br><i>H. neapolitanus</i>         | Nucleoid      | McdA             | McdB              | McdA              | Yes            | [14,15]    |
| Magnetosome   | <i>M. gryphiswaldense</i> MSR-1,<br><i>M. Magneticum</i> AMB-1 | Cell Membrane | MamY             | MamJ              | MamK              | Yes            | [12,27,30] |
| PHB Granule   | <i>C. necator</i> ( <i>R. eutropha</i> H16)                    | Nucleoid      | PhaM             |                   | PhaM/?            | Unknown        | [11,47,50] |
|               | <i>P. putida</i>                                               |               | PhaF             |                   | PhaF/?            |                | [48,56]    |
|               | <i>C. crescentus</i>                                           |               | PhaH             |                   | PhaH/?            |                | [52]       |
| PolyP Granule | <i>P. aeruginosa</i>                                           | Nucleoid      | AlgP             |                   | AlgP/?            | Unknown        | [58]       |

**Table 1.** Summary of scaffold binding, organelle binding, and organelle spacing components of bacterial organelle positioning systems reviewed here.

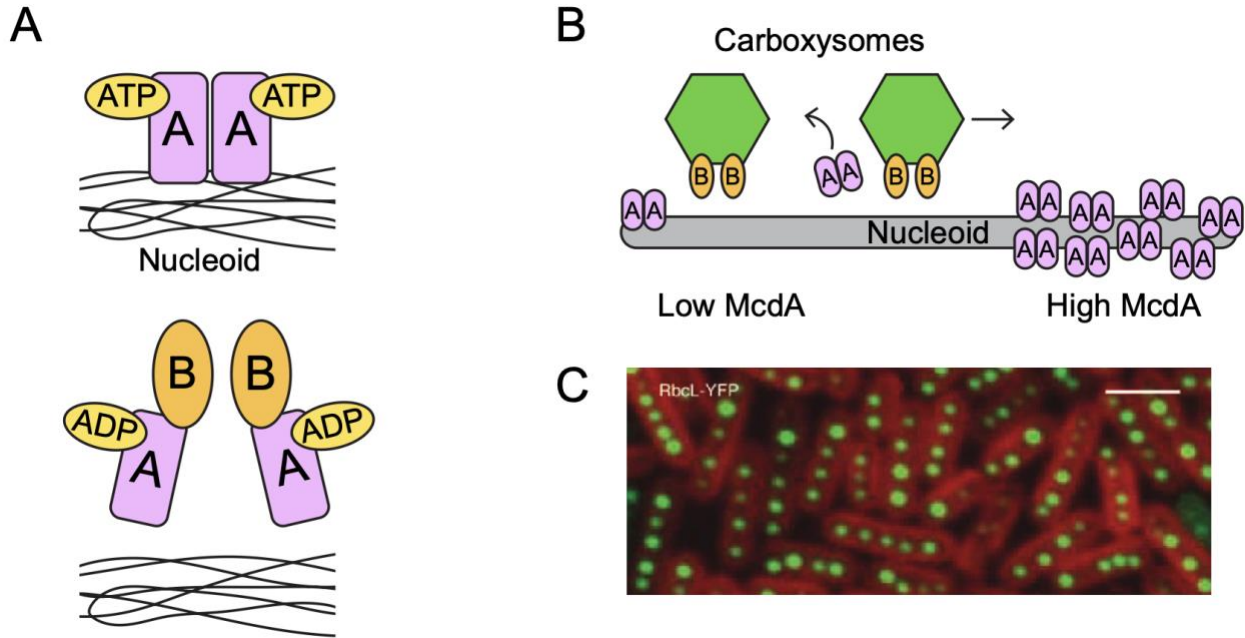


**Figure 1:** Carboxysomes are equally distributed along the nucleoid by McdA and McdB. **(A)** McdA dimerizes and binds to DNA nonspecifically when bound to ATP. McdB triggers the ATPase activity of McdA, which causes McdA to dissociate from DNA [14]. **(B)** McdB-bound carboxysomes create zones of depleted McdA while migrating towards areas of greater McdA concentration [14]. Note that oligomerization of McdB in some species is not depicted [15]. **(C)** Carboxysomes labeled by RbcL-YFP are equally spaced as a result of the McdAB system (reprinted with permission from [10]) [10,14].

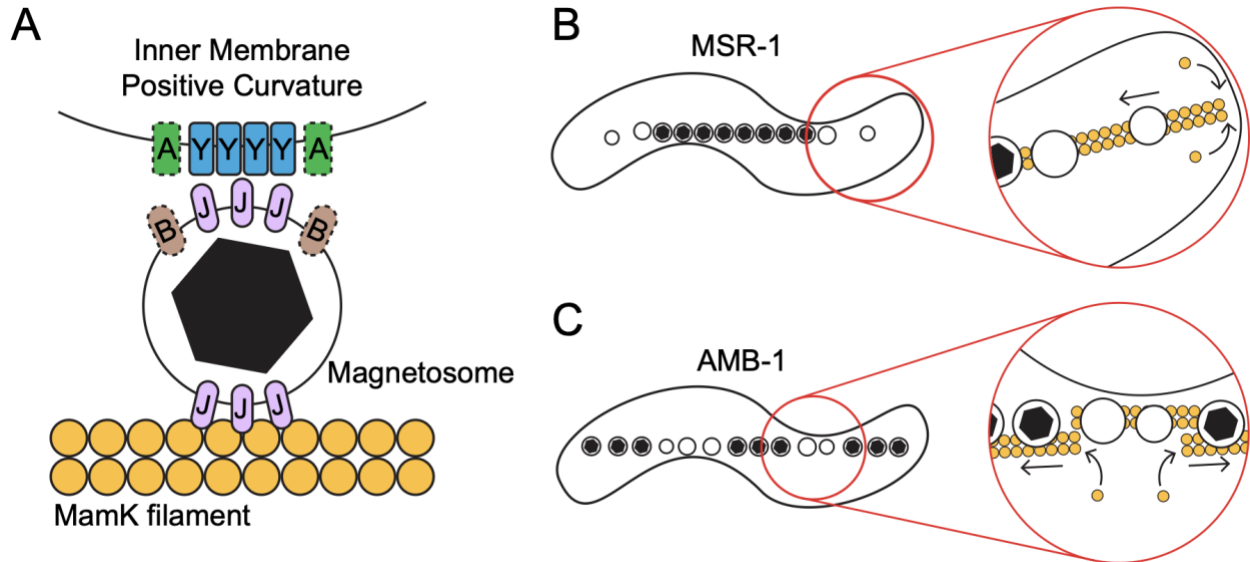


**Figure 2:** Magnetosomes are positioned by a cytoskeletal system. **(A)** The lipid-bound magnetosome in MSR-1 and AMB-1 associates with the MamK filament and positive curvature-localized MamY through MamJ [12,27]. In AMB-1, McaA is found at the positive curvature and McaB localizes to crystal-containing magnetosomes [34]. **(B)** In MSR-1, magnetosomes without magnetic crystals are at the chain periphery. MamK polymerization at the cell poles pushes magnetosomes towards the midcell, maintaining a continuous, linear chain [30]. **(C)** In AMB-1, McaA and McaB organize the magnetosome chain such that there is an alternating pattern of older magnetosomes (with magnetic crystals) and newer magnetosomes (without magnetic crystals). This pattern is speculated to arise from MamK pushing older magnetosomes into subchains, opening up space for new magnetosomes to form in the gaps [34].

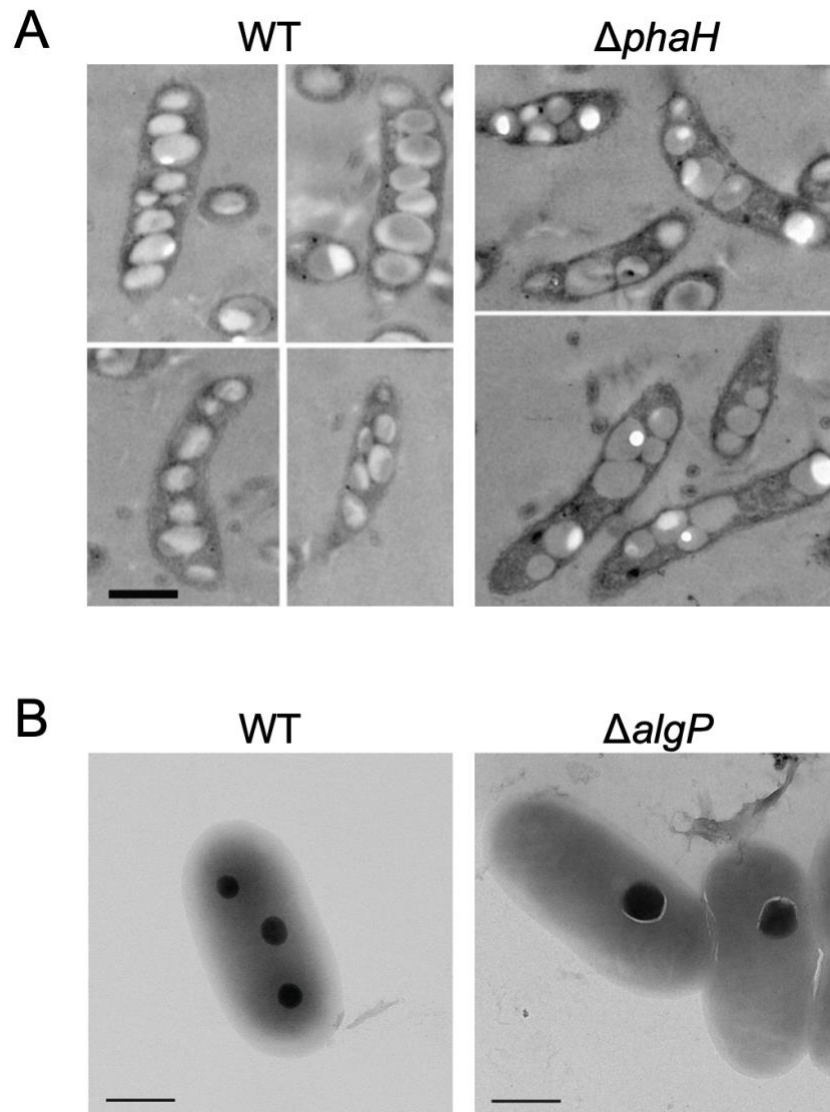


**Figure 3:** PHB and polyP granules are positioned by DNA-binding proteins. **(A)** Electron microscopy images of WT *C. crescentus* show PHB granules linearly positioned along the cell length. When *phaH* is deleted, the PHB granules are disorganized and have more aggregations [52]. Scale bar represents 1 $\mu$ m (reprinted from [52]). **(B)** Transmission electron microscopy of WT *P. aeruginosa* shows several, equally-spaced polyP granules. In contrast, the  $\Delta algP$  strain has fewer, enlarged polyP granules [58]. Scale bar represents 0.5 $\mu$ m (reprinted from [58]).

## Chapter 2

Magnetosome organelles are organized through interactions between McaA and McaB that alter the dynamics of the bacterial actin-like protein MamK

Yein Ra<sup>1</sup>, Yuanyuan Pan<sup>2</sup>, Tansy Chen<sup>1</sup>, Kayla Dixon<sup>1</sup>, Azuma Taoka<sup>2</sup>, Arash Komeili<sup>1</sup>

<sup>1</sup>Department of Plant and Microbial Biology, University of California, Berkeley, CA, USA

<sup>2</sup>Institute of Science and Engineering, Kanazawa University, Kanazawa, Ishikawa, Japan

## ABSTRACT

Magnetotactic bacteria (MTB) are a group of Gram-negative species that produce a lipid-bounded organelle, the magnetosome, in which a magnetic crystal is biomineralized. MTB use magnetosomes to align with the geomagnetic field for improved navigation of their environment. To optimize this alignment, these species organize their magnetosomes in a linear fashion using a handful of factors including the bacterial actin-like protein MamK. Despite these shared features, there is a broad diversity of species-specific linear magnetosome chain arrangements within MTB, but the molecular mechanisms behind these phenotypic variations are unclear. This chapter features our new finding of how magnetosome positioning systems can be directed to produce a different magnetosome organization. Recently, genetic analyses showed that two proteins - McaA and McaB - interface with the chain organization machinery of *Magnetospirillum magneticum* AMB-1 to arrange magnetite crystals in a series of subchains rather than the single cohesive chains found in closely related MTB. Here, we use *in vivo* co-immunoprecipitation in AMB-1 to demonstrate protein-protein interactions between McaA, McaB, and MamK. Experiments with McaA truncation mutants and conditional control of McaB localization determined that McaA-McaB interactions are dependent on amino acids 530-665 of McaA and McaB localization to the magnetosome chain. We further show that disrupting the McaA-McaB interaction alters the spatial dynamics of MamK *in vivo*. We present a model in which protein-protein interactions between McaA, McaB, and MamK drive changes in MamK behavior to establish AMB-1's magnetosome chain organization.

## IMPORTANCE

Magnetosomes serve as a model for understanding the cell biology of bacterial organelles and the mechanisms of bacterial cell organization. MamK, one of the best-studied bacterial actins, is a notable player in magnetosome chain assembly. This work explores how MamK dynamics are altered by two potential bacterial actin binding proteins, McaA and McaB. This system illustrates how changes in bacterial cytoskeleton regulation result in different organization of subcellular compartments. The conclusions from this research also have implications for understanding the broader evolutionary strategies for regulation of actin-like proteins and compartment organization diversification in bacteria.

## INTRODUCTION

Similar to eukaryotes, many bacterial species produce one or more organelles that carry out a range of biochemical and behavioral activities [51,72,73,74]. One of the best studied bacterial organelles is the magnetosome [75]. Produced by magnetotactic bacteria (MTB), these organelles start as a lipid-bounded compartment wherein a magnetic crystal of magnetite ( $\text{Fe}_3\text{O}_4$ ) or greigite ( $\text{Fe}_3\text{S}_4$ ) is biomineralized [40,76]. Magnetosomes allow MTB to align with the Earth's magnetic field, which is advantageous for navigating their aquatic environment with greater efficiency. Because MTB are typically motivated to find favorable oxygen environments, this behavior is termed magnetoaerotaxis [75]. The ability to form magnetosomes makes MTB an attractive bacterial system to study biomineralization, organelle biogenesis, and compartment localization.

Magnetosomes exemplify how the function of an organelle can be closely tied to its subcellular localization. A prerequisite to magnetoaerotaxis is the organization of

magnetosomes into a chain; various MTB mutants that produce magnetosomes but fail to organize them into a linear chain have dramatically lowered abilities to align with and navigate along a magnetic field [12,25,34,41,77]. In the model MTB strains *Magnetospirillum magneticum* AMB-1 (hereafter AMB-1) and *Magnetospirillum gryphiswaldense* MSR-1 (hereafter MSR-1), the magnetosome chain has two important characteristics: it follows the positive curvature of the cell and is continuous, features that are controlled by MamY and MamK, respectively [26,27]. AMB-1 and MSR-1 maintain a linear magnetosome chain in a spiral-shaped body by positioning its chain along the positive cell curvature, thereby ensuring that the chain is parallel to the axis of cell motility. MamY assembles into a scaffold at the positive cell curvature onto which magnetosomes attach through a second protein, MamJ [27]. The magnetosome chain is made continuous (i.e. there are no large gaps between magnetosomes) by MamK. MamK is a bacterial actin and in its absence the magnetosome chain is fragmented, not centered, and unevenly segregated during cell division [26,28,30,31,32,78]. Like eukaryotic actin, monomers of MamK polymerize into filaments when bound to ATP [29,79,80]. Such MamK filaments are observed in AMB-1 and MSR-1 using cryo-electron tomography [12,26]. While within the filament, MamK monomers hydrolyze their ATP into ADP, favoring dissociation from the filament [29]. The ATP-dependent polymerization and depolymerization of monomers are required for the dynamic quality and function of MamK. When MamK dynamics are perturbed by disrupting its ATPase activity, both chain assembly and magnetosome segregation are severely hindered [30]. MamJ, and its paralog LimJ, connect magnetosomes to MamK filaments and are required for MamK dynamics in AMB-1 [12,32,81]. MamJ is among the few known accessory factors of a bacterial actin, alongside ParR, AlfB, Alp7R, and others which partner with distinct actin-like proteins to carry out diverse functions [82–84]. Identifying additional proteins that regulate MamK can provide further insights to how bacterial cytoskeleton can be controlled.

Recently, two additional proteins that alter MamK behavior have been identified. In AMB-1 strains missing *mcaA* and *mcaB*, MamK dynamics are changed when observed by fluorescence recovery after photobleaching (FRAP). Additionally, McaA and McaB are required for AMB-1's magnetosome chain organization. At first glance with transmission electron microscopy (TEM), the WT AMB-1 magnetosome chain appears fragmented (Fig. 1A). Imaging with cryo-electron tomography, however, reveals that the gaps between crystal subchains are populated with magnetosome membranes that have not yet initiated crystal biomineralization [34]. Thus, AMB-1 magnetosomes are organized in subgroups of crystal-containing magnetosomes or empty magnetosomes that together form a continuous chain. In the absence of *mcaA*, *mcaB*, or both, the mutant chain resembles that of WT MSR-1, where magnetite crystals are continuous and at midcell while empty magnetosomes are at the chain periphery (Fig. 1A, B) [12,27,34]. This has collectively led to the hypothesis that McaA and McaB create a magnetosome chain with crystal subchains by influencing MamK behavior [34]. Investigating how the Mca proteins contribute to magnetosome chain organization will resolve the mechanism behind how AMB-1 and MSR-1, two organisms that share ~96% identity in their 16S rRNA genes [85], pattern their magnetosomes in a strikingly different manner. Currently, how McaA and McaB work together at the protein level, and whether they directly affect MamK, is unknown.

Here, we discovered that McaA, McaB, and MamK form protein-protein interactions with each other. The McaA-McaB interaction is mediated through a conserved region on McaA, and primarily occurs when McaB is localized to the magnetosome chain. Disrupting the McaA-McaB interactions by truncating McaA or altering McaB localization is accompanied by a shift in MamK dynamics. Altogether, the protein-protein interactions of McaA, McaB, and MamK affect MamK dynamics and ultimately organize magnetic crystals into subchains in AMB-1.

## RESULTS

### McaA and McaB interact with each other.

The genetic studies of *mcaA* and *mcaB* suggest that there is a machinery dedicated to producing the crystal subchain magnetosome organization [34]. To gain insight on the mechanism behind this magnetosome positioning system, we set out to determine which proteins interact with McaA and McaB *in vivo* in AMB-1. We separately expressed GFP-tagged McaA and McaB on replicative plasmids in AMB-1, which have previously been shown to complement their respective gene deletions [34], and used GFP-trap agarose beads for co-immunoprecipitation paired with mass spectrometry (IP-MS). The experiment was also performed with GFP alone to determine which interactions were due to the tag or non-specific binding to the agarose beads (Tables 1-3).

We first attempted to identify other magnetosome proteins involved in magnetosome chain organization by looking for proteins that co-eluted with both McaA-GFP and McaB-GFP. The McaB-GFP IP-MS results were poor due to low protein abundance, but we still identified peptides from four magnetosome proteins: MamA, MamQ, MamJ, and Mms6 (Table 2). MamA and MamQ were also identified in the IP-MS with McaA-GFP (Table 1). To assess whether MamA has a role in magnetosome chain organization, *mamA* deletion strain was imaged with TEM. No deviation in magnetosome chain organization was observed (Fig. S1A, B), suggesting that MamA is not necessary for the crystal subchain phenotype. Because AMB-1  $\Delta$ *mamQ* does not produce magnetosomes, magnetosome arrangement could not be assessed in this strain.

The IP-MS results from McaA-GFP also revealed that McaA and McaB may interact in AMB-1 (Table 1). To verify this result, the co-immunoprecipitation (co-IP) using GFP-trap beads was repeated in triplicate with a  $\Delta$ *mcaAB* strain expressing McaB-GFP and McaA-Halo. These tagged proteins can complement their respective deletion when assessed by TEM and coefficient of magnetism ( $C_{mag}$ ) measurements (Fig. S2), which measures the degree of magnetic field alignment by MTB cells in a liquid culture [40]. The elution was probed for the presence of both proteins using Western blotting. Both proteins co-eluted (Fig. 1C, D; Fig. S3A), indicating that indeed, McaA and McaB interactions occur in AMB-1. Co-IPs with McaB-GFP/Halo and GFP/McaA-Halo pairs expressed in AMB-1  $\Delta$ *mcaAB* confirmed that the GFP and Halo tags, or nonspecific interactions with beads, were not responsible for the interaction (Fig. 1D, Fig. S3B). Samples containing McaA-Halo almost always had a series of smaller bands in the anti-Halo Western blot (Fig. 1D). These appear to be specific to McaA-Halo, since they are not present in samples expressing other Halo-tagged proteins (Fig. 1D, Fig. S3B, Fig. S8D, Fig. S13C). These bands could represent McaA-Halo degradation products or be a result of post-translational processing of McaA-Halo. In the anti-GFP blots of McaB-GFP samples, there is a strong signal from a protein that is around the size of GFP (26.8kDa). In the *mcaB*-

*gfp* fusion, the start codon of GFP was not removed. Thus, there might be an internal ribosome binding site that drives the translation of free GFP. Given that our negative control co-IP with free GFP and McaA-Halo does not detect an interaction (Fig. 1D, Fig. S3B), it is unlikely that this free GFP is driving the interaction in the McaA-Halo/McaB-GFP co-IP.

Having observed McaA-McaB interactions in AMB-1, we investigated whether the interaction between them was direct by using purified proteins. Because McaA and McaB are both predicted to contain transmembrane domains [34], the domain architectures of both proteins were closely studied to identify a soluble, cytoplasmic portion that can be purified with minimal impact on potentially important domains (Fig. 3A, B). Analysis with DeepCoil2 revealed a high probability that McaB amino acids 81-112 forms a coiled-coil domain (Fig. S4A) [86]. Coiled-coil domains are alpha-helices that oligomerize by burying their hydrophobic residues, commonly referred to as “a” and “d” position amino acids, against each other. We mapped the “a” and “d” position amino acids predicted from DeepCoil2 onto the AlphaFold3-predicted McaB monomer, dimer, and trimer (Fig. S4B-D) [87]. We found that in a trimer, the “a” and “d” position residues are indeed predicted to form a hydrophobic core (Fig. S4D). Truncating the N-terminal end up to the transmembrane domain of McaB (amino acids 1-27) was not predicted by AlphaFold to disrupt this coiled-coil structure (Fig. S4E).

McaA is also predicted to contain a transmembrane domain [34]. It has a predicted periplasmic signal peptide and von Willebrand factor type A (VWA) domain at its N-terminus (Fig. 3A) that has been previously demonstrated to be necessary for its localization to the positive inner curvature of the cell [34]. Within the C-terminal cytoplasmic side of the transmembrane domain (aa392 and onward), McaA has a stretch of amino acids from 530 to 665 (named here the “530 region”) that is required for its function in magnetosome organization [34]. Given that McaB has only 4 amino acids predicted to be on the N-terminal side of its transmembrane domain (Fig. 3B), it seemed unlikely that McaA and McaB interact at the periplasm or magnetosome lumen. We thus reasoned that domains necessary for interaction with McaB and McaA function are on the cytoplasmic side of McaA. Therefore, McaA aa400-776 (McaA<sub>cyto</sub>) and McaB aa28-219 (McaB<sub>cyto</sub>) were chosen to be purified.

To that end, we constructed McaA<sub>cyto</sub> with an N-terminal Strep-tag and McaB<sub>cyto</sub> with an N-terminal maltose binding protein (MBP) tag and purified them using affinity chromatography and size exclusion chromatography (Fig. S5A). We performed *in vitro* pull downs with Strep-McaA<sub>cyto</sub> and MBP-McaB<sub>cyto</sub> using streptavidin beads. If there is an interaction between them, then both proteins should be found in the elution fraction. However, the elution only contained Strep-McaA<sub>cyto</sub> (Fig. S5B). Similarly, bacterial two hybrid assays did not detect an interaction between full-length McaB and full-length McaA or McaA<sub>cyto</sub> [34] (Fig. S6B). It is possible that the interaction between the two proteins requires proper localization to the membrane. Alternatively, the McaA-McaB interaction observed *in vivo* might be bridged by one or more proteins. To test whether any magnetosome proteins fulfills this role, we attempted to repeat the McaB-GFP/McaA-Halo co-IP in AMB-1  $\Delta$ MAI, a strain missing the magnetosome island (MAI) that encodes the majority of AMB-1 magnetosome genes (27). However, McaB-GFP is expressed poorly or unstable in this strain, as indicated by a weak signal in the lysate anti-GFP Western

blot (Fig. 2C, Fig. S8A), perhaps because a MAI-encoded protein is required for McaB expression or stability. Thus, we were unable to assess the interaction in this background.

### **MamK interacts with McaA and McaB *in vivo*.**

McaA-GFP IP-MS data showed that MamK may also interact with McaA (Table 1). As discussed above, MamK is a bacterial actin that is responsible for assembling magnetosomes into a linear chain [26,30]. Furthermore, MamK dynamics are altered in  $\Delta mcaA$  and  $\Delta mcaB$  strains [34]. A co-IP with McaA-GFP and MamK-Flag confirmed the interaction between these two proteins (Fig. 2A, Fig. S7A). Co-IPs with GFP/MamK-Flag and McaA-GFP/Halo-Flag combinations ensured that the observed interactions were not due to the GFP or Flag tags (Fig. S7A). To address whether this interaction is bridged by any other magnetosome protein, including McaB, we assessed whether McaA-GFP and MamK-Flag will interact in  $\Delta MAI\Delta MIS$ , a strain missing the magnetotactic islet (MIS) in addition to the MAI [35]. We found that MamK-Flag still elutes with McaA-GFP, signifying that the McaA-MamK interaction does not require other magnetosome proteins (Fig. 2A, Fig. S8B).

Since the signal from the McaB-GFP IP-MS experiment was poor, we also considered the possibility that an McaB interaction with MamK might have been missed. We therefore checked for McaB-MamK interactions using co-IP with McaB-GFP and MamK-Flag. Indeed, the two proteins co-eluted (Fig. 2B, Fig. S7B). This interaction is also independent of McaA, as the two proteins still interacted in an *mcaA* deletion background (Fig. 2B, Fig. S8C). The interaction is also not driven by the GFP and Flag tags, since co-IP with GFP/MamK-Flag and McaB-GFP/Halo-Flag in a  $\Delta mcaB\Delta mamK$  background did not result in co-elution of the protein pairs (Fig. S7B). The McaB-MamK interaction was not tested in  $\Delta MAI\Delta MIS$  since we previously found McaB expression to be unstable in strains missing the MAI (Fig. 2C, Fig. S8A). Finally, we asked whether MamK is necessary for the McaA-McaB interaction. We repeated the McaB-GFP and McaA-Halo co-IP in AMB-1  $\Delta mamK\Delta mamK-like$ , a double mutant missing both *mamK* and its homolog *mamK-like* that is located within the MIS [35]. McaB-GFP was stable in this background and interacted with McaA-Halo (Fig. 2C, Fig. S8A). In summary, we used a series of co-IP experiments to show that McaA, McaB, and MamK all have independent pairwise interactions with one another in AMB-1.

### **MamK directly interacts with McaA and McaB.**

The protein-protein interactions between McaA, McaB, and MamK paired with the previously observed effect of McaA and McaB on MamK dynamics raised the possibility that the Mca proteins are direct regulators of MamK behavior. To investigate direct interactions between these proteins, untagged recombinant MamK was expressed in *Escherichia coli* BL21 and purified using ammonium sulfate precipitation and size exclusion chromatography (Fig. S5A). Purified MamK was tested for interactions against recombinant McaA<sub>cyto</sub> and McaB<sub>cyto</sub> *in vitro*. MamK forms filaments when bound to ATP, which can be separated from MamK monomers using a pelleting assay (Fig. 3D). We used this assay to assess whether McaA or McaB co-pellets with MamK filaments. MBP-McaB<sub>cyto</sub> co-pelleted with MamK in an ATP-dependent manner while the MBP tag alone predominantly remained in the supernatant (Fig. 3D), signifying that McaB<sub>cyto</sub> directly binds MamK filaments. Strep-McaA<sub>cyto</sub> pellets on its own with ATP (Fig. 3C). Therefore,

we could not use this assay to determine its direct interaction with MamK. We instead turned to bacterial two hybrid assays, which detected an interaction between McaA<sub>cyto</sub> and MamK (Fig. S6A). This interaction was missed in a previous bacterial two hybrid assay where LB medium was used instead of M63 [34]. Thus, MamK interacts with both McaA and McaB directly as observed by pelleting and bacterial two hybrid assays.

### **Interaction between McaA and McaB is mediated through a conserved domain of McaA.**

Next, we asked whether disrupting the McaA-McaB interaction will have an impact on their function *in vivo*. To that end, we searched for possible domains necessary for the McaA-McaB interaction. The 530 region (aa530-665) of McaA is more conserved compared to the rest of the C-terminal side (Fig. S9B) and required for WT magnetosome chain organization. It has been shown that McaA without the 530 region (McaAΔ530) is unable to complement the *mcaA* deletion; Δ*mcaA* expressing McaAΔ530-GFP on a plasmid still produces a continuous chain of magnetite crystals despite having the same localization as full-length McaA-GFP [34]. McaAΔ530-GFP is also the only truncation made on the cytoplasmic side of McaA that cannot complement the Δ*mcaA* deletion [34]. Guided by AlphaFold3 structure predictions [87], the 530 region was further divided into three smaller segments and each was removed from McaA-GFP (Fig. S10A). Each GFP-tagged truncation had the same localization pattern as full length McaA-GFP (Fig. S10F). However, none of the three truncated McaA-GFP restored the crystal subchain magnetosome organization when expressed in the *mcaA* deletion, and the C<sub>mag</sub> of these strains was similar to Δ*mcaA* with an empty vector (Fig. S10C-E). Thus, the entire 530 region is necessary for McaA's function. We hypothesized that the loss-of-function of McaAΔ530 could be due to loss of interaction with McaB or MamK. To test this, we expressed McaB-GFP and McaAΔ530-Halo in Δ*mcaAB* and tested for interaction between the proteins using a co-IP assay. Indeed, McaAΔ530-Halo did not elute with McaB-GFP (Fig. 4A, Fig. S3A). On the other hand, McaAΔ530-GFP retained its interaction with MamK-Flag in Δ*mcaA*Δ*mamK* and ΔMAIΔMIS strains (Fig. S7A, Fig. S8B). Thus, the 530 region is necessary for McaA function and its interaction with McaB, and the loss-of-function of McaAΔ530 is not due to lack of interaction with MamK.

To determine whether the 530 region is sufficient for interaction with McaB, we cloned a fragment of McaA spanning this region and tagged it with GFP (530-GFP). We then expressed this construct in WT, Δ*mcaA*, and Δ*mcaB*. In WT, the majority of cells displayed 530-GFP with a magnetosome chain localization (Fig. 4B, C). This localization is not dependent on full-length McaA; though there was an increase in cells with a diffuse 530-GFP signal, a large proportion of the population still had 530-GFP localized to the magnetosome chain (Fig. 4B, C). This magnetosome chain localization is almost completely lost in the absence of *mcaB*; the vast majority of Δ*mcaB* cells have a diffuse 530-GFP signal (Fig. 4B, C). One explanation for this is that the 530 region is sufficient to interact with McaB, and this interaction draws 530-GFP to the magnetosome chain. To confirm this claim, we conducted a co-IP using the Δ*mcaAB* strain expressing McaB-GFP and a Halo-tagged 530 region (530-Halo) (Fig. S8D). Indeed, the 530-Halo co-eluted with McaB-GFP, signifying that the 530 region is sufficient for interaction with McaB.

### **McaA 530 region is necessary for WT MamK dynamics.**

It has been previously shown that MamK dynamics are impacted by McaA and McaB. In prior FRAP experiments with WT AMB-1 expressing MamK-GFP, the photobleached region was always static and recovered in place [34]. In  $\Delta mcaA$  and  $\Delta mcaB$ , on the other hand, the photobleached region was motile in a portion of the cells, indicating that MamK dynamics is influenced by the Mca proteins [34]. We hypothesized that the interaction between McaA and McaB, which appears critical for magnetosome chain patterning, also contributes to the dynamics of MamK recovery in FRAP.

To test this, we first made an in-frame deletion of the 530 region at the native *mcaA* locus. Like  $\Delta mcaA$ , this strain has a magnetosome chain with crystal-containing magnetosomes at the chain middle (Fig. 4D, E), and its  $C_{mag}$  is higher than that of WT (Fig. S10B). We then expressed MamK-GFP in WT,  $\Delta mcaA$ , and  $\Delta 530$ , and observed MamK dynamics using FRAP. We found MamK-GFP to be dynamic in all strain backgrounds (Fig. 5A-C; Fig. S11). We first compared the half-time of fluorescence recovery ( $t_{1/2}$ ), the time it takes for the bleached region to regain 50% fluorescence intensity of the whole filament intensity (Table 4). When examining the entire population of recovered cells across WT,  $\Delta mcaA$ , and  $\Delta 530$ , the  $t_{1/2}$  did not substantially vary ( $3.53 \pm 3.15$  min,  $3.02 \pm 2.22$  min,  $3.65 \pm 3.67$  min, respectively). In most WT cells that recovered, the bleach spot was stationary and recovered in place. However, the bleach spot moved during recovery in about 15% of WT cells observed. In a  $\Delta mcaA$  strain, the number of cells with a MamK-GFP moving bleach spot more than doubled (38% of recovered cells observed), consistent with prior observations [34]. Similarly, 38% of recovered  $\Delta 530$  cells exhibited the moving bleach spot pattern (Fig. 5A, B). MamK-GFP behavior thus shifted more towards moving-bleach-spot dynamics in  $\Delta 530$ , as in  $\Delta mcaA$  (Fig. 5C). When we separated the recovered cell population based on MamK recovery behavior (non-moving vs. moving bleach spot), we can see that in general, cells with a moving bleach spot recover faster than those with non-moving bleach spots (Table 4). Recovery times were independent of cell length, with the exception of  $\Delta 530$  cells in which those with non-moving bleach spots showed a slight positive correlation (Fig. S12A). The directions of the moving bleach spots were not consistent from cell to cell; the moving bleach spot moved towards the cell pole in some cells and towards midcell in others (Fig. S12B). Altogether, change in magnetosome organization and altered MamK dynamics correlates with the loss of the McaA-McaB interaction.

### **Non-biomineralization conditions affect McaB-GFP localization, McaA-McaB interaction, and MamK dynamics.**

McaAB-mediated change of MamK dynamics appears to be dependent on McaA-McaB interactions. This led us to consider that MamK behavior might be locally controlled based on where McaA-McaB interactions occur along the magnetosome chain. We further speculated that McaA-McaB interactions are limited to where the two proteins co-localize. To assess the role of localization on McaA-McaB interactions, we perturbed McaB localization to crystal-containing magnetosomes.

McaB's localization pattern resembles that of another magnetosome protein, Mms6. Mms6 is involved in the biomineralization of the magnetite crystal, and like McaB, is present on magnetosomes that contain magnetite [88,89]. Furthermore, Mms6 was identified in the IP-MS with McaB-GFP (Table 2). Therefore, we hypothesized that McaB

is recruited to crystal-containing magnetosomes through an interaction with Mms6. We tested this by comparing McaB-GFP localization in WT and  $\Delta mms6$ . At first glance, the McaB-GFP signal seems diffuse in  $\Delta mms6$  compared to WT. However, upon closer inspection, we noted that a subpopulation of McaB-GFP is still found at the magnetosome chain in most cells (Fig. S13A, B). Additionally, we could not confirm the Mms6-McaB interaction through co-IPs with Mms6-Halo and McaB-GFP in WT AMB-1 (Fig. S13C). A co-IP of McaB-GFP and McaA-Halo determined that the two proteins still interact in  $\Delta mms6$  (Fig. S13D) and TEM images of AMB-1  $\Delta mms6$  revealed only a minimal change in magnetosome arrangement in this strain (Fig. S13E, F). Therefore, Mms6 is not necessary for recruitment of McaB to the magnetosome chain, the McaA-McaB interaction, and proper chain formation.

We next turned to previous studies which showed a conditional change in McaB-GFP localization. When AMB-1 cells are shifted to an iron-limited growth medium, they produce a chain of magnetosome membranes that are devoid of magnetite crystals [40,90]. In previous work, we found that McaB-GFP localization appears diffuse under these non-biomineralizing conditions [34]. We first quantified the difference in McaB-GFP localization in both biomineralization and non-biomineralization conditions in WT AMB-1. Indeed, in non-biomineralization growth, the majority of AMB-1 cells show diffuse McaB-GFP signal (Fig. 6A, B). We then tested whether McaB still interacts with McaA when it is no longer localized to the magnetosome chain. Compared to biomineralizing conditions, McaB interactions with full-size McaA are notably reduced in non-biomineralizing conditions (Fig. 6C). Interestingly, shorter McaA-Halo bands still co-eluted with McaB-GFP (Fig. S14A). We speculate that some degradation products of McaA-Halo have lost their transmembrane domain. This soluble McaA-Halo truncation can then interact with the diffuse McaB-GFP, which further implies that protein localization, not growth condition, is the key contributor to the McaA-McaB interaction. Altogether, McaB localization to the magnetosome chain is a prerequisite for its interaction with McaA.

Since McaA-McaB interactions are missing in non-biomineralizing conditions, we predicted that MamK dynamics would also be impacted. We subjected WT AMB-1 expressing MamK-GFP to non-biomineralization conditions, then conducted FRAP. We found that in general, MamK-GFP recovery was much slower when cells are grown in non-biomineralization conditions with an average  $t_{1/2}$  of  $5.11 \pm 3.56$  min, and 22% of cells did not recover in the observed timeframe (Table 4). The longer recovery times may reflect a broader metabolic constraint from growing in iron-limited medium, demonstrated by the decreased growth observed under non-biomineralization conditions (Fig. S14B). Despite the overall increase in  $t_{1/2}$ , we observed that 36% of recovering cells displayed the moving-bleach-spot dynamics, similar to  $\Delta mcaA$  and  $\Delta 530$  (Fig. 6D-F, Table 4). This is consistent with our hypothesis that the effect of McaA and McaB on MamK dynamics is dependent on their ability to interact with each other, which is in part driven by McaB's localization to the magnetosome chain.

## DISCUSSION

### **McaA-McaB interactions drive magnetosome organization through MamK dynamics.**

In this study, we set out to determine how McaA and McaB produce the subchain organization of magnetosomes in AMB-1. Through both *in vivo* and *in vitro* work, we

discovered that McaA and McaB have protein-protein interactions with each other and the bacterial actin MamK. We further demonstrated that each pairwise interaction occurs independently of the third protein. Disrupting one of these interactions (McaA-McaB) leads to a change in magnetosome organization from subchains of magnetite crystals to having a singular crystal chain. This points to a model where a network of McaA, McaB, and MamK interactions are required for magnetosome chain organization.

The effect of McaAB on magnetosome organization is closely tied with MamK dynamics. Two types of MamK dynamics were observed through our FRAP imaging: moving-bleach-spot dynamics, where the bleach spot migrated from its original position while recovering, and recovery-in-place dynamics, where the bleach spot recovered without moving. It is challenging to extract the behavior of individual MamK filaments from this data because during FRAP, many overlapping 200nm-sized MamK filaments are simultaneously photobleached [26]. Consequently, our interpretation of the FRAP data is focused on bulk MamK filament behavior. We predict that the moving-bleach-spot dynamic is due to a global organization of individual MamK filaments in which the majority of MamK filaments move or polymerize in the same direction (Fig. 7A). We currently do not know how the movement is coordinated or how the movement direction is determined. In contrast, the recovery-in-place dynamics is perhaps a result of individual MamK filaments moving or polymerizing in either direction along the cell length (Fig. 7A). Though the directionality of individual filaments might be regulated locally, the movement direction is not uniform throughout the entire population of MamK filaments. Interestingly, MamK dynamics are not identical within an entire population. Even in populations that had more cells with moving-bleach dynamics, the majority of cells still showed recovery-in-place dynamics. This implies that there are additional controls to MamK dynamics in AMB-1 that are left to be discovered.

We further demonstrated that the interaction between McaA and McaB is correlated to changes in MamK dynamics. When the McaA-McaB interaction is removed through protein truncation or localization changes, MamK dynamics shift such that there are more cells exhibiting the moving-bleach-spot dynamics. This led us to hypothesize that MamK dynamics are locally controlled based on where McaAB interactions occur. McaB is at the crystal-containing magnetosomes in WT AMB-1 under normal biomineralization conditions while McaA localizes from cell pole-to-pole along the positive curvature in a dashed pattern in all mutants and growth conditions tested [34]. Previous co-localization studies of McaA-Halo and McaB-GFP in WT AMB-1 showed that McaB-GFP tends to localize within the gaps of the McaA-Halo signal [34]. The inverted localization pattern suggests that McaA and McaB only interact at the interface of their territories along the magnetosome chain. Alternatively, the inverted localization pattern could be a result of dynamic McaA-McaB-MamK activity that results in minimized McaA-McaB interactions. In either case, there is a confinement of McaA-McaB interactions, which in turn generates localized zones of MamK regulation.

Altogether, we propose the following model for magnetosome chain organization in AMB-1 (Fig. 7B). McaB is recruited to a magnetosome that has begun biomineralization. This increases the proximity of McaB to the positive curvature membrane-localized McaA and MamK filaments at the magnetosome chain, allowing for protein-protein interactions. The interactions between the three proteins activate McaA and McaB to alter MamK filament directionality locally, which consequently moves the

attached magnetosomes. The shifting of these crystal-containing magnetosomes causes some to join into a subchain and others to dislodge from an existing subchain. This in turn opens up space between crystal-containing magnetosomes for new, empty magnetosomes to form. The resulting magnetosome chain therefore has subchains of crystal-containing magnetosomes that are interrupted by empty magnetosomes.

Several pieces of this model remain to be resolved. Firstly, are there other proteins involved in patterning magnetosomes in AMB-1? Though we have presented evidence that McaA and McaB directly interact with MamK, we could not prove a direct interaction between McaA and McaB *in vitro*. This leaves the possibility that there are other protein contributors to magnetosome organization not identified here. Secondly, we still do not know how MamK is altered by McaAB at the biochemical level. Possibilities include changes in filament polarity, sites of polymerization, or ATPase activity. Lastly, our model relies on McaAB interactions to specifically occur at the crystal-containing magnetosomes, which we have not demonstrated here. Future work should aim to address these unknowns.

### **Changing MamK dynamics as a strategy to diversify magnetosome chain organization.**

Across MTB, we find diverse types of magnetosome chains. For example, there are species with multiple chains that are parallel, or strikingly, perpendicular to each other [91,92]. How did different types of magnetosome chain arrangements emerge across MTB species? One way is to change the machinery that controls magnetosome positioning. In *Desulfovibrio magneticus* RS-1, for example, a second bacterial actin Mad28 and a suite of coiled-coil proteins are required for magnetosome chain assembly and organization in addition to MamK [41]. Our work here reveals a possible second strategy where MamK is regulated to produce different magnetosome chains. This serves as a possible explanation for the difference in the magnetosome chain patterning between AMB-1 and its close relative *M. gryphiswaldense* MSR-1 (MSR-1). Both organisms use MamK, MamY, and MamJ to build a magnetosome chain, yet WT MSR-1 has a magnetosome chain with all crystal-containing magnetosomes at the chain center, just like AMB-1  $\Delta mcaAB$  [12,30]. Furthermore, in MSR-1, MamK polymerization has been reported to occur at the cell poles, thus pushing the MamK filaments towards midcell, resulting in a moving-bleach-spot dynamic when observed with FRAP [30]. The ability to produce a singular crystal chain is not a unique property of MSR-1 MamK since MSR-1  $\Delta mamK$  expressing AMB-1 *mamK* produces a similar magnetosome chain observed by TEM [93]. Collectively, it seems likely that the difference in magnetosome chain patterning between AMB-1 and MSR-1 is due to different MamK behavior mediated by McaA and McaB. This case might be one example of a broader mechanism underlying organization diversification of magnetosomes and other subcellular structures in bacteria.

### **McaA and McaB - bacterial actin binding proteins?**

Eukaryotic cells are known to have many actin binding proteins that regulate all aspects of actin's properties, ranging from capping proteins that prevent further actin polymerization to cross-linking proteins that stabilize higher order filament structures [94]. The massive collection of actin binding proteins allows actin to be a multi-functional filament that can have different architectures and dynamics depending on the subset of

actin binding proteins that it is interacting with. Compared to the impressive repertoire of eukaryotic actin binding proteins characterized, the discovery of analogous proteins in bacterial systems have lagged behind [95]. One aspect is the lack of conservation between eukaryotic and bacterial actin binding proteins, which is largely driven by the divergence of actin filament structure across bacterial actins [95,96], hindering the computational identification of potential regulators. McaA and McaB interaction with MamK, and MamK's behavioral change in their absence, opens the possibility that McaA and McaB are bacterial actin binding proteins. Further studies on the biochemical mechanism of McaAB influence on MamK behavior would not only reveal whether they are true actin binding proteins, but also serve as an example that will guide discoveries to other bacterial actin regulators.

## MATERIALS AND METHODS

**Bacterial strains and growth.** *Magnetospirillum magneticum* sp. AMB-1 was cultured as previously described (10). Briefly, AMB-1 was grown on MG medium supplemented with 30 $\mu$ M ferric malate and 1x Wolfe's vitamin solution. Solid medium is prepared by adding 0.7% agar before autoclaving. AMB-1 colonies grown on MG plates were used to inoculate 1.5mL liquid MG in 1.5mL microcentrifuge tubes, then grown at 30°C for 2-3 days. These stock cultures were maintained at room temperature for a maximum duration of 2 weeks and diluted 100-fold into fresh MG medium for growth and  $C_{mag}$  measurements, TEM, and fluorescence microscopy. AMB-1 growth for co-IP experiments are detailed in a different section. Unless indicated otherwise, AMB-1 was cultured in glass culture tubes at 30°C in 10% oxygen without shaking for 1-2 days in a microaerobic glove box. If anaerobic medium was used, the medium was prepared by bubbling MG with N<sub>2</sub> gas for at least 10 minutes before autoclaving in sealed Balch tubes. Ferric malate, Wolfe's vitamin solution, and inoculum were introduced through a sterile needle and syringe. When needed, kanamycin was added for a final concentration of 7 $\mu$ g/mL and 10 $\mu$ g/mL for liquid and solid medium, respectively.

OD and coefficient of magnetism ( $C_{mag}$ ) readings for AMB-1 were taken on a Spectronic 20D+ at 400nm.  $C_{mag}$  was determined by taking OD<sub>400</sub> readings with a stirbar magnet held parallel and perpendicular to the sample.  $C_{mag}$  was calculated by taking the ratio of the two OD<sub>400</sub> readings. A  $C_{mag}$  of 1 indicates no magnetic alignment.

*Escherichia coli* DH5 $\alpha$ , WM3064, and BL21 were grown in standard LB medium at 37°C, with 50 $\mu$ g/mL kanamycin, 25 $\mu$ g/mL chloramphenicol, 100 $\mu$ g/mL carbenicillin, and/or 5 $\mu$ M diaminopimelic acid (DAP) when needed, unless stated otherwise. Growth of *E. coli* strains used for bacterial two hybrids are explained in a different section.

**Non-biomineralization conditions.** All non-biomineralization cultures were grown in MG medium without the ferric malate solution in glassware treated overnight with 30mM oxalic acid then rinsed with MilliQ water three times to remove residual iron. To reduce existing magnetite in AMB-1 cell, AMB-1 cultures were passaged in 10mL non-biomineralization MG in acid-washed tubes three times, where each passage received a 100 $\mu$ L inoculum of the previous passage and incubated for 2 days microaerobically at 30°C. The final culture was inoculated at a 1:100 dilution and grown at 30°C with constant stirring in the microaerobic glove box to incorporate 10% oxygen to further inhibit biomineralization.

**Genetic Manipulation.** All strains and plasmids used in this study can be found in the Supplementary Materials. Plasmids used to express genes in AMB-1 or *E. coli* were cloned in *E. coli* DH5 $\alpha$  as previously described [34], with a few adjustments. Oligonucleotides were purchased from Integrated DNA technologies and PCR was done using Q5 High Fidelity DNA Polymerase (New England Biolabs). Constructed plasmids were sequence verified through the UC Berkeley DNA sequencing facility or Plasmidsaurus (www.plasmidsaurus.com). Briefly, to generate deletions in AMB-1, a suicide plasmid carrying two homologous regions flanking the target gene was transformed into *E. coli* WM3064. The plasmid is introduced into AMB-1 through conjugation, then kanamycin and 2% sucrose are used to select for the first and second recombination events, respectively. Deletions are PCR amplified and sequence verified.

**Cell lysis and *in vivo* co-immunoprecipitation.** 2L glass bottles with 2L of MG were inoculated with 2 10mL seed cultures of AMB-1 grown microaerobically for 2 days. These 2L cultures were incubated for 2 days at 30°C in 10% oxygen without shaking. If applicable, 10mL of the culture was taken to determine C<sub>mag</sub> and perform microscopy. To harvest, cells were centrifuged in 500mL centrifuge bottles at 11,000 x g with a JA-10 rotor and Beckman J2-21M centrifuge or GS-3 rotor and Sorvall RC-5B centrifuge. Cells were resuspended in 3-5mL medium and transferred to a 15mL falcon tube. The residual medium was removed through a final centrifugation at 8000 x g for 6min. Cell pellet mass was recorded, flash frozen in liquid nitrogen, and stored at -80°C until use. AMB-1 was subject to chemical lysis as previously reported [97]. Briefly, cell pellets were resuspended in 1mL Lysis Buffer A (10mM Tris-HCl, 150mM NaCl, 0.5mM EDTA, 0.5% NP-40, pH 7.5 supplemented with 2 $\mu$ g/mL pepstatin A, 2 $\mu$ g/mL leupeptin, 2mM PMSF, and 0.5mg/mL lysozyme) and incubated at RT for 15min without rotation. 3mL of Lysis Buffer B (20mM HEPES-KOH, 50mM NaCl, 1.25mM CaCl<sub>2</sub>, pH 7.5 with 2mM DTT and 5 $\mu$ g/mL DNase I) was added and samples were incubated at 4°C while rotating end-to-end for 45min. Samples were then centrifuged at 14,600 x g for 30min at 4°C. The supernatant was collected into a new 15mL Falcon tube and either used for co-IP immediately or flash frozen in liquid nitrogen and stored at -80°C until use.

For co-IPs to be evaluated by Western blot, the volume of lysate used was normalized according to the cell pellet mass harvested, then diluted two-fold with Wash Buffer (10mM Tris-HCl, 150mM NaCl, pH 7.4). 20 $\mu$ L of GFP-Trap agarose beads (ChromoTek) were equilibrated three times by resuspending in 500 $\mu$ L Wash Buffer followed by centrifugation. All centrifugation steps here were carried out at 2500 x g at 4°C, unless indicated otherwise. The diluted lysate was applied to the equilibrated beads in a 15mL Falcon tube and rotated end-to-end for 1hr at 4°C. The solution was centrifuged for 6min. The beads were transferred to a 1.5mL microcentrifuge tube and washed three times with 500 $\mu$ L Wash Buffer, centrifuged for 3min between each wash. To elute, the beads were resuspended in 100 $\mu$ L of 2x Laemmli sample buffer and incubated at 10min at 95°C. The samples were centrifuged for 3min and the supernatant was collected as the elution.

The co-IP protocol was adjusted for samples to be sent for mass spectrometry. 10 2L cultures were pooled (20L total). Cell lysis was done with 5mL Lysis Buffer A and 15mL Lysis Buffer B in a 50mL Falcon tube. The lysate was clarified by being split evenly into 6 15mL falcon tubes before centrifugation to minimize debris contamination. 30 $\mu$ L of the

GFP-Trap agarose bead slurry was used for the co-IP. Elution was done by resuspending the samples in 50 $\mu$ L of 200mM glycine, pH 2.5 and vigorously pipetting up and down for 30s. The sample was centrifuged at 2500 x g for 3min at 4°C. The supernatant was transferred to a new tube and neutralized with 5 $\mu$ L of 1M Tris, pH 10.4. The beads were then eluted a second time to increase protein abundance.

**Mass spectrometry.** Proteins were precipitated using trichloro acetic acid (TCA) precipitation. 100% TCA was added to the co-IP elution for a final TCA concentration of 20%. Samples were incubated on ice for 1hr, then centrifuged at 16,000 x g for 10min at 4°C. The protein pellet was washed three times with cold 100 $\mu$ L 0.01M HCL in 90% acetone, then allowed to dry at RT. Protein pellet mass was measured before proceeding. Precipitated protein samples were resuspended in 80 $\mu$ L 100mM Tris-HCl, 8M urea, pH 8.5. 100mM TCEP was added for a final concentration of 5mM, then incubated at RT for 20min. Fresh 500mM iodoacetamide was added for a 10mM final concentration, then incubated at RT for 15min in the dark. Samples were diluted 4-fold using 100mM Tris-HCl, pH 8.5. Filter-sterilized 100mM CaCl<sub>2</sub> was added for a final concentration of 1mM before adding 1 $\mu$ L of 0.5 $\mu$ g/ $\mu$ L sequence-grade modified trypsin (Promega). Samples were incubated overnight at 37°C in the dark, then formic acid was added for a 5% final concentration. Samples were then desalted using C18 Spec tips (Agilent). The Spec tip was washed with 200 $\mu$ L HPLC-grade MeOH, followed by three washes with 20 $\mu$ L 5% acetonitrile/5% formic acid. Sample was pushed through the tip, washed three times with 200 $\mu$ L 5% acetonitrile/5% formic acid, eluted with 2 passes of 100 $\mu$ L 80% acetonitrile/5% formic acid, then dried.

Mass spectrometry was performed at the Proteomics/Mass Spectrometry Laboratory at University of California, Berkeley. A nano LC column was packed in a 100- $\mu$ m inner diameter glass capillary with an integrated pulled emitter tip. The column consisted of 10cm of Polaris c18 5- $\mu$ m packing material (Varian). The column was loaded and conditioned using a pressure bomb. The column was then coupled to an electrospray ionization source mounted on a Thermo-Fisher LTQ XL linear ion trap mass spectrometer. An Agilent 1200 HPLC equipped with a split line so as to deliver a flow rate of 1 $\mu$ L/min was used for chromatography. Peptides were eluted with a 90-minute gradient from 100% buffer A to 60% buffer B. Buffer A was 5% acetonitrile/0.02% heptafluorobutyric acid (HBFA); buffer B was 80% acetonitrile/0.02% HBFA. Collision-induced dissociation and electron transfer dissociation spectra were collected for each *m/z*. Protein identification and quantification and analysis were done with Integrated Proteomics Pipeline-IP2 (Bruker Scientific LLC, Billerica, MA, <http://www.bruker.com>) using ProLuCID/Sequest [98], DTASelect2 [99,100], and Census [101,102]. Spectrum raw files were extracted into ms1 and ms2 files from raw files using RawExtract 1.9.9 (<http://fields.scripps.edu/downloads.php>) 10, and the tandem mass spectra were searched against MIT database [100,103]. Proteins encoded in AMB-1 magnetosome islet (MIS) were downloaded from NCBI on Sept. 1, 2022 and included for analysis.

**Western Blotting.** Western blots were done with protein samples separated via SDS-PAGE using Mini-PROTEAN TGX Stain-Free (BioRad). Prior to transfer, gels were imaged for total protein content using Stain-Free imaging. The primary antibodies and their dilutions used in this study are as follows: anti-GFP (Abcam ab 6556, 1:2500 and

Invitrogen GF28R, 1:2500), anti-HaloTag (Promega G921A, 1:1000), and anti-Flag (Sigma F3165, 1:2500). The secondary antibodies and their dilutions are as follows: anti-mouse goat HRP conjugate (Invitrogen A24512, 1:5000), anti-rabbit goat HRP conjugate (Biorad, 170-5046, 1:10,000). Blots were developed using Western Lighting Plus-ECL (PerkinElmer) and imaged with the BioRad ChemiDoc MP Imaging System.

**Epifluorescence microscopy.** 10mL of AMB-1 microaerobically grown for 2 days at 30°C until mid-to-late exponential phase ( $OD_{400}$  of 0.200-0.280). 1-1.5mL of this culture was centrifuged for 4min at 8000 x g. The pellet was resuspended in 10 $\mu$ L of residual medium and 0.7 $\mu$ L was applied on a glass slide. Imaging was done on a Zeiss Axio Observer Inverted microscope with Zen software (Zeiss), and images were handled using Fiji. To score images for localization, images were randomized using the Cell Counter plugin.

**Fluorescence recovery after photobleaching.** 10mL AMB-1 cultures were grown microaerobically until  $OD_{400}$  reached ~0.1. The cultures were concentrated to 10 $\mu$ L, of which 0.7 $\mu$ L was applied between the bottom of a glass bottom dish (MatTek) and a 450 $\mu$ L 2% agarose gumdrop that minimizes cell movement. Cells were imaged on an inverted Carl Zeiss LSM880 FCS laser scanning confocal microscope with an objective lens Plan-Apochromat 100x/1.40 oil DIC at RT. Cells were imaged with a 488nm laser at 3% power every 30s for 15min with Definite Focus autofocus (Zeiss). At t=1min, a small region of the cell was photobleached with the 488nm laser at 100% power. Images were acquired through LSM880 Zen software (Zeiss). Fiji was used to analyze the images and generate kymographs.

**Transmission electron microscopy.** AMB-1 cells to be used for TEM were grown in 10mL cultures microaerobically for 2 days at 30°C. 1-1.5mL of the culture was concentrated to 10 $\mu$ L and applied to Formvar/Carbon 300 Mesh copper grid (Electron Microscopy Sciences) that have been glow discharged with Pelco Easiglow. After 5min incubation at RT, excess cells were removed by washing the grid three times in MilliQ water. Cells were imaged on an FEI Tecnai 12 transmission electron microscope with an accelerating voltage of 120 kV. Images were captured using a Rio 16 4K CMOS camera and Gatan Digital Micrograph software. Grids carrying cells to be used for scoring were randomized before imaging and scoring.

**Protein purification.** To purify McaA and McaB, the predicted cytoplasmic side of both proteins were tagged, then expressed in BL21 *E. coli*. McaA (aa400-776) was N-terminally tagged with a Strep-tag and McaB (aa 28-219) was N-terminally tagged with maltose binding protein (MBP). MBP was also expressed from BL21. BL21 harboring the recombinant protein expression plasmids were inoculated as a 1:100 dilution from an overnight seed culture grown in 2xYT without inducers and grown at 37°C shaking at 200rpm. When  $OD_{600}$  reached ~0.5, Strep-McaA and MBP expression was induced with 0.1mM IPTG and MBP-McaB expression was induced with 0.5mM IPTG. Cultures were incubated for another 3-4hrs at 37°C shaking at 200rpm. Cells were harvested at 10,800 x g for 20min at 4°C, flash frozen with liquid nitrogen, and stored at -80°C until use. To lyse, cell pellets were resuspended in 35mL 10mM Tris-HCl, 150mM NaCl, pH7.4

supplemented with 1 µg/ml pepstatin A, 2 µg/ml leupeptin, and 1 mM PMSF, and lysed with a french press at 18,000 PSI. Lysate was clarified 14,600 x g for 30 min at 4°C, and stored at 4°C overnight. Lysates were filtered through a 0.22 µm PES syringe filter and applied to either a StrepTrap HP (GE Healthcare) or MBPTrap HP (Cytiva) column using an ÄKTA pure FPLC system with a 5 ml/min flow rate. Samples were washed with 20 column volumes (CV) with 10 mM Tris-HCl, 150 mM NaCl, pH 7.4 and eluted with 5 CV with either 10 mM Tris-HCl, 150 mM NaCl, 2.5 mM desthiobiotin, pH 7.4 for StrepTrap or 10 mM Tris-HCl, 150 mM NaCl, 10 mM maltose, pH 7.4 for MBPTrap. Fractions were evaluated with SDS-PAGE and those containing the protein of interest were pooled and concentrated with Amicon Ultra 10K centrifugal filter units. Samples were further purified via size exclusion chromatography using a HiLoad 16/600 Superdex 75 pg column (GE Healthcare) with 10 mM Tris-HCl, 150 mM NaCl, pH 7.4. Fractions with pure proteins were flash frozen and stored at -80°C until use. Protein concentrations were determined using the Micro BCA Protein Assay Kit.

Untagged recombinant MamK was purified from *E. coli* using ammonium sulfate precipitation as previously described [29], with a few adjustments. After the second 20% ammonium sulfate cut, MamK samples are dialyzed into depolymerization buffer (10 mM CAPs, 10 mM EDTA, 2 mM DTT, pH 9.6) at 4°C for approximately 8 h. The sample was further purified using anion exchange chromatography (DEAE TOYOPEARL, 2.3 cm × 12 cm, Tosoh, Japan) and gel filtration chromatography (Superdex 200 Increase 10/300 GL, Cytiva, USA) with depolymerization buffer. The monomeric MamK fractions were pooled, and aliquots were stored at -80°C. For experimental use, purified MamK is exchanged into polymerization buffer (10 mM Tris-HCl, 25 mM KCl, 2 mM MgCl<sub>2</sub>, 1 mM DTT, pH 7.5) using a desalting spin column (Zaba Spin Desalting Column, Thermo Fisher, USA).

**Pelleting Assay.** Protein samples were diluted to 1 µM in polymerization buffer (10 mM Tris-HCl pH 7.5, 25 mM KCl, 2 mM MgCl<sub>2</sub>, 1 mM DTT). Proteins in mixtures each had a concentration of 1 µM. Final concentration of 1 mM ATP was added if needed. Samples were incubated at 25°C for 10 min, then centrifuged at 100,000 x g for 30 min at 4°C. The supernatant and pellet fractions were recovered and analyzed by SDS-PAGE.

**In vitro pull down.** 10 µg of each protein was mixed into a total of 500 µL of equilibration buffer (10 mM Tris-HCl, 150 mM NaCl, pH 7.4) and incubated at 4°C for 1 hr with end-to-end mixing. All following steps were carried out at 4°C. For negative controls, 10 µg of each protein were incubated separately. 20 µL Strep-tactin resin (IBA Lifesciences) was equilibrated by washing three times with 100 µL equilibration buffer and centrifuging at 2500 x g for 3 min at 4°C between each wash. Protein samples were centrifuged at 16,000 x g for 5 min to remove any aggregates. The supernatant was added to the equilibrated Strep-tactin resin in a 1.5 mL microcentrifuge tube and incubated for 1 hr while mixing end-to-end. The sample was centrifuged at 2500 x g for 3 min, and the flowthrough supernatant was removed. The resin was washed three times using 500 µL equilibration buffer, then eluted with 50 µL 10 mM Tris-HCl, 150 mM NaCl, 2.5 mM desthiobiotin, pH 7.4 by pipetting vigorously for 30 s. The elution was collected by centrifuging for 3 min at 2500 x g. Samples were evaluated with SDS-PAGE and Coomassie staining.

**Bacterial adenylate cyclase two hybrids assay (B2H).** *E. coli* DHM1 were transformed with two plasmids, each carrying either the T18 or T25 fusion and incubated at 30°C overnight with 50µg/mL kanamycin and 100µg/mL carbenicillin for selection. A single colony was used to inoculate 80µL LB with kanamycin, carbenicillin, and 0.5mM IPTG in a 96 well plate, and incubated at 30°C overnight shaking at 200rpm. 3µL of the culture was spotted onto M63 agar supplemented with 25µg/mL kanamycin, 50µg/mL carbenicillin, 0.5mM IPTG, and 40µg/mL X-gal and incubated at 30°C for 5-8 days before imaging with an iPhone 15 camera.

**Multiple sequence alignment.** McaA homologs were first identified through a BlastP search of AMB-1 McaA on Dec. 27, 2024. Homolog sequences with an expectation value below 1e-5 from this initial search were downloaded from NCBI on the same day. The homolog sequences were then aligned on Jan. 14, 2025 using MAFFT (Version 7.511) using the iterative refinement algorithm L-INS-i [104]. The alignment was visualized using Jalview (version 2.11.4.1) [105].

**McaA and McaB protein sequence analysis.** McaA protein structure was predicted using AlphaFold3 on the AlphaFold Server (<https://alphafoldserver.com/>) on Nov. 12, 2022 [87]. Deepcoil2 (<https://toolkit.tuebingen.mpg.de/tools/deepcoil2>) was used to predict coiled-coil domains, “a” position amino acids, and “d” position amino acids of McaB [86]. The predicted domains and amino acids were mapped onto predicted McaB structures generated by AlphaFold3 on Oct. 20, 2025. Protein structures were visualized using UCSF ChimeraX [106].

## ACKNOWLEDGEMENTS

We thank Lori Kohlstaedt and Robert Maxwell at the Vincent J. Coates Proteomics/Mass Spectrometry Laboratory at UC Berkeley for performing the mass spectrometry for this study. We thank Denise Schichnes, Juliana Cho, and Steve Ruzin for their training and support with confocal imaging at the Biological Imaging Facility at UC Berkeley. We thank Danielle Jorgens, Reena Zalpuri, and Misun Kang for training and assistance with TEM at the Electron Microscope Lab at UC Berkeley. A. K. is supported by NIH National Institute of General Medical Sciences (R35GM127114). Y. R. was supported by NSF Graduate Research Fellowship Program (2020254971) and the Berkeley Fellowship for Graduate Study.

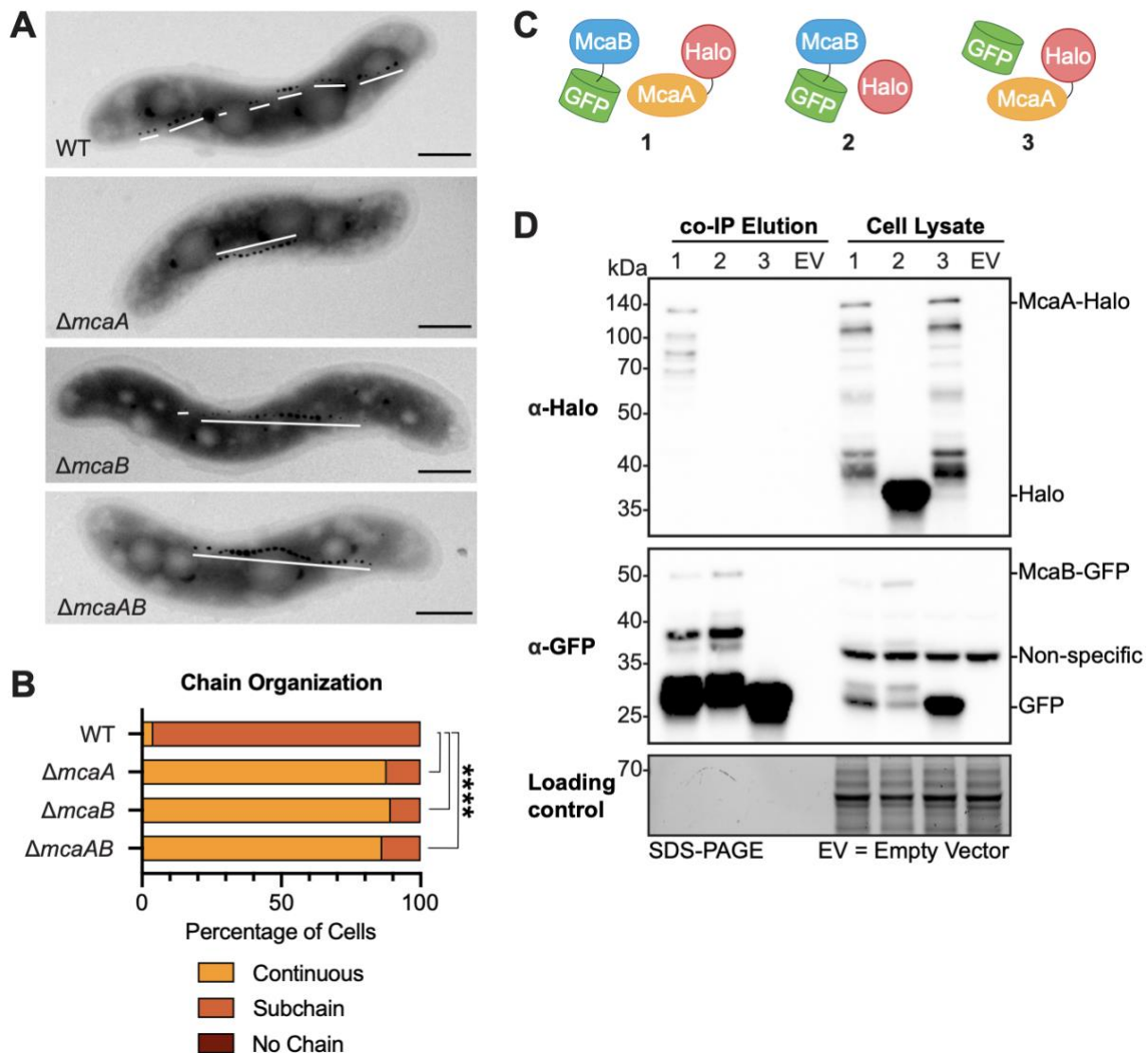


**Figure 1:** McaA and McaB contribute to magnetosome organization and interact in vivo. **(A)** Transmission electron microscopy images of a representative cell for AMB-1 WT,  $\Delta mcaA$ ,  $\Delta mcaB$ , and  $\Delta mcaAB$  showing the magnetosome chain. White lines are drawn to show the placement of crystal-containing magnetosomes. The WT magnetosome chain has many subchains of crystal-containing magnetosomes while  $\Delta mcaA$ ,  $\Delta mcaB$ , and  $\Delta mcaAB$  do not. Scale bar = 0.5  $\mu$ m. **(B)** Quantification of magnetosome chain organization using TEM images. Cells were categorized based on their magnetosome chain phenotype and the x-axis represents the percentage of cells that displayed the indicated chain organization. WT n = 109,  $\Delta mcaA$  n = 168,  $\Delta mcaB$  n = 165,  $\Delta mcaAB$  n = 75. Fisher's exact test was used to determine p-values (\*\*\*\* P < 0.0001). **(C)** Schematics of the combination of proteins used for the *in vivo* co-IP in (D). **(D)** Western blots of elutions from co-IP with GFP-Trap agarose beads and cell lysates illustrating that McaA-Halo and McaB-GFP interact, and this interaction is not driven by their tags. The co-IP was done using the  $\Delta mcaAB$  strain. The loading control is a stain-free SDS-PAGE gel of the same samples. This result is a representative of three biological replicates (see Fig. S3).

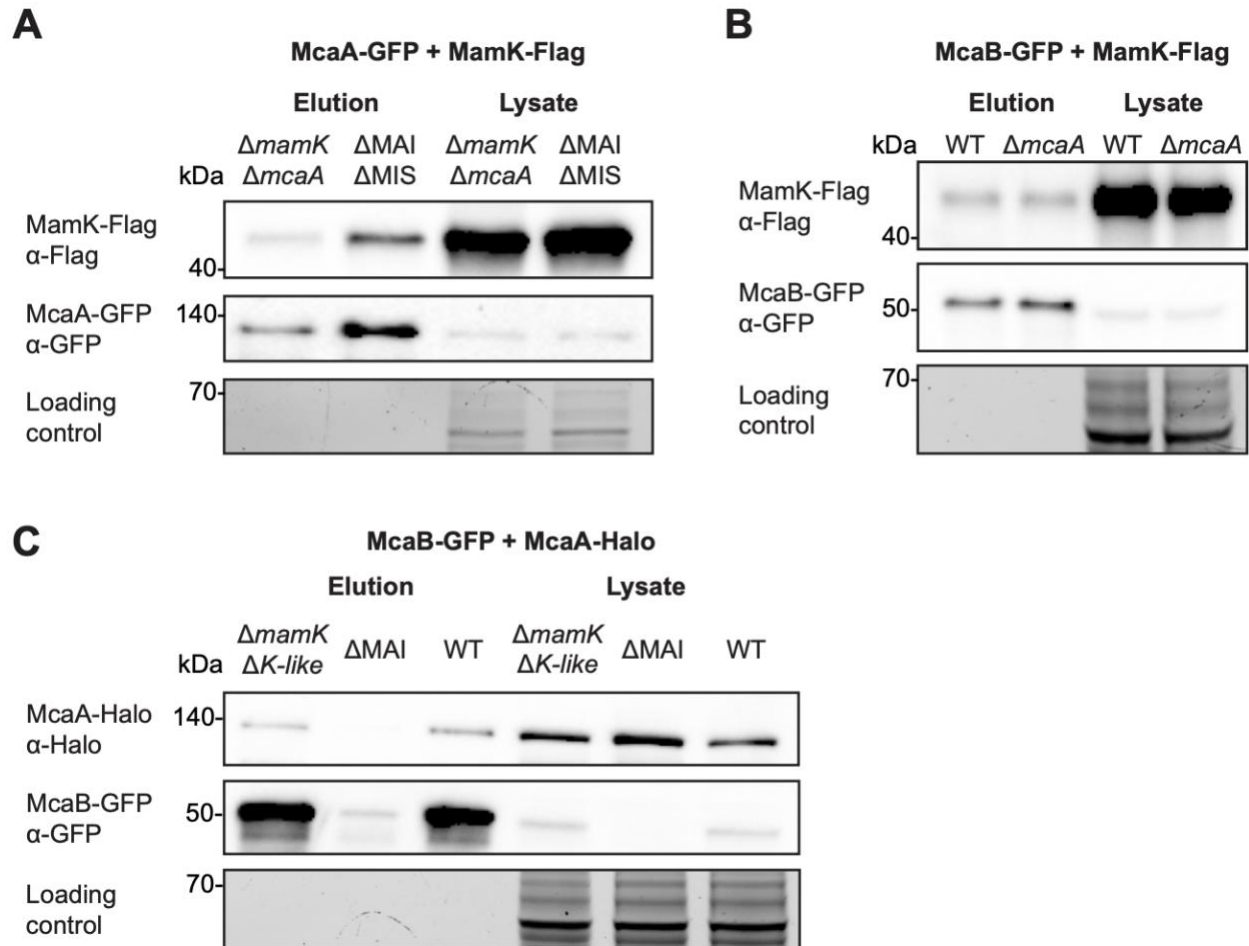


**Figure 2:** MamK interacts with both McaA and McaB *in vivo*. **(A)** Western blots of a co-IP with McaA-GFP and MamK-Flag done in both  $\Delta mamK \Delta mcaA$  and  $\Delta MAI \Delta MIS$ . **(B)** Western blots of a co-IP with McaB-GFP and MamK-Flag in WT and  $\Delta mcaA$ . **(C)** Western blots of a co-IP with McaB-GFP and McaA-Halo done in  $\Delta mamK \Delta mamK$ -like,  $\Delta MAI$ , and WT. In all cases, GFP-Trap agarose beads were used for co-IPs and a stain-free SDS-PAGE serves as the loading control.

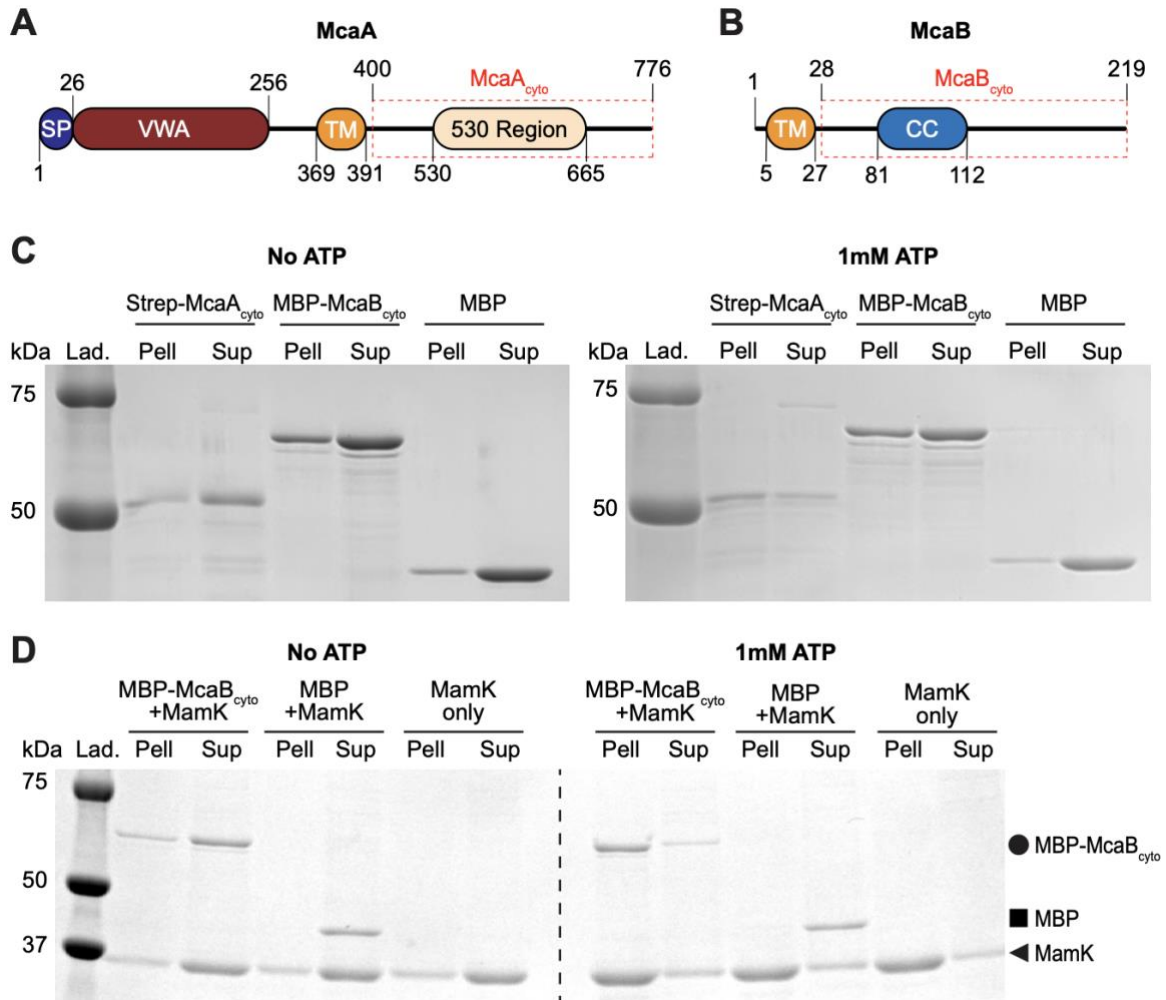


**Figure 3:** Purified McaB<sub>cyto</sub> interacts with MamK filaments. **(A)** Schematic of McaA showing the predicted signal peptide (SP), von Willebrand factor type A (VWA) domain, transmembrane domain (TM), and the conserved 530 region. The amino acid positions are indicated and the red dashed box represents the segment purified as McaA<sub>cyto</sub>. **(B)** Schematic of McaB showing the predicted transmembrane domain (TM) and coiled-coil domain (CC). The amino acid positions are indicated and the red dashed box represents the segment purified as McaB<sub>cyto</sub>. **(C)** SDS-PAGE of pelleting assay samples. Proteins were incubated with or without 1mM ATP, then separated into the pellet (Pell) and supernatant (Sup) fractions. **(D)** SDS-PAGE of co-pelleting assay samples. MamK was mixed with either MBP-McaB<sub>cyto</sub> or MBP and incubated with or without 1mM ATP. The sample was separated into the pellet (Pell) and supernatant (Sup) fractions. MamK polymerizes into filaments in the presence of ATP, demonstrated by the shift into the pellet fraction when incubated with ATP. MBP-McaB<sub>cyto</sub> shifts into the pellet fraction in the presence of MamK filaments, indicating an interaction between MBP-McaB<sub>cyto</sub> and MamK. MBP alone remains predominantly in the supernatant in the parallel experiment. MBP-McaB<sub>cyto</sub> (65.0kDa), MBP (43.2kDa), and MamK (37.6kDa) bands are indicated with a circle, square, and triangle respectively. Lanes with protein ladders (Lad.) are also included.

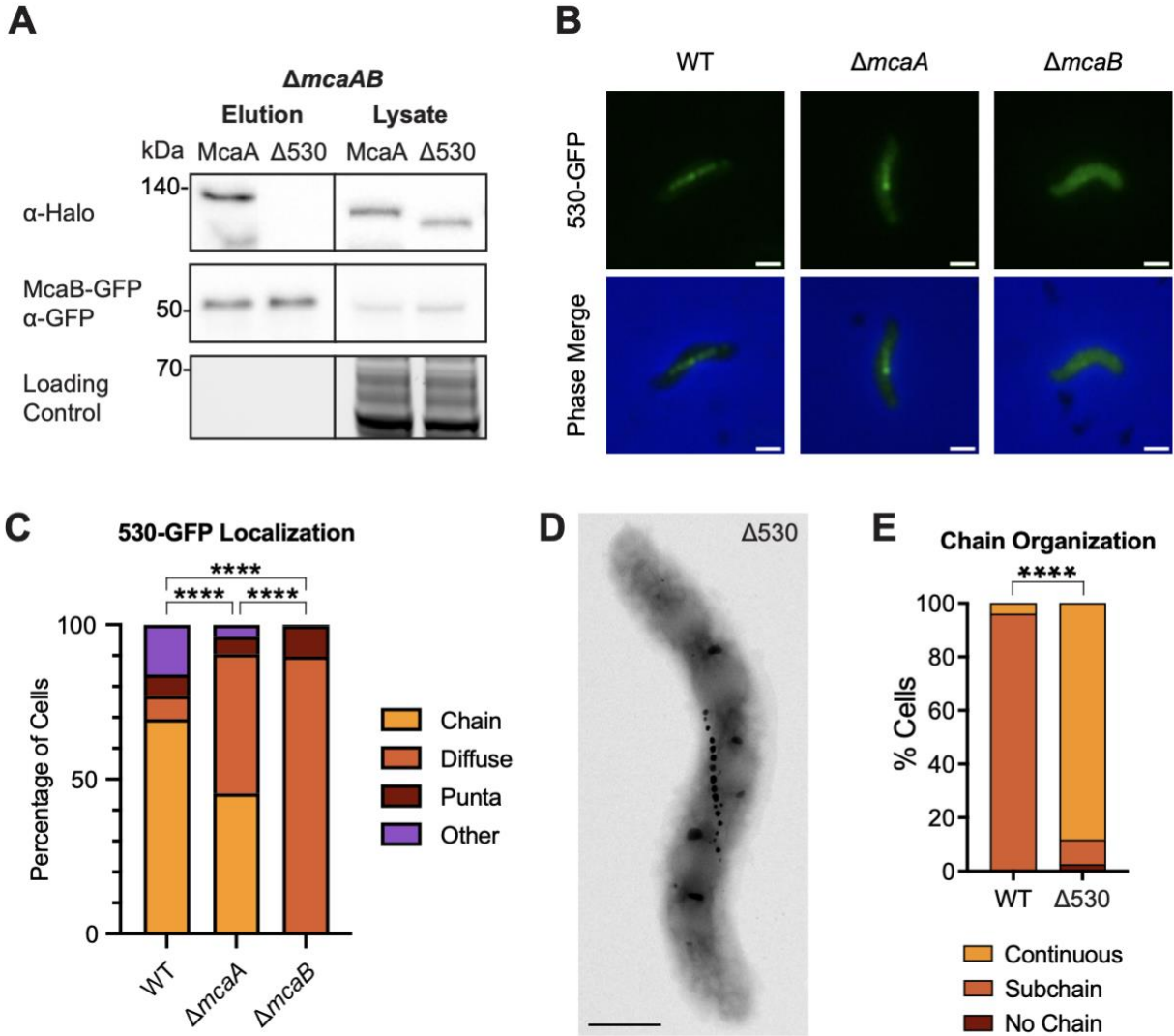


**Figure 4:** The 530 region of McaA is necessary for interaction with McaB and crystal subchain phenotype. **(A)** Western blots of co-IPs with McaB-GFP and either full-length McaA-Halo or McaA-Halo missing the 530 region ( $\Delta 530$ ) in a  $\Delta mcaAB$  strain using GFP-Trap agarose beads. The loading control is a stain-free SDS-PAGE gel. **(B)** The 530 region fragment was tagged with GFP and expressed in WT,  $\Delta mcaA$ , and  $\Delta mcaB$ , then imaged with epifluorescence microscopy. Representative cells for each expression strain are shown. Scale bar = 1  $\mu$ m. **(C)** Quantification of 530-GFP localization from fluorescent microscopy images. Cells were categorized based on their localization pattern and the y-axis represents the percentage of cells that displayed the indicated localization. WT n = 390,  $\Delta mcaA$  n = 390,  $\Delta mcaB$  n = 332. Fisher's exact test was used to determine p-values (\*\*\*\* P < 0.0001). **(D)** Representative TEM image of AMB-1 with the 530 region deleted at the native locus. Scale bar = 0.5  $\mu$ m. **(E)** Quantification of magnetosome chain organization using TEM images. Cells were categorized based on their magnetosome chain phenotype and the y-axis represents the percentage of cells that displayed the indicated chain organization. WT n = 75,  $\Delta 530$  n = 77. Fisher's exact test was used to determine p-values (\*\*\*\* P < 0.0001).

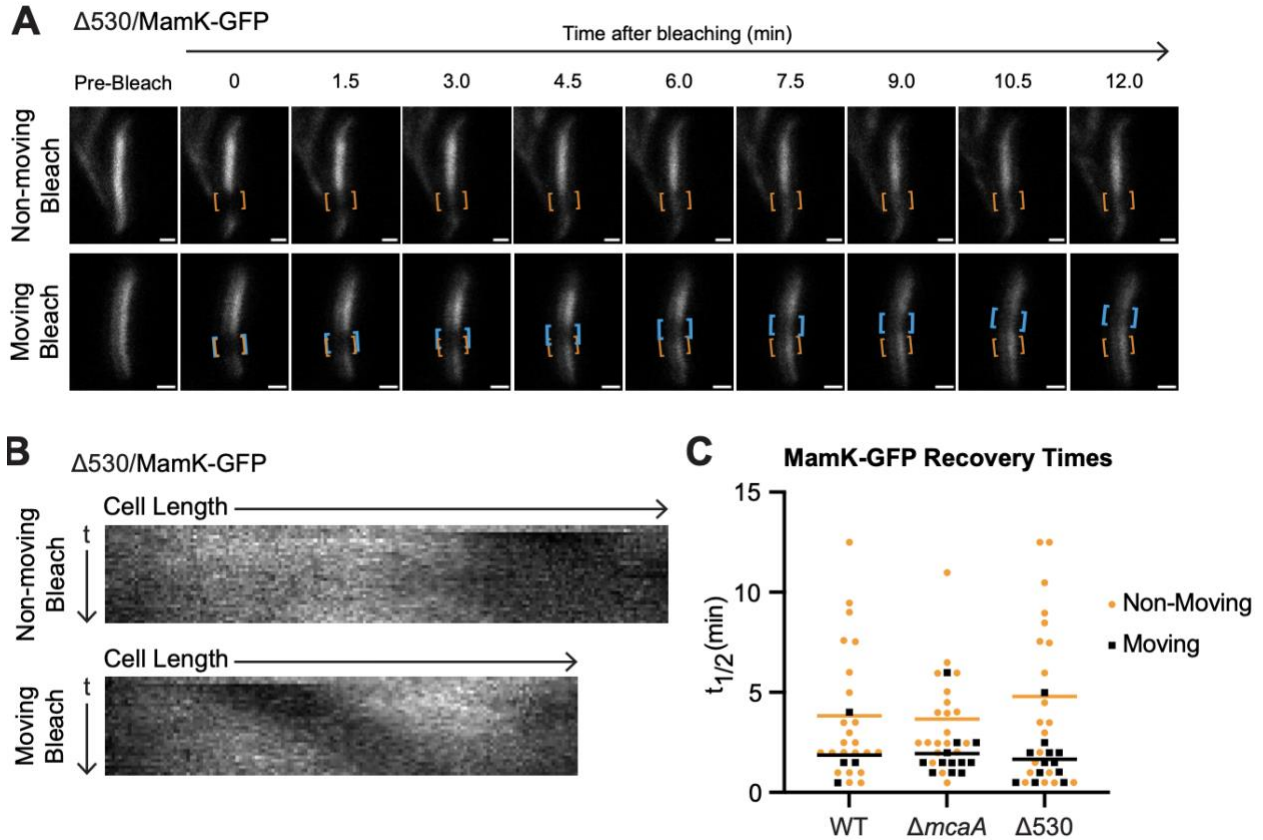


**Figure 5:** The 530 region is required for WT MamK dynamics. **(A)** Two representative cells from FRAP of MamK-GFP in  $\Delta 530$  strain imaged using confocal microscopy. Orange brackets indicate the original bleaching location. Blue brackets track bleach spot movements. The top cell has a bleach spot that does not move while the bottom cell has a moving bleach spot. Scale bar =  $0.5\mu\text{m}$ . **(B)** Kymographs of the cells in (A). **(C)** MamK-GFP half-time of fluorescence recovery ( $t_{1/2}$ ) across different AMB-1 strains. Each point represents one cell. Yellow circles indicate cells with non-moving bleach spots while black triangles represent those with a moving bleach spot. Cells that did not recover were not included. Bars represent the average recovery half-time of each population.

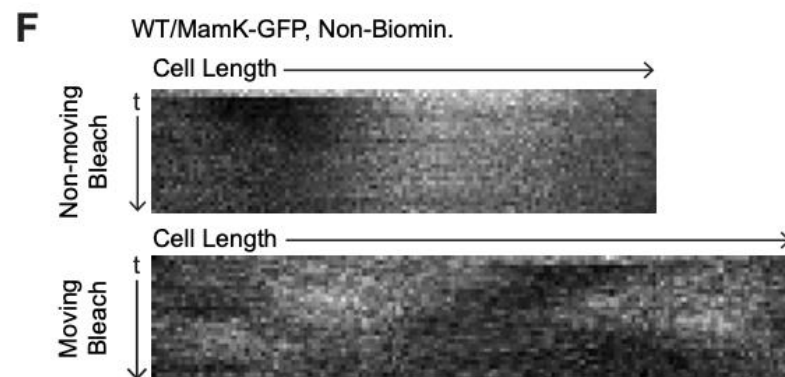
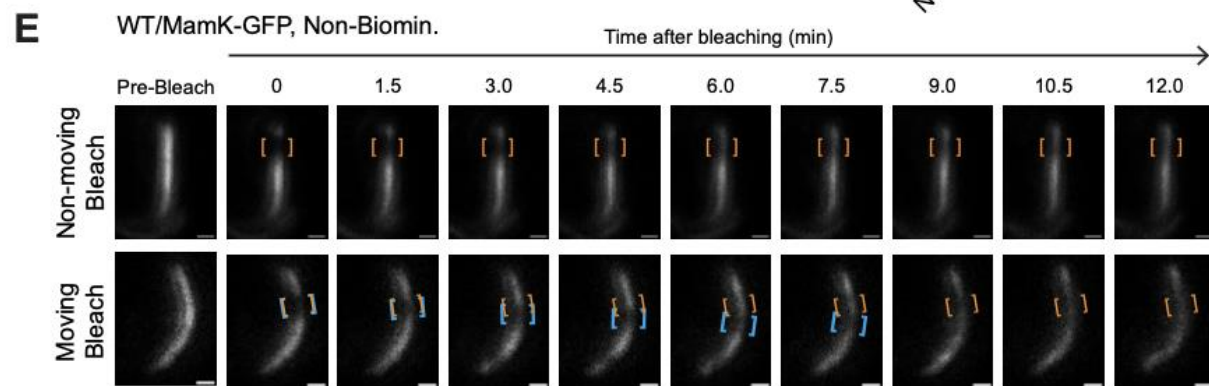
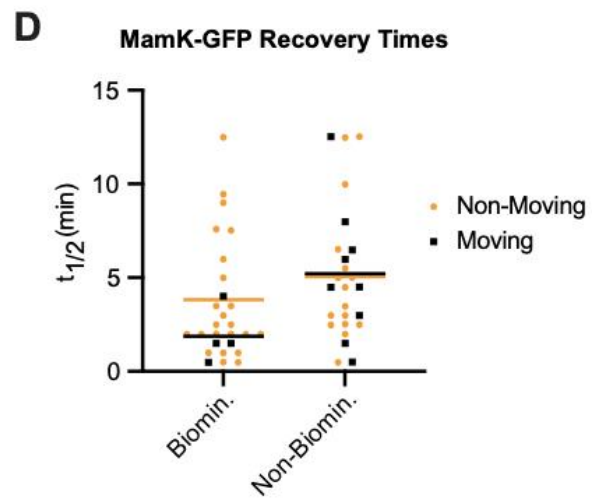
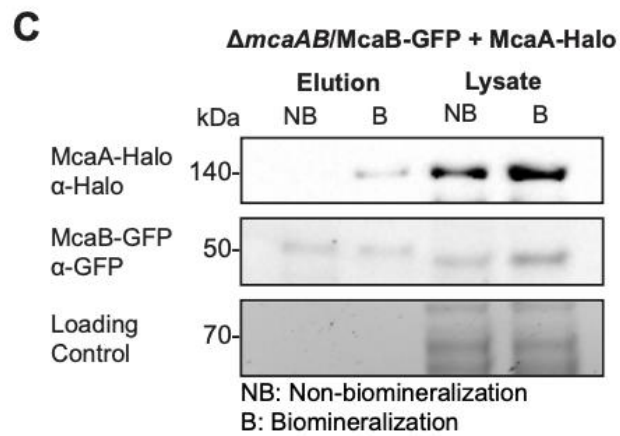
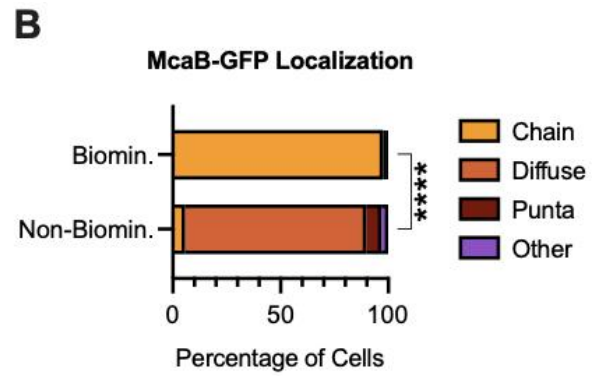
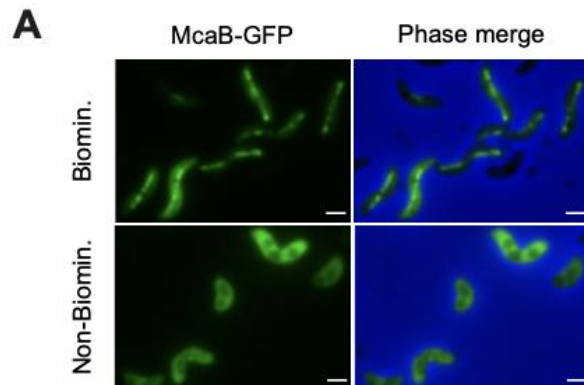


**Figure 6:** Changes to McaB localization correlates with its ability to interact with McaA and shift in MamK dynamics. **(A)** Representative cells showing McaB-GFP localization in biomineralization and non-biomineralization conditions in the same strain used for co-IP in (C). Images were taken with epifluorescence microscopy. Scale bar = 1 $\mu$ m. **(B)** Quantification of McaB-GFP localization using fluorescent microscopy images. Cells were categorized based on their localization pattern and the y-axis represents the percentage of cells that displayed the indicated chain organization. Biomineralization n = 845, non-biomineralization n = 4243. Chi-squared test of independence was used to determine p-values (\*\*\*\* P < 0.0001). **(C)** Western blots of co-IP with McaB-GFP and McaA-Halo in non-biomineralization (NB) and biomineralization (B) conditions in a  $\Delta mcaAB$  background using GFP-Trap agarose beads. The loading control is a stain-free SDS-PAGE gel. **(D)** Recovery half-times ( $t_{1/2}$ ) of MamK-GFP in WT AMB-1 under biomineralization (same as WT from Fig. 5C) or non-biomineralization conditions. Each point represents one cell. Yellow dots indicate cells with a non-moving bleach spot while black squares represent those with a moving bleach spot. Cells that did not recover were not included. Bars represent the average recovery half-time of each population. **(E)** Two representative cells from FRAP of MamK-GFP in WT grown under non-biomineralizing conditions imaged using confocal microscopy. Orange brackets indicate the original bleaching location. Blue brackets track bleach spot movements. The top cell has a bleach spot that does not move while the bottom cell has a moving bleach spot. Scale bar = 0.5 $\mu$ m. **(F)** Kymographs of the same cells depicted in (E).

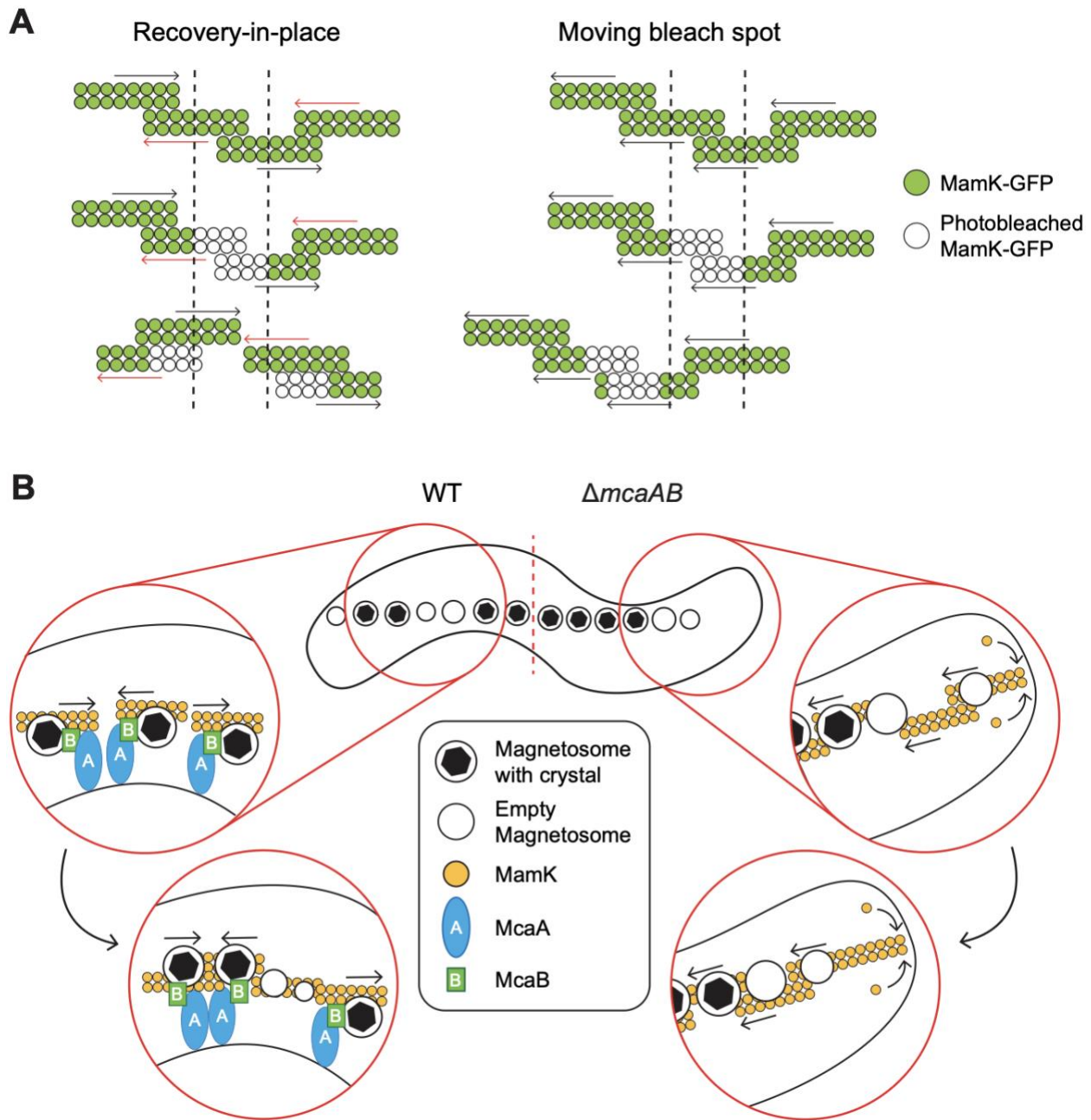


**Figure 7:** Model for how the magnetosome chain organization is established in WT AMB-1. **(A)** Schematic displaying how the dynamics of MamK filaments produced the two observed FRAP behaviors. Green circles depict fluorescent MamK-GFP while white circles represent photobleached MamK-GFP during the FRAP experiments. The dashed lines indicate the location of photobleaching. In situations where the MamK-GFP bleach spot does not move and is recovering-in-place (left), nearby MamK filaments are moving or polymerizing in either direction, represented by the arrows. The bleached filaments may move away while unbleached filaments move into the region, causing the gradual recovery of the bleached location in place. In cells where the bleach spot is moving (right), nearby MamK filaments have coordinated movement in a single direction. The bleach

spot also recovers during its movement, likely because the individual filaments undergo polymerization while moving. **(B)** Model for how McaA and McaB produce crystal subchains by altering MamK dynamics in AMB-1. In WT (left), McaA-McaB interactions occur along the magnetosome chain where crystal-containing magnetosomes are present. This allows the McaAB complex to interact with MamK filaments to alter their directionality, resulting in subgroups of crystal-containing magnetosomes. Concentrating crystal-containing magnetosome into subchains creates space throughout the cell for new magnetosomes to form. In the absence of *mcaAB* (right), MamK filaments move in a single direction, pushing magnetosomes to the midcell. The oldest magnetosomes - most of which have crystals - are at the middle of the chain and the newest are at the chain periphery.

| <b><u>Protein Hit</u></b>                                    | <b><u># Sequences</u></b> | <b><u>Spectrum Count</u></b> |
|--------------------------------------------------------------|---------------------------|------------------------------|
| McaA-GFP                                                     | 25                        | 122                          |
| Magnetosome protein MamA                                     | 5                         | 7                            |
| Uncharacterized protein, Amb3721                             | 3                         | 5                            |
| Magnetic particle membrane specific GTPase, Mms16 (amb0546)  | 2                         | 3                            |
| Actin-like protein MamK                                      | 4                         | 7                            |
| Uncharacterized protein, Amb2913                             | 1                         | 1                            |
| Uncharacterized protein, Amb3442                             | 1                         | 2                            |
| Cell shape-determining protein MreB                          | 5                         | 6                            |
| Phasin_2 domain-containing protein, Amb0571                  | 1                         | 1                            |
| Dihydrolipoyl dehydrogenase, Amb3963                         | 4                         | 6                            |
| Nitrous oxide reductase, Amb3086                             | 6                         | 8                            |
| Uncharacterized protein, Amb3082                             | 1                         | 1                            |
| 627aa long 2-oxoacid-ferredoxin oxidoreductase alpha subunit | 4                         | 5                            |
| EF-hand domain-containing protein, Amb1981                   | 3                         | 4                            |
| Cytochrome c-type protein, Amb2686                           | 1                         | 2                            |
| ATPase involved in chromosome partitioning, Amb1961          | 2                         | 2                            |
| Ferrous iron transport protein B, Amb1024 (FeoB?)            | 3                         | 9                            |
| Uncharacterized protein, Amb0319                             | 2                         | 3                            |
| Glycosyltransferase, Amb0142                                 | 1                         | 1                            |
| Cytochrome c-type biogenesis protein cycH, Amb4203           | 1                         | 3                            |
| Enoyl-[acyl-carrier-protein] reductase [NADH], Amb1591       | 1                         | 2                            |
| D-3-phosphoglycerate dehydrogenase, Amb3193                  | 2                         | 2                            |
| NADH dependant phenylglyoxylate, Amb2923                     | 2                         | 2                            |
| LysM domain-containing protein, Amb0606                      | 1                         | 1                            |
| Enoyl-CoA hydratase/carnithine racemase, Amb2242             | 1                         | 1                            |
| Predicted membrane protein, Amb1418                          | 3                         | 4                            |
| Uncharacterized protein, Amb1207                             | 1                         | 2                            |
| Poly(3-hydroxyalkanoate) synthetase, Amb3725                 | 2                         | 2                            |
| Magnetosome protein MamQ                                     | 1                         | 1                            |
| K(+)-insensitive proton pump, HppA, Amb2348                  | 2                         | 3                            |
| Predicted signal transduction protein, Amb3093               | 2                         | 2                            |
| Cellulose synthase catalytic subunit, Amb3053                | 2                         | 2                            |
| Cell division protein FtsZ                                   | 1                         | 1                            |
| Histidine kinase, Amb1466                                    | 3                         | 4                            |
| UDP-N-acetylmuramate-L-alanine ligase, MurC                  | 1                         | 1                            |
| Uncharacterized protein, Amb1075                             | 1                         | 2                            |
| McaB                                                         | 3                         | 3                            |

**Table 1:** IP-MS results with McaA-GFP. This table only shows the most relevant hits; for the full dataset, see [doi.org/10.1128/mbio.00276-26](https://doi.org/10.1128/mbio.00276-26).

| <b><u>Protein Hit</u></b>                   | <b><u># Sequences</u></b> | <b><u>Spectrum Count</u></b> |
|---------------------------------------------|---------------------------|------------------------------|
| Mms6                                        | 1                         | 1                            |
| McaB-GFP                                    | 4                         | 7                            |
| Oxidative damage repair enzyme, Amb3941     | 1                         | 1                            |
| Cytochrome c-552, Amb1398                   | 3                         | 5                            |
| Transcriptional regulator, Amb3180          | 1                         | 1                            |
| Uncharacterized protein, a=Amb1710          | 1                         | 2                            |
| Peptidoglycan-associated protein, Pal       | 1                         | 2                            |
| Chaperone protein DnaK                      | 2                         | 2                            |
| Magnetosome protein, MamA                   | 1                         | 2                            |
| Acyl-CoA dehydrogenase, Amb2024             | 1                         | 1                            |
| Magnetosome protein, MamQ                   | 1                         | 2                            |
| D-3-phosphoglycerate dehydrogenase, Amb3193 | 1                         | 1                            |
| Magnetosome-associated protein, MamJ        | 1                         | 3                            |

**Table 2:** IP-MS results with McaB-GFP.

| <b><u>Protein Hit</u></b>                             | <b><u># Sequences</u></b> | <b><u>Spectrum Count</u></b> |
|-------------------------------------------------------|---------------------------|------------------------------|
| GFP                                                   | 20                        | 117                          |
| Peptidoglycan-associated protein, Pal                 | 4                         | 8                            |
| Nitrogen regulatory protein P-II, Amb0013             | 2                         | 3                            |
| ATP synthase subunit alpha, AtpA                      | 10                        | 15                           |
| ATP synthase subunit beta, AtpD                       | 6                         | 10                           |
| 50S ribosomal protein L4, RplD                        | 2                         | 3                            |
| 50S ribosomal protein L2, RplB                        | 3                         | 5                            |
| Hypothetical PE-PGRS family protein, Amb0025          | 6                         | 14                           |
| ATP synthase subunit delta, AtpH                      | 2                         | 3                            |
| 50S ribosomal protein L21, RplU                       | 1                         | 1                            |
| ATP synthase gamma chain, AtpG                        | 2                         | 4                            |
| 30S ribosomal protein, RpsL                           | 1                         | 2                            |
| Aspartate-semialdehyde dehydrogenase, Asd             | 2                         | 3                            |
| Tol-Pal system protein, TolB                          | 2                         | 2                            |
| Uncharacterized protein, Amb0165                      | 1                         | 2                            |
| Uncharacterized protein with OMP domain, Amb1085      | 2                         | 3                            |
| NADH-quinone oxidoreductase, Amb2781                  | 3                         | 3                            |
| Dihydrolipoyl dehydrogenase, Amb2321                  | 2                         | 3                            |
| 50S ribosomal protein, RplM                           | 1                         | 1                            |
| 3-hydroxyacyl-CoA dehydrogenase, Amb3902              | 1                         | 1                            |
| Dehydrogenase with different specificities, Amb0843   | 1                         | 2                            |
| Nitrite reductase, Amb1395                            | 2                         | 3                            |
| ADP-ribosylglycohydrolase, Amb1627                    | 2                         | 2                            |
| Malate dehydrogenase, Mdh                             | 1                         | 1                            |
| Alginate_exp domain-containing protein, Amb3421       | 1                         | 1                            |
| Pyruvate dehydrogenase E1 component, Amb2317          | 1                         | 1                            |
| Acetyl-CoA acetyltransferase, Amb0842                 | 1                         | 1                            |
| Outer membrane protein, Amb0602                       | 1                         | 1                            |
| Chaperonin GroEL                                      | 1                         | 2                            |
| Cytochrome c-552, Amb1398                             | 1                         | 1                            |
| Succinate dehydrogenase flavoprotein subunit, Amb3952 | 1                         | 1                            |
| PglZ domain-containing protein, Amb2046               | 1                         | 1                            |
| Methyl-accepting chemotaxis protein, Amb4076          | 1                         | 1                            |
| ALGX domain-containing protein, Amb1064               | 1                         | 2                            |

**Table 3:** IP-MS results with GFP.

|                         |          |                    |                                     | <b>t<sub>1/2</sub> (min) average ± SD</b> |                                |                            |
|-------------------------|----------|--------------------|-------------------------------------|-------------------------------------------|--------------------------------|----------------------------|
| <b>Strain</b>           | <b>n</b> | <b>% recovered</b> | <b>% moving bleach of recovered</b> | <b>All cells</b>                          | <b>Non-moving bleach cells</b> | <b>Moving bleach cells</b> |
| WT/MamK-GFP             | 31       | 87 (n = 27)        | 15 (n = 4)                          | 3.53 ± 3.15                               | 3.82 ± 3.29                    | 1.87 ± 1.50                |
| Δ <i>mcaA</i> /MamK-GFP | 32       | 100 (n = 32)       | 38 (n = 12)                         | 3.02 ± 2.22                               | 3.65 ± 2.41                    | 1.95 ± 1.37                |
| Δ530/MamK-GFP           | 35       | 91 (n = 32)        | 38 (n = 12)                         | 3.65 ± 3.67                               | 4.79 ± 4.14                    | 1.66 ± 1.24                |
| WT/MamK-GFP, nonbiomin  | 32       | 78 (n = 25)        | 36 (n = 9)                          | 5.11 ± 3.56                               | 5.06 ± 3.64                    | 5.21 ± 3.63                |

**Table 4:** MamK-GFP dynamics observed by FRAP.

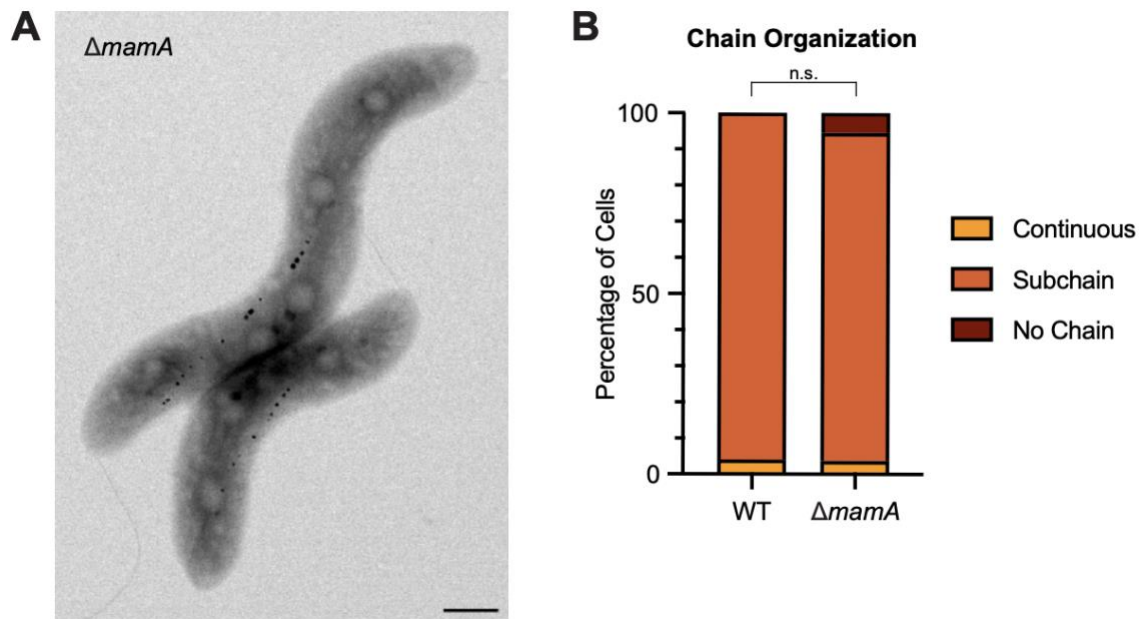


**Figure S1:** MamA is not necessary for crystal subchains. **(A)** Representative TEM image of AMB-1 *ΔmamA* showing the magnetosome chain organization. Scale bar = 0.5μm. **(B)** Quantification of magnetosome chain organization using TEM images. Cells were categorized based on their magnetosome chain phenotype and the y-axis represents the percentage of cells that displayed the indicated chain organization. WT n = 75, *ΔmamA* n = 54. Fisher's exact test was used to determine p-values (n.s. not significant).

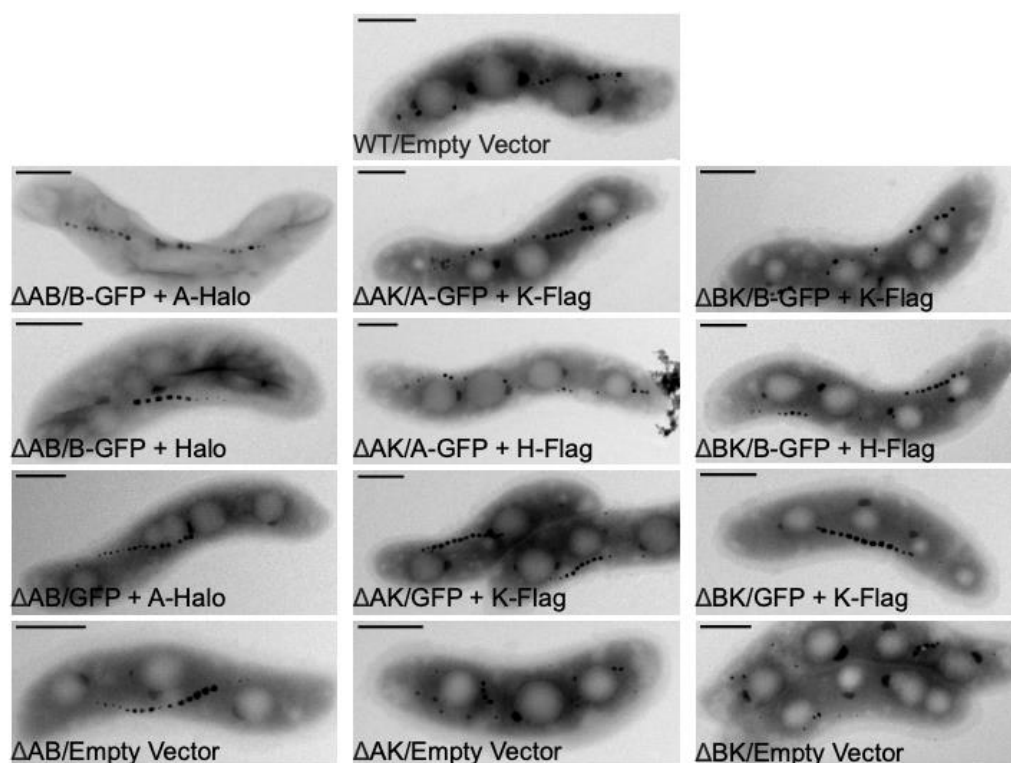
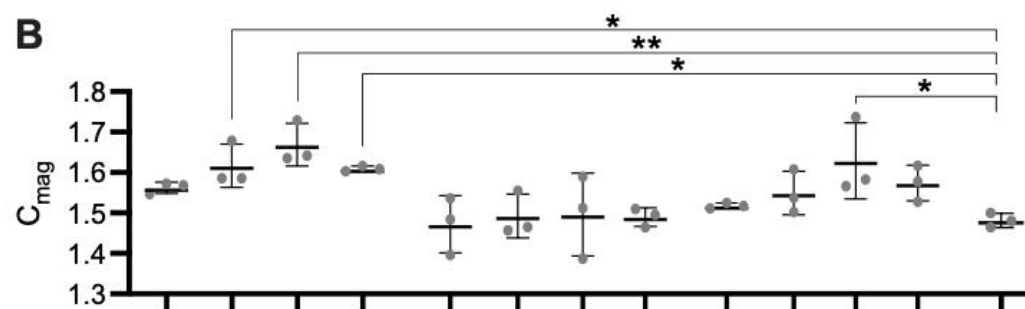
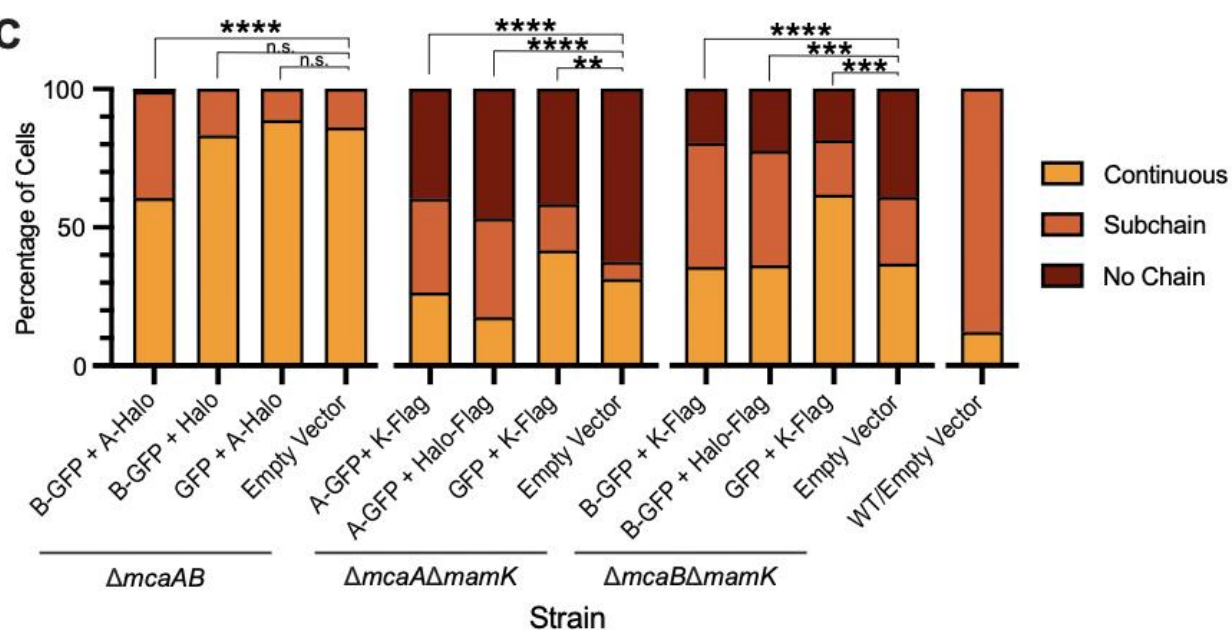
**A****B****C**

**Figure S2:** Tagged constructs of McaA, McaB, and MamK can partially complement their respective deletions. **(A)** Representative TEM image of different strains used for co-IPs. Scale bar = 0.5 $\mu$ m. **(B)** The coefficient of magnetism ( $C_{mag}$ ) of the different co-IP strains. The column labels are the same as those in **(C)**. Each point represents one independent culture, and the average and standard deviation is shown. One-way ANOVA with Dunnett's multiple comparisons test was used to compare each strain to both its respective empty vector and WT/empty vector. Comparisons without stars did not have a significant difference. **(C)** Quantification of magnetosome chain organization using TEM images. Cells were categorized based on their magnetosome chain phenotype and the y-axis represents the percentage of cells that displayed the indicated chain organization. n from left to right: 104, 125, 115, 93, 106, 109, 137, 96, 143, 210, 81, 182, 91. Fisher's exact test was used to determine p-values (\*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\* $P < 0.001$ , \*\*\*\*  $P < 0.0001$ , n.s. Not significant). A = *mcaA*, McaA; B = *mcaB*, McaB; K = *mamK*, MamK; H = Halo-tag.



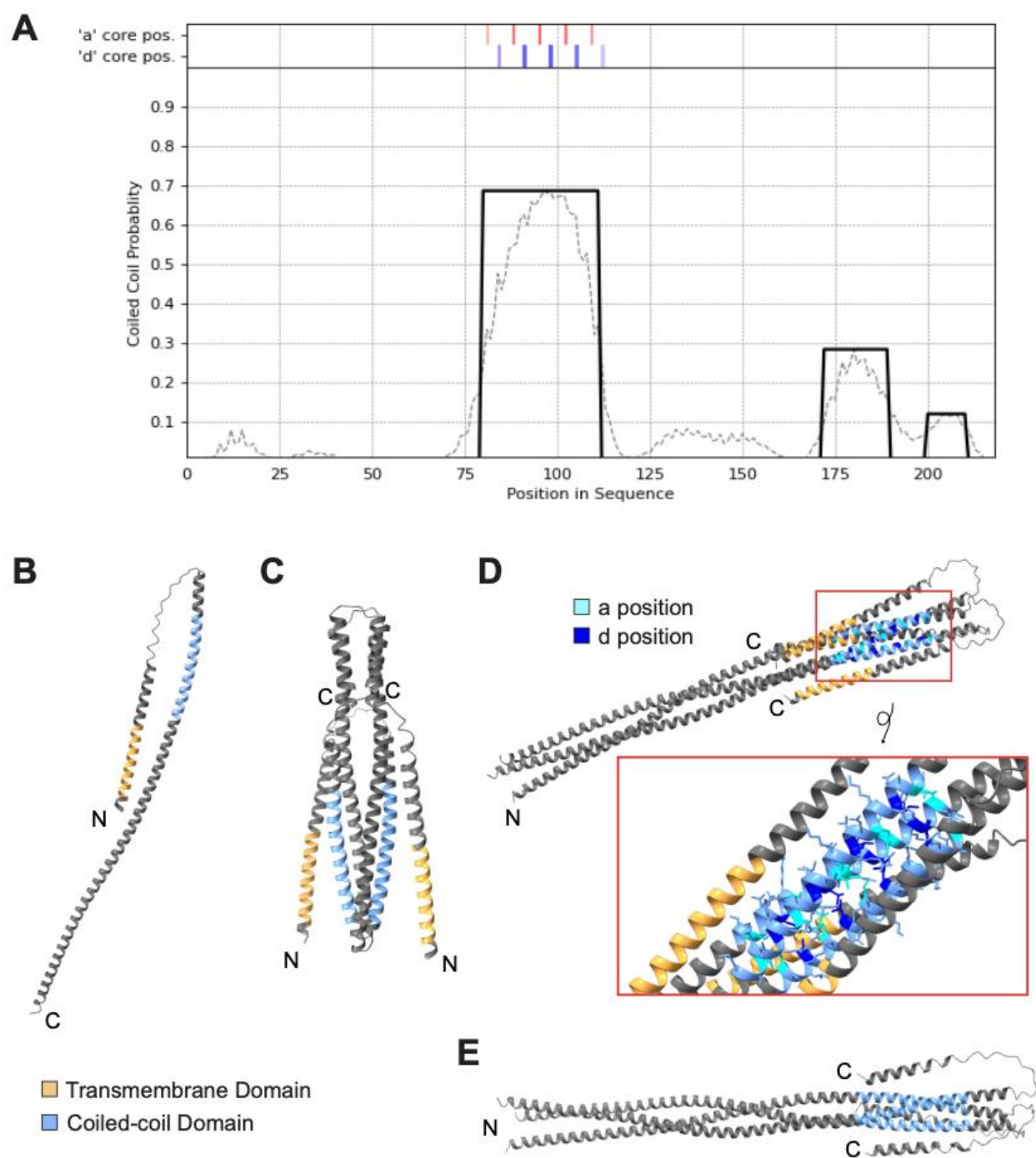


**Figure S4:** McaB is a predicted coiled-coil protein. (A) Probability of coiled-coil domains in McaB determined by DeepCoil2. The x-axis represents the amino acid positions and the y-axis represents the probability of coiled-coils. The red and blue colored bars at the top represent predicted “a” position and “d” position amino acids respectively, where hydrophobic residues occupy in a coiled-coil. AlphaFold3 predictions of McaB as a monomer (B), dimer (C), and trimer (D). (D) includes the predicted “a” position (cyan) and

“d” position (dark blue) amino acids and the inset highlights these amino acids forming a hydrophobic core typical to coiled-coils. (**E**) AlphaFold3 prediction of McaB<sub>cyto</sub> as a trimer suggests that the coiled-coil domain interactions are not disrupted by the truncation. All AlphaFold3-predicted structures have their transmembrane domain (yellow), predicted coiled-coil domain (blue), N-terminus (N), and C-terminus (C) labeled.

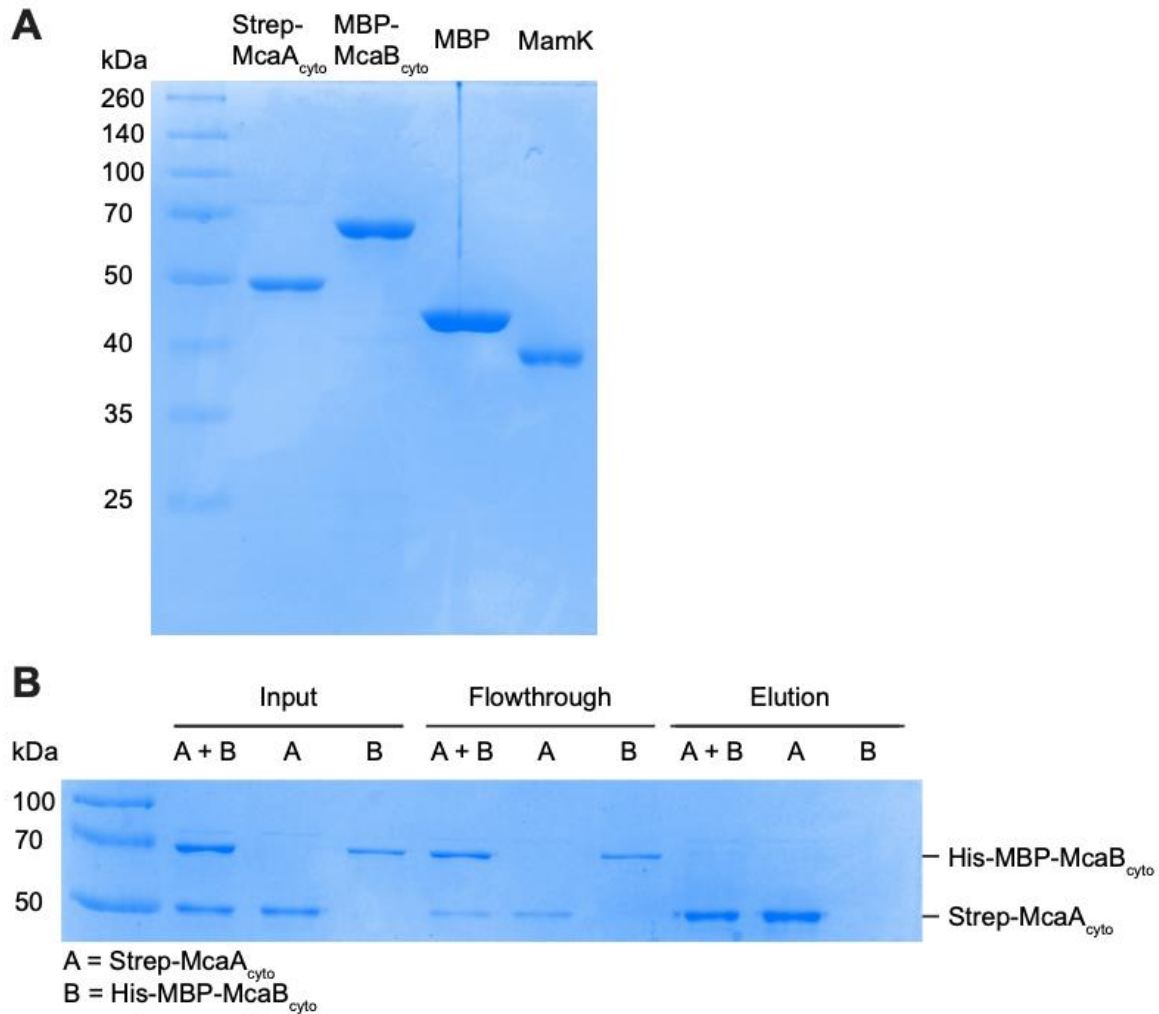


**Figure S5:** Purified cytoplasmic domains of McaA and McaB do not directly interact *in vitro*. **(A)** SDS-PAGE with purified Strep-McaA<sub>cyto</sub>, MBP-McaB<sub>cyto</sub>, MBP, and untagged MamK. **(B)** SDS-PAGE of an *in vitro* pull down with purified Strep-McaA<sub>cyto</sub> and His-MBP-McaB<sub>cyto</sub>. Strep-McaA<sub>cyto</sub> and His-MBP-McaB<sub>cyto</sub> were applied onto Strep-Tactin resin either individually or mixed together (input). Unbound proteins were washed off (flowthrough) and the resin was washed with a buffer. Elution shows that Strep-McaA<sub>cyto</sub> and His-MBP-McaB<sub>cyto</sub> do not co-elute. Details of the *in vitro* pull down can be found in the materials and methods. A, Strep-McaA<sub>cyto</sub>; B, His-MBP-McaB<sub>cyto</sub>. Lad, ladder.

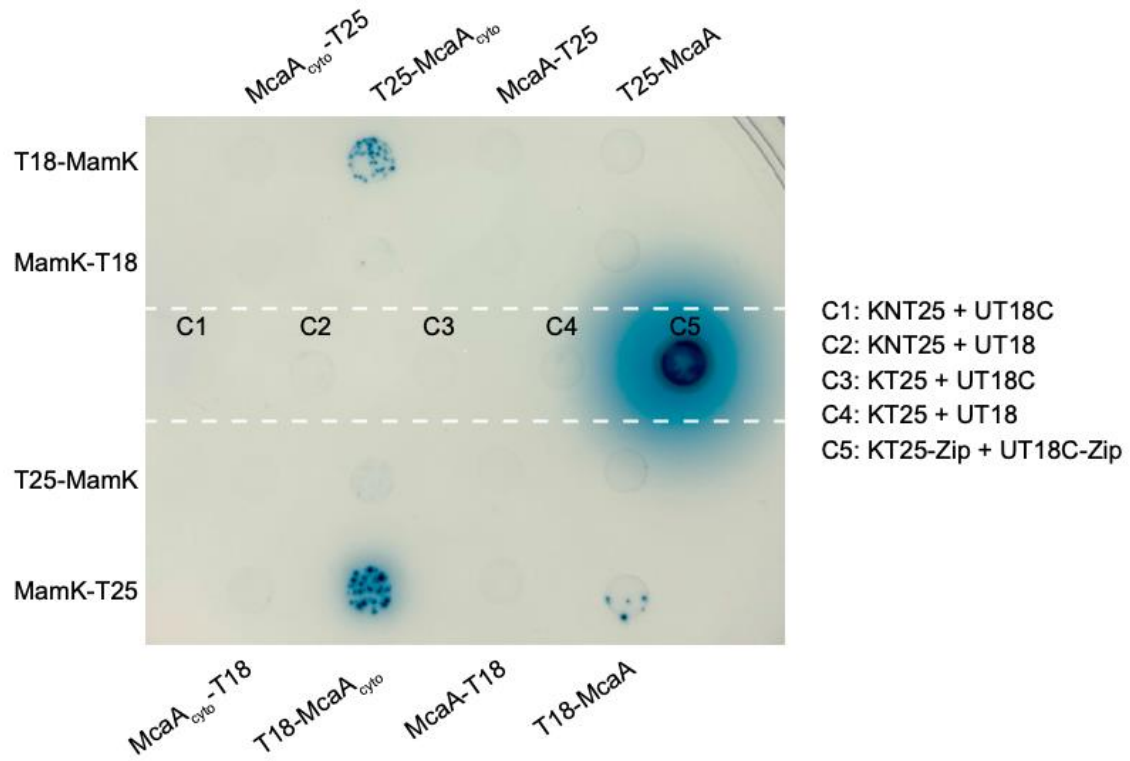
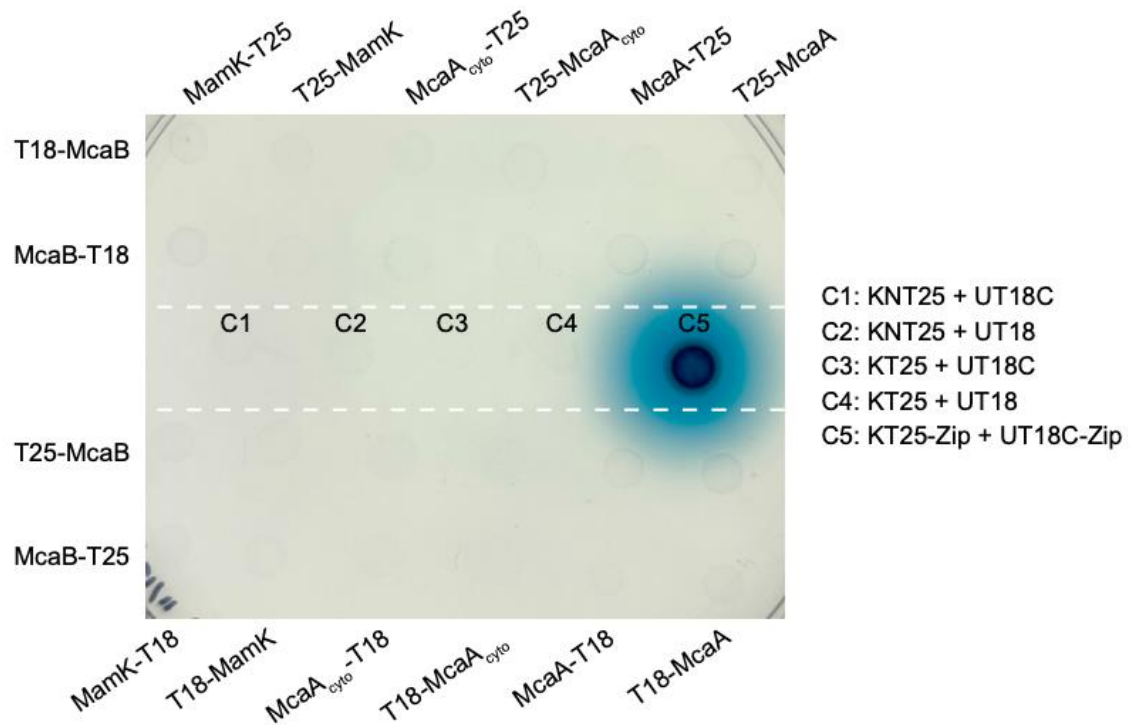
**A****B**

**Figure S6:** Bacterial two-hybrids (B2H) show interaction between MamK and the cytoplasmic side of McaA. **(A)** B2H between MamK and either full length McaA or the cytoplasmic side of McaA (McaA<sub>cyto</sub>) tagged with B2H fragments T18 or T25. The co-expression strains are spotted on M63 agar with IPTG and X-gal. A blue spot indicates an interaction between the two co-expressed proteins. Untagged T18 and T25 negative controls (C1-4) and a leucine zipper positive control (C5) is shown in the center of the plate. **(B)** B2H between McaB and MamK, McaA, and McaA<sub>cyto</sub>. Experimental set up is similar to (A).

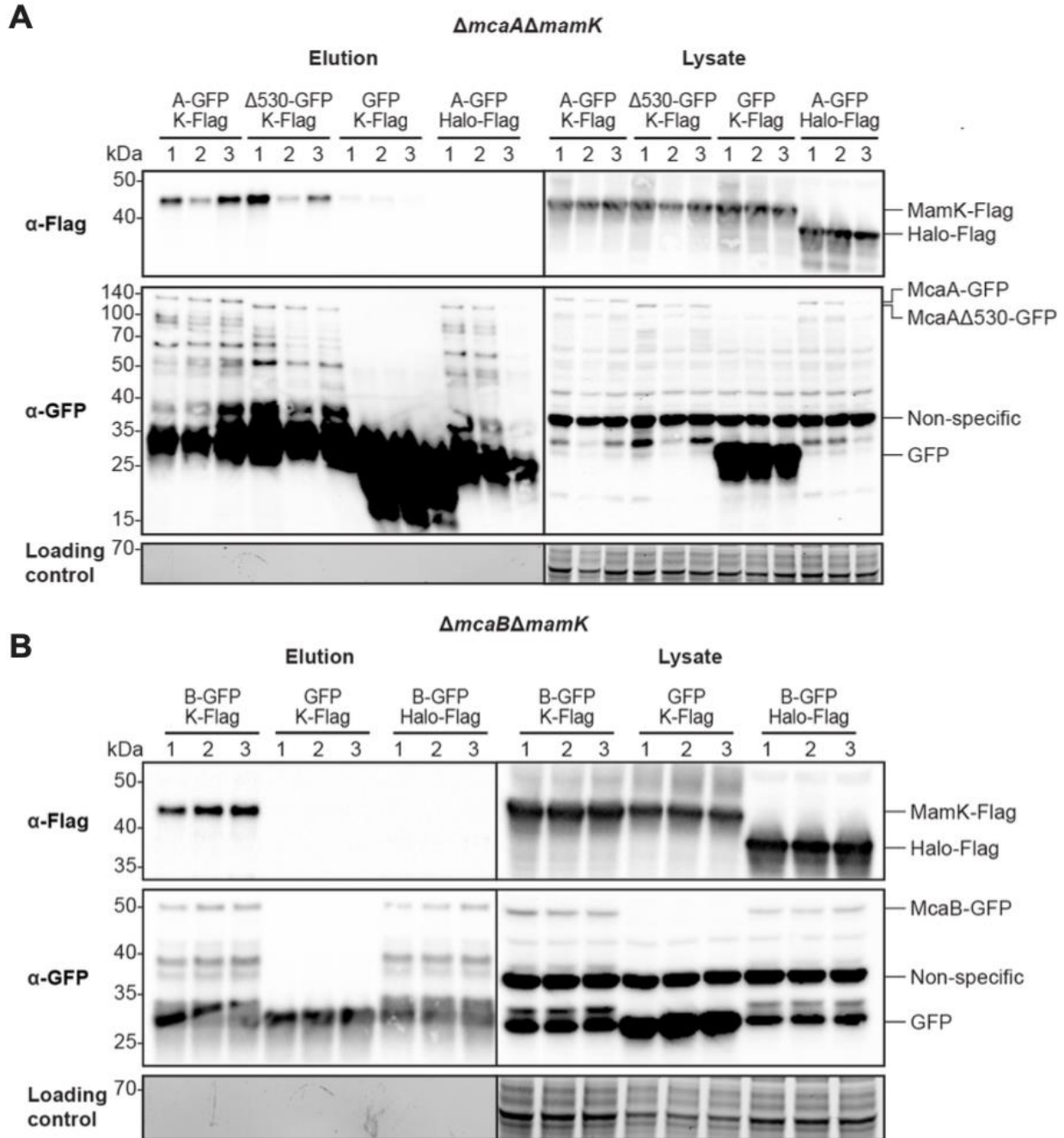


**Figure S7:** MamK interacts with both McaA and McaB *in vivo*. **(A)** Western blots of co-IP elutions and lysates illustrating that MamK-Flag (K-Flag) interacts with both McaA-GFP (A-GFP) and McaA $\Delta 530$ -GFP ( $\Delta 530$ -GFP). The GFP and Flag tags on McaA and MamK, respectively, play no or minimal role in driving the interaction. **(B)** Western blots of co-IP elutions and lysates illustrating that MamK-Flag (K-Flag) interacts with both McaB-GFP (B-GFP). The GFP and Flag tags on McaB and MamK, respectively, do not drive the interaction. In both (A) and (B), the co-IP was done using GFP-Trap agarose beads and three independent biological replicates are shown. The loading control is a stain-free SDS-PAGE gel.

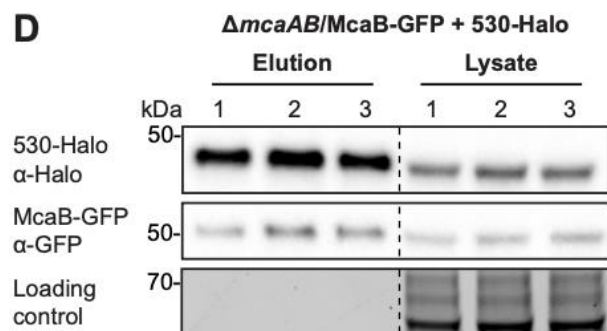
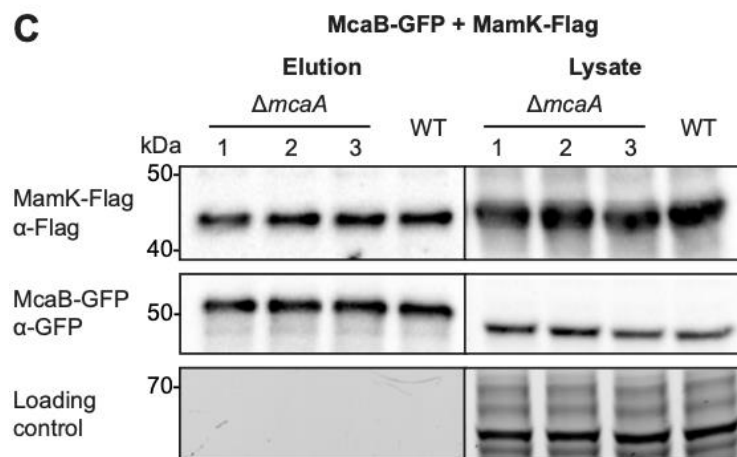
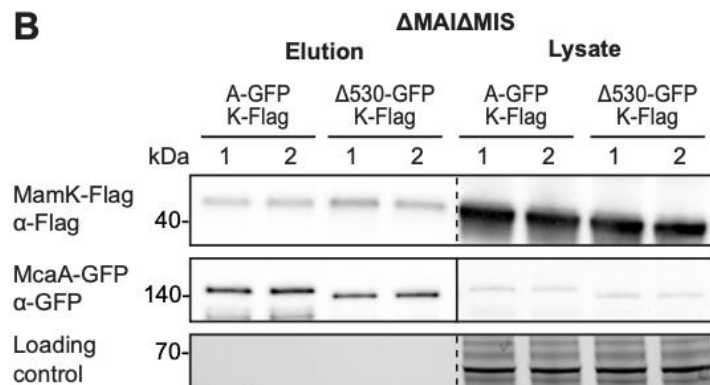
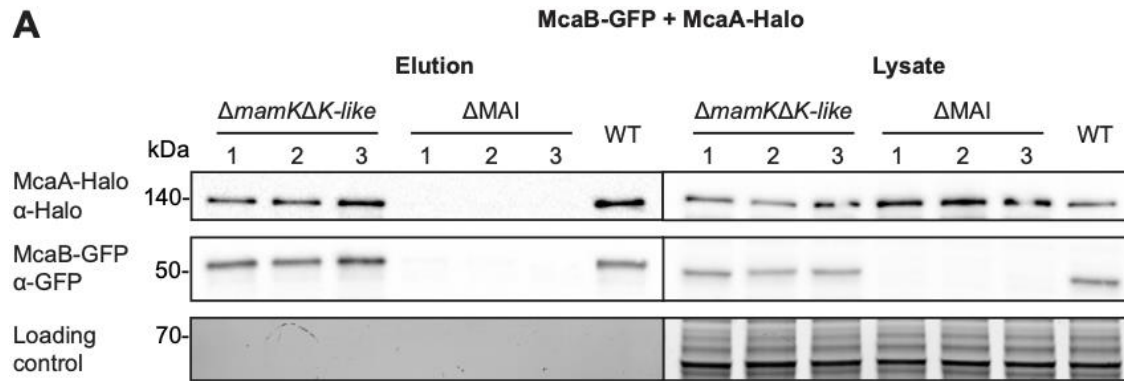
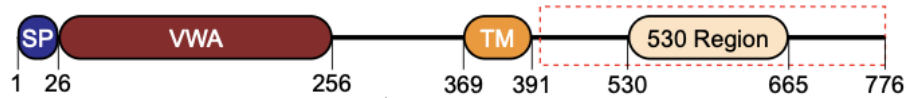


**Figure S8:** McaA, McaB, and MamK form pairwise interactions independent of the third protein. **(A)** Western blots of co-IP elutions and lysates of McaB-GFP and McaA-Halo in  $\Delta mamK \Delta mamK-like$  and  $\Delta MAI$  show that the McaA-McaB interaction does not require MamK or MamK-like. McaB-GFP expression is drastically reduced in  $\Delta MAI$ , evidenced by the lack of McaB-GFP bands in the lysate. WT samples serve as a positive control. **(B)** Western blots of co-IP elutions and lysates of MamK-Flag (K-Flag) with McaA-GFP (A-GFP) or McaA $\Delta$ 530-GFP ( $\Delta$ 530-GFP) in  $\Delta MAI \Delta MIS$ . The results demonstrate that MamK interacts with McaA independent of the 530 region and all other magnetosome proteins. **(C)** Western blots of co-IP elutions and lysates of MamK-Flag with McaB-GFP in  $\Delta mcaA$  show that MamK interacts with McaB independent of McaA. WT samples serve as a positive control. **(D)** Western blots of co-IP elutions and lysates of 530-Halo and McaB-GFP in  $\Delta mcaAB$  show that the 530 region is sufficient for interaction with McaB. All co-IPs were done using GFP-Trap agarose beads. All blots have independent biological replicates numbered and a stain-free SDS-PAGE gel serves as the loading control.

A



B

|                                |     |                                                |                                |     |
|--------------------------------|-----|------------------------------------------------|--------------------------------|-----|
| AMB-1 McaA                     | 400 | QKAAQAAARQSAVVTAASHPPQKVEAPVIRTKPPVIVPGARAAAMI | PALKSSESLGQTLPPVPNATILPSADPIPE | 477 |
| AMB-1 McaA-like                | 352 | --SAKAG--                                      | --PAIPL--                      | 361 |
| UT-4                           | 352 | --SAKAG--                                      | --PAVPL--                      | 361 |
| <i>P. marisnigri</i>           | 352 | --SAKAG--                                      | --PAIPL--                      | 361 |
| Alphaproteobacterium           | 368 | --SAKAG--                                      | --PAIPL--                      | 377 |
| SS-4                           | 348 | --TAKAE--                                      | --PAVPV--                      | 357 |
| <i>P. caucaseum</i>            | 348 | --TAKAE--                                      | --PAVPV--                      | 357 |
| <i>Candidatus T. magnetica</i> | 348 | --TAKAE--                                      | --PAVPV--                      | 357 |
| LM-5                           | 348 | --AAKAE--                                      | --PAVPV--                      | 357 |
| XM-1                           | 348 | --AAKAE--                                      | --PAVPV--                      | 357 |
| Deltaproteobacterium           | 371 | --AARAE--                                      | --EAEVETEIRKMKQGLAEMEAEEVAA--  | 420 |

|                                |     |                                                                              |     |
|--------------------------------|-----|------------------------------------------------------------------------------|-----|
| AMB-1 McaA                     | 478 | QPEVGTALLAAMEAPEEVLPSPTAVVYLPRSVDL--LEIEADESEVSLSDHMLVAEFIDGKVDIISAEALLRQK   | 552 |
| AMB-1 McaA-like                | 362 | --                                                                           | 385 |
| UT-4                           | 362 | --                                                                           | 385 |
| <i>P. marisnigri</i>           | 362 | --                                                                           | 385 |
| Alphaproteobacterium           | 378 | --                                                                           | 401 |
| SS-4                           | 358 | --                                                                           | 381 |
| <i>P. caucaseum</i>            | 358 | --                                                                           | 381 |
| <i>Candidatus T. magnetica</i> | 358 | --                                                                           | 381 |
| LM-5                           | 358 | --                                                                           | 381 |
| XM-1                           | 358 | --                                                                           | 381 |
| Deltaproteobacterium           | 421 | QSKSGEQEVKAEEKRRMRELEAREIEMVSTIEANSVERKKLEAELEAEI--GRMKEQGLAEMEAEEVAAKEKMRQG | 495 |

|                                |     |                                                                  |     |
|--------------------------------|-----|------------------------------------------------------------------|-----|
| AMB-1 McaA                     | 553 | LLTRTSGVIDGEFQFLLFVAAPLGSIEV---YQCRDGYERHGSALDVALQGLSVGVNAKDIQSI | 626 |
| AMB-1 McaA-like                | 386 | LLVNESGAVNVERRFSLRVSVPPGAMV---                                   | 459 |
| UT-4                           | 386 | LLVNESGAVNVERRFSLRVSVPPGAMV---                                   | 459 |
| <i>P. marisnigri</i>           | 386 | LLVNESGAVNVERRFSLRVSVPPGAMV---                                   | 459 |
| Alphaproteobacterium           | 402 | LLVNESGAVNVERRFSLRVSVPPGAMV---                                   | 475 |
| SS-4                           | 382 | LLIAESEAVKVERRFSLRVAVPTGAMEI---                                  | 455 |
| <i>P. caucaseum</i>            | 382 | LLIAESEAVKVERRFSLRVAVPTGAMEI---                                  | 455 |
| <i>Candidatus T. magnetica</i> | 382 | LLIAESEAVKVERRFSLRVAVPTGAMEI---                                  | 455 |
| LM-5                           | 382 | LLIAESEAVKVERRFSLRVAVPTGAMEI---                                  | 455 |
| XM-1                           | 382 | LLIAESEAVKVERRFSLRVAVPTGAMEI---                                  | 455 |
| Deltaproteobacterium           | 496 | ---QGLVEMAEVAAEKEKRMQFEAQLAEYKWSVE--QVEAEKERRMQELAEI             | 586 |

|                                |     |                                              |     |
|--------------------------------|-----|----------------------------------------------|-----|
| AMB-1 McaA                     | 627 | HIEHANVMAYGPDSTVIG---FFQFHDNINSWMNWI         | 701 |
| AMB-1 McaA-like                | 460 | AVKKFEEIRRDGSR                               | 503 |
| UT-4                           | 460 | AVKKFEEIRRDGSR                               | 503 |
| <i>P. marisnigri</i>           | 460 | AVKKFEEIRRDGSR                               | 503 |
| Alphaproteobacterium           | 476 | AVKKFEEIRRDGSR                               | 519 |
| SS-4                           | 456 | GVKKFQEIIRRDGSR                              | 499 |
| <i>P. caucaseum</i>            | 456 | GVKKFQEIIRRDGSR                              | 499 |
| <i>Candidatus T. magnetica</i> | 456 | GVKKFQEIIRRDGSR                              | 499 |
| LM-5                           | 456 | GVKKFQEIIRRDGSR                              | 499 |
| XM-1                           | 456 | GVKKFQEIIRRDGSR                              | 499 |
| Deltaproteobacterium           | 567 | RICEQRMVEMAEVAAEKEKRMQFEAQLAEYKWSVELEVAEKGRK | 613 |

|                                |     |                                            |     |
|--------------------------------|-----|--------------------------------------------|-----|
| AMB-1 McaA                     | 702 | WPVVPGRSARDRDSARIEDVQKELVSTPVRSDVPVQGLTTGI | 776 |
| AMB-1 McaA-like                | 504 | --                                         | 504 |
| UT-4                           | 504 | --                                         | 504 |
| <i>P. marisnigri</i>           | 504 | --                                         | 504 |
| Alphaproteobacterium           | 520 | --                                         | 520 |
| SS-4                           | 500 | --                                         | 501 |
| <i>P. caucaseum</i>            | 500 | --                                         | 501 |
| <i>Candidatus T. magnetica</i> | 500 | --                                         | 501 |
| LM-5                           | 500 | --                                         | 501 |
| XM-1                           | 500 | --                                         | 501 |
| Deltaproteobacterium           | 614 | --                                         | 615 |

**Figure S9:** The 530 region of AMB-1's McaA is a conserved region of the cytoplasmic side of McaA. **(A)** Schematic of full length McaA. The amino acid positions are shown. The N-terminus has a putative signal peptide (SP) and a von Willebrand factor type A domain (VWA), followed by a transmembrane domain (TM). The cytoplasmic side of McaA is indicated by a red dashed box, which includes the 530 region (amino acids 530-665). **(B)** Amino acid sequence alignment of the cytoplasmic C-terminal side of McaA across different MTB. Both AMB-1 McaA and its homolog McaA-like is included in the alignment. Residues are colored using the Clustal coloring scheme, which assigns colors based on the chemical properties of the amino acid. The schematic from (A) is shown along the alignment to indicate where the 530 region is. Species name abbreviations: UT-4, *Magnetospirillum* sp. UT-4; *P. marisnigri*, *Paramagnetospirillum marisnigri*; SS-4, *Magnetospirillum* sp. SS-4; *P. caucaseum*, *Paramagnetospirillum caucaseum*; *Candidatus T. magnetica*, *Candidatus Terasakilla magnetica*; LM-5, *Magnetospirillum* sp. LM-5; XM-1, *Magnetospirillum* sp. XM-1.

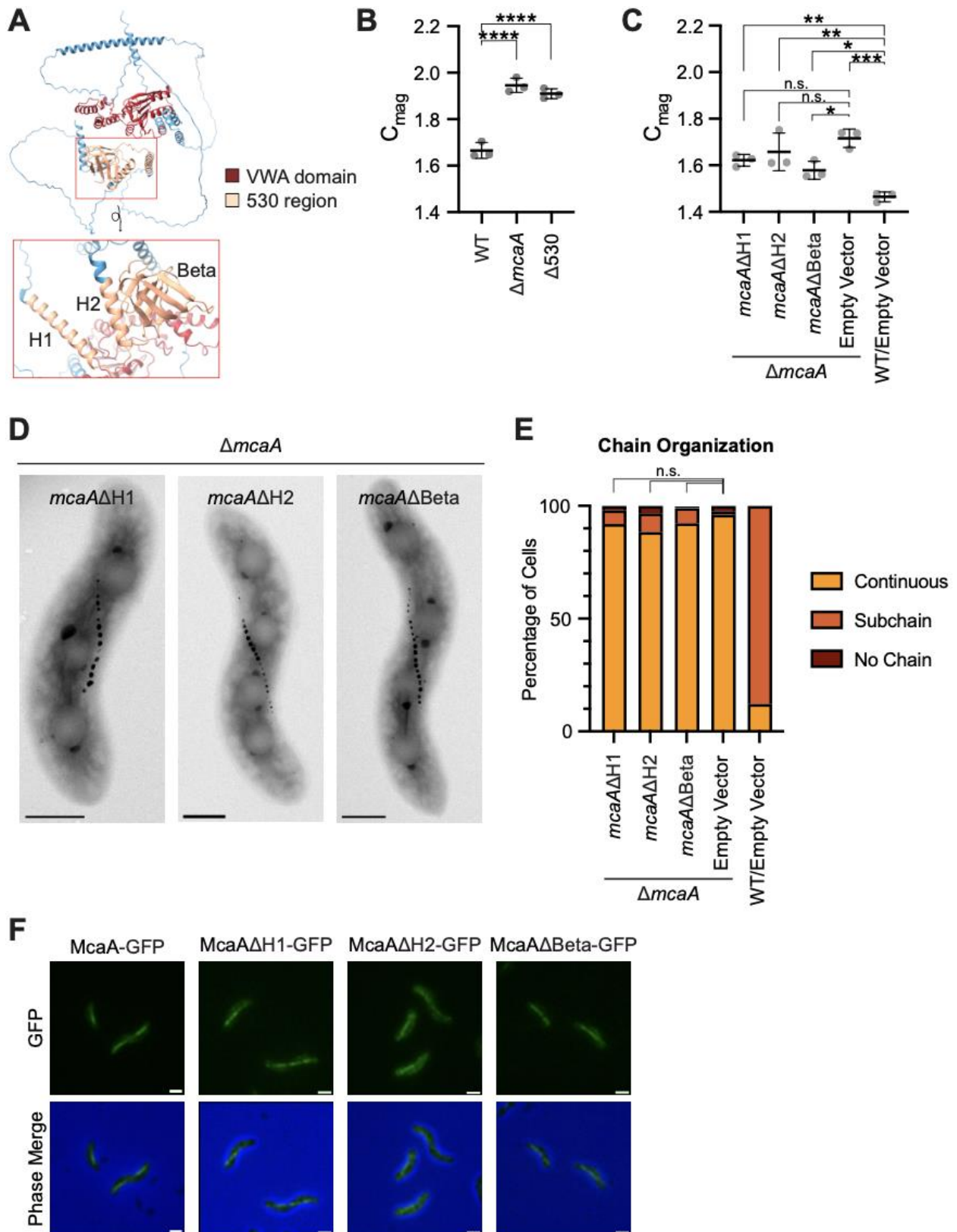


**Figure S10:** The entire 530 region is necessary for crystal subchain formation. **(A)** Predicted protein structure of McaA according to Alphafold3. The VWA domain and 530 domain are colored red and light yellow, respectively. The inset shows three distinct structural elements of the 530 region: Helix 1 (H1), helix 2 (H2) and a beta sheet (Beta). **(B)** Coefficient of magnetism ( $C_{mag}$ ) measurements of WT,  $\Delta mcaA$ , and  $\Delta 530$  demonstrate that removing the 530 region increases the  $C_{mag}$  to levels similar to that of  $\Delta mcaA$ . **(C)**  $C_{mag}$  measurements of strains expressing McaA $\Delta$ H1-GFP, McaA $\Delta$ H2-GFP, McaA $\Delta$ Beta-GFP, or an empty vector in a  $\Delta mcaA$  background and compared to a WT with an empty vector. It is common for WT/empty vector grown with kanamycin to have a lower  $C_{mag}$  compared to WT grown without kanamycin. For both (B) and (C), each point represents one independent culture, and the averages and standard deviations are shown. One-way ANOVA with Dunnett's multiple comparisons test was used to determine p-values (\*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ , \*\*\*\*  $P < 0.0001$ , n.s. Not significant). **(D)** Representative TEM image of  $\Delta mcaA$  strains expressing McaA with different truncations in the 530 region. All constructs have a C-terminal GFP tag. Scale bar = 0.5 $\mu$ m. **(E)** Quantification of magnetosome chain organization using TEM images indicates that H1, H2, and Beta are all required for McaA function. Cells were categorized based on their magnetosome chain phenotype and the y-axis represents the percentage of cells that displayed the indicated chain organization. n from left to right: 50, 60, 103, 126, 91. Fisher's exact test was used to determine p-values (n.s. Not significant). All groups were significantly different from WT/Empty Vector ( $P < 0.0001$ ). **(F)** Representative cells expressing GFP-tagged McaA or McaA truncation, showing that each truncated McaA construct is expressed and localized similarly to full-length McaA-GFP. Images were taken using epifluorescence microscopy. Scale bar = 1 $\mu$ m.

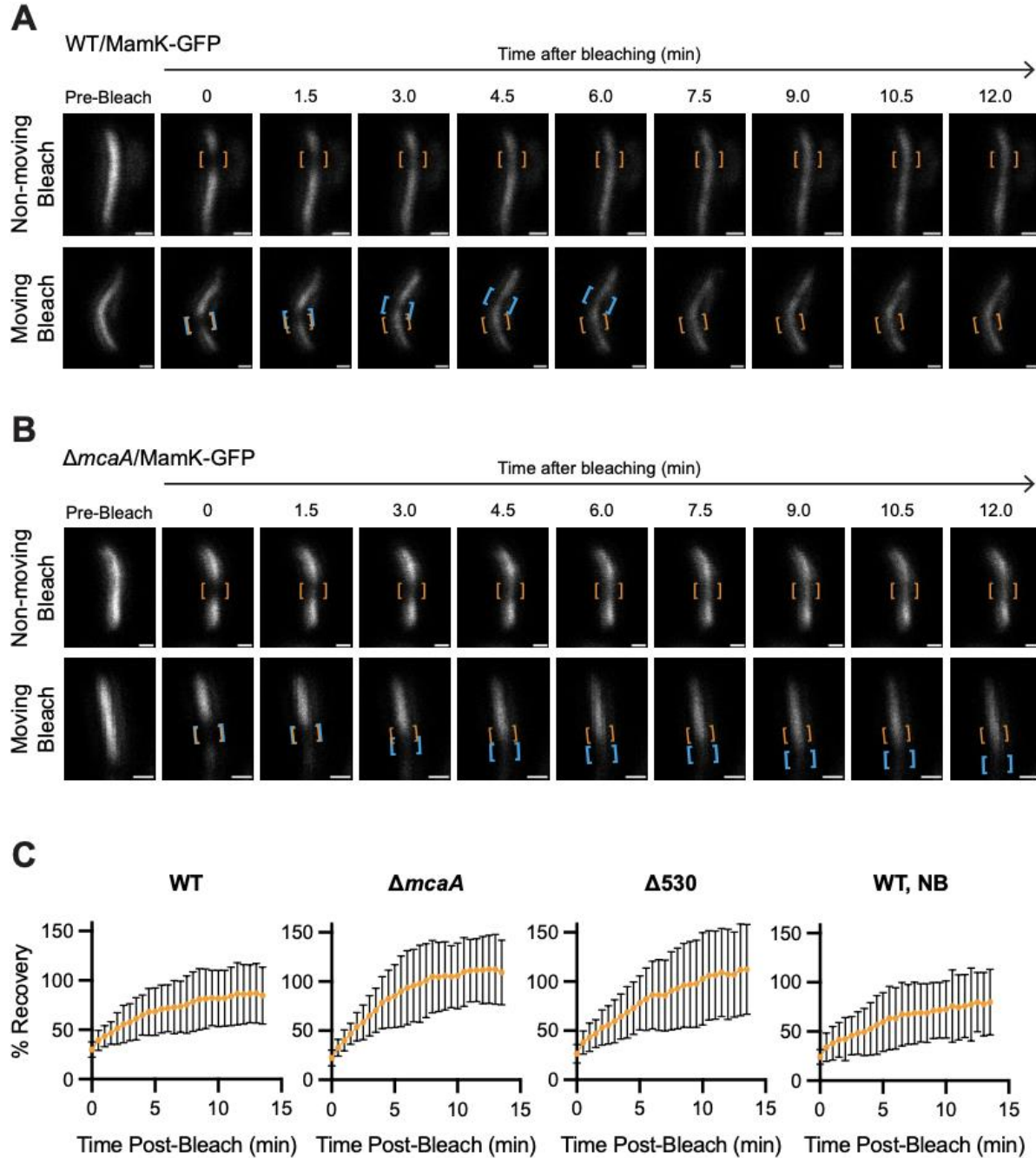


**Figure S11:** MamK-GFP dynamics in WT,  $\Delta mcaA$ , and  $\Delta 530$ . Two representative cells from FRAP of MamK-GFP in WT (**A**) and  $\Delta mcaA$  (**B**) imaged using confocal microscopy. Orange brackets indicate the original bleaching location. Blue brackets track bleach spot movements. The top cell has a bleach spot that does not move while the bottom cell has a moving bleach spot. Scale bar =  $0.5\mu\text{m}$ . (**C**) Percent recovery of MamK-GFP photobleached region in WT,  $\Delta mcaA$ ,  $\Delta 530$ , and WT grown in non-biomineralization conditions (WT, NB). The percent recovery is normalized to the whole filament fluorescence at every time point. The percent recovery averages  $\pm$  standard deviations across all cells are shown.

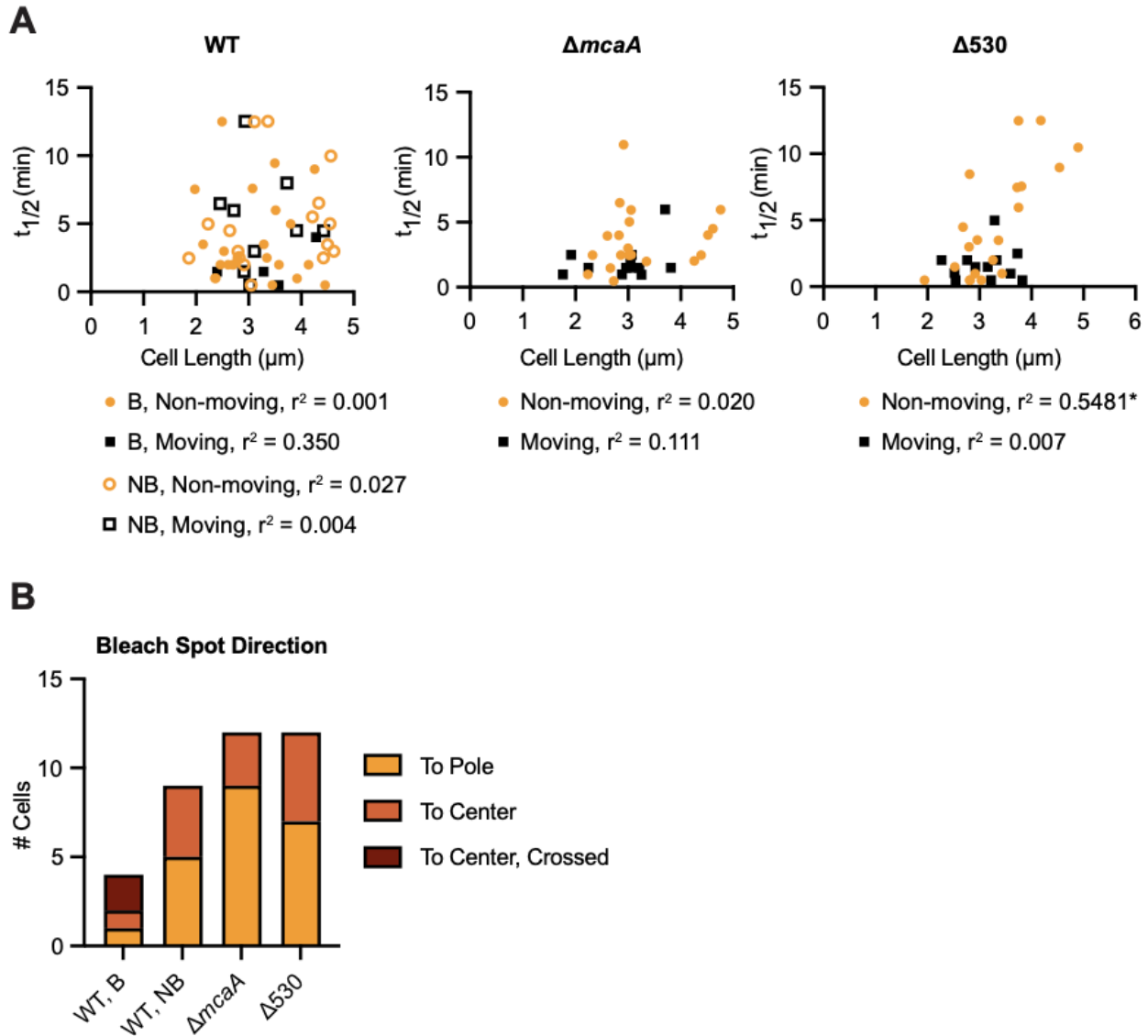


**Figure S12:** MamK-GFP recovery half-times are mostly independent from cell length and bleach spot recovery direction is inconsistent. **(A)** The recovery half-times ( $t_{1/2}$ ) from MamK-GFP FRAP experiments are plotted against the cell length. Each point represents one cell. Yellow circles and black squares represent cells with non-moving bleach spots and with a moving bleach spot, respectively. For WT, filled circles and squares represent cells grown in biomineralization conditions (B) while empty ones represent those grown in non-biomineralization conditions (NB). Cells that did not recover were excluded from the plot. The  $r^2$  values and significance generated from simple linear regression are shown (\*  $P < 0.05$ ). **(B)** For each cell with a moving bleach spot during the FRAP experiments, the movement direction was categorized as going towards a cell pole or towards the cell center. Cells with bleach spots that moved towards the cell center and crossed through it during the FRAP observation time frames were categorized separately (“To Center, Crossed”). The numbers of cells with each moving direction across each strain and biomineralization condition are shown.

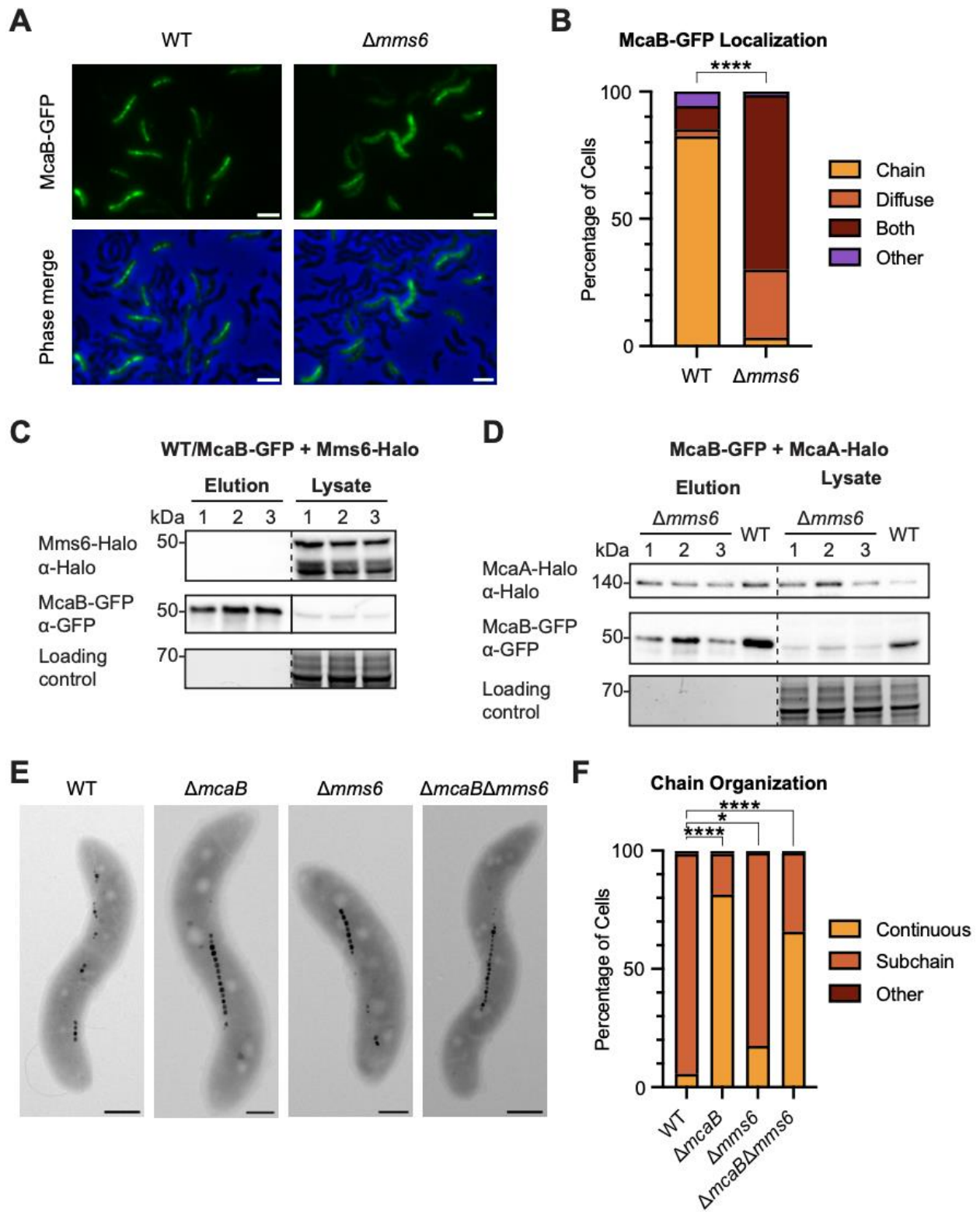


**Figure S13:** Mms6 is not required for crystal subchain formation or McaA-McaB interactions. **(A)** Representative cells showing McaB-GFP localization in WT and  $\Delta mms6$  strains. Images were taken using epifluorescence microscopy. Scale bar = 2 $\mu$ m. **(B)** Quantification of McaB-GFP localization using fluorescent microscopy images. Cells were categorized based on their localization pattern and the y-axis represents the percentage of cells that displayed the indicated localization. WT n = 2316,  $\Delta mms6$  n = 3663. Fisher's exact test was used to determine p-values (\*\*\*\* P < 0.0001). **(C)** Western blots of co-IP elutions and lysates illustrating that Mms6-Halo and McaB-GFP do not interact. **(D)** Western blots of co-IP elutions and lysates illustrating that McaA-Halo interacts with McaB-GFP in  $\Delta mms6$ . WT samples serve as a positive control. In both (C) and (D), GFP-Trap agarose beads were used for the co-IP. Three independent biological replicates are shown and a SDS-PAGE gel serves as a loading control. **(E)** Representative TEM images of WT,  $\Delta mcaB$ ,  $\Delta mms6$ , and  $\Delta mcaB\Delta mms6$  to show the magnetosome chain organization. Strains were grown anaerobically since AMB-1 strains missing *mms6* makes poor crystals in microaerobic conditions. Scale bar = 0.5 $\mu$ m. **(F)** Quantification of magnetosome chain organization using TEM images. Cells were categorized based on their magnetosome chain phenotype and the y-axis represents the percentage of cells that displayed the indicated chain organization. WT n = 71,  $\Delta mcaB$  n = 146,  $\Delta mms6$  n = 103,  $\Delta mcaB\Delta mms6$  n = 108. Fisher's exact test was used to determine p-values (\* P < 0.05, \*\*\*\* P < 0.0001).

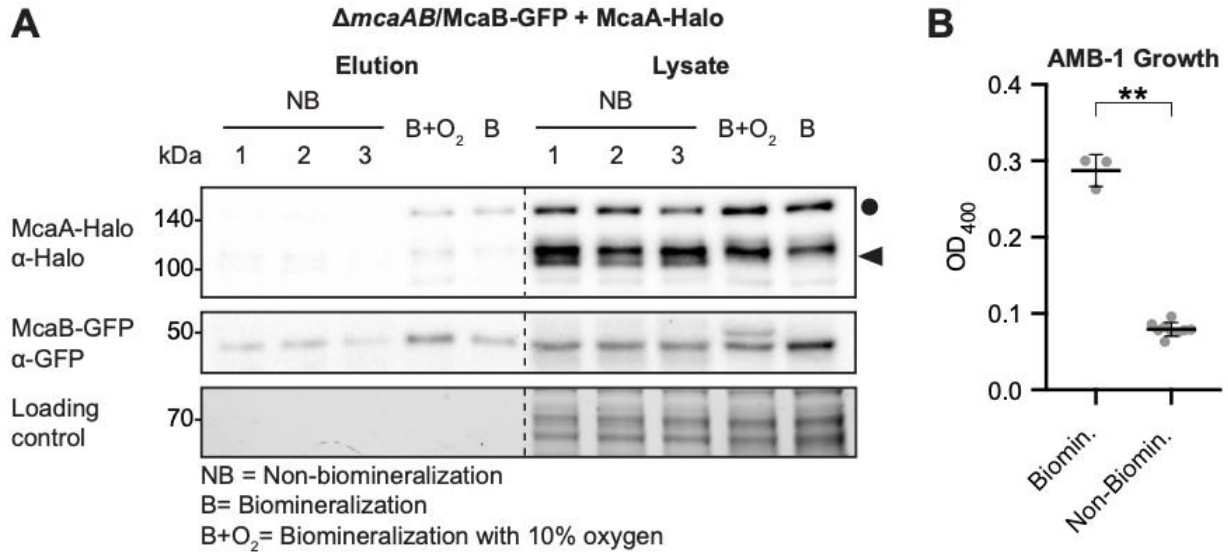


**Figure S14:** McaA and McaB do not interact in non-biomineralizing conditions. **(A)** Western blots of elutions from co-IP with GFP-Trap agarose beads and lysates illustrating that full-length McaA-Halo does not interact with McaB-GFP in the  $\Delta mcaAB$  strain grown under non-biomineralizing conditions (NB). Biomineralization conditions with 10% O<sub>2</sub> (B + O<sub>2</sub>) and normal biomineralization conditions (B) serve as positive controls. The circle and triangle indicate the positions of full-length McaA-Halo and shorter McaA bands, respectively. Three independent biological replicates are shown and the loading control is a stain-free SDS-PAGE gel. **(B)** OD<sub>400</sub> of 10mL liquid cultures of AMB-1  $\Delta mcaAB$  expressing McaA-Halo and McaB-GFP microaerobically grown for 2 days in either biomineralization or non-biomineralization conditions. Each point represents an independent culture and the average and standard deviation are shown. Mann-Whitney *U* test was used to determine p-values (\*\* *P* < 0.01).

| Organism                     | Details                       | Reference  |
|------------------------------|-------------------------------|------------|
| <i>M. magneticum</i> AMB-1   | WT                            | [90]       |
|                              | $\Delta mcaA$                 | [34]       |
|                              | $\Delta mcaB$                 | [29]       |
|                              | $\Delta mcaAB$                | [29]       |
|                              | $\Delta mcaA\Delta mamK$      | This study |
|                              | $\Delta mcaB\Delta mamK$      | This study |
|                              | $\Delta MAI\Delta MIS$        | [29]       |
|                              | $\Delta mamK\Delta mamK-like$ | [36]       |
|                              | $\Delta MAI$                  | [90]       |
|                              | $\Delta 530$                  | This study |
|                              | $\Delta mamA$                 | [40]       |
|                              | $\Delta mms6$                 | [107]      |
|                              | $\Delta mcaB\Delta mms6$      | This study |
| <i>Escherichia coli</i> DH5a | Standard cloning strain       | [90]       |
| <i>Escherichia coli</i> WM   | Conjugation Strain            | [85]       |
| <i>Escherichia coli</i> BL21 | Protein expression Strain     | Agilent    |
| <i>Escherichia coli</i> DHM1 | Reporter strain for B2H assay | Euromedex  |

**Supplementary Table 1:** Strains used in this study.

| <b>Plasmid</b> | <b>Content</b>                    | <b>Backbone</b> | <b>Ref</b> |
|----------------|-----------------------------------|-----------------|------------|
| pAK35          | <i>mamK</i> deletion              | pAK31           | [26]       |
| pAK196         | MamK-GFP                          | pAK22           | [21]       |
| pAK420         | MamK                              | pET             | [29]       |
| pAK566         | <i>mms6</i> deletion              | pAK31           | [107]      |
| pAK1255        | McaB-GFP + McaA-Halo              | pAK22           | [34]       |
| pAK1270        | McaB-GFP + Mms6-Halo              | pAK22           | [34]       |
| pAK1430        | McaA-GFP + MamK-Flag              | pAK22           | This work  |
| pAK1431        | McaB-GFP + Halo                   | pAK22           | This work  |
| pAK1434        | GFP + McaA-Halo                   | pAK22           | This work  |
| pAK1437        | 530 region deletion               | pAK31           | This work  |
| pAK1438        | McaB-GFP + McaA $\Delta$ 530-Halo | pAK22           | This work  |
| pAK1478        | His-MBP-McaBcyto                  | pET             | This work  |
| pAK1492        | McaA $\Delta$ H2-GFP              | pAK22           | This work  |
| pAK1493        | McaA $\Delta$ H1-GFP              | pAK22           | This work  |
| pAK1494        | McaA $\Delta$ Beta-GFP            | pAK22           | This work  |
| pAK1496        | GFP + MamK-Flag                   | pAK22           | This work  |
| pAK1497        | McaA $\Delta$ 530-GFP + MamK-Flag | pAK22           | This work  |
| pAK1499        | MBP-McaBcyto                      | pET             | This work  |
| pAK1500        | MBP                               | pET             | This work  |
| pAK1501        | McaA-GFP + Halo-Flag              | pAK22           | This work  |
| pAK1502        | Strep-McaAcyto                    | pET             | This work  |
| pAK1522        | 530-GFP                           | pAK22           | This work  |
| pAK1587        | McaB-GFP + Halo-Flag              | pAK22           | This work  |
| pAK1588        | McaB-GFP + MamK-Flag              | pAK22           | This work  |
| pBBR1MCS-2     | Empty Vector                      | N/A             | [108]      |

**Supplementary Table 2:** Plasmids used in this study

| <b>Plasmid</b> | <b>Content</b>                           | <b>Reference</b> |
|----------------|------------------------------------------|------------------|
| pAK324         | T25-MamK                                 | [36]             |
| pAK325         | MamK-T25                                 | [36]             |
| pAK326         | MamK-T18                                 | [36]             |
| pAK327         | T18-MamK                                 | [36]             |
| pAK1240        | T25-McaA                                 | [34]             |
| pAK1241        | McaA-T25                                 | [34]             |
| pAK1242        | McaA-T18                                 | [34]             |
| pAK1243        | T18-McaA                                 | [34]             |
| pAK1249        | T25-McaB                                 | [34]             |
| pAK1250        | McaB-T25                                 | [34]             |
| pAK1251        | McaB-T18                                 | [34]             |
| pAK1252        | T18-McaB                                 | [34]             |
| pAK1363        | T25-McaA[aa400-end]                      | This work        |
| pAK1364        | McaA-T25[aa400-end]                      | This work        |
| pAK1365        | McaA-T18[aa400-end]                      | This work        |
| pAK1366        | T18-McaA[aa400-end]                      | This work        |
| pAK318         | pKT25 (empty vector)                     | Euromedex        |
| pAK319         | pKNT25 (empty vector)                    | Euromedex        |
| pAK320         | pUT18 (empty vector)                     | Euromedex        |
| pAK321         | pUT18C (empty vector)                    | Euromedex        |
| pAK322         | pKT25-Zip (Leu zipper positive control)  | Euromedex        |
| pAK323         | pUT18C-Zip (Leu zipper positive control) | Euromedex        |

**Supplementary Table 3:** Plasmids used for bacterial two hybrids in this study.

| Name  | Sequence (5'→ 3')                                       | Amplicon            | In Plasmid          |
|-------|---------------------------------------------------------|---------------------|---------------------|
| YR53  | GCGCACGCGGCGGGCTGCAGGACAAAAGGCGGCTCAGGCG                | McaA[aa400-end]     | pAK1363             |
| YR54  | GTACCCGGGGATCCTCTAGATCACTTTTTGAAAATGGCATTCTCTGC         |                     |                     |
| YR55  | GCCAAGCTTGCATGCCTGCAGACAAAAGGCGGCTCAGGCG                | McaA[aa400-end]     | pAK1364,<br>pAK1365 |
| YR56  | GGTACCCGGGGATCCTCTAGAGTCTTTTTGAAAATGGCATTCTCTGC<br>T    |                     |                     |
| YR57  | GCAGTGGAAACGCCACTGCAGACAAAAGGCGGCTCAGGCG                | McaA[aa400-end]     | pAK1366             |
| YR54  | GTACCCGGGGATCCTCTAGATCACTTTTTGAAAATGGCATTCTCTGC         |                     |                     |
| YR153 | TAGACTAGTGTGATCACGGAGGAACGTAATGAGTGAAGGTGAAG<br>GCCA    | MamK-Flag           | pAK1430,<br>pAK1431 |
| YR124 | TGGCGGCCGCTCTAGAACTA                                    | GFP                 | pAK1434             |
| YR175 | CTAGACTAGTGTGATCACGGAGGAACGTATGAGTAAAGGAGAAG<br>AACT    |                     |                     |
| YR124 | TGGCGGCCGCTCTAGAACTA                                    | 530-downstream      | pAK1437             |
| YR171 | CTGCAGCCCGGGGATCCACTAGTGCAGGCTCTGCACACTTGAT             |                     |                     |
| JW20  | CTCCGACCACATGGCGAAAGGAACTGAGCACGATG                     | 530-upstream        | pAK1437             |
| YR172 | CAGTTCCTTTCGCCATGTGGTCGGAGAGTGAGA                       |                     |                     |
| YR173 | TGGCGGCCGCTCTAGAACTAGTATTTGATGCCACGCGTTGCC              | McaA-delta-530      | pAK1438             |
| YR169 | ACTAGTGTGATCACGGAGGAACGTAGTGGTAATTCGAGGGGGG             |                     |                     |
| JW24  | GTACCGATTTCTGCGGATCCCTTTTTGAAAATGGCATTCC                | McaB[28-end]        | pAK1478             |
| YR180 | AACCTGTACTTCCAATCCTGGAAGCTGACTGCCGCG                    |                     |                     |
| YR181 | CCAAGGGGTTATGCTAGCTACTCGAGCTTAGAAAGGAT                  | MBP                 | pAK1478             |
| YR184 | ATGGGTTCTTCTCACCATCA                                    |                     |                     |
| YR176 | GGATTGGAAGTACAGGTTTT                                    | McaA-deltaH2        | pAK1492             |
| YR174 | ACATTAATTGCGTTGCGCTC                                    |                     |                     |
| YR192 | TCCATGACGCGAAAGGAACTGAGCACGA                            | McaA-deltaH2        | pAK1492             |
| YR58  | GAGCGCAACGCAATTAATGT                                    |                     |                     |
| YR193 | CCTTTCGCGTCATGGAATTGAAAGAACCC                           | McaA-deltaH1        | pAK1493             |
| YR174 | ACATTAATTGCGTTGCGCTC                                    |                     |                     |
| YR190 | TCCGACCACCGCACATCTGGCGTGATCG                            | McaA-deltaH1        | pAK1493             |
| YR58  | GAGCGCAACGCAATTAATGT                                    |                     |                     |
| YR191 | ATGTGCGGTGGTCGGAGAGTGAGACTT                             | McaA-deltaB         | pAK1494             |
| YR174 | ACATTAATTGCGTTGCGCTC                                    |                     |                     |
| YR188 | TTGGTTCAGACAATATTAATTCATGGATGAA                         | McaA-deltaB         | pAK1494             |
| YR58  | GAGCGCAACGCAATTAATGT                                    |                     |                     |
| YR189 | ATATTGTCTGAACCAAGCGGAGCAGCAA                            | GFP                 | pAK1496             |
| YR163 | ACTAGTTCTAGAGCGGCC                                      |                     |                     |
| YR22  | TGTTTCATATGATCTGGGTA                                    | MamK-Flag           | pAK1496,<br>pAK1497 |
| YR23  | CAGATCATATGAAACAGCAT                                    |                     |                     |
| YR124 | TGGCGGCCGCTCTAGAACTA                                    | McaA-delta530-GFP   | pAK1497             |
| YR58  | GAGCGCAACGCAATTAATGT                                    |                     |                     |
| YR22  | TGTTTCATATGATCTGGGTA                                    | McaB[28-end]        | pAK1499             |
| YR186 | AAAACCTGTACTTCCAATCC                                    |                     |                     |
| YR202 | CATGGTATATCTCCTTCTTA                                    | MBP                 | pAK1499,<br>pAK1500 |
| YR203 | AAGGAGATATACCATGAAAATCGAAGAAGGTAA                       |                     |                     |
| YR176 | GGATTGGAAGTACAGGTTTT                                    | Halo-Flag           | pAK1501,<br>pAK1587 |
| YR159 | TAGACTAGTGTGATCACGGAGGAACGTAATGGCAGAAATCGGTA<br>CTGGC   |                     |                     |
| YR124 | TGGCGGCCGCTCTAGAACTA                                    | Strep-McaA[400-end] | pAK1502             |
| YR205 | CCACCCGCAGTTCGAAAAGGAAAACCTGTACTTCCAATCC                |                     |                     |
| YR179 | CAAGGGGTTATGCTAGTCACTTTTTGAAAATGGCATT                   | 530-GFP             | pAK1522             |
| YR217 | AATTTACACAGGAAACAGAATTCATGGTTGCTGAATTCATTGATG           |                     |                     |
| JW22  | GAAAAGTTCTTCTCCTTACTCATGGATCCAATTCTGCTAACAACCT<br>CGATC | MamK-Flag           | pAK1588             |
| YR160 | GATGAACTATACAAATAGACTAGTGTGATCACGG                      |                     |                     |
| YR124 | TGGCGGCCGCTCTAGAACTA                                    |                     |                     |

**Supplementary Table 4:** Primers used for cloning in this study.

| <b>Name</b> | <b>Sequence (5'→ 3')</b>  | <b>For Sequencing</b>                           |
|-------------|---------------------------|-------------------------------------------------|
| YR17        | ATTTGATGCCACGCGTTGCC      | AMB-1 $\Delta$ 530                              |
| YR18        | GCCAACTGCAGTGGTTTATC      | AMB-1 $\Delta$ 530                              |
| YR27        | TCTTTGGGTCGTGCCTATTT      | AMB-1 $\Delta$ 530                              |
| YR44        | TGCTCACTGATGGATGGCCG      | AMB-1 $\Delta$ 530                              |
| YR183       | TTTATGATCGGCAGGAAGGG      | AMB-1 $\Delta$ 530                              |
| JW27        | CTTCGGTCCAGCTGTTGAC       | AMB-1 $\Delta$ 530                              |
| JW30        | GCTGTAAGATGATGATTC        | AMB-1 $\Delta$ 530                              |
| YR115       | TCCTTACTCACTCCAAAGCC      | AMB-1 $\Delta$ <i>mcaA</i> $\Delta$ <i>mamK</i> |
| YR208       | TCGACAACATGACTTCATCG      | AMB-1 $\Delta$ <i>mcaA</i> $\Delta$ <i>mamK</i> |
| YR114       | AAGACCTTCTCGGTGGAATT      | AMB-1 $\Delta$ <i>mcaB</i> $\Delta$ <i>mamK</i> |
| YR207       | ATTGTCCTTTGATGGTGGAG      | AMB-1 $\Delta$ <i>mcaB</i> $\Delta$ <i>mamK</i> |
| YR208       | TCGACAACATGACTTCATCG      | AMB-1 $\Delta$ <i>mcaB</i> $\Delta$ <i>mamK</i> |
| mmsfREV     | CGCATCATCGGGTCCGAAGATCAGA | AMB-1 $\Delta$ <i>mcaB</i> $\Delta$ <i>mms6</i> |

**Supplementary Table 5:** Sequencing primers used in this study.

### **Chapter 3**

Magnetosome protein MamA regulates size of another organelle, the PHB granule, in *Magnetospirillum magneticum* AMB-1

Yein Ra, Tansy Chen, Jude Alexis, Lindsey Way, and Arash Komeili

Department of Plant and Microbial Biology, University of California, Berkeley, CA, USA

## ABSTRACT

Magnetotactic bacteria (MTB) are a diverse group of species that are best known for their production of the magnetosome organelle, which they use to navigate their environment. However, many MTB produce other organelles as well. Whether there is any relationship between magnetosomes and other such compartments remains mostly unknown. *Magnetospirillum magneticum* AMB-1 produces polyhydroxybutyrate granules (PHBGs) in addition to magnetosomes. In this chapter, we demonstrate that the magnetosome protein MamA also regulates the size of PHBG in AMB-1, and that MamA self-oligomerization is required for both functions. We provide preliminary data that MamA interacts with both magnetosome and PHBG proteins, and that the protein composition of the PHBG may be altered in the absence of MamA. MamA controls aspects of both magnetosomes and PHBGs, illustrating that inter-organelle interactions, a common feature of eukaryotic cells, can also occur in bacteria.

## INTRODUCTION

One key feature of a eukaryotic cell is that it contains many diverse types of organelles with seemingly distinct functions. However, eukaryotic organelles often communicate and coordinate their activities in the form of metabolite exchange, protein trafficking, and even physical association [4–6]. Some bacterial species also form multiple organelles in a single cell, but whether bacterial organelles also undergo inter-organelle communication remains largely unexplored. Better understanding bacterial inter-organelle communication will reveal the conserved mechanisms of organelle cross-talk, inspire methods of controlling organelles in engineering settings, and uncover novel principles of bacterial organelle biology.

There are a few cases where bacterial inter-organelle communication has been observed. One example is the close association between carboxysomes, a proteinaceous bacterial microcompartment wherein carbon fixation occurs, and polyphosphate (polyP) granules, an excess phosphate storage compartment, in *Halothiobacillus neapolitanus* using cryo-electron tomography [13,73,109,110]. Strikingly, some carboxysome-polyP granule associations appeared to be mediated by a lattice structure [73]. Carboxysomes and polyP granules also occasionally contact in the model cyanobacteria *Synechococcus elongatus*, and such interactions increase in frequency when the McdAB carboxysome positioning system (reviewed in Chapter 1) is disrupted [111]. Furthermore, the disruption of polyP granules leads to the expansion of the nucleoid, which in turn increases carboxysome number and dynamics [111]. Another example is in magnetotactic bacteria (MTB). MTB are often studied for their magnetosomes, membrane-bound magnetic crystals that aid the cell's navigation of the environment [75,112]. In addition to magnetosomes, polyhydroxybutyrate (PHB) granules - compartments that store excess carbon in the form of a PHB polymer - and polyP granules are found in the model MTB *Magnetospirillum magneticum* AMB-1 (hereafter AMB-1) [44,113]. Previous studies identified a negative correlation between magnetosome production and PHB granule formation when growing MTB on different carbon source or oxygen concentrations [113,114]. However, whether the correlation between PHB granules and magnetosomes is due to a direct organelle interaction or a consequence of different growth conditions, and the possible mechanism behind PHB granule-magnetosome relationship, was not resolved.

Here, we explore the potential magnetosome-PHB granule communication in MTB. We demonstrate that the magnetosome protein MamA affects both magnetosome crystal number and PHB granule size. In cells lacking MamA, the total number of magnetite crystals per cell decreases, as does the average PHB granule size. We use co-immunoprecipitation and proteomics to show that MamA interacts with various PHB granule proteins, and that the protein content of PHB granules and magnetosomes changes in the absence of MamA. Altogether, we propose a mechanism of bacterial inter-organelle communication by which a single protein influences two distinct organelles in MTB.

## RESULTS

### **MamA affects both magnetosome crystal number and PHB granule size.**

A previous study that measured magnetosome, PHB granule (PHBG), and polyP granule abundances in AMB-1 grown in different oxygen concentrations determined that conditions that favored magnetosome biosynthesis also disfavored PHBG formation [113]. To further investigate the relationship between magnetosomes and PHBGs, we first looked for any changes to the PHBG in a strain of AMB-1 that is missing the *mamAB* operon ( $\Delta mamAB_{op}$ ), one of the key operons in the magnetosome gene island that is required for magnetosome synthesis [90], using transmission electron microscopy (TEM). Our initial observations showed that AMB-1  $\Delta mamAB_{op}$  has smaller PHBGs (Fig. 1). The *mamAB* operon contains 18 *mam* genes that play various roles in magnetosome synthesis and positioning [90]. To determine whether there is a single gene that is responsible for the PHBG phenotype, single deletion strains of each of the 18 *mam* genes were observed using TEM. We identified that the deletion of *mamA* was the only single gene deletion that produced the smaller PHBG phenotype (Fig. 2B, C). PHBG size can be complemented with MamA-GFP expressed using a constitutive Tac promoter on a replicative plasmid (Fig. 2B, C). AMB-1  $\Delta mamA$  cells also formed fewer magnetite crystals than WT and had a lower  $C_{mag}$ , a metric for magnetic alignment taken with a spectrometer, consistent with previous observations (Fig. 2D, E) [40]. Both magnetosome phenotypes were complemented by the addition of MamA-GFP (Fig. 2D, E).

MamA has six tetra-trico-peptide repeat (TPR) domains (Fig. 2A), which are known to facilitate protein complex formation [115,116]. To assess the role of the MamA TPR domains in regards to PHBG size, we created two GFP-tagged MamA truncation mutants, missing either the first ( $\Delta 41$ ) or last ( $\Delta c34$ ) TPR domain (Fig. 2A). We first checked the localization of the truncations in a  $\Delta mamA$  background to see whether they were expressed and localized as expected. Full length MamA-GFP appeared to localize to the magnetosome chain, in line with previous observations (Fig. 2F) [40,116]. MamA $\Delta 41$ -GFP had a diffuse localization, also as previously observed (Fig. 2F) [116]. Prior structural analysis of MamA determined that the N-terminal TPR domain of MamA is required for self-oligomerization into large complexes [116], and such self-oligomerization and possibly interactions with other magnetosome proteins leads to the localization of MamA to the magnetosome membrane [116,117]. MamA $\Delta 41$ -GFP expressed in AMB-1  $\Delta mamA$  could not complement  $C_{mag}$ , magnetite number, or PHB size, indicating that the first TPR domain - and likely the ability to self-interact - is required for all three aspects of MamA function (Fig. 2B-E). MamA $\Delta c34$ -GFP localized into several, distinct puncta in most cells observed (Fig. 2F). Despite this aberrant localization, MamA $\Delta c34$ -GFP partially

complemented both magnetite number and PHB size, but not  $C_{mag}$  (Fig. 2B-E). Closer examination of MamA $\Delta$ c34-GFP localization showed that some MamA $\Delta$ c34-GFP puncta were adjacent to PHBGs (Fig. 2F). However, this observation was not consistent from cell to cell. Further investigation is necessary to determine whether the MamA $\Delta$ c34-GFP puncta co-localizes with the magnetosome chain and/or PHBGs, and why  $C_{mag}$  remains low despite WT-amounts of crystal-containing magnetosomes.

### **AMB-1 encodes both PHB-synthesizing proteins and PHBG-associating phasins.**

Though the formation of PHBGs in AMB-1 has been documented [113], the genes required for production and regulation of these organelles have not been thoroughly characterized. To speculate how PHBG size might be controlled by MamA, we first needed to identify PHBG proteins as they might be contributors to this potential inter-organelle interaction. PHBG synthesis requires three proteins: PhaA, PhaB, and PhaC [118]. Using genome annotations, we identified *phaC*, *phaA*, and *phaB* organized in an operon predicted to encode these proteins in the genome (*amb0841*, *amb0842*, and *amb0843*, respectively) in AMB-1 (Fig. 3A). Interestingly, this operon is predicted to have an additional gene of unknown function (*amb0840*) upstream of *phaC*. Upstream of the *amb0840-0843* operon is a putative *phaR*, which encodes a transcriptional regulator known to repress expression of phasin genes in *Cupriavidus necator* (previously *Ralstonia eutropha*) [119]. We attempted to delete *phaC* alone or the entire *amb0840-0843* operon to assess whether these gene(s) are essential for PHBG formation, but our efforts were unsuccessful. We suspected that this operon is essential for AMB-1 under the standard lab culturing method. Indeed, a closer look at the AMB-1 Tn-seq library previously constructed revealed that the 23 out of 24 transposon insertions in *amb0840-phaCAB* operon were in the outer 10% of the genes [120], suggesting that disrupting the middle of these genes is largely deleterious for AMB-1 growth. AMB-1 has a second copy of *phaC* at another locus (*amb3725*) (Fig. 3A). Amb3725-sfGFP localizes to PHBG (Fig. 3B). We named this putative PHB polymerase PhaC2. Deleting *phaC2* had no observable effect on PHBG (Fig. 3C), likely because its function is at least partially redundant to *phaC*.

PHBGs rely on another group of proteins called phasins. Phasins are not directly involved in the synthesis or depolymerization of the PHB polymer, but localize to the PHBG and often regulate the granule size, number, and position [11,43,48,52,121]. In AMB-1, there are 5 genes annotated to encode phasins: *mms16* (*amb0546*), *amb0571*, *amb1096*, *amb1121*, and *amb3386*. Mms16 from another MTB species, *Magnetospirillum gryphiswaldense* MSR-1, has been determined to localize to the PHBG and activate the PHB depolymerase [122,123], but its contributions to PHBG in AMB-1 has not been explored. To determine whether the 5 putative phasins localize to the PHB granule in AMB-1, we tagged each gene with a C-terminal sfGFP and expressed them in WT AMB-1. Attempts to clone *amb1121* were unsuccessful, but the remaining four sfGFP fusions co-localized to the PHBGs seen in the phase channel (Fig. 3B). Therefore, we confirmed that Mms16, Amb0571, Amb1096, and Amb3386 are indeed PHBG-localized phasin proteins. Next, we deleted each of the 5 phasin genes. In WT AMB-1, PHBGs tend to be uniformly distributed along the cell length. In the  $\Delta$ *mms16* strain, this distribution is lost; PHBGs tend to be adjacent to each other and found more often at the cell poles than at midcell (Fig. 3C). Single deletions of *amb0571*, *amb1097*, *amb1121*, and *amb3386* did

not result in a noticeable phenotype (Fig. 3C). One possibility is that some phasins have redundant roles, and so the contributions of these phasins cannot be elucidated from single gene deletions.

### **MamA interacts with PHB granule proteins.**

MamA affects the size of PHBGs, but the mechanism by which it does is unknown. One possibility is that MamA interacts with PHBG proteins. To test this hypothesis, we conducted a preliminary co-immunoprecipitation experiment paired with mass spectrometry (IP-MS) using cell lysate from AMB-1  $\Delta mamA$  expressing MamA-GFP. The experiment was conducted with GFP nanobodies bound to agarose beads, as detailed in the Materials and Methods. The final elution was analyzed with mass spectrometry to identify proteins that were bound to MamA-GFP. Proteins identified in the MamA-GFP IP-MS were cross-checked with the IP-MS completed with GFP in Chapter 2, which identified proteins that had affinity for GFP and/or the agarose beads, as a preliminary negative control. The IP-MS demonstrated that MamA-GFP interacts with a cohort of both magnetosome proteins and PHB granule proteins (Table 1). Mms6 has been previously shown to interact with MamA [117], and indeed Mms6 peptides were identified in our IP-MS with MamA-GFP. Additionally, the two most abundant magnetosome proteins in the elution were MamK, a bacterial actin-like protein involved in arranging magnetosomes into a linear chain [26,28], and MamE, a protease that regulates magnetosome membrane growth [124]. A subset of PHB granule proteins co-eluted with MamA-GFP. Peptides from PhaA and PhaC2, and to a lesser extent PhaB, were identified in the IP-MS. Mms16, Amb1121, and Amb0571 also co-eluted with MamA-GFP, signifying that MamA interacts with phasins. Additionally, peptides of the putative PhaR were identified in the elution. Altogether, this preliminary data suggests that MamA interacts with both magnetosome and PHB granule proteins in AMB-1.

### **MamA influences the protein content of magnetosomes and PHBGs.**

MamA is localized to the magnetosome (Fig. 2F) [40,125]. How then, does MamA affect the size of the PHBG while it is at the magnetosome? The IP-MS data indicates that MamA interacts with various PHBG proteins. One possibility is that while MamA is at the magnetosome, it interacts with PHBG proteins to either regulate their activity or to sequester them from the PHBG. Another possibility is that while the majority of MamA is localized to the magnetosome, a small population of MamA is at the PHBG and/or the cytoplasm and interacting with the PHBG proteins at these locations. The first hypothesis is supported by a previous magnetosome proteomics, which identified PHBG proteins, including Mms16, Amb0571, Amb1121, and Amb3386, to be enriched on purified magnetosomes [124]. One explanation for this enrichment is the interaction between MamA and phasins sequesters a subpopulation of phasins at the magnetosome. If this is true, then we would expect phasins to be depleted from the magnetosome in the absence of MamA. Therefore, we purified magnetite crystal-containing magnetosomes from both WT and  $\Delta mamA$  and assessed the presence of PHBG proteins on magnetosomes using mass spectrometry.

The magnetosome fraction had a unique protein profile compared to whole cell lysate (Fig. S1A). As expected, many magnetosome proteins were enriched in the magnetosome fraction in both WT and  $\Delta mamA$  strains (Fig. S1B, Table 2, 3). To compare

protein enrichment and depletion on the magnetosome between the two strains, the magnetosome-to-lysate fold-difference abundance for each protein in each strain was plotted against the other (Fig. 4A). Some magnetosome proteins were more enriched on WT magnetosomes compared to those in  $\Delta mamA$ . This observation suggests that though MamA is not required for the targeting of most magnetosome proteins to crystal-containing magnetosomes, it may be required for further protein recruitment, retainment, and stability at the magnetosome. Of the PHBG proteins, most of those involved in PHB synthesis (PhaA and PhaB) are less abundant in the magnetosome compared to the cell lysate. PhaC2 was slightly enriched in the magnetosome fraction in both WT and  $\Delta mamA$ . Interestingly, Mms16, Amb0571, Amb1121, Amb3386, and the putative PhaR were enriched on the magnetosome fraction in WT, and to a higher degree in  $\Delta mamA$ . This was surprising as we were expecting the abundance of PHBG proteins to decrease due to the lack of MamA. This prompted us to more closely examine the magnetosome fraction with TEM. Alongside the crystal-containing magnetosomes, we also found round, electron-sparse entities that resemble PHBGs in intact AMB-1 cells (Fig. 4B). The magnetosome purification method used here was very similar to that used in [124], thus it is likely that the original magnetosome proteomics that inspired this experiment [124] also used a magnetosome fraction that co-purified similar structures. The presence of PHBG proteins in the magnetosome fraction is then unsurprising, assuming that the structures visualized with TEM are indeed PHBGs. Since the magnetosome fraction is a mixture of magnetosome and PHBGs, the enrichment of phasins in the magnetosome fraction in the  $\Delta mamA$  strain could be attributed to a change in PHBG protein content. In other words, one possible interpretation of the proteomics result is that phasins become more abundant in the PHBG of  $\Delta mamA$  compared to WT.

### **Overexpressing phasins lead to smaller, misplaced PHBGs.**

When MamA is missing, PHBGs are smaller and they may have an increased abundance of phasins. In *C. necator*, overexpressing phasins can affect PHBG size and abundance [11,47,121]. This led us to consider that excess phasins may cause PHBG size to decrease. To test this hypothesis, we overexpressed different phasins (excluding *amb1121*, which could not be successfully cloned) in AMB-1 by expressing each one constitutively on a replicative plasmid in a WT background. Although the effect of phasin overexpression varied from strain to strain, we observed cells that had clusters of many, smaller PHBGs in strains overexpressing *mms16*, *amb0571*, and *amb1096* (Fig. 5). The clustering phenotype implies that the cell is not only overproducing smaller PHBGs, but has also lost the ability to properly spatially distribute the granules. The increased observation of clustering PHBGs when *mms16*, *amb0571*, and *amb1096* are overexpressed suggests that PHBGs are sensitive to phasin abundance. Interestingly, overexpressing *amb3386* did not lead to clustering PHBGs (Fig. 5). This suggests that though many phasins appear to have redundant functions, they may also have distinct roles as well.

## DISCUSSION

### **MamA is a dual organelle regulator that impacts PHBG size by sequestering phasins.**

This study explored how the magnetosome protein MamA affects the size of another organelle, the PHB granule, in the model MTB *Magnetospirillum magneticum* AMB-1. We found that in a strain lacking MamA, both the magnetosome and the PHBG is altered: There are fewer crystal-containing magnetosomes per cell and the average PHBG size decreases. Both phenotypes can be complemented with the addition of MamA-GFP. MamA affecting two organelles leads us to hypothesize that MamA is a mediator of inter-organelle interactions in AMB-1.

How does MamA influence PHBG size? MamA appears to have protein-protein interactions with some PHBG proteins. Additionally, we found that there is an increase in the abundance of phasin proteins in the magnetosome fraction of the cell, which likely contains co-purified PHBGs. We further illustrated that overexpressing some phasins led some cells to overproduce PHBGs that are smaller and group into a cluster. Altogether, we present the following model for how the magnetosome protein MamA affects the size of the PHBG (Fig. 6). MamA localizes to the magnetosome, which is dependent on its ability to self-oligomerize [116]. At the magnetosome, MamA can bind to and sequester phasins. This reduces the amount of phasins that can localize to the PHBG. In the  $\Delta mamA$  strain, the loss of phasin sequestration at the magnetosome increases the phasin abundance at the PHBG. The increased amount of phasins causes PHBGs to become smaller. Thus, in this model, MamA - a magnetosome protein that controls magnetosome crystal numbers - also acts as a regulator of PHBG size through its interaction with phasins.

Why would MTB evolve a mechanism where one protein regulates both the magnetosome and the PHBG? Considering that the PHBG can take up to close to 90% of the cell volume [113], it is necessary to control PHBG size to ensure that other cellular processes are not hindered. Indeed, we frequently observe breaks in the magnetosome chain in cells with particularly large PHBGs (Fig. S2). Uncontrolled PHBG growth could compromise the magnetosome chain and make the cell unable to align with a magnetic field. Therefore, it may be advantageous to have a system where the development of the magnetosome chain and the growth of the PHBGs are coordinated. Whether this multi-organelle coordination is achieved through MamA alone or through a network of proteins remains unknown.

There are several limitations of this present investigation. Firstly, our model asserts that the PHBG size is ultimately caused by a change in phasin content of the PHBG. However, whether there is a change in the protein content of the PHBG between WT and  $\Delta mamA$  strains was not directly tested. PHBGs should be purified from both strains and analyzed using proteomics to confirm this hypothesis. The proteomics experiment will also resolve whether MamA influences the PHBG while localized at the magnetosome, or whether there is a subpopulation of MamA that is found at the PHBG. Next, the exact mechanism of how the phasins influence PHBG remains unknown. According to biochemical studies with Mms16 from *M. gryphiswaldense* MSR-1, Mms16 activates the PHB depolymerase that degrades the PHB polymer [122]. Furthermore, PHBGs in *Escherichia coli* produced by heterologously expressing *phaCAB* were larger with MSR-1's Mms16 tagged with EGFP compared to no phasin addition [123]. In AMB-1, deleting

*mms16* alone did not change PHBG size. Whether this is because Mms16 has a redundant role with another phasin, or because AMB-1's Mms16 does not impact PHBG size is unknown. Furthermore, AMB-1  $\Delta mms16$  had mispositioned PHBGs. However, it is unclear whether the putative PHB depolymerase activation activity is involved in Mms16's apparent function in spatially distributing PHBGs in AMB-1. The remaining four AMB-1 phasins are completely unexplored outside of this study to our knowledge.

How then, might phasins be regulating the size of PHBGs in AMB-1? One possibility is that the phasins form a rigid structure around the PHB polymer, restricting its maximum size. Another is that PHB synthesis is altered by the phasins. In *C. necator*, the phasin PhaM activates PhaC, the PHB polymerase [53]. Overexpressing PhaM leads to the formation of many, smaller PHBGs and it is speculated that this occurs due to increased activity of PhaC [53,54]. It is possible that a similar mechanism is causing the PHBG clustering phenotype when we overexpress Mms16, Amb0571, or Amb1096 in AMB-1. However, we did not observe similar PHBG clustering in the  $\Delta mamA$  strain, a mutant where we predict an over-abundance of phasins at the PHBG. Thus, there may be additional controls to PHBG positioning that are perturbed when phasins are overexpressed, but preserved in the  $\Delta mamA$  strain.

### **AMB-1 phasins regulate PHBG positioning through a novel mechanism.**

Previous knowledge about PHBG positioning comes from studies done in *C. necator*, *Pseudomonas putida*, and *Caulobacter crescentus* [11,48,52]. As reviewed in Chapter 1, each of these species uses a different phasin - PhaM, PhaF, and PhaH respectively - to position their PHBGs linearly along the cell length. One conserved feature of PhaM, PhaF, and PhaH is a non-specific DNA binding domain. The current model states that the non-specific binding to the chromosome spaces out the PHBGs to prevent undesired fusion of the granules [11,48,52]. AMB-1 also has a protein that regulates PHBG position: In the absence of Mms16, PHBGs are no longer uniformly distributed. Interestingly, Mms16 lacks a DNA binding domain. Instead, it has a GTPase domain and can hydrolyze GTP [126]. Currently, the mechanism by which Mms16 influences PHBG localization in AMB-1 and the precise role of the GTPase is unknown, but could be distinct from the mode of action by PhaM, PhaF, and PhaH. Alternatively, PhaM, PhaF, and PhaH may work with an undiscovered Mms16-like protein in their respective systems. Other organelle positioning mechanisms, such as the cytoskeletal MamK system and the ParAB-like machinery, have an energy expenditure via ATP hydrolysis [14,29]. Perhaps Mms16 similarly powers PHBG positioning through GTP hydrolysis. Further investigation of PHBG localization in AMB-1 and other organisms will resolve these possibilities.

## **MATERIALS AND METHODS**

**Bacterial Growth and Strains.** Standard AMB-1 and *Escherichia coli* culturing were done identically to what is described in Chapter 2. The growth of AMB-1 to be used for magnetosome proteomics is explained in a different section.

**Genetic manipulation.** Genetic manipulation was done identically to the protocols detailed in Chapter 2.

**Epifluorescence microscopy.** Epifluorescence microscopy was done identically to the protocol detailed in Chapter 2.

**Transmission electron microscopy.** Transmission electron microscopy of bacterial cell samples was done identically to the protocol detailed in Chapter 2. To image the magnetosome fraction with TEM, the magnetosome sample was thoroughly mixed by pipetting. Approximately 20 $\mu$ L was applied to the glow-discharged copper grid and imaged using the same protocol.

**Co-immunoprecipitation.** Co-immunoprecipitation (co-IP) was done as detailed in Chapter 2 with a few changes. 5 2L cultures of AMB-1 (10L total) were harvested using GS-3 rotor and Sorvall RC-5B centrifuge for each sample. All buffers used in co-IP, with those used for the elution step, contained cOmplete™, Mini, EDTA-free Protease Inhibitor Cocktail (Roche) following manufacturer's protocols.

**Mass spectrometry for co-IP.** Samples were trypsin-digested in preparation for mass spectrometry as described in Chapter 2. Mass spectrometry was done by the Proteomics/Mass Spectrometry Laboratory at University of California, Berkeley as described in Chapter 2.

**Magnetosome purification for proteomics.** Protocol to purify magnetosomes was adapted from [124]. To grow AMB-1 for magnetosome purification, MG supplemented with HEPES (5.96g per liter MG) and at pH 7.2 was used instead of standard MG for enhanced magnetosome production. For each biological replicate, 4L cultures (2x 2L) of AMB-1 grown in a microaerobic chamber with 10% O<sub>2</sub> for two days were used. Cells were harvested by centrifuging at 8000 x g for 17min with a GS-3 rotor and Sorvall RC-5B centrifuge. Cells were resuspended in residual liquid, divided into 2 15mL centrifuge tubes, and centrifuged at 8000 x g for 6min. Residual liquid was removed and the sample was flash frozen using liquid nitrogen. Frozen samples were stored at -80°C until use. To lyse, the cell pellets were thawed on ice and resuspended in 3.5mL standard MG medium supplemented with cOmplete™, Mini, EDTA-free Protease Inhibitor Cocktail (Roche). PMSF was added to a final concentration of 1mM just prior to lysing the cells with a French press at 10,000psi [127]. Cells were passed through the French press only one time to minimize disrupting the magnetosome membranes (Fig. S3). Lysates were collected into a fresh 15mL centrifuge tube. Samples from the same biological replicate were combined and 75 $\mu$ L was saved as the lysate fraction. A series of magnets were taped onto the vertical side of the falcon tube and left overnight at 4°C to separate the magnetic fraction (See Fig. S3A for an illustration). The following day, the non-magnetic supernatant was carefully removed with a pipette. The magnetic pellet was vigorously washed with 3mL of cold MG supplemented with the protease inhibitor cocktail. The magnetic pellet was allowed to reform for 45min at 4°C. The non-magnetic fraction was carefully discarded, the magnets were removed, and the final magnetic pellet was resuspended in 500 $\mu$ L of cold MG.

All of the following steps were taken at 4°C, unless indicated otherwise. To remove any unlysed cells and other debris, the magnetic fraction was separated with a Percoll gradient. To prepare the gradient, 5mL of Percoll and 5mL of MG were mixed in a 15mL

centrifuge tube, then centrifuged at 8000 x g for 30min using a fixed angle rotor. All 500 $\mu$ L of the magnetic fraction was carefully added to the top of the Percoll gradient, then centrifuged at 500 x g for 4hrs using a swinging bucket rotor. A dark pellet near the bottom of the tube contained magnetosomes (the lighter brown pellet contains unlysed cells). To retrieve the magnetosomes, the gradient was carefully removed with a pipette. When < 1mL remained, a P200 pipette was used to remove as much Percoll as possible before collecting the magnetosomes into a fresh 1.5mL microcentrifuge tube. The magnetosome fraction was washed three times with 1mL MG to remove the Percoll via centrifugation at 8000 x g for 3min.

To further remove any contaminants, the magnetosome fraction from the Percoll gradients were passed through a MACS MS magnetic separation column (Miltenyi Biotec). The column was equilibrated with 1 column volume (CV) of MG. Magnets were added to the column. The magnetosome pellet was resuspended through vigorous pipetting and added to the column. The flowthrough was collected and reapplied to the column once to maximize magnetosome binding. The column was washed with 1CV MG three times. To elute, the magnets were removed and 250 $\mu$ L MG was added to the column. The plunger supplied with the column was used to push the magnetic elution out of the column. The elutant was reapplied to the column and passed through three additional times to maximize magnetosome yield. 30 $\mu$ L of the elution was set aside for Coomassie gel and protein concentration assays. The elutions were stored at 4°C overnight to be processed for proteomics the next day (otherwise, storing at -80°C is recommended).

**Sample preparation for proteomics.** The protein concentration of elution and lysate samples were determined using the Bio-Rad Protein Assay in triplicate. To ensure that the protein concentrations are within range of the assay, diluting the lysate 100-fold and the elution 5-fold is recommended. Samples were either diluted with MG or concentrated using a 0.5mL Ultracel 3K centrifugal protein concentrator (Amicon) to normalize the protein concentration to 1000 $\mu$ g/mL. 50 $\mu$ L of the normalized sample was added to a fresh microcentrifuge tube. 10 $\mu$ L of fresh 100mM  $\text{NH}_4\text{HCO}_3$ , pH 7.5, followed by 25 $\mu$ L of 0.2% solution of RapiGest SF Surfactant (Waters), was added and the samples were vortexed. The samples were heated at 80°C for 15min, then briefly centrifuged to gather the sample to the bottom of the tube. 2.5 $\mu$ L of 100mM dithiothreitol was added, then the sample was vortexed and incubated at 60°C for 30min. The samples were cooled to RT and briefly centrifuged. 7.5 $\mu$ L of 100mM iodoacetamide was added, then the samples were vortexed and incubated in the dark at RT for 30min. 5 $\mu$ L of 1 $\mu$ g/ $\mu$ L Trypsin/Lys-C Mix (Mass Spec Grade; Promega V5073) was added, then the samples were vortexed and incubated at 37°C overnight. The following day, 10 $\mu$ L of 5%v/v trifluoroacetic acid was added. The samples were vortexed, then incubated at 37°C for 90min. The samples were then centrifuged at max rpm on a table top centrifuge for 30min at 4°C. 30 $\mu$ L of the supernatant was provided to the mass spectrometry facility for analysis in a standard sample vial.

**Mass spectrometry for proteomics.** Trypsin-digested magnetosome and lysate samples were each analyzed in triplicate using an Acquity M-class ultra-performance liquid chromatography (UPLC) system that was connected in line with a Synapt G2-Si mass spectrometer (Waters, Milford, MA) as described elsewhere [128]. Data acquisition

and analysis were performed using MassLynx (version 4.1, Waters) and Progenesis QI for Proteomics software (version 4.2, Waters Nonlinear Dynamics). Data were searched against the *M. magneticum* AMB-1 reference proteome [129] that has been supplemented with proteins encoded in the AMB-1 magnetotaxis islet [35]. Proteomics data was visualized using custom Python code written with a Jupyter notebook [130].

**SDS-PAGE and Western blotting.** SDS-Page and Western blots were done identically to the protocol detailed in Chapter 2. The generation of anti-MamE antibodies has been previously described [131].

## **ACKNOWLEDGEMENTS**

We thank Anthony Iavarone at the QB3 Mass Spectrometry Facility at UC Berkeley for advising and performing the mass spectrometry for magnetosome proteomics. We thank Robert Maxwell at the Vincent J. Coates Proteomics/Mass Spectrometry Laboratory at UC Berkeley for performing the mass spectrometry for the MamA co-IP. We thank Danielle Jorgens, Reena Zalpuri, and Misun Kang for training and assistance with TEM at the Electron Microscope Lab at UC Berkeley. Y. R. thanks her community on Instagram for assisting on color schematic decisions. A. K. is supported by NIH National Institute of General Medical Sciences (R35GM127114).

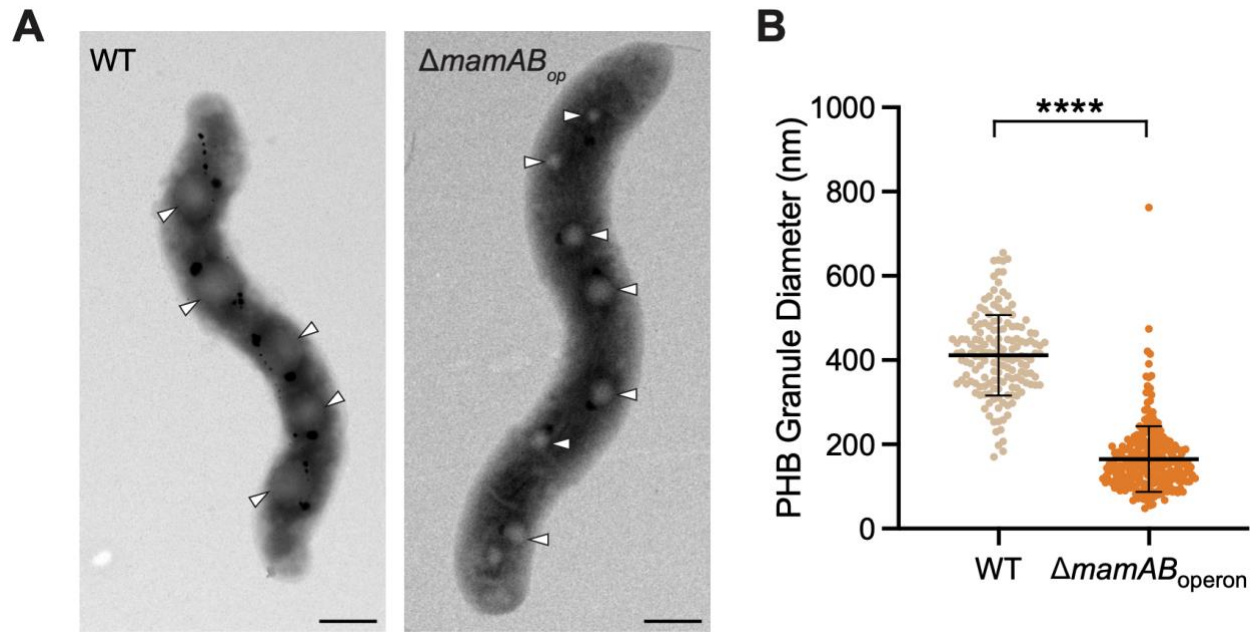


**Figure 1.** AMB-1 missing the *mamAB* operon has smaller PHB granules. **(A)** Representative transmission electron microscopy (TEM) images of WT (left) and  $\Delta mamAB_{op}$  (*mamAB* operon; right) AMB-1. White triangles point out the PHB granules. Scale bar = 0.5 $\mu$ m. **(B)** Distribution of PHB granule diameter (nm) measured from TEM images of WT and  $\Delta mamAB_{op}$ . Each point represents one granule. The mean and standard deviation is indicated. The Mann-Whitney U test was used to determine significance ( $P < 0.0001$ , \*\*\*\*).

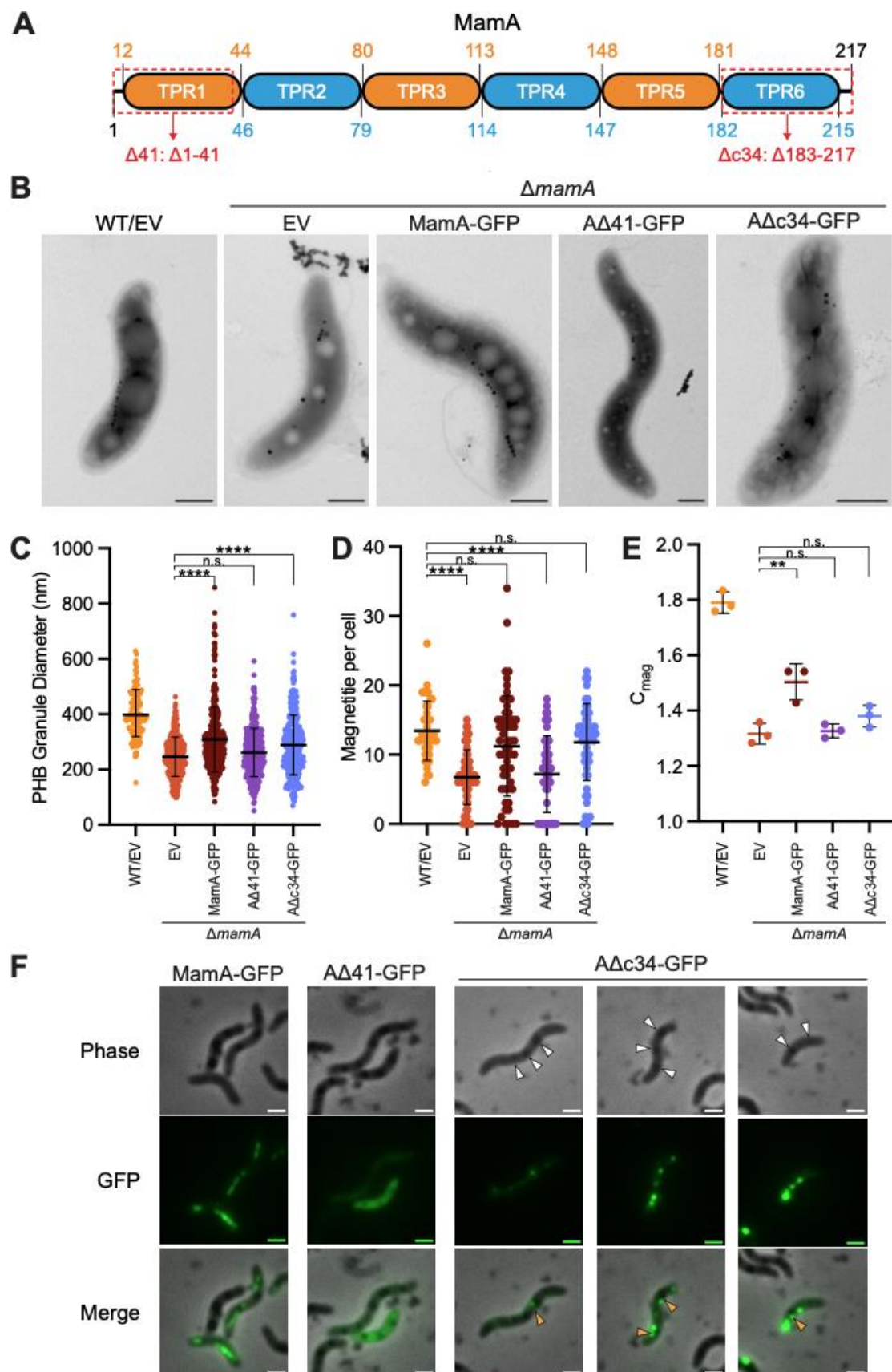


**Figure 2.** MamA is required for normal PHBG size, and its N-terminal TPR domain is necessary for this phenotype. **(A)** Schematic of MamA, showing its six tetra-trico-peptide repeat (TPR) domains [116]. Amino acid positions for where each TPR domain starts and ends are indicated. Red dashed boxes show the amino acids deleted to make the  $\Delta 41$  and  $\Delta c34$  truncation. **(B)** Representative TEM images of WT with empty vector (EV) and  $\Delta mamA$  with EV, MamA-GFP, MamA $\Delta 41$ -GFP (A $\Delta 41$ -GFP), or MamA $\Delta c34$ -GFP (A $\Delta c34$ -GFP). Scale bar = 0.5 $\mu$ m. Distribution of PHBG diameters **(C)**, number of magnetite crystals per cell **(D)**, and  $C_{mag}$  measurements of three independent cultures **(E)** for the same strains in **(B)**. In **(C-E)**, the means and standard deviations are shown, and the Kruskal-Wallis with Dunnett's multiple comparisons test was used to determine significance (\*\*  $P < 0.01$ , \*\*\*\*  $P < 0.0001$ , n.s. Not significant). In **(C)** and **(E)**, all comparisons to WT/EV were significantly different (\*\*\*\*  $P < 0.0001$ ) **(F)** Representative cells expressing GFP-tagged MamA or MamA truncation in a  $\Delta mamA$  background. PHBGs can be seen in the phase channel and are indicated with white arrows. Orange arrows indicate potential contact points between a MamA focus and a PHBG. Images were taken using epifluorescence microscopy. Scale bar = 1 $\mu$ m.

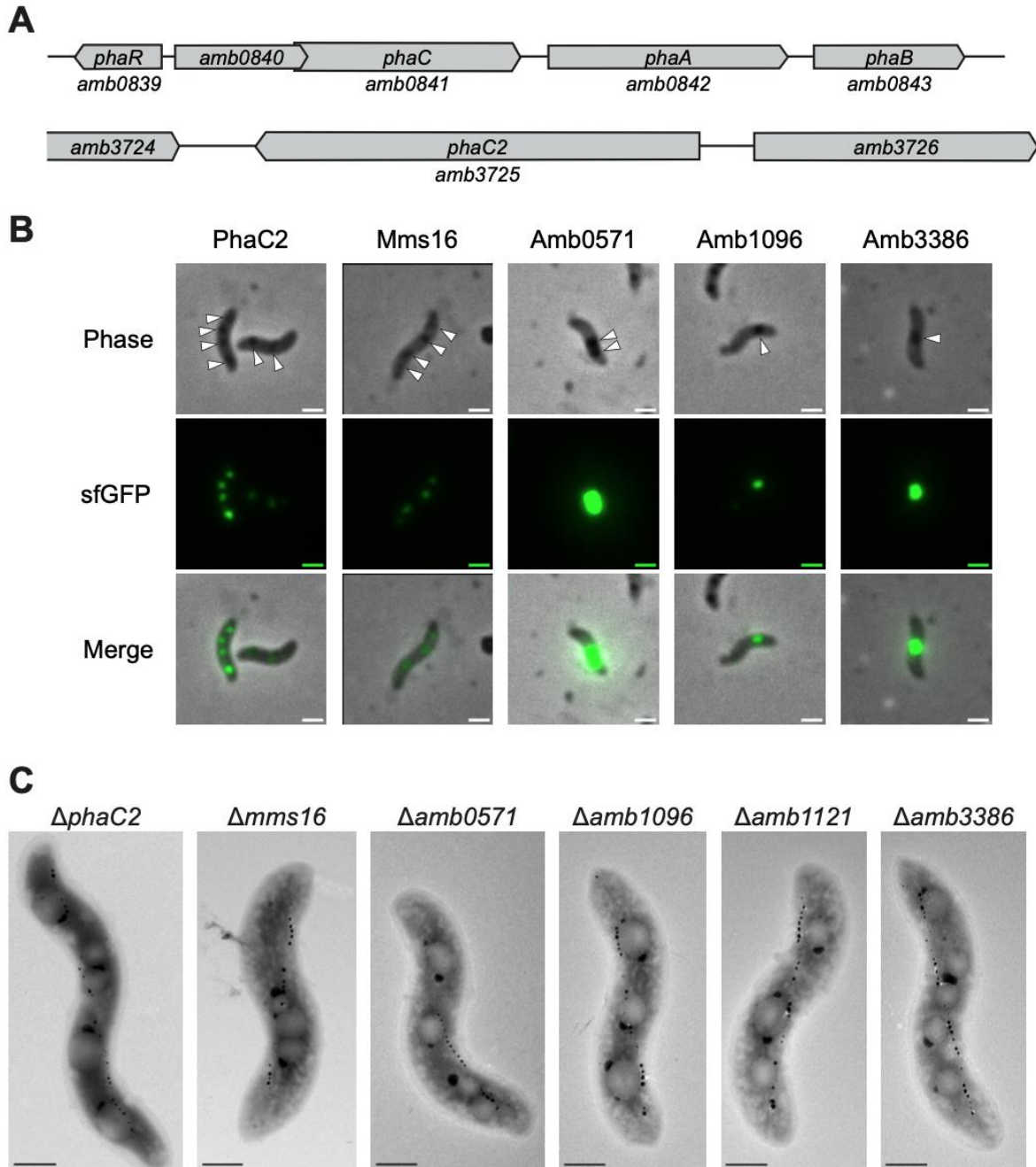


**Figure 3.** AMB-1 encodes proteins that localize to the PHB granule. **(A)** Schematic of two loci that encode genes annotated to be involved in PHBG synthesis. The top locus encodes a putative *phaCAB* in an operon with *amb0840*. A putative *phaR* is encoded upstream. AMB-1 also has a second copy of *phaC* (*amb3725*) at another locus. **(B)** Representative WT cells expressing PhaC2, Mms16, Amb0571, Amb1096, or Amb3386 with a C-terminal sfGFP tag. The PHBGs in the phase channel are indicated with a white arrow. Scale bar = 1  $\mu$ m. **(C)** Representative TEM images of AMB-1 strains missing *phaC2*, *mms16*, *amb0571*, *amb1096*, *amb1121*, or *amb3386*. Scale bar = 0.5  $\mu$ m.

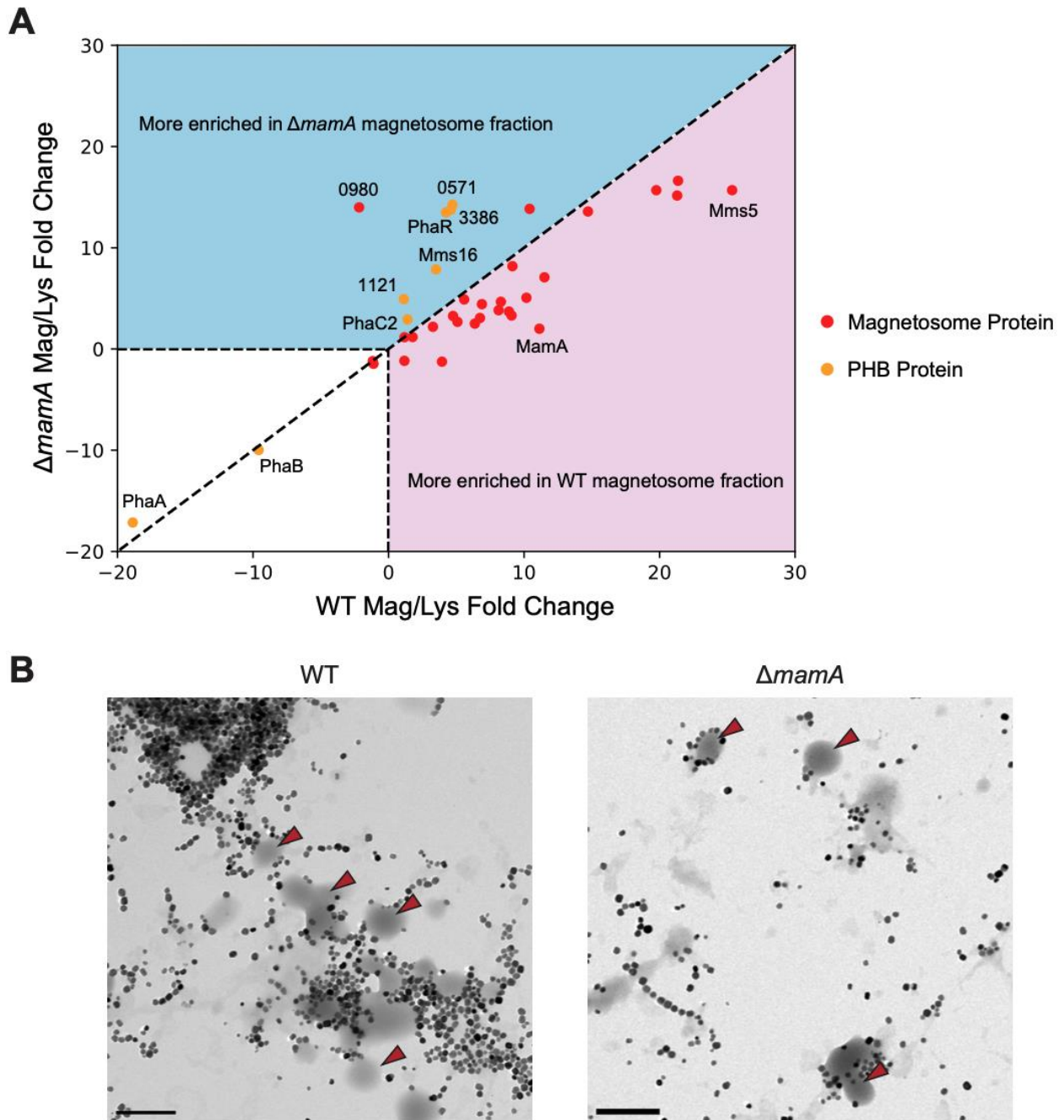


**Figure 4.** Some phasins and PhaR are further enriched in the magnetosome fraction of  $\Delta mamA$  compared to that of WT. **(A)** Proteomics data from biological triplicates comparing the magnetosome fraction and whole lysate of WT and  $\Delta mamA$  strains. Magnetosome proteins (red) and PHB granule proteins (orange) are enriched in the magnetosome fraction. The x- and y-axes show the fold-change difference in protein abundance between the magnetosome fraction and lysate for WT and  $\Delta mamA$ , respectively, where positive values indicate magnetosome enrichment and negative values indicate higher abundance in the lysate. Proteins with equal enrichment between

both strains are plotted near the diagonal dashed line (slope = 1); proteins above this line are more enriched in  $\Delta mamA$  than WT. Only magnetosome and PHB granule proteins are shown here. **(B)** Representative TEM images of the magnetosome fraction collected from AMB-1 WT or  $\Delta mamA$ . Few of the PHBG-like structures are indicated with a red arrow. Scale bar = 0.5 $\mu$ m.

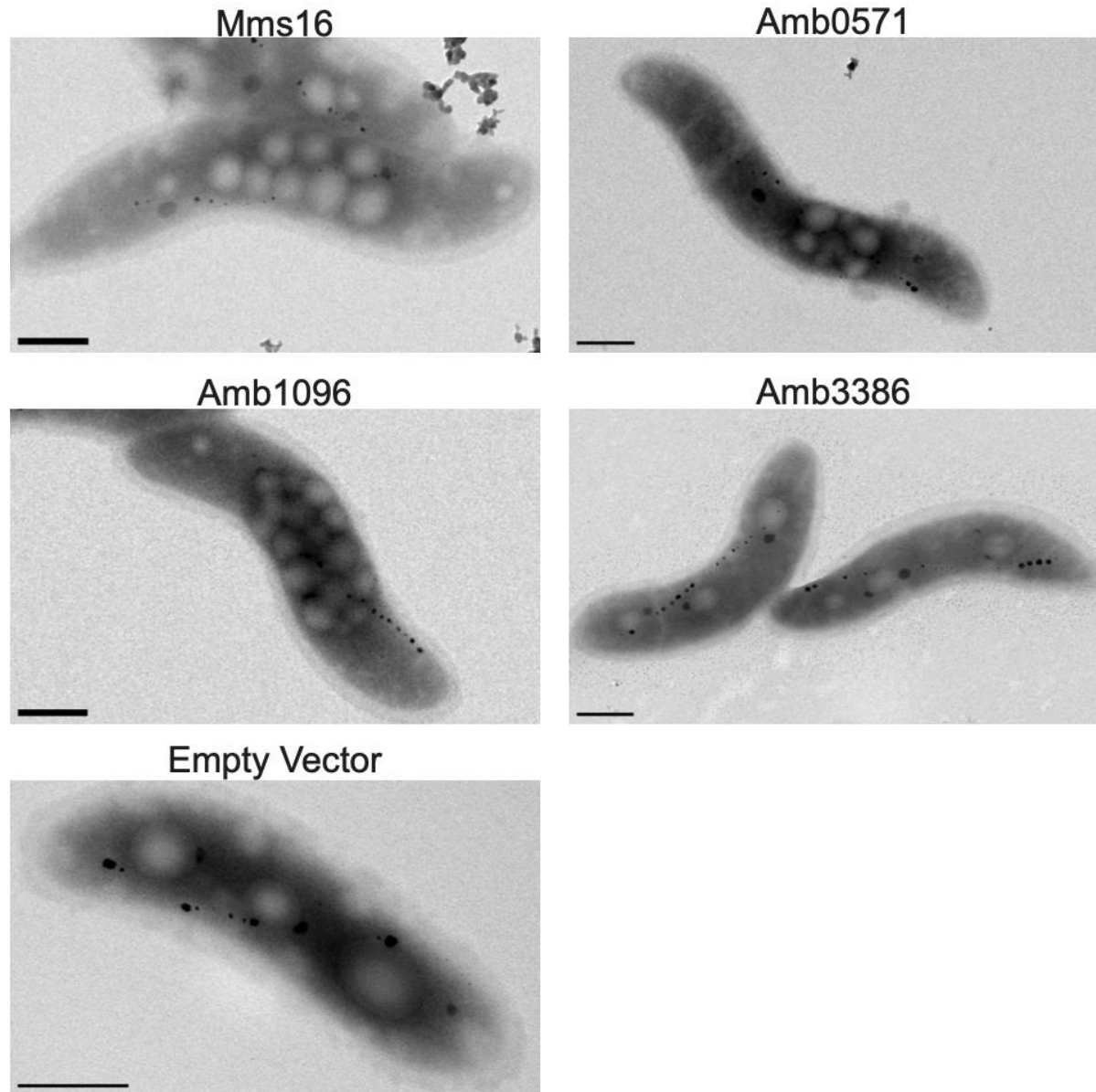


**Figure 5.** Strains overexpressing some phasins have smaller PHBGs that cluster. TEM images of WT expressing Mms16, Amb0571, Amb1096, Amb3386, or an empty vector. All phasins are tagged with a C-terminal sfGFP and expression is driven with a constitutive Tac promoter on a replicative plasmid. Cells overexpressing Mms16, Amb0571, and Amb1096 have PHBGs that aggregate into a single cluster. PHBGs in these clusters are generally smaller than PHBGs in WT with an empty vector. No such PHBG clustering was observed in cells overexpressing Amb3386. Scale bar = 0.5 $\mu$ m.

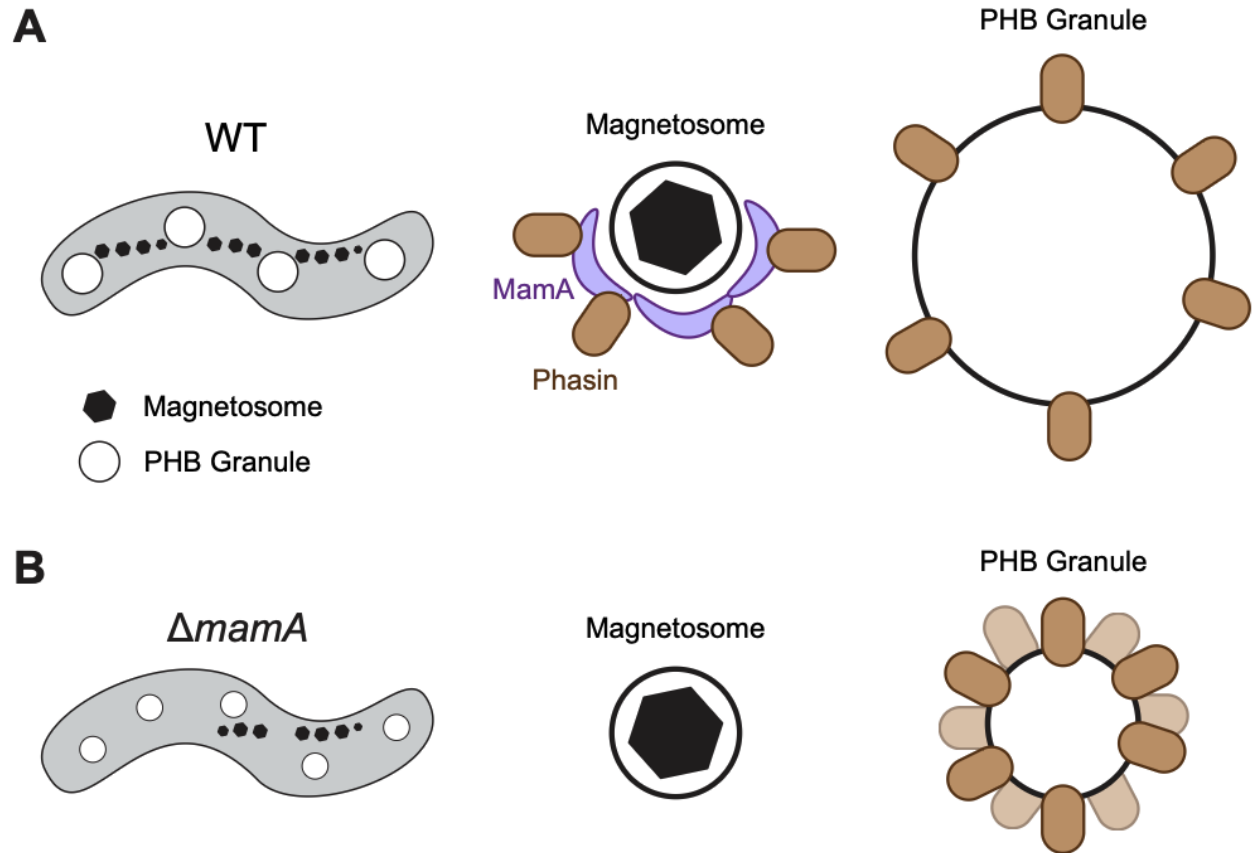


**Figure 6.** Model for how MamA regulates PHBG size. **(A)** In WT AMB-1, MamA (purple) localizes to the magnetosome. MamA can interact with phasins (brown), and therefore sequesters a subpopulation of phasins away from the PHBG. Lower abundance of phasins at the PHBG allows the granule to grow to a larger size. **(B)** In the absence of *mamA*, the phasin sequestration by MamA is lost. Consequently, more phasins localize to the PHBG, restricting the granule size.

| <b>Magnetosome proteins</b> | <b>Gene number</b> | <b>Name</b>                                  | <b># peptide hits</b> | <b># unique peptide hits</b> | <b># of supporting peptides</b> |
|-----------------------------|--------------------|----------------------------------------------|-----------------------|------------------------------|---------------------------------|
|                             | amb0971            | MamA-linker-GFP                              | 97                    | 97                           | 149                             |
|                             | amb0965            | MamK                                         | 26                    | 26                           | 35                              |
|                             | amb0963            | MamE                                         | 20                    | 19                           | 32                              |
|                             | amb0969            | MamO                                         | 12                    | 5                            | 19                              |
|                             | amb0972            | MamQ1                                        | 11                    | 11                           | 16                              |
|                             | amb1005            | MamQ2                                        | 11                    | 11                           | 16                              |
|                             | amb1018            | MamY                                         | 9                     | 9                            | 14                              |
|                             | amb1004            | MamO-like                                    | 8                     | 1                            | 13                              |
|                             | MIS                | MamK-like                                    | 8                     | 8                            | 9                               |
|                             | amb0956            | Mms6                                         | 7                     | 7                            | 11                              |
|                             | amb0952            | MamD                                         | 6                     | 6                            | 8                               |
|                             | amb0967            | MamM                                         | 6                     | 6                            | 6                               |
|                             | amb1002            | LimE                                         | 5                     | 4                            | 6                               |
|                             | amb0954            | MamG                                         | 4                     | 4                            | 6                               |
|                             | amb0964            | MamJ                                         | 4                     | 4                            | 5                               |
|                             | amb0400,           | MamD-like                                    | 4                     | 4                            | 4                               |
|                             | amb0974/           | MamB1/MamB2                                  | 3                     | 3                            | 3                               |
|                             | 1007               |                                              |                       |                              |                                 |
|                             | amb0976            | MamT                                         | 3                     | 3                            | 3                               |
|                             | amb0970            | MamP                                         | 2                     | 2                            | 2                               |
|                             | amb1017            | MamX                                         | 2                     | 2                            | 3                               |
|                             | amb0951            | MamC                                         | 1                     | 1                            | 1                               |
|                             | amb0953            | MamF                                         | 1                     | 1                            | 1                               |
|                             | amb0975            | MamS                                         | 1                     | 1                            | 2                               |
|                             | amb0977            | MamU                                         | 1                     | 1                            | 1                               |
|                             | amb0978            | MamV                                         | 1                     | 1                            | 1                               |
|                             | amb1027            | Mms5                                         | 1                     | 1                            | 1                               |
|                             | MIS                | MamQ-like                                    | 1                     | 1                            | 1                               |
|                             | MIS                | MamE-like                                    | 1                     | 1                            | 1                               |
|                             | MIS                | McaA                                         | 2                     | 2                            | 2                               |
|                             | amb0908            | McaA-like                                    | 2                     | 2                            | 2                               |
|                             | MIS                | McaB                                         | 1                     | 1                            | 1                               |
| <b>PHB proteins</b>         |                    |                                              |                       |                              |                                 |
|                             | amb0546            | Mms16                                        | 19                    | 19                           | 31                              |
|                             | amb0842            | PhaA                                         | 12                    | 12                           | 20                              |
|                             | amb3725            | PhaC2                                        | 11                    | 11                           | 12                              |
|                             | amb1121            | Phasin                                       | 8                     | 8                            | 11                              |
|                             | amb0571            | Phasin                                       | 2                     | 2                            | 3                               |
|                             | amb0843            | PhaB                                         | 2                     | 2                            | 2                               |
|                             | amb0840            | Uncharacterized protein in <i>pha</i> operon | 1                     | 1                            | 1                               |

**Table 1.** Magnetosome and PHBG proteins from MamA-GFP IP-MS. MIS refers to the magnetotaxis gene islet [35].

| Description                                                                       | WT fold change Mag/Lys | $\Delta A$ fold change Mag/Lys |
|-----------------------------------------------------------------------------------|------------------------|--------------------------------|
| MamF                                                                              | 19.7613418             | 15.6869908                     |
| Uncharacterized protein Amb0958                                                   | 4.76114521             | 3.25993619                     |
| MamO                                                                              | 8.29264598             | 4.66537222                     |
| MamD                                                                              | 14.7178068             | 13.5816972                     |
| Uncharacterized membrane-bound protein Amb0959                                    | 3.27785859             | 2.19118345                     |
| Mms5                                                                              | 25.3569736             | 15.6885945                     |
| MmsF                                                                              | 21.3656544             | 16.6186281                     |
| MamE                                                                              | 6.88993183             | 4.42865917                     |
| MamJ                                                                              | 5.58315244             | 4.89302208                     |
| MamR                                                                              | 11.5071451             | 7.08245773                     |
| MamY                                                                              | 10.4092397             | 13.842954                      |
| MamM                                                                              | 6.74826312             | 3.06261399                     |
| MamD                                                                              | 9.14496678             | 8.1811533                      |
| MamT                                                                              | 9.07973994             | 3.30788558                     |
| Mms6                                                                              | 8.1278634              | 3.82971936                     |
| MamP                                                                              | 6.36915883             | 2.50710452                     |
| Periplasmic protein involved in high affinity Fe <sup>2+</sup> transport, Amb0940 | -37.223146             | -270.22239                     |
| MamF                                                                              | 21.301791              | 15.1587136                     |
| MamQ                                                                              | 5.08698202             | 2.68349385                     |
| MamZ                                                                              | 10.177353              | 5.05862958                     |
| CheY-like receiver, Amb0980                                                       | -2.1731063             | 13.9913976                     |
| MamH                                                                              | -1.1114078             | -1.4612112                     |
| MamA                                                                              | 11.1322882             | 2.00055945                     |
| MamC                                                                              | 8.88524642             | 3.69868255                     |
| Mms67                                                                             | -1.165727              | -1.1989011                     |
| MamV                                                                              | 3.94291435             | -1.2537625                     |
| MamN                                                                              | 1.77561134             | 1.1785481                      |
| High-affinity Fe <sup>2+</sup> /Pb <sup>2+</sup> permease, Amb0937                | 1.17034021             | -1.170315                      |
| MamK                                                                              | 1.174646               | 1.15954977                     |

**Table 2.** Magnetosome gene island (MAI) protein enrichment on WT vs.  $\Delta mamA$  ( $\Delta A$ ) magnetosome fraction. Positive values represent enrichment in the magnetosome fraction; negative values represent higher abundance in the lysate compared to the magnetosome fraction.

| <b>Description</b> | <b>WT fold change Mag/Lys</b> | <b><math>\Delta A</math> fold change Mag/Lys</b> |
|--------------------|-------------------------------|--------------------------------------------------|
| PhaA, Amb0842      | -18.87588292                  | -17.14204165                                     |
| Phasin, Amb3386    | 4.584766673                   | 13.75504545                                      |
| PhaB, Amb0843      | -9.58257443                   | -9.982977324                                     |
| Mms16              | 3.501449593                   | 7.85901942                                       |
| Phasin, Amb0571    | 4.721263423                   | 14.24739498                                      |
| PhaR, Amb0839      | 4.241128995                   | 13.50429746                                      |
| Phasin, Amb1121    | 1.129152338                   | 4.927823004                                      |
| PhaC2, Amb3725     | 1.39905093                    | 2.921473422                                      |

**Table 3.** PHB granule protein enrichment on WT vs.  $\Delta mamA$  ( $\Delta A$ ) magnetosome fraction. Positive values represent enrichment in the magnetosome fraction; negative values represent higher abundance in the lysate compared to the magnetosome fraction.

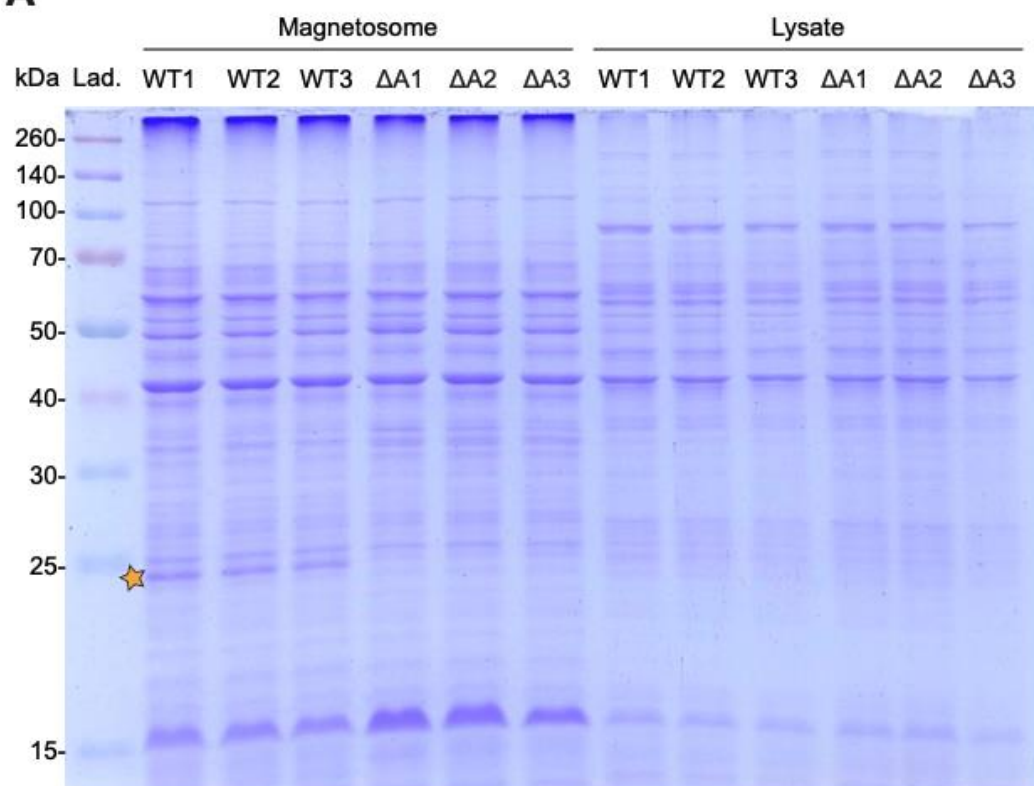
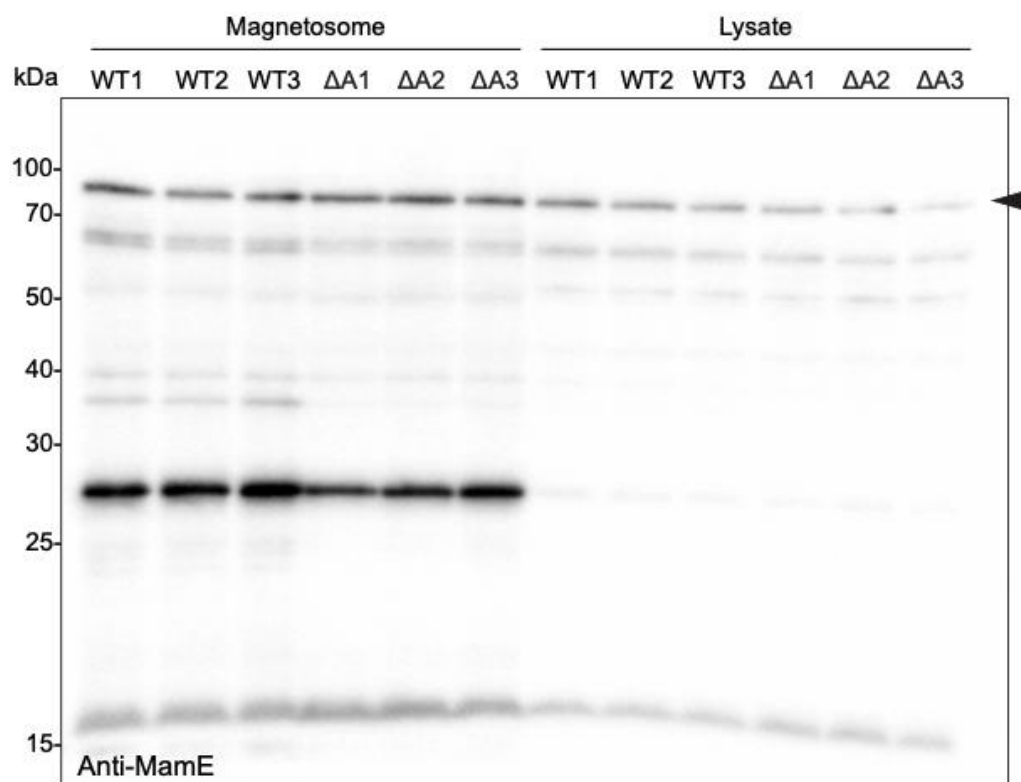
**A****B**

**Figure S1.** The magnetosome fraction from WT and  $\Delta mamA$  strains have a distinct protein content. **(A)** Coomassie blue staining of an SDS-PAGE gel with magnetosome fraction and whole-cell lysate samples from WT and  $\Delta mamA$  ( $\Delta A$ ) strains. Protein abundance was normalized across all samples and three biological replicates are shown. Comparison between the magnetosome fraction and lysate lanes show that there is a distinct cohort of proteins that are enriched in the magnetosome fractions. The star indicates the band that likely represents MamA (24.4kDa). **(B)** Western blot with the same samples in (A) using an anti-MamE primary antibody, showing an enrichment of MamE in the magnetosome fractions, as expected. The black triangle indicates the bands that represent full length MamE (73.5kDa).

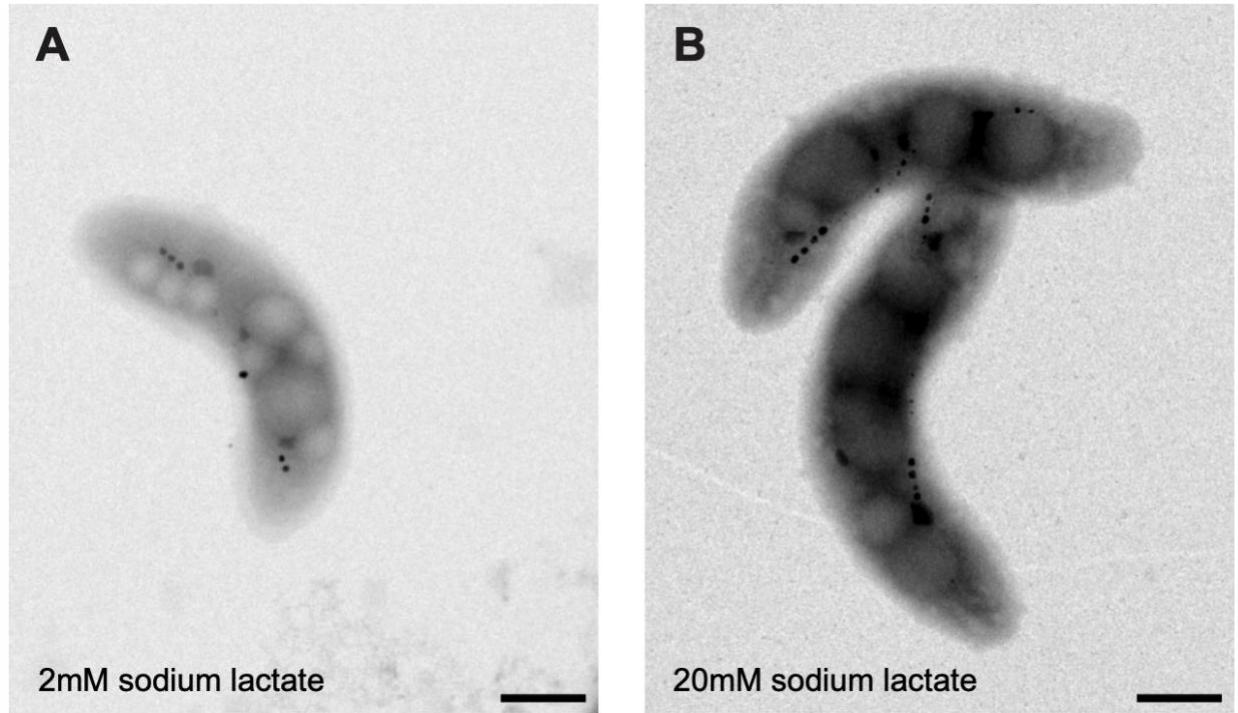


**Figure S2.** PHBGs can interrupt the magnetosome chain. WT AMB-1 was grown in MG where the normal carbon sources were replaced with 2mM (**A**) or 20mM (**B**) sodium lactate to promote PHBG growth, then imaged with TEM. These cells demonstrate how enlarged PHBGs can cause deformation of the magnetosome chain. Scale bar = 0.5 $\mu$ m.

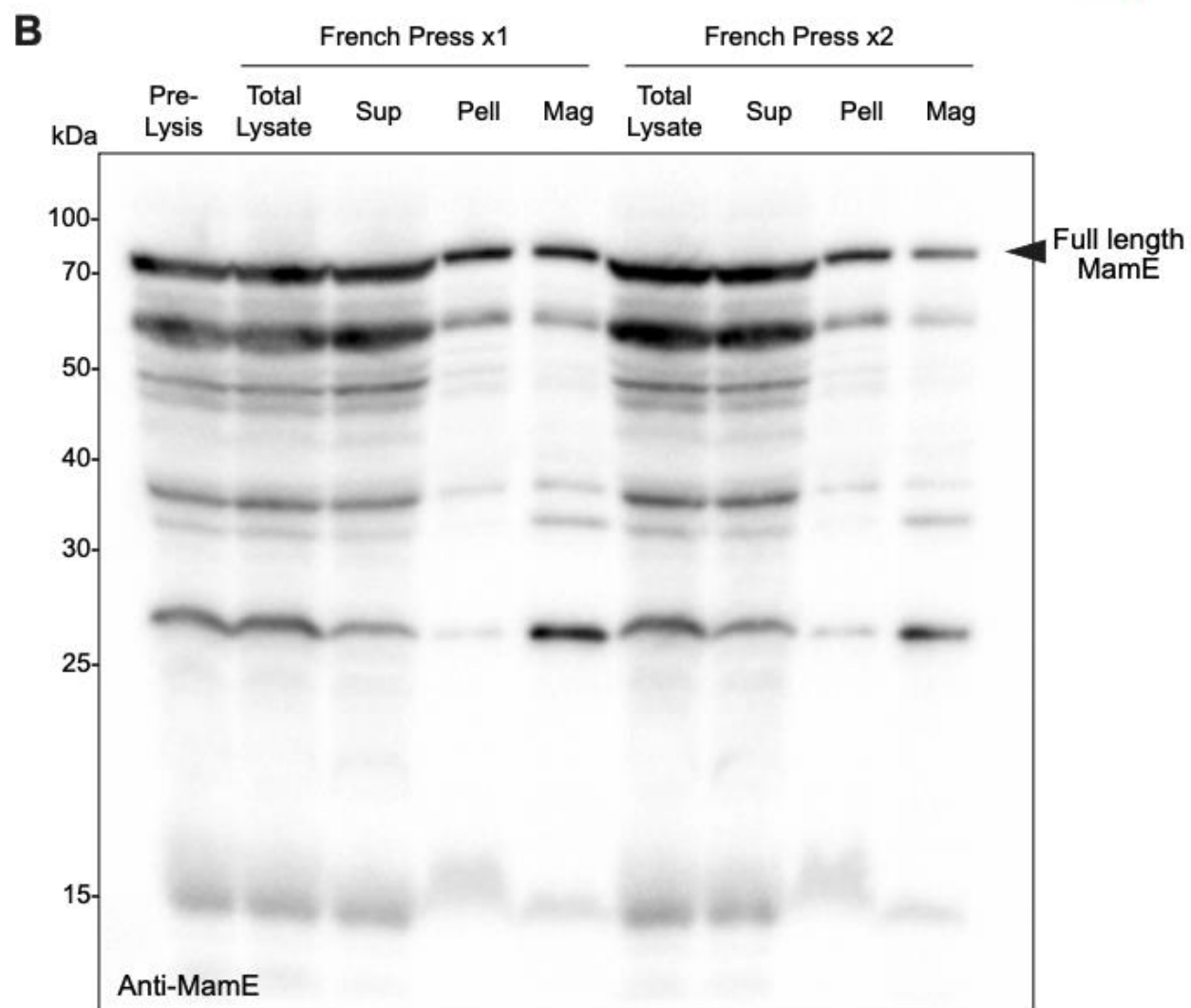
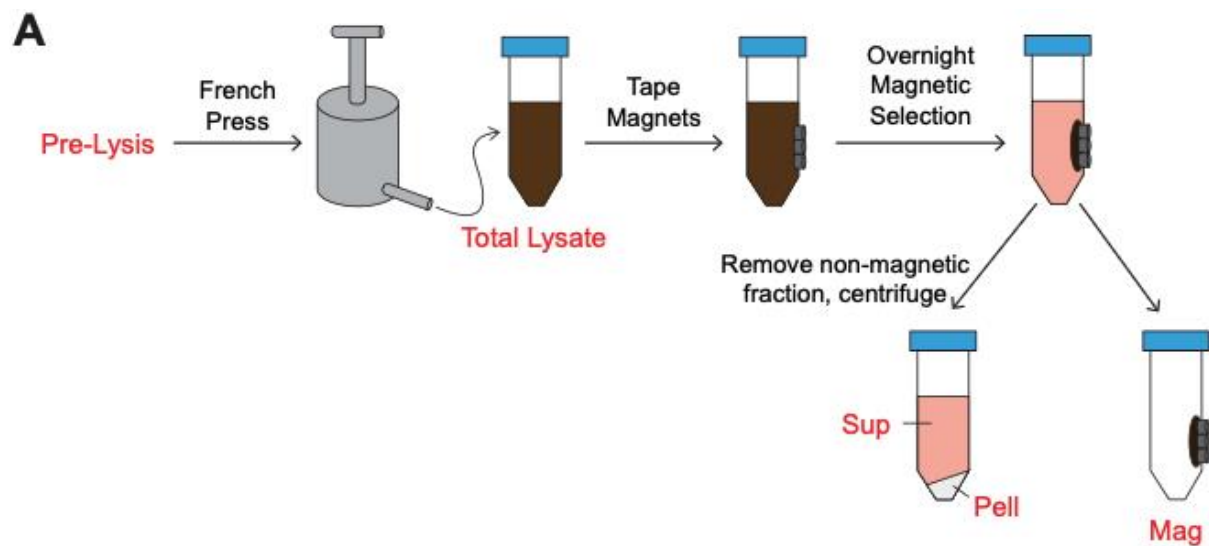


**Figure S3.** Additional passages through the French press likely disrupts the magnetosome membrane. **(A)** Experimental method used to lyse AMB-1 to assess MamE abundance in different fractions in **(B)**. Total lysate from the French press is separated into a magnetic (Mag) and non-magnetic fraction with an overnight magnetic selection. The non-magnetic fraction is centrifuged to separate the supernatant (Sup) and pellet (Pel) fraction. The lysis and magnetic separation are identical to the procedure used for magnetosome purification. Further details can be found in the Materials and Methods section. **(B)** Western blot of the samples collected via the procedure depicted in **(A)** using WT AMB-1. MamE is used as a marker for magnetosome membrane proteins. Samples were passed through the French press (FP) either once or twice. Decreased MamE signal in the magnetic fraction of the second FP pass compared to the first FP pass suggests that there is a loss of magnetosome membrane proteins with subsequent FP passages. The black triangle indicates bands that correspond to full length MamE (73.5kDa).

## **Chapter 4**

Conclusions and Future Directions

Yein Ra

Department of Plant and Microbial Biology, University of California, Berkeley, CA, USA

The overall aim for this thesis work was to identify new mechanisms of organelle organization in bacterial species. To that end, we used *Magnetospirillum magneticum* AMB-1 (hereafter AMB-1) to investigate the regulation of two of its organelles, the magnetosome and the polyhydroxybutyrate granule (PHBG). Chapter 2 presents our discovery that two proteins controlling magnetosome chain organization, McaA and McaB, directly interact with the bacterial actin MamK. I further show using FRAP that the behavior of MamK filaments change when McaA-McaB interactions are prevented. These findings point to a system where precise organelle positioning can be achieved by controlling the dynamics of a cytoskeleton. Chapter 3 details an early investigation on how bacteria organize multiple organelles in a single cell. We discovered that MamA affects the size of the PHBG in addition to regulating the number of mature, crystal-containing magnetosomes. We characterize the PHBG synthesis proteins and phasins, and present preliminary data on how MamA interacts with a subset of PHBG proteins. We use this information to speculate a model of how MamA directs changes in PHBG size by modulating phasin availability. Altogether, the presented work advanced knowledge on two distinct strategies of organelle organization in bacteria.

Many questions remain regarding how McaA and McaB control MamK dynamics. Firstly, the behavior of individual MamK filaments with and without the Mca proteins has not been resolved. To address this gap, single-molecule studies using speckle labeling of MamK monomers can be used to examine changes in MamK dynamics at higher resolution. Furthermore, the effect of McaAB on MamK at the biochemical and structural level has not been addressed. Biochemical assays using purified McaA, McaB, and MamK should be done to identify which behavior(s) of MamK is altered in the presence and absence of the Mca proteins. Additionally, any changes in the higher order structure of MamK filaments caused by McaA and/or McaB can be observed using electron microscopy. Continuing the investigation on how AMB-1 controls MamK will not only inform magnetosome cell biology, but also add to the growing knowledge of how bacterial actins can be regulated.

Additionally, further experimentation is required to fully characterize the MamA-mediated relationship between magnetosomes and PHBGs. More evidence is needed to determine if MamA does indeed sequester phasins away from the PHBG. Proteomics of purified PHBG from WT and  $\Delta mamA$  AMB-1 should be prioritized. Secondly, whether the PHBGs influence any aspect of magnetosomes remains an open question. Given that PHB synthesis appears essential, such studies will likely require knock downs or gene expression repression strategies to deplete expression of the *pha* operon genes. Furthermore, the functions of individual phasin proteins in AMB-1 still remain unknown. These functions can be assessed by either making multi-phasin deletion strains in AMB-1 or using heterologous expression in another species. Lastly, an unexplored part of AMB-1 cell biology is the relationship between PHBG and polyphosphate granules. The two organelles are frequently observed to be physically associated with each other in AMB-1 [113], but whether this is genetically controlled and what the purpose of this association might be is completely unknown. While the magnetosome is no doubt one of the most definitive features of MTB, there are other exciting avenues of studying cell biology using AMB-1. I hope that this work will inspire future researchers in this field to not just study the magnetosome in isolation, but in the context of other cellular processes as well.

## REFERENCES

1. Hug LA, Baker BJ, Anantharaman K, Brown CT, Probst AJ, Castelle CJ, Butterfield CN, Hernsdorf AW, Amano Y, Ise K, et al.: **A new view of the tree of life.** *Nat Microbiol* 2016, **1**:16048.
2. Greening C, Lithgow T: **Formation and function of bacterial organelles.** *Nat Rev Microbiol* 2020, **18**:677–689.
3. Müller FD, Schüler D, Pfeiffer D: **A Compass To Boost Navigation: Cell Biology of Bacterial Magnetotaxis.** *J Bacteriol* 2020, **202**.
4. Gottschling DE, Nyström T: **The Upsides and Downsides of Organelle Interconnectivity.** *Cell* 2017, **169**:24–34.
5. Jain A, Zoncu R: **Organelle transporters and inter-organelle communication as drivers of metabolic regulation and cellular homeostasis.** *Molecular Metabolism* 2022, **60**:101481.
6. Murley A, Nunnari J: **The Emerging Network of Mitochondria-Organelle Contacts.** *Molecular Cell* 2016, **61**:648–653.
7. Ra Y, Komeili A: **Organizing organelles: bacterial strategies for localizing intracellular compartments.** *Current Opinion in Microbiology* 2025, **86**:102614.
8. Byrne ME, Ball DA, Guerquin-Kern J-L, Rouiller I, Wu T-D, Downing KH, Vali H, Komeili A: ***Desulfovibrio magneticus* RS-1 contains an iron- and phosphorus-rich organelle distinct from its bullet-shaped magnetosomes.** *Proceedings of the National Academy of Sciences* 2010, **107**:12263–12268.
9. Giessen TW: **Encapsulins.** *Annual Review of Biochemistry* 2022, **91**:353–380.
10. Savage DF, Afonso B, Chen AH, Silver PA: **Spatially Ordered Dynamics of the Bacterial Carbon Fixation Machinery.** *Science* 2010, **327**:1258–1261.
11. Pfeiffer D, Wahl A, Jendrosseck D: **Identification of a multifunctional protein, PhaM, that determines number, surface to volume ratio, subcellular localization and distribution to daughter cells of poly(3-hydroxybutyrate), PHB, granules in *Ralstonia eutropha* H16: Function of PhaM in *R. eutropha*.** *Molecular Microbiology* 2011, **82**:936–951.
12. Scheffel A, Gruska M, Faivre D, Linaroudis A, Plitzko JM, Schüler D: **An acidic protein aligns magnetosomes along a filamentous structure in magnetotactic bacteria.** *Nature* 2006, **440**:110–114.
13. Rae BD, Long BM, Badger MR, Price GD: **Functions, Compositions, and Evolution of the Two Types of Carboxysomes: Polyhedral Microcompartments That Facilitate CO<sub>2</sub> Fixation in Cyanobacteria and Some Proteobacteria.** *Microbiology and Molecular Biology Reviews* 2013, **77**:357–379.
14. MacCready JS, Hakim P, Young EJ, Hu L, Liu J, Osteryoung KW, Vecchiarelli AG, Ducat DC: **Protein gradients on the nucleoid position the carbon-fixing organelles of cyanobacteria.** *eLife* 2018, **7**:e39723.
15. MacCready JS, Tran L, Basalla JL, Hakim P, Vecchiarelli AG: **The McdAB system positions  $\alpha$ -carboxysomes in proteobacteria.** *Molecular Microbiology* 2021, **116**:277–297.
16. Jalal ASB, Le TBK: **Bacterial chromosome segregation by the ParABS system.** *Open Biology* 2020, **10**:200097.

17. Schumacher MA, Henderson M, Zhang H: **Structures of maintenance of carboxysome distribution Walker-box McdA and McdB adaptor homologs.** *Nucleic Acids Research* 2019, **47**:5950–5962.
18. Hakim P, Hoang Y, Vecchiarelli AG: **Dissection of the ATPase active site of McdA reveals the sequential steps essential for carboxysome distribution.** *MBoC* 2021, **32**:ar11.
19. Alberti S: **Phase separation in biology.** *Current Biology* 2017, **27**:R1097–R1102.
20. MacCready JS, Basalla JL, Vecchiarelli AG: **Origin and Evolution of Carboxysome Positioning Systems in Cyanobacteria.** *Molecular Biology and Evolution* 2020, **37**:1434–1451.
21. Basalla JL, Mak CA, Byrne JA, Ghalmi M, Hoang Y, Vecchiarelli AG: **Dissecting the phase separation and oligomerization activities of the carboxysome positioning protein McdB.** *eLife* 2023, **12**:e81362.
22. Basalla JL, Ghalmi M, Hoang Y, Dow RE, Vecchiarelli AG: **An invariant C-terminal tryptophan in McdB mediates its interaction and positioning function with carboxysomes.** *MBoC* 2024, **35**:ar107.
23. Rillema R, Hoang Y, MacCready JS, Vecchiarelli AG: **Carboxysome Mispositioning Alters Growth, Morphology, and Rubisco Level of the Cyanobacterium *Synechococcus elongatus* PCC 7942.** *mBio* 2021, **12**:e02696-20.
24. Yang M, Wenner N, Dykes GF, Li Y, Zhu X, Sun Y, Huang F, Hinton JCD, Liu L-N: **Biogenesis of a bacterial metabosome for propanediol utilization.** *Nat Commun* 2022, **13**:2920.
25. Paulus A, Ahrens F, Schraut A, Hofmann H, Schiller T, Sura T, Becher D, Uebe R: **MamF-like proteins are distant Tic20 homologs involved in organelle assembly in bacteria.** *Nat Commun* 2024, **15**:10657.
26. Komeili A, Li Z, Newman DK, Jensen GJ: **Magnetosomes Are Cell Membrane Invaginations Organized by the Actin-Like Protein MamK.** *Science* 2006, **311**:242–245.
27. Toro-Nahuelpan M, Giacomelli G, Raschdorf O, Borg S, Plitzko JM, Bramkamp M, Schüler D, Müller F-D: **MamY is a membrane-bound protein that aligns magnetosomes and the motility axis of helical magnetotactic bacteria.** *Nat Microbiol* 2019, **4**:1978–1989.
28. Katzmann E, Scheffel A, Gruska M, Plitzko JM, Schüler D: **Loss of the actin-like protein MamK has pleiotropic effects on magnetosome formation and chain assembly in *Magnetospirillum gryphiswaldense*.** *Molecular Microbiology* 2010, **77**:208–224.
29. Ozyamak E, Kollman J, Agard DA, Komeili A: **The Bacterial Actin MamK: IN VITRO ASSEMBLY BEHAVIOR AND FILAMENT ARCHITECTURE.** *Journal of Biological Chemistry* 2013, **288**:4265–4277.
30. Toro-Nahuelpan M, Müller FD, Klumpp S, Plitzko JM, Bramkamp M, Schüler D: **Segregation of prokaryotic magnetosomes organelles is driven by treadmilling of a dynamic actin-like MamK filament.** *BMC Biol* 2016, **14**:88.
31. Cornejo E, Subramanian P, Li Z, Jensen GJ, Komeili A: **Dynamic Remodeling of the Magnetosome Membrane Is Triggered by the Initiation of Biomineralization.** *mBio* 2016, **7**:e01898-15.

32. Draper O, Byrne ME, Li Z, Keyhani S, Barrozo JC, Jensen G, Komeili A: **MamK, a bacterial actin, forms dynamic filaments in vivo that are regulated by the acidic proteins MamJ and LimJ: Dynamic MamK filaments are regulated by MamJ and LimJ.** *Molecular Microbiology* 2011, **82**:342–354.
33. Jogler C, Wanner G, Kolinko S, Niebler M, Amann R, Petersen N, Kube M, Reinhardt R, Schöler D: **Conservation of proteobacterial magnetosome genes and structures in an uncultivated member of the deep-branching *Nitrospira* phylum.** *Proceedings of the National Academy of Sciences* 2011, **108**:1134–1139.
34. Wan J, Monteil CL, Taoka A, Ernie G, Park K, Amor M, Taylor-Cornejo E, Lefevre CT, Komeili A: **McaA and McaB control the dynamic positioning of a bacterial magnetic organelle.** *Nat Commun* 2022, **13**:5652.
35. Rioux J-B, Philippe N, Pereira S, Pignol D, Wu L-F, Ginot N: **A Second Actin-Like MamK Protein in *Magnetospirillum magneticum* AMB-1 Encoded Outside the Genomic Magnetosome Island.** *PLOS ONE* 2010, **5**:e9151.
36. Abreu N, Mannoubi S, Ozyamak E, Pignol D, Ginot N, Komeili A: **Interplay between Two Bacterial Actin Homologs, MamK and MamK-Like, Is Required for the Alignment of Magnetosome Organelles in *Magnetospirillum magneticum* AMB-1.** *J Bacteriol* 2014, **196**:3111–3121.
37. Lefèvre CT, Bazylinski DA: **Ecology, Diversity, and Evolution of Magnetotactic Bacteria.** *Microbiol Mol Biol Rev* 2013, **77**:497–526.
38. Uzun M, Koziaeva V, Dziuba M, Alekseeva L, Krutkina M, Sukhacheva M, Baslerov R, Grouzdev D: **Recovery and genome reconstruction of novel magnetotactic *Elusimicrobiota* from bog soil.** *The ISME Journal* 2023, **17**:204–214.
39. Rahn-Lee L, Byrne ME, Zhang M, Sage DL, Glenn DR, Milbourne T, Walsworth RL, Vali H, Komeili A: **A Genetic Strategy for Probing the Functional Diversity of Magnetosome Formation.** *PLOS Genetics* 2015, **11**:e1004811.
40. Komeili A, Vali H, Beveridge TJ, Newman DK: **Magnetosome vesicles are present before magnetite formation, and MamA is required for their activation.** *Proceedings of the National Academy of Sciences* 2004, **101**:3839–3844.
41. Russell VV, Iavarone AT, Ozyamak E, Grant C, Komeili A: **A network of coiled-coil and actin-like proteins controls the cellular organization of magnetosome organelles in deep-branching magnetotactic bacteria.** *Nat Commun* 2025, **16**:11453.
42. Pohl A, Berger F, Sullan RMA, Valverde-Tercedor C, Freindl K, Spiridis N, Lefèvre CT, Menguy N, Klumpp S, Blank KG, et al.: **Decoding Biomineralization: Interaction of a Mad10-Derived Peptide with Magnetite Thin Films.** *Nano Lett* 2019, **19**:8207–8215.
43. Maestro B, Sanz JM: **Polyhydroxyalkanoate-associated phasins as phylogenetically heterogeneous, multipurpose proteins.** *Microbial Biotechnology* 2017, **10**:1323–1337.
44. Jendrossek D: **Polyhydroxyalkanoate Granules Are Complex Subcellular Organelles (Carbonosomes).** *Journal of Bacteriology* 2009, **191**:3195–3202.
45. Kutralam-Muniasamy G, Corona-Hernandez J, Narayanasamy R-K, Marsch R, Pérez-Guevara F: **Phylogenetic diversification and developmental**

- implications of poly-(R)-3-hydroxyalkanoate gene cluster assembly in prokaryotes. *FEMS Microbiology Letters* 2017, **364**:fnx135.
46. Obruca S, Sedlacek P, Slaninova E, Fritz I, Daffert C, Meixner K, Sedrlova Z, Koller M: **Novel unexpected functions of PHA granules.** *Appl Microbiol Biotechnol* 2020, **104**:4795–4810.
  47. Wahl A, Schuth N, Pfeiffer D, Nussberger S, Jendrosseck D: **PHB granules are attached to the nucleoid via PhaM in *Ralstonia eutropha*.** *BMC Microbiol* 2012, **12**:262.
  48. Galán B, Dinjaski N, Maestro B, de Eugenio LI, Escapa IF, Sanz JM, García JL, Prieto MA: **Nucleoid-associated PhaF phasin drives intracellular location and segregation of polyhydroxyalkanoate granules in *Pseudomonas putida* KT2442.** *Molecular Microbiology* 2011, **79**:402–418.
  49. Hauf W, Watzer B, Roos N, Klotz A, Forchhammer K: **Photoautotrophic Polyhydroxybutyrate Granule Formation Is Regulated by Cyanobacterial Phasin PhaP in *Synechocystis* sp. Strain PCC 6803.** *Applied and Environmental Microbiology* 2015, **81**:4411–4422.
  50. Bresan S, Jendrosseck D: **New Insights into PhaM-PhaC-Mediated Localization of Polyhydroxybutyrate Granules in *Ralstonia eutropha* H16.** *Appl Environ Microbiol* 2017, **83**:e00505-17.
  51. Beeby M, Cho M, Stubbe J, Jensen GJ: **Growth and Localization of Polyhydroxybutyrate Granules in *Ralstonia eutropha*.** *Journal of Bacteriology* 2012, **194**:1092–1099.
  52. Salinas AL, Osorio A, Legorreta-Hissner T, Lara-Martinez R, Jimenez-Garcia LF, Camarena L, Poggio S: **A new type of phasin characterized by the presence of a helix-hairpin-helix domain is required for normal polyhydroxybutyrate accumulation and granule organization in *Caulobacter crescentus*.** *Molecular Microbiology* 2023, **120**:307–323.
  53. Pfeiffer D, Jendrosseck D: **PhaM Is the Physiological Activator of Poly(3-Hydroxybutyrate) (PHB) Synthase (PhaC1) in *Ralstonia eutropha*.** *Applied and Environmental Microbiology* 2014, **80**:555–563.
  54. Juengert JR, Patterson C, Jendrosseck D: **Poly(3-Hydroxybutyrate) (PHB) Polymerase PhaC1 and PHB Depolymerase PhaZa1 of *Ralstonia eutropha* Are Phosphorylated In Vivo.** *Applied and Environmental Microbiology* 2018, **84**:e00604-18.
  55. Choi SY, Oh J, Jung J, Park Y, Lee SY: **Three-dimensional label-free visualization and quantification of polyhydroxyalkanoates in individual bacterial cell in its native state.** *Proc Natl Acad Sci USA* 2021, **118**:e2103956118.
  56. Tarazona NA, Hernández-Arriaga AM, Kniewel R, Prieto MA: **Phasin interactome reveals the interplay of PhaF with the polyhydroxyalkanoate transcriptional regulatory protein PhaD in *Pseudomonas putida*.** *Environmental Microbiology* 2020, **22**:3922–3936.
  57. Kelly S, Tham J-L, McKeever K, Dillon E, O'Connell D, Scholz D, Simpson JC, O'Connor K, Narancic T, Cagney G: **Comprehensive Proteomics Analysis of Polyhydroxyalkanoate (PHA) Biology in *Pseudomonas putida* KT2440: The**

- Outer Membrane Lipoprotein OprL is a Newly Identified Phasin.** *Mol Cell Proteomics* 2024, **23**:100765.
58. Chawla R, Klupt S, Patsalo V, Williamson JR, Racki LR: **The Histone H1-Like Protein AlgP Facilitates Even Spacing of Polyphosphate Granules in *Pseudomonas aeruginosa*.** *mBio* 2022, **13**:e02463-21.
  59. Deretic V, Konyecsni WM: **A procaryotic regulatory factor with a histone H1-like carboxy-terminal domain: clonal variation of repeats within *algP*, a gene involved in regulation of mucoidy in *Pseudomonas aeruginosa*.** *J Bacteriol* 1990, **172**:5544–5554.
  60. Henry JT, Crosson S: **Chromosome replication and segregation govern the biogenesis and inheritance of inorganic polyphosphate granules.** *MBoC* 2013, **24**:3177–3186.
  61. Frank C, Pfeiffer D, Aktas M, Jendrosseck D: **Migration of Polyphosphate Granules in *Agrobacterium tumefaciens*.** *Microbial Physiology* 2022, **32**:71–82.
  62. Pallerla SR, Knebel S, Polen T, Klauth P, Hollender J, Wendisch VF, Schoberth SM: **Formation of volutin granules in *Corynebacterium glutamicum*.** *FEMS Microbiology Letters* 2005, **243**:133–140.
  63. Werner JN, Chen EY, Guberman JM, Zippilli AR, Irgon JJ, Gitai Z: **Quantitative genome-scale analysis of protein localization in an asymmetric bacterium.** *Proceedings of the National Academy of Sciences* 2009, **106**:7858–7863.
  64. Christen B, Fero MJ, Hillson NJ, Bowman G, Hong S-H, Shapiro L, McAdams HH: **High-throughput identification of protein localization dependency networks.** *Proceedings of the National Academy of Sciences* 2010, **107**:4681–4686.
  65. Zhang R, Huang Y, Li M, Wang L, Li B, Xia A, Li Y, Yang S, Jin F: **High-throughput, microscopy-based screening and quantification of genetic elements.** *mLife* 2023, **2**:450–461.
  66. Hümpfer N, Thielhorn R, Ewers H: **Expanding boundaries – a cell biologist’s guide to expansion microscopy.** *Journal of Cell Science* 2024, **137**:jcs260765.
  67. Kunz TC, Rühling M, Moldovan A, Paprotka K, Kozjak-Pavlovic V, Rudel T, Fraunholz M: **The Expandables: Cracking the Staphylococcal Cell Wall for Expansion Microscopy.** *Front Cell Infect Microbiol* 2021, **11**.
  68. Castagnini D, Palma K, Jara-Wilde J, Navarro N, González MJ, Toledo J, Canales-Huerta N, Scavone P, Härtel S: ***Proteus mirabilis* biofilm expansion microscopy yields over 4-fold magnification for super-resolution of biofilm structure and subcellular DNA organization.** *Journal of Microbiological Methods* 2024, **220**:106927.
  69. Correa SS, Schultz J, Zahodnik-Huntington B, Naschberger A, Rosado AS: **Carboxysomes: The next frontier in biotechnology and sustainable solutions.** *Biotechnology Advances* 2025, **79**:108511.
  70. Wang J, Xing Y, Ngatio M, Bies P, Xu LL, Xing L, Zarea A, Makela AV, Contag CH, Li J: **Engineering Magnetotactic Bacteria as Medical Microrobots.** *Advanced Materials* 2025, **37**:2416966.
  71. Ra Y, Pan Y, Chen T, Dixon K, Taoka A, Komeili A: **Magnetosome organelles are organized through interactions between McaA and McaB that alter the dynamics of the bacterial actin-like protein MamK.** *mBio* 2026, n/a:e00276-26.

72. Greening C, Lithgow T: **Formation and function of bacterial organelles**. *Nat Rev Microbiol* 2020, **18**:677–689.
73. Iancu CV, Morris DM, Dou Z, Heinhorst S, Cannon GC, Jensen GJ: **Organization, Structure, and Assembly of  $\alpha$ -Carboxysomes Determined by Electron Cryotomography of Intact Cells**. *Journal of Molecular Biology* 2010, **396**:105–117.
74. Dobro MJ, Oikonomou CM, Piper A, Cohen J, Guo K, Jensen T, Tadayon J, Donermeyer J, Park Y, Solis BA, et al.: **Uncharacterized Bacterial Structures Revealed by Electron Cryotomography**. *Journal of Bacteriology* 2017, **199**:10.1128/jb.00100-17.
75. Bazylinski DA, Frankel RB: **Magnetosome formation in prokaryotes**. *Nat Rev Microbiol* 2004, **2**:217–230.
76. Blakemore R. **Magnetotactic Bacteria**. *Science* 1975, **190**:377-379.
77. Pfeiffer D, Schüler D: **Quantifying the Benefit of a Dedicated “Magnetoskeleton” in Bacterial Magnetotaxis by Live-Cell Motility Tracking and Soft Agar Swimming Assay**. *Appl Environ Microbiol* 2020, **86**:e01976-19.
78. Taoka A, Kiyokawa A, Uesugi C, Kikuchi Y, Oestreich Z, Morii K, Eguchi Y, Fukumori Y: **Tethered Magnets Are the Key to Magnetotaxis: Direct Observations of *Magnetospirillum magneticum* AMB-1 Show that MamK Distributes Magnetosome Organelles Equally to Daughter Cells**. *mBio* 2017, **8**:e00679-17.
79. Löwe J, He S, Scheres SHW, Savva CG: **X-ray and cryo-EM structures of monomeric and filamentous actin-like protein MamK reveal changes associated with polymerization**. *Proceedings of the National Academy of Sciences* 2016, **113**:13396–13401.
80. Pan Y, Kikuchi Y, Saito T, Fukumori Y, Taoka A: **Visualizing the dynamic polymerization of the bacterial actin-like cytoskeleton for magnetic organelle positioning**. *Sci Rep* 2025, **15**:44508.
81. Scheffel A, Schüler D: **The Acidic Repetitive Domain of the *Magnetospirillum gryphiswaldense* MamJ Protein Displays Hypervariability but Is Not Required for Magnetosome Chain Assembly**. *J Bacteriol* 2007, **189**:6437–6446.
82. Garner EC, Campbell CS, Weibel DB, Mullins RD: **Reconstitution of DNA Segregation Driven by Assembly of a Prokaryotic Actin Homolog**. *Science* 2007, **315**:1270–1274.
83. Polka JK, Kollman JM, Mullins RD: **Accessory factors promote AlfA-dependent plasmid segregation by regulating filament nucleation, disassembly, and bundling**. *Proceedings of the National Academy of Sciences* 2014, **111**:2176–2181.
84. Petek-Seoane NA, Rodriguez J, Derman AI, Royal SG, Lord SJ, Lawrence R, Pogliano J, Mullins RD: **Polymer dynamics of Alp7A reveals how two critical concentrations govern assembly of dynamically unstable actin-like proteins**. *MBoC* 2024, **35**:ar145.
85. Burgess JG, Kawaguchi R, Sakaguchi T, Thornhill RH, Matsunaga T: **Evolutionary relationships among *Magnetospirillum* strains inferred from phylogenetic analysis of 16S rDNA sequences**. *J Bacteriol* 1993, **175**:6689–6694.

86. Ludwiczak J, Winski A, Szczepaniak K, Alva V, Dunin-Horkawicz S: **DeepCoil—a fast and accurate prediction of coiled-coil domains in protein sequences.** *Bioinformatics* 2019, **35**:2790–2795.
87. Abramson J, Adler J, Dunger J, Evans R, Green T, Pritzel A, Ronneberger O, Willmore L, Ballard AJ, Bambrick J, et al.: **Accurate structure prediction of biomolecular interactions with AlphaFold 3.** *Nature* 2024, **630**:493–500.
88. Arakaki A, Kikuchi D, Tanaka M, Yamagishi A, Yoda T, Matsunaga T: **Comparative Subcellular Localization Analysis of Magnetosome Proteins Reveals a Unique Localization Behavior of Mms6 Protein onto Magnetite Crystals.** *Journal of Bacteriology* 2016, **198**:2794–2802.
89. Bickley CD, Wan J, Komeili A: **Intrinsic and extrinsic determinants of conditional localization of Mms6 to magnetosome organelles in *Magnetospirillum magneticum* AMB-1.** *Journal of Bacteriology* 2024, **206**:e00008-24.
90. Murat D, Quinlan A, Vali H, Komeili A: **Comprehensive genetic dissection of the magnetosome gene island reveals the step-wise assembly of a prokaryotic organelle.** *Proceedings of the National Academy of Sciences* 2010, **107**:5593–5598.
91. Schüller D: **Genetics and cell biology of magnetosome formation in magnetotactic bacteria.** *FEMS Microbiol Rev* 2008, **32**:654–672.
92. Bidaud CC, Monteil CL, Menguy N, Busigny V, Jézéquel D, Viollier É, Travert C, Skouri-Panet F, Benzerara K, Lefevre CT, et al.: **Biogeochemical Niche of Magnetotactic Cocci Capable of Sequestering Large Polyphosphate Inclusions in the Anoxic Layer of the Lake Pavin Water Column.** *Front Microbiol* 2022, **12**.
93. Awal RP, Müller FD, Pfeiffer D, Monteil CL, Perrière G, Lefèvre CT, Schüller D: **Experimental analysis of diverse actin-like proteins from various magnetotactic bacteria by functional expression in *Magnetospirillum gryphiswaldense*.** *mBio* 2023, **14**:e01649-23.
94. Pollard TD: **Actin and Actin-Binding Proteins.** *Cold Spring Harb Perspect Biol* 2016, **8**:a018226.
95. Charles-Orszag A, Petek-Seoane NA, Mullins RD: **Archaeal actins and the origin of a multi-functional cytoskeleton.** *Journal of Bacteriology* 2024, **206**:e00348-23.
96. Ozyamak E, Kollman JM, Komeili A: **Bacterial Actins and Their Diversity.** *Biochemistry* 2013, **52**:6928–6939.
97. Hershey DM, Browne PJ, Iavarone AT, Teyra J, Lee EH, Sidhu SS, Komeili A: **Magnetite Biomineralization in *Magnetospirillum magneticum* Is Regulated by a Switch-like Behavior in the HtrA Protease MamE.** *Journal of Biological Chemistry* 2016, **291**:17941–17952.
98. Xu T, Park SK, Venable JD, Wohlschlegel JA, Diedrich JK, Cociorva D, Lu B, Liao L, Hewel J, Han X, et al.: **ProLuCID: An improved SEQUEST-like algorithm with enhanced sensitivity and specificity.** *Journal of Proteomics* 2015, **129**:16–24.
99. Cociorva D, L. Tabb D, Yates JR: **Validation of Tandem Mass Spectrometry Database Search Results Using DTASelect.** *Current Protocols in Bioinformatics* 2006, **16**:13.4.1-13.4.14.

100. Tabb DL, McDonald WH, Yates JR: **DTASelect and Contrast: Tools for Assembling and Comparing Protein Identifications from Shotgun Proteomics.** *J Proteome Res* 2002, **1**:21–26.
101. Park SK, Venable JD, Xu T, Yates JR: **A quantitative analysis software tool for mass spectrometry–based proteomics.** *Nat Methods* 2008, **5**:319–322.
102. Park SKR, Aslanian A, McClatchy DB, Han X, Shah H, Singh M, Rauniyar N, Moresco JJ, Pinto AFM, Diedrich JK, et al.: **Census 2: isobaric labeling data analysis.** *Bioinformatics* 2014, **30**:2208–2209.
103. Eng JK, McCormack AL, Yates JR: **An approach to correlate tandem mass spectral data of peptides with amino acid sequences in a protein database.** *J Am Soc Mass Spectrom* 1994, **5**:976–989.
104. Katoh K, Standley DM: **MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability.** *Mol Biol Evol* 2013, **30**:772–780.
105. Waterhouse AM, Procter JB, Martin DMA, Clamp M, Barton GJ: **Jalview Version 2—a multiple sequence alignment editor and analysis workbench.** *Bioinformatics* 2009, **25**:1189–1191.
106. Meng EC, Goddard TD, Pettersen EF, Couch GS, Pearson ZJ, Morris JH, Ferrin TE: **UCSF ChimeraX: Tools for structure building and analysis.** *Protein Science* 2023, **32**:e4792.
107. Murat D, Falahati V, Bertinetti L, Csencsits R, Körnig A, Downing K, Faivre D, Komeili A: **The magnetosome membrane protein, MmsF, is a major regulator of magnetite biomineralization in *Magnetospirillum magneticum* AMB-1.** *Molecular Microbiology* 2012, **85**:684–699.
108. Kovach ME, Elzer PH, Steven Hill D, Robertson GT, Farris MA, Roop RM, Peterson KM: **Four new derivatives of the broad-host-range cloning vector pBBR1MCS, carrying different antibiotic-resistance cassettes.** *Gene* 1995, **166**:175–176.
109. Racki LR, Tocheva EI, Dieterle MG, Sullivan MC, Jensen GJ, Newman DK: **Polyphosphate granule biogenesis is temporally and functionally tied to cell cycle exit during starvation in *Pseudomonas aeruginosa*.** *Proceedings of the National Academy of Sciences* 2017, **114**:E2440–E2449.
110. Hamm CW, Gray MJ: **Inorganic polyphosphate and the stringent response coordinately control cell division and cell morphology in *Escherichia coli*.** *mBio* 2024, **16**:e03511-24.
111. Dudley CE, Foust DJ, Savage DF, Biteen JS, Vecchiarelli AG: **Polyphosphate acts as an architectural regulator of carbon fixation and nucleoid structure in cyanobacteria.** 2026, doi:10.64898/2026.04.09.717567.
112. Blakemore R: **Magnetotactic bacteria.** *Science* 1975, **190**:377–379.
113. Su Q, Andersen HR, Bazyliński DA, Jensen MM: **Effect of oxic and anoxic conditions on intracellular storage of polyhydroxyalkanoate and polyphosphate in *Magnetospirillum magneticum* strain AMB-1.** *Front Microbiol* 2023, **14**.
114. Fernández-Castané A, Li H, Thomas ORT, Overton TW: **Development of a simple intensified fermentation strategy for growth of *Magnetospirillum gryphiswaldense* MSR-1: Physiological responses to changing environmental conditions.** *New Biotechnology* 2018, **46**:22–30.

115. Okuda Y, Denda K, Fukumori Y: **Cloning and sequencing of a gene encoding a new member of the tetratricopeptide protein family from magnetosomes of *Magnetospirillum magnetotacticum***. *Gene* 1996, **171**:99–102.
116. Zeytuni N, Ozyamak E, Ben-Harush K, Davidov G, Levin M, Gat Y, Moyal T, Brik A, Komeili A, Zarivach R: **Self-recognition mechanism of MamA, a magnetosome-associated TPR-containing protein, promotes complex assembly**. *Proceedings of the National Academy of Sciences* 2011, **108**:E480–E487.
117. Nguyen HV, Suzuki E, Oestreicher Z, Minamide H, Endoh H, Fukumori Y, Taoka A: **A protein-protein interaction in magnetosomes: TPR protein MamA interacts with an Mms6 protein**. *Biochemistry and Biophysics Reports* 2016, **7**:39–44.
118. Meier DAO, Carlson HansK, Coates JD: **Polyhydroxyalkanoates as ecological currencies across the microbial tree of life**. In *Advances in Microbial Physiology*. Elsevier; 2026:S0065291126000032.
119. Pötter M, Madkour MH, Mayer F, Steinbüchel A: **Regulation of phasin expression and polyhydroxyalkanoate (PHA) granule formation in *Ralstonia eutropha* H16**. *Microbiology* 2002, **148**:2413–2426.
120. McCausland HC, Wetmore KM, Arkin AP, Komeili A: **Global Analysis of Biomineralization Genes in *Magnetospirillum magneticum* AMB-1**. *mSystems* 2022, **7**:e01037-21.
121. Wieczorek R, Pries A, Steinbüchel A, Mayer F: **Analysis of a 24-kilodalton protein associated with the polyhydroxyalkanoic acid granules in *Alcaligenes eutrophus***. *J Bacteriol* 1995, **177**:2425–2435.
122. Handrick R, Reinhardt S, Schultheiss D, Reichart T, Schüler D, Jendrossek V, Jendrossek D: **Unraveling the Function of the *Rhodospirillum rubrum* Activator of Polyhydroxybutyrate (PHB) Degradation: the Activator Is a PHB-Granule-Bound Protein (Phasin)**. *J Bacteriol* 2004, **186**:2466–2475.
123. Schultheiss D, Handrick R, Jendrossek D, Hanzlik M, Schüler D: **The Presumptive Magnetosome Protein Mms16 Is a Poly(3-Hydroxybutyrate) Granule-Bound Protein (Phasin) in *Magnetospirillum gryphiswaldense***. *J Bacteriol* 2005, **187**:2416–2425.
124. Wan J, Browne PJ, Hershey DM, Montabana E, Iavarone AT, Downing KH, Komeili A: **A protease-mediated switch regulates the growth of magnetosome organelles in *Magnetospirillum magneticum***. *Proceedings of the National Academy of Sciences* 2022, **119**:e2111745119.
125. Taoka A, Asada R, Sasaki H, Anzawa K, Wu L-F, Fukumori Y: **Spatial Localizations of Mam22 and Mam12 in the Magnetosomes of *Magnetospirillum magnetotacticum***. *J Bacteriol* 2006, **188**:3805–3812.
126. Okamura Y, Takeyama H, Matsunaga T: **A Magnetosome-specific GTPase from the Magnetic Bacterium *Magnetospirillum magneticum* AMB-1\***. *Journal of Biological Chemistry* 2001, **276**:48183–48188.
127. Masó-Martínez M, Fryer B, Aubert D, Peacock B, Lees R, Rance GA, Fay MW, Topham PD, Fernández-Castané A: **Evaluation of cell disruption technologies on magnetosome chain length and aggregation behaviour from *Magnetospirillum gryphiswaldense* MSR-1**. *Front Bioeng Biotechnol* 2023, **11**:1172457.

128. Grant CR, Amor M, Trujillo HA, Krishnapura S, Iavarone AT, Komeili A: **Distinct gene clusters drive formation of ferrosome organelles in bacteria.** *Nature* 2022, **606**:160–164.
129. Matsunaga T, Okamura Y, Fukuda Y, Wahyudi AT, Murase Y, Takeyama H: **Complete Genome Sequence of the Facultative Anaerobic Magnetotactic Bacterium *Magnetospirillum* sp. strain AMB-1.** *DNA Res* 2005, **12**:157–166.
130. Kluyver T, Ragan-Kelley B, P#233, Rez F, Granger B, Bussonnier M, Frederic J, Kelley K, Hamrick J, Grout J, et al.: **Jupyter Notebooks – a publishing format for reproducible computational workflows.** In *Positioning and Power in Academic Publishing: Players, Agents and Agendas*. IOS Press; 2016:87–90.
131. Hershey DM, Ren X, Melnyk RA, Browne PJ, Ozyamak E, Jones SR, Chang MCY, Hurley JH, Komeili A: **MamO Is a Repurposed Serine Protease that Promotes Magnetite Biomineralization through Direct Transition Metal Binding in Magnetotactic Bacteria.** *PLOS Biology* 2016, **14**:e1002402.