Magnetotactic bacteria (MTB) are Gram-negative bacteria that can navigate along geomagnetic fields. This ability is a result of a unique intracellular organelle, the magnetosome. These organelles are composed of membrane-enclosed magnetite (Fe₃O₄) or greigite (Fe₃S₄) crystals ordered into chains along the cell. Magnetosome formation, assembly, and magnetic nano-crystal biomineralization are controlled by magnetosome-associated proteins (MAPs). Most MAP-encoding genes are located in a conserved genomic region – the magnetosome island (MAI). The MAI appears to be conserved in all MTB that were analyzed so far, although the MAI size and organization differs between species. It was shown that MAI deletion leads to a non-magnetic phenotype, further highlighting its important role in magnetosome formation. Today, about 28 proteins are known to be involved in magnetosome formation, but the structures and functions of most MAPs are unknown. To reveal the structure–function relationship of MAPs we used bioinformatics tools in order to build homology models as a way to understand their possible role in magnetosome formation. Here we present a predicted 3D structural models’ overview for all known Magnetospirillum gryphiswaldense strain MSR-1 MAPs.

Keywords: magnetosome, structure prediction, Protein structure–function, magnetotactic bacteria, membrane biogenesis

INTRODUCTION

Magnetotactic bacteria (MTB) are a group of Gram-negative aquatic prokaryotes that can synthesize a unique prokaryotic organelle, called a magnetosome (Bazylinski et al., 2004). The magnetosome contains magnetic crystals enclosed within membrane vesicles, which are aligned as intracellular chains along the cell (Balkwill et al., 1980; Komedi et al., 2006). The magnetosome membrane creates an isolated environment in the cell which is important for mineral crystal nucleation and growth (Komedi et al., 2004). The magnetosome chain forces the bacteria to align passively to the geomagnetic field and the bacteria then swim accordingly with the use of their flagella, a behavior called magnetotaxis (Balkwill et al., 1980; Lower and Bazylinski, 2013). Magnetotaxis is believed to aid MTB to reach regions of optimal oxygen concentrations without long, random movements (Gorby et al., 1988).

The magnetic crystals consist of magnetite (Fe₃O₄) or greigite (Fe₃S₄). Their size typically ranges between 35 and 120 nm and are in the size range of a single-magnetic-domain (SD; Frankel et al., 1979; Mann et al., 1990; Kirschvink, 1992). Each bacteria has a defined species-dependent crystal size and shape and contains one or more magnetosome chains (Balkwill et al., 1980; Bazylinski and Frankel, 2004). The consistent morphology in different species or strains of the magnetic crystals indicates that mineral formation is a highly controlled process (Frankel and Bazylinski, 2009).

It was found that magnetosome formation is under strict control of specific gene expression (Schübbe et al., 2003). Most of these genes are located on a genomically conserved region, known as the magnetosome island (MAI; Schübbe et al., 2003). The MAI is found to be highly conserved in almost all MTB species and includes a highly conserved and essential operon (mamAB), as well as three less conserved operons (mamGFCD, mms6, and mamXY; Fukuda et al., 2006). It was shown that MAI or mamAB operon deletion causes the loss of magnetosome formation (Murat et al., 2010; Lohse et al., 2011).

MamAB operon in M. gryphiswaldense MSR-1 includes 17 open reading frames which correspond to ~16 kb of DNA (Schübbe et al., 2006). This operon contains genes that are essential for magnetosome formation and have important functions such as membrane invagination, iron transport, and magnetite biomineralization (Lohse et al., 2011). The mamAB cluster encodes proteins that are essential for membrane invagination and magnetosome biogenesis (mamB, E, I, L, and Q), magnetosomal iron transport (mamB and M), magnetite biomineralization (mamD, O, T, P, and S), and magnetosome chain assembly (mamK and mamJ; Murat et al., 2010; Yang et al., 2010; Quinlan et al., 2011; Uebe et al., 2011).

In M. gryphiswaldense MSR-1, the mamXY operon is a ~4.9 kb segment which is located ~28 kb downstream of the mamAB operon and consists of mamV, mamX, mamZ (mamH-like), and fhuD-like genes (Ding et al., 2010). MamXY is conserved among all Magnetotrichium bacteria. It was shown in both M. gryphiswaldense MSR-1 and M. magnetoautotrophicus AMB-1 that mamXY-encoding proteins are associated with the magnetosome membrane (Lohse et al., 2011). Deletion of M. gryphiswaldense MSR-1 mamXY causes short magnetosome chains with regular shape but smaller particles (Lohse et al., 2011).

The mamCD (2.1 kb) and mms6 (3.6 kb) operons are not essential for biomineralization but they encode genes which control the size and shape of magnetite particles (Scheffel et al., 2008; Murat et al., 2010; Lohse et al., 2011).
et al., 2010; Lohsse et al., 2011; Tanaka et al., 2011; Murat et al., 2012). Deletion of the mamCD operon – which contains four genes (mamC, mamD, mamF, and mamG) – results in crystals with approximately 75% of the wild type size (Scheffel et al., 2008). The mms6 operon is located upstream of the mamCD operon and contains five genes (mms6, mmsf, mgr4070, mgr4072, and mgr4074; Fairve and Schuler, 2008). Co-deletion of both operons results in further reduction in the shape regularity and alignment of magnetite crystals (Lohsse et al., 2011).

Based on current scientific data it is suggested that magnetosome formation occurs via several steps that can act simultaneously. These include, (i) protein sorting and inner membrane invagination, (ii) alignment of the magnetosome into chains, (iii) iron uptake and crystal nucleation, and (iv) crystal maturation (Murat et al., 2010). Today, a general model for magnetosome formation and protein involvement is based on genetic approaches but most of the protein functions have yet to be determined (Murat et al., 2010).

One of the most studied strains is the magnetotactic α-proteobacterium Magnetospirillum gryphiswaldense MSR-1, which contains all four magnetosome operons (Lohsse et al., 2011). M. gryphiswaldense MSR-1 creates more than 60 cubo-octahedral magnetite-containing magnetosomes (Schleifer et al., 1991). In M. gryphiswaldense MSR-1 there are over 28 different proteins that are involved in magnetosome formation and most of the corresponding genes are located on the MAI (Schubbe et al., 2006; Richter et al., 2007). Some of these proteins are homologous to known protein families, such as: tetratricopeptide repeat (TPR) proteins, CDF transporters, PDZ proteins, proteases, and more (Okuda et al., 1996).

In order to understand the function of each protein during magnetosome formation it is essential to find their structure-function relationships. Nowadays there are only a few known MTB protein structures that promote understanding of the protein roles during magnetosome formation. MamA and MamP are the only proteins from the mamAB operon whose 3D structures have been determined (Zeytuni et al., 2011, 2012; Siponen et al., 2013). In this work we predicted and analyzed the 3D models of magnetosome proteins from M. gryphiswaldense MSR-1 strain as a way of elucidating their functions during the processes of magnetosome formation and present them based on their main predicted function or their association with protein families.

**PROTEIN SORTING AND MAGNETOSOME Membrane INVAGINATION**

*MamI*

MamI is a small, 77 residue protein with two predicted integral membrane α-helices (Komeili, 2012). MamI deletion results in the loss of the magnetosome membrane, which might be indicative of MamI involvement in magnetosome membrane invagination (Komeili, 2012). MamI-GFP was shown to be localized to the magnetosome and indicates the presence and position of the magnetosome in the cell (Murat et al., 2010). MamI has no homologous domains or proteins except for its homologues in other MTB species. MamI secondary structure prediction yields a short loop of three amino acids (Thr35, Glu36, and Leu37) between the transmembrane helices (Figure 1; Slabinski et al., 2007). This loop is not predicted to interact with the magnetic particle, which may indicate that MamI has the ability to bend the magnetosome membrane.

*MamQ*

MamQ deletion in AMB-1 cells results in the complete loss of magnetosome formation (Murat et al., 2010). MamQ is predicted to be an integral membrane protein with 273 amino acids and is homologous to the LemA protein family (Greene and Komeili, 2012). LemA proteins are predicted to have a membrane-spanning domain on their N-terminal in which the non-membrane C-terminal domain (CTD) will be extracellular (pointing towards the magnetosome lumen); yet, its role is still unknown (Lenz et al., 1996).

MamQ secondary structure prediction using XtalPred and PsiPred servers show that MamQ contains a cytosolic unstructured N-terminal followed by an integral membrane helix and a CTD with a helix-turn-helix fold (Figure 1; Slabinski et al., 2007). The model structure of MamQ was based on the LemA template (PDB ID: 2ETD; Arnold et al., 2006). The model presents only the cytosolic-terminal domain (Asn70 to Thr223, Figure 1).

Multiple sequence alignment shows high similarities between anchoring transmembrane helices are in gray. Black bars define the protein size in angstroms. M. gryphiswaldense MSR-1 MamA structure was determined by X-ray crystallography.

**FIGURE 1** | Protein structure predictions that are involved in protein sorting, magnetosome membrane invagination, and magnetosome chain assembly. Structures are in ribbon representation. Predictions of
MamQ proteins from other MTB species and good conservation to important residues in Listeria monocytogenes LemA (Li, 2003). The MamQ model presents a magnetosomal domain with a negatively charged surface similar to the LemA structure, whose function is still unknown (Figure 2).

**MamL**

The mamL gene is located within the mamAB operon and its deletion results in the loss of the magnetosome membrane in M. magneticum AMB-1 (Murat et al., 2010). MamL is a small, 123 amino acid protein containing two predicted integral membrane α-helices and is very similar to MamQ (Figure 1; Komeili et al., 2012). MamL is found only in MTB species and there are no homologous proteins or domains in other organisms (Komeili et al., 2012).

**MamA**

MamA is one of the most conserved MAPs and exists in all MTB species or domains in other organisms (Komeili, 2012). It was shown that MamA-GFP expressed in M. magneticum AMB-1 cells were localized to the magnetosome chain and surrounded the magnetosome vesicles (Yamamoto et al., 2010; Zeytuni et al., 2011). MamA contains several TPR domains and self-assembles into stable homo-oligomeric complexes (Okuda et al., 1996; Zeytuni et al., 2011). TPR motifs are known to be involved in protein–protein interactions and are present in a number of proteins that are functionally unrelated (Blatch and Lisde, 1999). TPR is a 34 amino acid structure arranged as repeats of antiparallel α-helices (Das et al., 1998).

It was shown that purified MamA forms round-shaped complexes with a central pore cavity (Zeytuni et al., 2011). Mutation in the first helix of the MamA TPR motif causes disassembly of MamA complexes, which indicates its involvement in complex formation (Zeytuni et al., 2011). The conserved salt bridge between Arg50-Asp79 in M. magneticum AMB-1 is responsible for stabilization of the N-terminal domain, which is important to MamA complex formation and localization to the magnetosome chain (Zeytuni et al., 2011). The structures of the MamA deletion mutant from M. magneticum AMB-1, M. gryphiswaldense MSR-1, and Magnetohacterium bavaricum are composed of 10 antiparallel α-helices that are folded as five TPR motifs and form a hook-shaped structure (PDB ID: 3AS4, 3AS8, and 2MUC; Figure 1; Zeytuni et al., 2011). It was suggested that MamA has three binding sites, two which are needed to create the round homo-oligomeric complexes and one to interact with the magnetosome chain (Zeytuni et al., 2011). Based on many TPR-ligand structures it was shown that the TPR protein family can bind unstructured peptides, helices, or entire TPR motifs (Zeytuni et al., 2011). Compared to other TPR proteins, which have a positive or negative binding pocket in their concave surface, MamA from M. magneticum AMB-1 displays a highly positive binding site in the concave surface similar to other MTB species (Figure 2; Zeytuni et al., 2011, 2012). The convex surface charge in Magnetospirillum species is negative, unlike in M. bavaricum strain that presents both positive and negative patches (Zeytuni et al., 2012). It was predicted that the MamA convex side may act as a binding site with other magnetosome-associated proteins (MAPs; Zeytuni et al., 2011). MamA was found to interact with four different proteins or protein fragments in vivo (of 26.8, 31.6, 34, and 63.5 kDa) which supports the suggested model in which the MamA convex surface faces the magnetosome membrane and acts as a multi-protein assembly site (Yamamoto et al., 2010; Zeytuni et al., 2011).

**MamU**

MamU is a 297 amino acid protein predicted to fold as a mixed α-helices–β-sheets structure (Slabinski et al., 2007). ΔmamU mutants did not yield any changes in their magnetic response (Murat et al., 2010). One MamU homologous protein family are the diacylglycerol kinases (DGKs), which phosphorylate the second-messenger diacylglycerol (DAG) to phosphatidic acid (PA); Van Bliertswijk and Houssa, 2008). The DGK pathway is known to be a major player in the regulation of cell response (Mérida et al., 2008). Nowadays there are nine known members of the DGK family which contain the conserved catalytic domains and two cysteine rich domains on the protein N-terminal (Van Bliertswijk and Houssa, 2000). The catalytic domain is known to have six conserved aspartate residues which play a major role in the enzyme’s activity (Van Bliertswijk and Houssa, 2000).

The MamU model structure is based on a DGK structure (ID: STSP chain L; Figure 1; Guer and Peritel, 1997). Sequence alignment between MamU and DGK shows that the conserved aspartates do not appear in the MamU sequence and there is only one cysteine residue on the MamU N-terminal domain. Changes in the electrostatic density map between MamU and DGK indicate that MamU has a different substrate and activity, or that the fold of MamU is different from the DGK protein family.

**MamY**

MamY is a 371 amino acid protein predicted to have two integral membrane helices at its N-terminal and a large cytosolic
domain at the C-terminal (Slabinski et al., 2007). MamY has a weak homology to BAR domain proteins, which are known to be involved in cellular membrane dynamics (Peter et al., 2004; Tanaka et al., 2010). Deletion of the mamY gene yielded an enlarged magnetosome vesicle phenotype with small magnetite crystals (Tanaka et al., 2010). In *M. magnetotacticum* AMB-1, MamY-GFP were localized near to the magnetosome vesicles with small magnetite crystals that are still attached to the inner membrane (Tanaka et al., 2010). It was suggested that MamY’s role is to constrict the magnetosome membrane during its invagination, followed by magnetite crystal growth (Tanaka et al., 2010).

While searching for MamY structural models, several proteins were suggested as templates (Artsios et al., 2006; Kelley and Sternberg, 2009). One of these templates was the cytoplasmic domain of a bacterial chemoreceptor from *Thermotoga maritima*, which has a structure of two anti-parallel helices that dimerize into a four-helix bundle with another methyl-accepting chemotaxis protein (MCAP) subunit (Park et al., 2006). MCP is a transmembrane kinase which is involved in the signaling network that controls bacterial chemotaxis (Park et al., 2006). In addition, MamY structural analysis using Phyre2 shows low conservation to the domain from talin protein (PDB ID: 1SJ8; Kelley and Sternberg, 2009). Talin is a cytoskeletal protein that is known as a linker between actin and the membrane via integrin proteins, which are involved in cell adhesions (Niggli et al., 1994). The talin domain is composed of an N-terminal five-helix bundle and a C-terminal four-helix bundle, which are connected by a short loop (Niggli et al., 1994). MamY model structure shows the cytosolic domain, which is composed of four $\alpha$-helices on its N-terminal followed by five $\alpha$-helices (Figure 1). The electrostatic density map of the MamY model presents a highly negatively charged surface, similar to talin’s surface charge distribution (Figure 2; Papagrigoriou et al., 2004). Sequence alignment between MamY and talin presents low identity, whilst the MamY model structure has high confidence (95.36%) with the talin structure (Kelley and Sternberg, 2009). From these results we can suggest that MamY may function similarly to the talin protein and plays a role in membrane invagination and magnetosome separation from the inner membrane (Tanaka et al., 2010).

**Magnetosome Arrangement into Chain Structure**

**MamJ**

*MamJ* is essential for the magnetosome chain structure and its deletion leads to a new magnetosome arrangement as 3D clusters instead of a linear chain (Scheffel and Schuler, 2007). MamJ is known as an acidic protein which contains 426 amino acids with a repetitive domain structure. It has a central acidic repetitive domain (CAR domain) which is composed of an 88 amino acid motif followed by tandemly arranged copies of a highly acidic, 28 amino acid motif, consisting primarily of Pro and Glu residues arranged in Glu-Pro (Scheffel et al., 2006; Scheffel and Schuler, 2007). The CAR domain is not required for magnetosome restoration in $\Delta$mamJ cells, unlike regions at the N- or C-terminal in MamJ (Scheffel and Schuler, 2007).

It is known that MamJ is associated with MamK filaments and magnetosome vesicles, thus creating a linear chain (Scheffel et al., 2006). The interactions between MamJ and MamK apparently are mediated by two protein–protein interaction domains, one located on the C-terminal and the other located in the MamJ N-terminal region (Scheffel and Schuler, 2007). Recently it was shown, by using FLIM-FRET technique, that co-expression of MamK_mCherry and eGFP_MamJ in *Escherichia coli* create a stable interaction between them which is important for magnetosome alignment (Carillo et al., 2013).

**Prediction of MamJ structure**

Scheffel and Schuler servers show that most of the protein is unstructured (Figure 1; Dourzayev et al., 2005; Slabinski, et al., 2007). Additional analysis of the MamJ sequence using the ProDom server has found two conserved domains which exist in TonB protein (Bru et al., 2005). TonB is a highly proline-rich protein that includes a segment consisting of multiple X-Pro dipeptide repeats with an anchor to the cytoplasmic membrane via a single N-terminal transmembrane helix (Harnavy et al., 1990). TonB also functions as a mechanical linkage between the inner and outer membranes through protein–protein interactions (Sill and Postle, 2012). These predictions support the previous evidence that MamJ can interact with MamK via protein–protein interactions and is involved in magnetosome chain assembly.

**MamK**

The mamK gene encodes a filamentous structure which is similar to prokaryotic MreB and ParM (Sonkaria et al., 2012). MreB-like proteins polymerized into helical filamentous structures that run along the cell length (Carballido-Lopez, 2006). The 3D structure of MreB shows high similarity to eukaryotic actin. The actin-like proteins, similar to actin, function in prokaryotic cells and are required for targeting and positioning proteins or molecular complexes (Carballido-Lopez, 2006).

In *M. magnetotacticum* AMB-1 strain, mamK deletion abolishes the filamentous structure near the magnetosome (Korneli et al., 2006), whereas $\Delta$mamK in *M. gyrophonomus MSR-1 results in shorter, fragmented, and off-center chains and was suggested to be involved in proper magnetosome chain positioning and segregation (Katzmann et al., 2010, 2011). The function of MamK is to organize the magnetosome chain along the cell axis and associate with MamJ, which may act as an anchor between MamK and the magnetosome membrane (Sonkaria et al., 2012). Previously, it was shown that the localization of MamK filaments in the cell does not depend on the presence of MamJ (Scheffel and Schuler, 2007).

Biochemical studies indicate that MamK polymerization is the result of ATP binding whilst filament disassembly happens during ATP hydrolysis (Ozyamak et al., 2013). It seems that ATP stabilizes MamK filaments and prevents their aggregation (Ozyamak et al., 2013). *M. magnetotacticum* AMB-1 MamK form dynamic filaments in vivo which depend on the ATPase active site and two proteins (MamM and LimJ) which are required for promoting MamK filament formation (Draper et al., 2011). Furthermore, MamK filament structure has an architecture of two parallel strands, unlike MreB filaments which are linked in an antiparallel arrangement (Ozyamak et al., 2013). The MamK homology model is based on MreB’s structure (PDB ID: 1FIA; Gues and Poitot, 1997; Sonkaria et al., 2012). The MamK model structure has four domains that are similar to the conserved secondary structure of...
IRON TRANSPORT AND NUCLEATION

MamO

MamO is a large, 65.3 kDa protein whose deletion leads to an empty magnetosome phenotype, suggesting that MamO might be involved in biomineralization, iron transport, and/or magnetite nucleation, or allows for a suitable environment for magnetite synthesis in the magnetosome (Murat et al., 2010). MamO is an integral membrane protein with eight predicted transmembrane α-helices. Based on ProDom prediction, MamO contains two domains: one is similar to the trypsin-like peptidase and the second is similar to integral membrane proteins. The latter are involved in the transport of anions across the cytoplasmic membrane during taurine metabolism as an exporter of sulfoacetate (Yang et al., 2010). Although it seems that MamO has a predicted trypsin-like peptidase domain, expression of MamO in E. coli does not show any protease activity. In addition, several single point mutations (T225A, H116A, and D149A) in the predicted active site of the MamO trypsin domain did not affect magnetite crystal formation (Yang et al., 2010; Quinlan et al., 2011).

Today there is no direct evidence that any part of MamO has trypsin or protease activity. To explore whether MamO can act as an active protease, we decided to build a homology model using the Swiss-Model server which can aid with understanding as an active protease, we decided to build a homology model using the Swiss-Model server which can aid with understanding of protein localization to the magnetosome membrane and is predicted to fold as a putative serine protease with two PDZ domains and a predicted membrane anchoring the hydrophobic helix on its N-terminal (Yang et al., 2010; Siponen et al., 2012). Recently it was discovered that MamE contains a putative cystochrome c-like domain with a CXXCH motif that acts as covalent thioether bonds to the heme vinyl groups (magnetochrome; Bowman and Bren, 2008; Siponen et al., 2012). Deletion of MamE in vivo leads to empty magnetosome vesicles and to the loss of magnetite synthesis (Murat et al., 2010). As a putative serine protease, MamE has a highly conserved catalytic triad. It was shown that site-directed mutagenesis of these residues in M. gryphiswaldense MSR-1 MamE (Figure 3). This, together with the lack of the PDZ domain, indicates that MamO is most likely missing the protease activity.

MamE

MSR-1 MamE is a large, 655 amino acid protein which is important for protein localization to the magnetosome membrane and is predicted to fold as a putative serine protease with two PDZ domains and a predicted membrane anchoring the hydrophobic helix on its N-terminal (Yang et al., 2010; Siponen et al., 2012). Recently it was discovered that MamE contains a putative cystochrome c-like domain with a CXXCH motif that acts as covalent thioether bonds to the heme vinyl groups (magnetochrome; Bowman and Bren, 2008; Siponen et al., 2012). Deletion of MamE in vivo leads to empty magnetosome vesicles and to the loss of magnetite synthesis (Murat et al., 2010). As a putative serine protease, MamE has a highly conserved catalytic triad. It was shown that site-directed mutagenesis of these residues in M. gryphiswaldense AMB-1 MamE (His198, Asp221, Ser297) does not affect magnetite crystal nucleation but results in a phenotype characterized by smaller magnetite crystals and the loss of the magnetic response (Quinlan et al., 2011). The same phenotype is similar to the deletion of the magnetochrome domain (Quinlan et al., 2011). There are few hypotheses regarding the functions of magnetochrome, such as electron donation to oxidized iron, extraction of electrons to maintain the magnetosome redox state or to act as a redox...
buffer to maintain the balance between maghemite and magnetite (Siponen et al., 2012).

To understand MamE function and how it is involved in magnetosome formation, we decided to create a homology model. The MSR-1 MamE model was built using the Swiss-PdbViewer program and is based on a serine protease structure (PDB ID: 2Z9M; Guex and Peitsch, 1997; Figure 3). From the 3D structure and the multiple sequence alignment we can locate the highly conserved catalytic triad of serine proteases, yet it was not shown experimentally whether MamE has protease activity (Figure 4).

Based on domain prediction by InterProScan server and the available 3D model of MamE, a PDZ domain is predicted. PDZ domains are usually known to be involved in protein–protein interactions and signaling complex (Lee and Zheng, 2010). In a previous study, it was shown that MamE deletion caused mislocalization of magnetosome proteins which could indicate the importance of the PDZ domain to protein–protein interactions (Murat et al., 2010). Prediction of a MamE PDZ domain shows high conservation to PDZ architecture, which consists of six anti-parallel β-strands and two α-helices (Sheng and Sala, 2001). Furthermore, the PDZ domain was shown to interact with the conserved PDZ binding signature at the C-terminal of MSR-1 MamB (Uebe et al., 2011). The major binding site of the MamE PDZ domain lies between α1 and β3 and contains a large number of hydrophobic amino acids, similar to the HtrA2-PDZ domains fold which is known to be a binding site for single peptide ligands or hydrophobic amino acids of other proteins (Zhang et al., 2007; Wang et al., 2010). Prediction of the electrostatic density map displays negative and positive patches on the PDZ surface, which is similar to other HtrA2 PDZ domains. Based on all the previous results, we can assume that MamE acts as a serine protease and that its PDZ domain can interact with other proteins that are involved in magnetosome formation.

MamH

MamH is a 428 amino acid protein and in most MTB it is located in the mshA operon, though its function and how it is involved in magnetosome formation are both unknown (Murat et al., 2010; Lobose et al., 2011). In MSR-1, MamH is highly similar to MamZ (Raschdorf et al., 2013). Deletion of MamH results in a decrease in the number and size of magnetosomes (Murat et al., 2010; Raschdorf et al., 2013). It was shown that mamH deletion in ΔmamZ mutants causes a strong effect on the crystals’ size, shape, and magnetic response (Raschdorf et al., 2013). Therefore, it was suggested that MamH may be involved in magnetite biomineralization (Raschdorf et al., 2013). By searching for homologous proteins or conserved domains with the BLAST server we found similarities to a conserved domain in the Major Facilitator Superfamily (MFS), members of which are known to function as membrane transporters (Pao et al., 1998; Marchler-Bauer et al., 2011; Raschdorf et al., 2013). MFS proteins are single-polypeptide secondary carriers that use the electrochemical potential of the transported substrates (Pao et al., 1998). Prediction of MamH secondary structure shows an organization of 12 transmembrane helices with short connecting loops and a longer loop connecting H6–H7 (Raschdorf et al., 2013; Figure 3). This organization is similar to MFS protein structures (Huang et al., 2003). The MamH predicted structural model displays a negative cavity which can bind positive ions or ligands like iron and transfer them through the magnetosome membrane (PDB ID: 2XUT). The hypothesis that MamH is an integral membrane protein cannot only be based on the sequence prediction but also on the model’s electrostatic density map. This contains the positive girdle envelope around the protein exterior, which is usually a characteristic feature of integral membrane proteins (Figures 3 and 5). Another structure of MFS proteins indicates a proton-coupled transporter which is essential for phosphate uptake in plants and fungi. The Piriformospora indica phosphate transporter (PiPT) is a high affinity phosphate transporter that is involved in improving phosphate nutrition levels in the host plant and is known to be a phosphate/H+ symporter from the MFS (Pao et al., 1998; Pedersen et al., 2013). By using Swiss-PdbViewer we created manually another MamH model structure based on PiPT structure which provided a different view for MamH function (PDB ID: 4D05; Guex and Peitsch, 1997). In the MamH PiPT-based electrostatic map we can detect a positive pocket in the magnetosomal side and a negative patch in the cytosolic side. These distinctions are similar to PiPT structure and may result in a new perspective.
on MamH function. Recently, it was shown in *M. gryphiswaldense* MSR-1 that there are two iron phases during the biomineralization process: ferrihydrate with high phosphorus content – similar to the bacterial ferritin core – and magnetite (Fdez-Gubieda et al., 2013). It has also been suggested by Baumgartner et al. (2013) that magnetite formation starts from a phosphate-rich ferric hydroxide phase, through a short-lived ferrihydrate phase followed by a final phase of magnetite. These results support our MamH model, which can function as a phosphate transporter clearing phosphate from the magnetosome while biomineralizing magnetite (Fdez-Gubieda et al., 2013).

### MamN

MamN is a 437 amino acid protein and its deletion leads to an empty magnetosome chain phenotype (Murat et al., 2010). The inability to synthesize magnetite within the magnetosomes can indicate MamN involvement in biomineralization, iron transport, magnetite nucleation, or chemical environment determination needed for magnetite synthesis in the magnetosome (Murat et al., 2010). However, its deletion does not affect the localization of other magnetosome proteins (Murat et al., 2010).

MamN has homology to a Na+/H+ antiporter; this activity can influence the pH within the magnetosome core (Komeili, 2012). It is known that magnetite synthesis requires a basic environment which raises the speculation that a H+/cation antiporter homologous protein did not result in a convincing structure.

**MamM and MamB**

MamM and MamB are large proteins – 34.4 and 31.9 kDa, respectively – and are magnetosome membrane proteins which may be involved in iron transport due to their similarity to the cation diffusion facilitator (CDF) protein family (Uebe et al., 2011). CDF proteins are found in all kingdoms of life and are involved in the transport of divalent metal cations (Paulsen and Saier, 1997). Most members of the CDF family contain six highly conserved transmembrane helices, organized as a transmembrane domain, with cytoplasmic N- and C-terminals (Cragg et al., 2002). It was shown that CDFs create a homodimeric structure and use a proton antiport mechanism to drive substrate translocation across the lipid membrane (Haney et al., 2005; Lu et al., 2009). MamM deletion causes the loss of magnetite crystal formation and results in empty magnetosomes (Uebe et al., 2011). Except for its suggested role in iron transport, MamM is also involved in crystallization initiation and proper localization of other magnetosome proteins (Uebe et al., 2011). In contrast to MamM, MamB deletion causes a lack of magnetosome vesicles (Murat et al., 2010; Uebe et al., 2011). Further experiments indicated that MamM and MamB can interact, and that MamM is required for MamB stabilization (Uebe et al., 2011). It has also been shown that the cytoplasmic CTD in MamM and MamB are involved in MamB stabilization (Uebe et al., 2011).

Replacement of the conserved cysteine residues (Cys119/138) with serine or alanine did not affect MamM function but similar mutations in MamB abolished its function (Uebe et al., 2011). Mutation of Cys138 in MamB blocked its oligomerization and magnetosome formation (Uebe et al., 2011). In MSR-1 the MamB C-terminal has a TPR recognition signature and was shown to interact with MamE’s PDZ domain (Quinlan et al., 2011; Uebe et al., 2011). In addition, point mutations in the MamM membrane putative metal binding site (Y46H, Y46D, D350A, H155A, and D159A) lead to changes in crystal size and morphology, which may be the result of a reduction in iron transport rates into the magnetosome vesicles or of the nucleation of magnetite crystals (Uebe et al., 2011). It seems that magnetite can only be stable in a pH range from ~7 to 14 (Bell et al., 1987). Therefore, if MamM is using a H+/cation antiport mechanism similar to other CDF proteins, it may explain why dysfunction of MamM causes defects in crystal formation (Uebe et al., 2011).
MamP and MamB secondary structure prediction indicates a transmembrane domain with six α-helices followed by a C-terminal cytosolic domain (Uebe et al., 2012). MamM and MamB 3D structures prediction is based on the FieF structure and built manually in SPDB viewer (PDB ID: 3H90; Figure 3). The cytochrome c oxidase structure has a highly negative pocket that may be involved in ion binding (Figure 4). MamP 3D structure presents a PDZ fold followed by a C-terminal cytosolic domain (Solmaz and Culotta, 2000). MamP is a 270 amino acid protein that is predicted to have a domain structure from cytochrome bc1 complex to cytochrome c oxidase (Solmaz and Culotta, 2000). The electrostatic density map shows similar characterization (Figure 3). It was shown that MamP structure has a highly negative pocket between the two monomers which includes conserved acidic residues (Glu91, His93, Glu98, Glu123, and Glu193) that were suggested to act as an ion-binding site (Figure 5). Mutation in these acidic residues to alanine in vivo led to magnetic response and crystal size defects (Siponen et al., 2013).

MamT
In M. gryphiswaldense MSR-1, MamT contains 174 amino acids and is predicted to have a double cytochrome c CXXCH motif and a membrane-anchoring hydrophobic helix on its N-terminal (Slabinski et al., 2007). MamT secondary structure prediction shows that the CTD is located on the magnetosome lumen and includes α-helices and β-sheets (Figure 3; Slabinski et al., 2007). A ΔmamT mutant shows defects in crystal maturation and the loss of magnetic response (Murat et al., 2010, Siponen et al., 2012).

MamZ
MamZ is a 661 amino acid protein and is known to have similarities to the ferric reductase-like transmembrane component (YedZ-like domain) on its C-terminal side, whilst its N-terminal has homology to the MFS (Richter et al., 2007; Raschdorf et al., 2013). Recently it was shown that MamZ interacts with the magnetosome membrane and was suggested to create an iron oxoreductase and transport complex with MamM and MamH (Raschdorf et al., 2013). MamZ deletion shows a slightly reduced magnetic response with two types of crystals that are still aligned into a chain (Raschdorf et al., 2013). Deletion of MamZ C-terminal, which shares homology with YedZ-like domain, reveals the same phenotype as the full MamZ deletion, suggesting that this domain has a critical role in the biomagnetization process (Raschdorf et al., 2013). MamZ 3D structure prediction is based on two different templates. The N-terminal is predicted based on the glycerol-3-phosphate transporter (GlpT) from E. coli, which belongs to the MFS transporter family (PDB ID: 1PW4; Kelley and Sternberg, 2009). GlpT is known to transport glycerol-3-phosphate into the cytoplasm and inorganic phosphate to the periplasm and is composed of 12 transmembrane helices (Huang et al., 2003). MamZ N-terminal model structure presents 12 transmembrane helices with a negative pore facing the magnetosomal side, similar to GlpT (Figure 5). The electrostatic density map of MamZ cytosolic side is different from GlpT which is highly positively charged and may indicate a different function (Figure 5; Huang et al., 2003). MamZ C-terminal is predicted to have a domain structure from cytochrome bc1 complex, which is known as ubiquinol-cytochrome c reductase (PDB ID: 3CX5; Figure 3). The cytochrome c's function is to transfer electrons from the cytochrome bc1 complex to cytochrome c oxidase (Solmaz and Hunte, 2008). MamZ C-terminal model structure includes three α-helices and two short β-sheets. The electrostatic density map of MamZ presents two highly charged patches – positive and negative – that are predicted to face the magnetosome lumen (Figures 3 and 5).
MamX
The mamX gene is conserved in most magnetite-producing α-proteobacteria (Abreu et al., 2011). It has a weak similarity to both the magnetosome serine-like protease MamH and to MamS (Richter et al., 2007; Lobue et al., 2011). Structure prediction of MamX presents a transmembrane helix on its N-terminal side, two cytochrome c-like domains and a DNA-binding domain on its C-terminal (Söding, 2005; Sabinski et al., 2007). As mentioned above, cytochrome c functions as an electron carrier between different redox partners and can also be found in MamT and MamS (Siponen et al., 2012). Deletion of these three proteins shows defects in crystal maturation (Siponen et al., 2012). It was shown that deletion of the cytochrome c domain in MamX abolishes its function (Ruschdorff et al., 2013). The two cytochrome c-like domains are predicted to be located between Tyr42 and Val111 and their structures were predicted based on four different templates of cytochrome c domains (PDB ID: 1OGY_B, 1JNI_A, 3ML1_B, and 1QO8_A; Figure 3; Söding, 2005). In all these templates the cytochrome c domain creates a ring-like shape around the heme groups, except for the MamX cytochrome c-like domain prediction. By using HHpred server the C-terminal is predicted to be shaped like an OB fold motif (PDB ID: 2QOA_A, 3KDF_D, 3KF6_A; Figure 3; Söding, 2005). This motif is observed as a binding domain for oligonucleotides or oligosaccharides (Murzin, 1993). The OB-fold has a five β-sheet coiled fold that forms a closed β-barrel with an α-helix located between the third and fourth strands (Murzin, 1993). The MamX C-terminal predicted structure contains three β-strands followed by an α-helix, similar to the OB fold (Söding, 2005).

CRYSTAL SHAPE AND SIZE
MamS
MamR was shown to be important for crystal number and size (Murat et al., 2010). FtsZ proteins are known to be involved in cell division and GTP-dependent polymerization into filaments (Kelley and Sternberg, 2009). The MamS structure model displays six β-sheets with two short α-helices, similar to the OB fold which is known to create a β-barrel structure that can bind oligonucleotides or oligosaccharides (Murzin, 1993).

Deletion of mamX abolishes its function (Ruschdorff et al., 2013). MamR model structure is based on the DNA-binding protein Rv2175c from M. tuberculosis (PDB ID: 2KFS; Cohen-Gonsaud et al., 2009). Rv2175c is activated by phosphorylation of its N-terminal domain (Cohen-Gonsaud et al., 2009). The MamR model presents a C-terminal with two α-helices followed by two β-sheets and a negative patch on the protein surfaces (Figures 6 and 7; Kelley and Sternberg, 2009).

MamS is a ~19 kDa protein and its deletion leads to a weak magnetic response due to defects in magnetite crystal size and morphology (Murat et al., 2010). In M. magneticum AMB-1 strain several small magnetic particles were clustered together in one magnetosome membrane, suggesting MamS involvement in the magnetite post-nucleation event (Murat et al., 2010; Kosmeli, 2012). According to BLAST server there is no conserved domain in the MamS sequence (Marchler-Bauer et al., 2013). Prediction of its secondary structure indicates a fold containing a transmembrane helix on the N-terminal followed by β-strands on the cytoplasmic side (Figure 6; Sabinski et al., 2007). Prediction of the MamS 3D structure is based on the structure of the hypothetical protein YgiW from E. coli with unknown function (PDB ID: 1NNX; Kelley and Sternberg, 2009). The MamS structure model displays six β-sheets with two short α-helices, similar to the OB fold which is known to create a β-barrel structure that can bind oligonucleotides or oligosaccharides (Murzin, 1993).

FtsZ-LIKE
The ftsZ-like gene was found to be a second copy of the gene encoding the tubulin-like FtsZ protein in Magnetospirillum species, but missing the 200 amino acid C-terminal tail (Ding et al., 2010). Deletion of ftsZ-like does not affect cell division but results in smaller magnetite crystals (Ding et al., 2010). It was shown that FtsZ-like displays both ATPase and GTase activities and also has GTP-dependent polymerization into filaments in vitro (Ding et al., 2010). FtsZ proteins are known to be involved in cell division and

Phage-encoded excisionase (Xis) proteins are involved in DNA architectural structure and are needed for recruiting integrase to a specific binding site for its excision (Sims et al., 2002). MamR secondary structure prediction shows a fold of four α-helices and two short β-strands (Sabinski et al., 2007).

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create the septum which allows the cell to divide into two daughter cells (Scheffers and Drössler, 2001). FtsZ is known to have GTPase activity and to create a filamentous structure within the cell (Mukherjee and Lutkenhaus, 1998). The FtsZ 3D structure contains two domains, one of which is the N-terminal domain, which has a Rossmann fold containing the GTP binding site and the other is the CTD, which is required for proper polymerization of FtsZ and interaction with other proteins (Din et al., 1998; Lowé and Amos, 1998).

Prediction of the FtsZ-like 3D structure shows high similarity to other FtsZ proteins (Figure 6; PDB ID: 2RHL; Arnold et al., 2006). The FtsZ-like structure model presents the two known FtsZ protein domains. The electrostatic density map fits the GTP binding pocket characteristics (Figure 7). It can be assumed from the structure and the high homology between the FtsZ proteins that FtsZ-like may be involved in cell division processes (Abschul et al., 1990).

**MamC**

MamC (also known as Mms13) is a small, 12.4 kDa protein and is strongly conserved in most magnetotactic α-proteobacteria (Abreu et al., 2011). Deletion of MamC does not present any defects in crystal size or shape and is tightly associated with the magnetosome membrane in *M. gryphiswaldense* MSR-1 strain (Wawer et al., 2001; Scheffel et al., 2008). ∆mamC cells show mislocalization of MamC-GFP to the magnetosome chain (Quinlan et al., 2011). Prediction of MamC secondary structure prediction shows that the N-terminal is located within the magnetosome lumen containing crystal nucleation during magnetite formation and directs the shape of magnetite crystals in vitro (Arakaki et al., 2003). Mms6, MamD and MamG have a common Leu-Gly repetitive motif to MamD and Mms6 (Scheffel et al., 2008). Prediction of its secondary structure in the XtalPred server displays two transmembrane helices and an unstructured C-terminal that faces the bacteria cytosol (Figure 6). Sequence analyses indicate that most of the protein’s amino acids are hydrophobic except for the last residues, which are negatively (Asp68, Glu78, and Glu82) and positively charged (Lys71, Arg73, and Lys74).

**MamD**

MamD is a 30.2 kDa protein which contains a transmembrane helix on its C-terminal and a Leu-Gly repeat domain on its N-terminal, which is located in the magnetosome lumen (Richter et al., 2007; Arakaki et al., 2008). MamD is one of the magnetosome membrane-associated proteins which controls magnetite crystal size (Scheffel et al., 2008). Its secondary structure prediction shows that the N-terminal is located within the magnetosome lumen with β-sheets and α-helices. Prediction of the MamD 3D structure shows only a small part of the magnetosomal side (Gly116 to ALA 158) and displays a β-sheets fold which is based on the CTD of FlgD protein from *Xanthomonas campestris* (PDB ID: 3C12; Figure 6, Kelley and Sternberg, 2009). FlgD is a scaffold protein and is required for flagellar hook assembly (Ohnishi et al., 1994). Sequence alignment between MamD and FlgD presents low sequence identity, which does not suggest a function for MamD. Most of the residues in the MamD structure are hydrophobic with a few polar or negatively charged residues (Kelley and Sternberg, 2009).

**MamF**

MamF is a small, 12.3 kDa protein which shares 61% identity with Mms6 (Lohse et al., 2011). MamF was shown to be protected against proteolytic degradation due to its integral membrane localization and its highly hydrophobic nature with three predicted transmembrane helices (Gru et al., 2004). In vivo localization of MamF-GFP was identified as linear spots along the cell axis in accordance with the magnetosome chain localization (Lang and Schüler, 2008). MamF secondary structure prediction shows three transmembrane helices with short connecting loops (Figure 6; Kelley and Sternberg, 2009). The first loop which predicted to be located in the magnetosomal side and includes highly charged residues (Arg39, Asp40, Asp41, and Glu42) which may indicate a possible interaction with the magnetite particles (McGuin et al., 2000; Kelley and Sternberg, 2009).

**Mms6**

In *M. gryphiswaldense* MSR-1 strain, Mms6 is a small, 136 amino acid protein that was suggested to undergo proteolytic cleavage from its proprotein (Gru et al., 2004). In *M. magneticum* AMB-1 strain, ∆mms6 strains showed smaller magnetite crystals with different shapes (Tanaka et al., 2011; Murat et al., 2012). The Mms6 C-terminal is highly acidic and the region between the middle and the C-terminal contains basic amino acids (Arakaki et al., 2005). It was shown that Mms6 possesses iron-binding activity and it was suggested that the C-terminal region can initiate crystal nucleation during magnetite formation and direct the shape of magnetite crystals in vitro (Arakaki et al., 2003). Mms6, MamD and MamG have a common Leu and Gly repetitive sequence...
et, neither the driving force nor the player were determined in the existence of such a helical sequence indicates that the predicted transmembrane helix (G91). Analysis of the Mms6 model structure and protein electrostatic density map of Mms6 3D structure model results in statistical terms in the energy function (Cheng et al., 2005). The predicted in 3Dpro server which use structural characterization and by one transmembrane helix and a C-terminal, which may form a terminal domain that is predicted to be unstructured followed to iron binding, a conformational change is induced (Feng et al., M. magneticum in January 2014 | Volume 5 | Article 9 | www.frontiersin.org

∼ the size of the membrane structure (with a highly curved concave turn followed by a convex membrane starts to fold into a vesicle – by a yet unknown mechanism – followed by the localization of magnetosome proteins to the vesicles. However, the diameter of the invagination membraneous neck is only about 40 Å or less (Konemi et al., 2006). By analyzing the size of these proteins some key questions regarding the magnetosome membrane invagination can be answered. One of the questions that still exist in the magnetosome field regards how the magnetosome membrane invaginates. In the literature, the invagination process is described as a curving of the inner membrane that creates a pocket shape. Yet, neither the driving force nor the player were determined in MTB (Konemi et al., 2006). One possible option is that the inner membrane starts to fold into a vesicle – by a yet unknown mechanism – followed by the localization of magnetosome proteins to the vesicles. However, the diameter of the invagination membraneous neck is only about 40 Å or less (Konemi et al., 2006) with a highly curved concave turn followed by a convex membrane structure (Figure 8). This geometry is very limited in size and shape, especially with the concave turn. Based on our model, many proteins have domains that are larger in size than the magnetosome invagination diameter. For example, MamB, which is essential to the magnetosome invagination, has a cytoplasmic domain with a size of ∼52 Å and a magnetosomal domain in the size of ∼37 Å (Figure 3). Other examples are MamO and MamE, both with large, CTD (∼36 Å in size; Figure 3). Even by looking at these few examples we can determine that such proteins cannot pass through this magnetosome invagination neck due to their size, especially if this part is held by other proteins, as suggested by Tanaka et al. (2010). A more probable mechanism that fits our structural data is that the magnetosome proteins are sorted prior to the magnetosome invagination and accumulate on the inner membrane as protein–protein–lipid complexes that might be mimicking lipid rafts (Figure 8). The formation of such a complex, together with the natural curvature of the integral magnetosome membrane helices, may lead to a natural invagination without a special protein support; a process that will gain more force as more proteins are targeted into the membrane complex. Based on the genomic data, deletion of several MAPs (MamB, MamQ, MamL, and MamL) leads to the abolishment of magnetosome invagination (Murat et al., 2010) and we can suggest these as hubs for the protein–protein interaction, or as proteins with correct curvatures needed for the magnetosome invagination.

From the prediction of structures it can also be shown that some of the proteins have protein–protein interaction domains (such as PDZ and TPR domains). Such domains can be indicative of the creation of possible protein networks that are involved in magnetosome formation. It has already been shown that such interactions exist between MAPs; for example, between the MamE PDZ domain and the CTD of MamB (Ube et al., 2011), between MamK and MamL, and even between MamA and other proteins (Yamamoto et al., 2010). Except for the protein–protein interaction domains there are other redundant predicted domains that appear in magnetosome proteins such as: proteases (MamO and MamE), cytochrome c domains (MamI, MamT, MamX, and MamZ), CDF (MamM and MamB), and transporters (MamO, MamN, MamH, and MamZ). This redundancy can be found in the function of all four genes in the mamCD and mms operons which control the size and shape of magnetite crystals (Schiefel et al., 2008). These repetitive functions and domains indicate the ability of the bacteria to ensure magnetosome formation and its activity.

Another question that is raised from protein structure predictions and protein orientations in the magnetosome membrane is why we do not see a gap between the magnetosome membranes and the mineral crystal in electron microscopy (EM) images. In EM experiments of magnetosome vesicles the magnetosome membrane is attached directly to the magnetic particle without a ∼3 nm gap that will fit our predicted protein domain (Murat et al., 2010; Abreu et al., 2013). The distance between the magnetosome membrane and the particle is not enough to contain the proteins that are directed to the magnetosome lumen (MamQ, MamE, MamO, MamP, MamX, MamY, and MamZ; Figures 1, 3, and 6). One explanation is that this may be the result of a similar density between the membrane and proteins together with a large contrast between the mineral and the membrane that hinders the detection protein gaps, as seen in EM. Another hypothesis is that protein degradation by magnetosome proteases is taking place during mineral maturation that reduces the proteins’ outer membrane domains. Yet, more experiments will need to be conducted in order to resolve this issue.
| Protein | Number of amino acid | Transmembrane helices | Domain | Predicted structure template | % Structure confidence or sequence identity (E value) | Major function in | Gene function |
|---------|----------------------|-----------------------|--------|----------------------------|------------------------------------------------------|------------------|--------------|
| MamE    | 591                  | 1 (27–48)             | Magnetochrome, trypsin and PDZ | 2Z3I | 100% | Biomineralization |
| MamT    | 171                  | 1 (10–28)             | Magnetochrome | Secondary structure only | – | Biomineralization |
| MamO    | 632                  | 1, 1 (N-terminal, 7-C-terminal) | Trypsin and anion transport | 1LCY | 17% | Biomineralization, iron transport |
| MamH    | 428                  | 12                    | MFS, similar to MamZ | 2XUT, 4JDD (EptP) | 100% | Biomineralization |
| MamI    | 77                   | 2                     | No conserved domain | Secondary structure only | – | Membrane invagination |
| MamP    | 270                  | 1 (N-terminal)        | PDZ, magnetochrome | 4U3 (MamP MID) | Structure was determined | Biomineralization |
| MamN    | 437                  | 11                    | Na⁺⁺/H⁺ antiporter | 4F36 (monomer) | 100% | Biomineralization, iron transport and nucleation |
| MamD    | 314                  | 1 (C-terminal)        | No conserved domain | 3C12 (Rhd) – only N-terminal | 77.10% | Crystal shape |
| MamM    | 318                  | 3                     | CDF, metal carrier | 3H90 (RufF) | 100% | Iron transport |
| MamB    | 297                  | 3                     | CDF, metal carrier | 3H90 (RufF) | 100% | Iron transport |
| MamQ    | 273                  | 1 (N-terminal)        | LamA protein | 2ETD (LamA) | 100% | MFS formation |
| MamR    | 72                   | –                     | DNA-binding | 2K5S (Rv2175c) | 95.90% | Crystal shape |
| MamG    | 180                  | 1 (N-terminal)        | OB fold | 1NNX (YgW6) | 91.80% | Post-nucleation |
| MamZ    | 661                  | 18                    | YesZ-like domain | 1PW4 (DjF), 3C0S | N-terminal (100%), C-terminal (32.86%) | Biomineralization |
| MamJ    | 426                  | –                     | CAF domain modified unstructured, TonB | Unstructured protein | – | Magnetosome assembly into chain structure |
| MamK    | 360                  | –                     | Filaments structure (MreB, ParM, actin-like, ATP binding site) | 1JCF (MreB) | 100% | Magnetosome assembly into chain structure |
| MamL    | 123                  | 2                     | Similar to MamL | Secondary structure only | – | Membrane invagination |

(Continued)
Table 1 | Continued

| Protein | Number of amino acid | Transmembrane helices | Domain | Predicted structure template | % Structure confidence or sequence identity (E value) | Major function in |
|---------|----------------------|-----------------------|--------|-----------------------------|-------------------------------------------------------|------------------|
| MamA    | 217                  | –                     | TPR    | 3AS4 (MamA, AMB-1), 3AS8 (MamA, MSR-1), 2KUC (MamA, M. bavaricum) | Structure was determined Surrounded the magnetosome vesicles (MamA, M. bavaricum) | |
| MamU    | 297                  | –                     | DGKs   | 2.00E-15                    | Membrane invagination                                   | |
| MamX    | 269                  | 1 (N-terminal)        | Similar to MamE and MamS, cytochrome, DNA binding domain | 1ODX, UNI, 3ML1, 1QO8 (cytochrome C), 2QOA, 3KDF (3QF6, Q6 fold) | 90% (N-terminal) Crystal maturation                     | |
| MamY    | 371                  | 2 (N-terminal)        | BAR domain | 1SJ8 (talin) | 95.40% | Membrane invagination |
| FtsZ like | 323                  | –                     | GTPase actin domain | 2PIH, 1QO8 | 100E-92 | Crystal size and cell division |
| MamC    | 125                  | 2                     | No conserved domain | Secondary structure only | – | Crystal size and shape |
| MamG    | 84                   | 2                     | Similar to MamD and Mms6 | Secondary structure only | – | Crystal size and shape |
| MamF    | 124                  | 3                     | Similar to MmsF | Secondary structure only | – | Crystal size and shape |
| Mms6    | 136                  | 1                     | C-terminal highly acidic involved in nucleation | Energy calculations of the sequence | – | Crystal size and shape |
We thank Mr. Samuel Cronin for his help with the manuscript.

METHODS

All MTB protein sequences were extracted from the NCBI website, followed by homologous protein and conserved domain searching with the BLAST server. To analyze the protein sequences and to predict their structures we used several different servers: XtalPred and PaIPred were used to identify protein secondary structure (McGuffin et al., 2005; Slabinski et al., 2007). Swiss-Model, HHpred, Phyre, and PaIPred servers were used to predict the protein structures and to identify their possible functions (Guex and Peitsch, 1997; McGuffin et al., 2000; Soding, 2005; Kelley and Sternberg, 2009). Each model structure was energetically minimized in the Swiss PDB Viewer program for a few energy minimization cycles (Guex and Peitsch, 1997). Electrostatic calculations were performed via the APBS tool and images were prepared using PyMOL (Baker et al., 2001; DeLano, 2002). All the obtained model structure files are available at http://fidsers-bga.ac.il/obisarivach/pages/research/predicted-structures.php.

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