Magnetotactic Bacteria
5th International Meeting

MTB 2016
Marseille
11-15 Sept 2016

Program

Sunday	Welcome
Monday	Magnetosome synthesis
Tuesday	Physico-chemical characterization
Wednesday	Biotechnology
Thursday	Biodiversity

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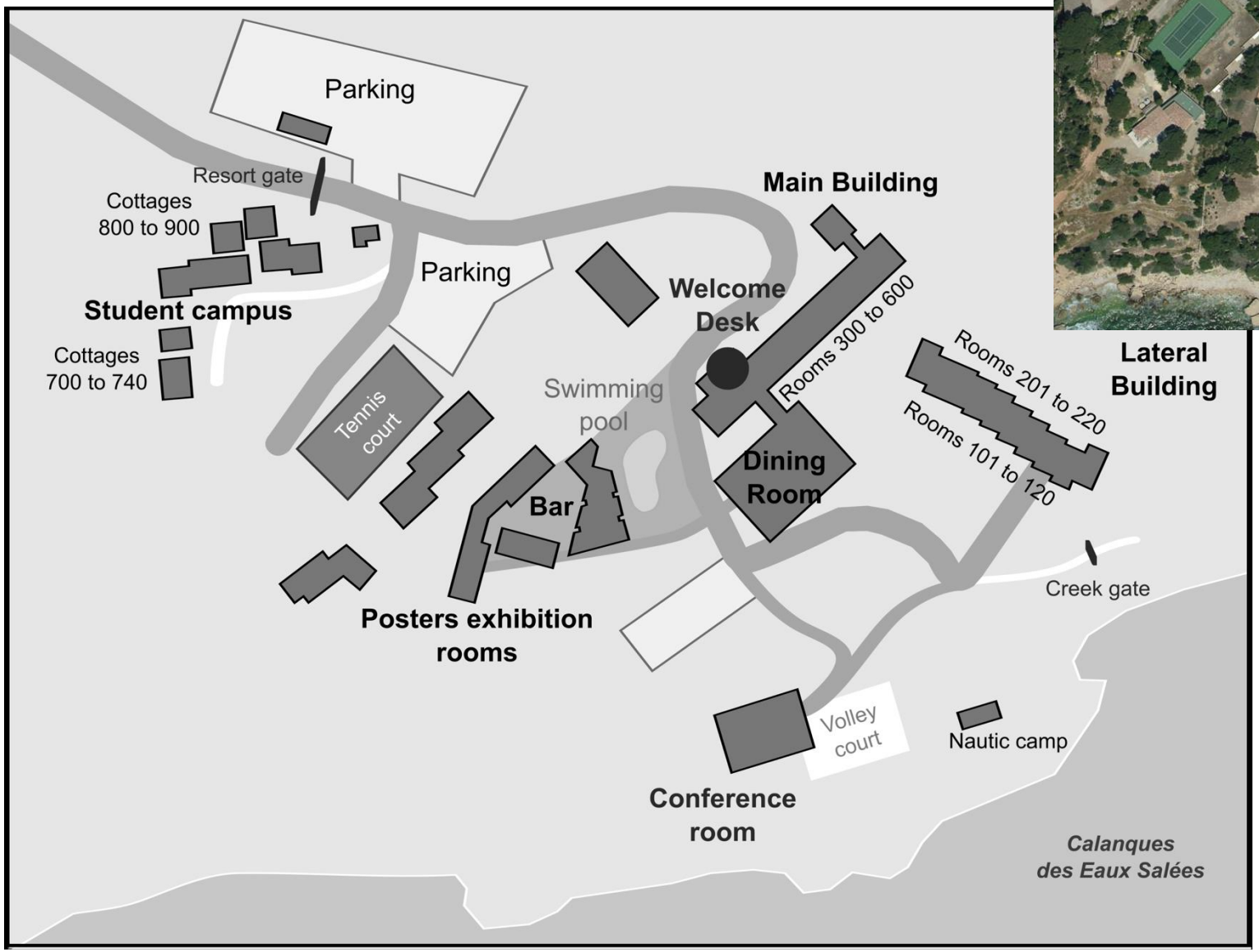

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SCIENTIFIC PROGRAM OF THE 2016 MTB MEETING

Sunday

18:00-18:30	Welcome Speech		
18:30-19:00	**R. Frankel**	What bacteria know about permanent magnets	Page 07
19:00-20:00	Aperitif		
20:00-21:30	Dinner		
21:30	Bar is open		

Monday

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09:35-09:55	**S. Barber-Zucker**	ZnT-10 disease-related mutation studied *via* magnetotactic bacteria shows a domain structural loss	Page 10
09:55-10:15	**R. Uebe**	Magnetosomal iron uptake proceeds through multiple cellular routes	Page 11
10:15-10:35	**H. Nudelman**	Structure and function analysis of MamC, a magnetite-associated protein from magnetotactic bacteria	Page 12
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10:55-11:25	**A. Komeili**	Beyond simple genetics: understanding the dynamic mechanisms of magnetosome formation in diverse organisms	Page 13
11:25-11:45	**P. Browne**	The Role of HtrA Proteins in magnetosome biomineralization	Page 14
11:45-12:05	**A. E. Rawlings**	Understanding MMS biomineralisation proteins to controlling the precise biomimetic/biokleptic synthesis of magnetic nanoparticles	Page 15
12:05-12:25	**A. Arakaki**	Unique localization behaviour of Mms6 protein during magnetite formation	Page 16
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14:00-16:00	Free-time		
16:00-16:30	**A. Taoka**	The actin-like cytoskeletal protein MamK and its ATPase activity play a role in the positioning of	Page 17

		magnetosomes	
16:30-16:50	**F. Müller**	A new magnetosome chain positioning determinant in *Magnetospirillum gryphiswaldense*	Page 18
16:50-17:10	**M. Toro-Nahuelpan**	Segregation of magnetosomes is driven by MamK filament treadmilling and its transient interaction with MamJ in *Magnetospirillum gryphiswaldense*	Page 19
17:10-17:30	**É. Bereczk-Tompa**	Flagellar nanotubes engineered with MamI and Mms6 magnetosome-associated proteins for magnetite binding	Page 20
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17:50-18:20	**R. Zarivach**	Structural studies of magnetosome-associated proteins: a key approach for basic science and biotechnology development	Page 21
18:20-18:40	**D. Pfeiffer**	The polar landmark proteins PopZ and TipN affect cell division and motility in *Magnetospirillum gryphiswaldense*	Page 22
18:40-19:00	**C. Grant**	Magnetsomes meet ferrosomes : characterization of an iron-accumulating microbial organelle	Page 23
19:00-19:20	**Y. Zhang**	A novel LysR type transcriptional regulator OxyR-Like impairs magnetosome maturation via controlling the expression of pyruvate dehydrogenase complex	Page 24
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21:30	**Poster session with a beer at the Bar**		

Tuesday

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09:15-09:35	**M. Amor**	Magnetite biomineralization in *Magnetospirillum magneticum* strain AMB-1: An iron isotope study	Page 26
09:35-09 55	**A. Muela**	Following the incorporation of new elements into the magnetosome structure	Page 27
09:55-10:15	**F. Guyot**	Insights into the magnetite formation medium in magnetotactic bacteria from measurements of trace and minor elements	Page 28
10:15-10:35	Coffee Break		
10:35-11:05	**T. Prozorov**	Magnetite magnetosome biomineralization *in situ*	Page 29

11:05-11:25	**J. Werckmann**	Mapping iron close to magnetosome surface using high resolution analytical transmission electron microscopy	Page 30
11:25-11:45	**E. Günther**	Probing the intracellular pH in magnetotactic bacteria	Page 31
11:45-12:05	**R. Le Fevre**	Magnetic characterization of magnetosomes before and after extraction from bacteria	Page 32
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14:00-16:00	Free-time		
16:00-16:30	**S. Klumpp**	Modeling magnetotactic bacteria	Page 33
16:30-16:50	**C. Fradin**	Taking the temperature of rotating magnetotactic bacteria	Page 34
16:50-17:10	**B. Kiani**	Elastic properties of magnetosome chains	Page 35
17:10-17:30	**A. Tay**	Directed evolution of *Magnetospirillum magneticum* (AMB-1) with magnetic ratcheting platform	Page 36
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21:30	**Poster session with a beer at the Bar**		

Wednesday

08:45-09:15	**S. Staniland**	From magnetosome to artificial magnetosomes for biomedical applications	Page 37
09:15-09:35	**M. Boucher**	Genetically functionalized magnetosomes used as MRI contrast agent for *in vivo* molecular imaging of brain tumor in U87 mice.	Page 38
09:35-09:55	**A. Fernández-Castané**	Bioprocessing magnetotactic bacteria and production of magnetosomes: challenges toward industrial applications	Page 39
09:55-10:15	**M. L. Fdez-Gubieda**	Optimal parameters for hyperthermia treatment of magnetosomes	Page 40
10:15-10:35	**S. Martel**	Clinical platform based on magnetotactic bacteria	Page 41
10:35-10:55	Coffee Break		
10:55-11:15	**Y. Geng**	Antitumor effect and translocation of drug-loaded magnetosomes	Page 42
11:15-11:35	**J-B Abbé**	Engineering of magnetotactic bacteria for cobalt accumulation	Page 43
11:35-11:55	**T. Song**	Applications of magnetotactic bacteria under the regulation of magnetic fields to combat	Page 44

		Staphylococcus aureus	
11:55-12:15	**L. Qi**	Preparation of the BMs based drug carrier with crossing blood-ocular barrier ability and its therapy of Choroidal melanoma	Page 45
12:15-12:35	**F. Mickoleit**	Generation of multifunctional bacterial nanoparticles by genetic engineering and surface display of peptides and reporter enzymes	Page 46
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20:00-21:30	Barbecue Party		
21:30	Bar still open		

Thursday

08:45-09:15	**D. Bazylinski**	Clues to culturing freshwater magnetococci using a metagenomic approach	Page 47
09:15-09:35	**U. Lins**	Culture-independent characterization of magnetotactic bacteria from acidic habitats	Page 48
09:35-09:55	**Y. Chen**	Large diversity and temporal variation of the multicellular magnetotactic prokaryotes in Sanya	Page 49
09:55-10:15	**D. Trubitsyn**	Transcriptional response of *Magnetovibrio blakemorei* MV-1 to different terminal electron acceptors and iron availability	Page 50
10:15-10:35	Coffee Break		
10:35-11:05	**C. Lefèvre**	Genomic insights into magnetotactic bacteria	Page 51
11:05-11:25	**W. Lin**	Metagenomics of magnetotactic bacteria	Page 52
11:25-11:45	**W. Zhang**	Characterization and genomic analysis of an uncultivated magnetotactic bacterium in the phylum *Nitrospirae*	Page 53
11:45-12:15	*Final Words*		
12:30-14:00	Lunch		
14:00-17:00	*Check-out and Shuttle*		

ORAL PRESENTATIONS

What bacteria know about permanent magnets

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In 1975, R. P. Blakemore published the first report of bacteria with motility directed by the geomagnetic field, i.e., magnetotaxis [1]. He proposed that magnetotactic bacteria (mtb) have an intracytoplasmic magnetic moment that causes mrb orientation in the geomagnetic field. This proposal was vetted with a number of experiments and observations, particularly between the years 1978 and 1982 [2-7]. The results were consistent with a permanent magnet that persists in the cell and descendants and is sufficiently robust so the cell is sufficiently oriented along the geomagnetic field in spite of thermal disorientation. Amazingly, the construction of this magnet satisfies a number of constraints to make an efficient product that enables the mtb to swim straight and not lose its heading and also allows mtb to pass on magnetic polarity during cell division. I will review this story and discuss what mtb had to get right to produce this wonderful permanent magnet.

[1] Blakemore RP. *Magnetotactic bacteria*. **Science.** 1975. 190, 377-379.
[2] Kalmijn AJ., Blakemore RP. *The magnetic behaviour of mud bacteria*. **Animal Migration and Homing**.1978. Edited by K. Schmidt-Koenig and Keeton WT, Springer Verlag, New York, 344-345.
[3] Frankel RB., Blakemore RP., Wolfe, R *Magnetite in freshwater bacteria*. **Science**. 1979. 203, 1355-1356.
[4] Frankel RB., Blakemore RP. *Navigational compass in magnetic bacteria*. **Journal of Magnetism and Magnetic Materials**. 1980. 15-18, 1562-1564.
[5] Denham CR., Blakemore RP., Frankel RB. *Bulk magnetic properties of magnetotactic bacteria.* **IEEE Transactions on Magnetics**. 1980. Mag-16, 1006-1007.
[6] Rosenblatt C., Torres de Araujo FF., Frankel RB. *Birefringence determination of magnetic moments of magnetotactic bacteria*. **Biophysical Journal**. 1982. 40, 83-85.
[7] Kalmijn AJ. *Biophysics of magnetic field detection*. **IEEE Transactions on Magnetics**. 1981. Mag-17, 1113-1124.

How magnetosome membranes are formed: Unveiling the early steps of magnetic organelle biogenesis

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Magnetosome biosynthesis is as stepwise process which is thought to begin with the formation of a dedicated compartment, the magnetosome membrane (MM). While the biomineralization of magnetic crystals and their subsequent assembly into magnetosome chains have become increasingly well studied, the molecular mechanisms and early stages involved in MM formation had remained poorly understood. By ultrastructural analysis of key mutant strains we now have identified the gene complement controlling MM formation in the alphaproteobacterium *Magnetospirillum gryphiswaldense* [1]. Whereas the magnetosomal iron transporter MamB was most crucial and caused the most severe MM phenotype upon gene deletion, MamM, MamQ and MamL were also required, but not sufficient for formation of MMs. A subset of seven magnetosome genes (*mamLQBIEMO*) combined within a synthetic operon was sufficient to restore the formation of intracellular membranes but not magnetite biomineralization in the absence of other genes from the key *mamAB* operon. Cryo-electron tomography also revealed previously unknown details of MM structure as well as the presence of novel atypical membrane vesicles, putatively representing defective or immature MM states. Tracking of *de novo* MM formation by genetic induction revealed that individual magnetosomes originate from random locations before their alignment into coherent chains. Overall our results indicate that MM formation is orchestrated by the cumulative action of several magnetosome proteins with multiple and partially overlapping functions.

[1] Raschdorf, O., Forstner, Y., Kolinko, I., Uebe, R., Plitzko, J. M., & Schüler, D. (2016). Genetic and Ultrastructural Analysis Reveals the Key Players and Initial Steps of Bacterial Magnetosome Membrane Biogenesis. *PLoS Genet*, *12*(6), e1006101–23

Lipid rafts in *Magnetospirillum gryphiswaldense* MSR-1

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Receptor-mediated endocytosis and similar pathways were widely used among eukaryotic cells when absorbed nutritions or even phagocytized viruses [1]. Up to now, at least seven receptor-mediated endocytic pathways have been reported. Although an endocytosis-like process was reported in *Gemmata obscuriglobus* recently, it is still in debate. The process of magnetosome membrane invagination is much more similar to the formation of endocytic vesicles in morphogenesis.

All eukaryotic endocytic pathways begin with the formation of lipid rafts or similar membrane structures. Lipid rafts are the special structures of cell membrane which are enriched in protein receptors and particular lipids. Rafts were once thought to be absent in prokaryotes, but recent reports confirmed the presence of detergent resistant membranes (DRMs), which were later proved to be lipid rafts in some model bacteria [2].

In this work, we investigated the presence of lipid rafts in the magnetotactic bacteria, *Magnetospirillum gryphiswaldense* MSR-1, from the perspectives of proteins and lipids, respectively. By LC-MS and GC-MS, we found the lipid and protein compositions in DRMs were different from other membrane regions. It is noteworthy that the majority of Mam proteins associated with DRMs. The lipid raft marker protein, flotillin were found on magnetosomes. We suggested that lipid rafts may play a role in the process of magnetosome biosynthesis.

[1] Gary J. Doherty and Harvey T. McMahon. *Mechanisms of endocytosis*. **Annual Review of Biochemistry.** 2009. 78, 857-902.
[2] Lopez, D. and R. Kolter. *Functional microdomains in bacterial membranes.* **Genes & Development.** 2010. 24, 1893-1902.

ZnT-10 disease-related mutation studied via magnetotactic bacteria shows a domain structural loss

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The cation diffusion facilitator (CDF) protein family is a highly conserved, metal ion efflux transporter family that maintains divalent transition metal cation homeostasis. Human CDF proteins are named Zinc Transporters (ZnT) 1-10. Different mutations within ZnT proteins were shown to enhance or cause a variety of diseases, such as zinc deficiency and parkinsonism. The missense mutation L349P in the manganese transporter ZnT-10 was shown to be related to high levels of whole-blood manganese (hypermanganesemia), hepatomegaly and dystonia. In this study, we characterized ZnT-10 L349P via a CDF protein from magnetotactic bacteria, MamM, an iron-transporter that can serve as a model system to study human CDF-related disease mechanisms. We used a variety of computational, biophysical and molecular techniques to study the synonymous mutation in MamM, M250P. Our results show that the M250P mutation causes severe structural changes to the MamM cytoplasmic domain and reduces MamM function in the MTB strain *Magnetospirillum gryphiswaldense* MSR-1. Based on this work, we suggest a mechanism for the effect of the ZnT-10 L349P mutation and explain how it leads to the related symptoms in humans.

Magnetosomal iron uptake proceeds through multiple cellular routes

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Magnetotactic bacteria (MTB) accumulate tremendous amounts of iron for the biomineralization of magnetite or greigite nanoparticles inside intracellular magnetosome vesicles. The α-proteobacterial model MTB *Magnetospirillum gryphiswaldense*, for example, incorporates approximately 200 times more iron than non-magnetotactic *Escherichia coli* cells. Several possible routes for the uptake of iron into magnetosomes have been proposed. For example, when the magnetosome membrane is still in physical contact with the cytoplasmic membrane iron might enter the magnetosome lumen by direct transport or diffusion from the periplasm. An alternative, but not mutually exclusive, route involves the uptake of iron into the cell by general cellular iron transport systems and subsequent iron transport across the magnetosome membrane by magnetosome-specific transporters. Interestingly, for *M. gryphiswaldense* it has been assumed that iron for magnetite biomineralization is processed throughout cell membranes directly to magnetosomes without iron transport through the cytoplasm, suggesting that pathways for magnetite formation and biochemical iron uptake are distinct [1]. In order to identify the iron uptake route for magnetite biomineralization and the underlying molecular mechanisms, we started to genetically dissect the iron metabolism of *M. gryphiswaldense.* Preliminary characterization of iron-responsive regulator mutants shows that, in agreement with previous studies, magnetite biomineralization is only poorly integrated into the cellular iron homeostasis systems. Additionally, we provide evidence for at least two distinct intracellular magnetosomal iron uptake routes. Furthermore, our deletion study suggests that a third, yet unidentified, iron uptake route exists. In conclusion we could show that iron uptake for magnetite biomineralization proceeds through multiple pathways

[1] Faivre et al. *Intracellular Magnetite Biomineralization in Bacteria Proceeds by a Distinct Pathway Involving Membrane-Bound Ferritin and an Iron(II)Species*. **Angew. Chem. Int. Ed.** 2007. 46(44), 8495–8499

Structure and function analysis of MamC, a magnetite-associated protein from magnetotactic bacteria

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Biomineralization process can be found in kingdoms of life. It was shown that this process is under highly biological control which also involves proteins. Magnetotactic bacteria (MTB), as a model system, can navigate through the Earth's magnetic field by synthesize organelles called "magnetosomes". This organelle contain a magnetic nanoparticle of magnetite (Fe_3O_4) or greigite (Fe_3S_4) surrounded by a lipid membrane. It was shown that the magnetosome membrane contains a specific set of proteins that are thought to direct the magnetite crystals biomineralization formation. One of the proteins involved in the magnetite crystals biomineralization is an integral membrane protein -MamC. It is a small protein (~12kDa) with two transmemebrane helices. In order to understand its function, we attached its magnetosomal loop (located between H1 to H2, which predicted to interact with the magnetite crystal) onto the C-terminal of MBP (maltose-binding protein). By using X-ray crystallography, we determined the MBP-MamC-loop structure from magnetotactic bacteria to a resolution of 2.8 Å. It has also been shown that it can interact with the magnetite particle using isothermal titration calorimetry (ITC) and affect magnetite crystal size and shape during iron precipitation *in vitro*. Based on these results, we identified a specific negatively charged patch that is highly conserved among other MamC-homologous structure models. This patch may be important for the process of magnetite biomineralization and affect its size and shape. Therefore, MamC loop can be used as an anchor between magnetite particle to any desirable protein.

Beyond simple genetics: understanding the dynamic mechanisms of magnetosome formation in diverse organisms

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Over the last two decades, genetic, proteomic and comparative genomic analyses have identified a large number of factors that participate in magnetosome formation. In particular, the model organisms *Magnetospirillum magneticum* AMB-1 and *Magnetospirillum gryphiswaldense* MSR-1 have been used to probe the functions of *mam* and *mms* genes using random and directed genetic techniques. As a result, a step-wise assembly model for magnetosome formation has been proposed. In this model, membrane biogenesis, protein sorting, chain organization and biomineralization are distinct phases of the development of a magnetosome chain. Here, I will present some of our recent work that are use this model as a starting point to understand magnetosomes and magnetotactic bacteria in greater detail. First, I will discuss our efforts towards disentagling the dynamic processes that link the various the steps of magnetosome formation. By following the properties of individual magnetosomes as they form and mature, we have discovered a previously unknown control step in magnetite biomineralization. Specifically, we find that in the absence of mineral formation an individual magnetosome membrane does not grow bigger than 50 nm. However, if magnetite nucleation has been initiated the membrane can grow to a larger size that accommodates the growth of a stable single-domain particle. Second, I will explore the biochemical mechanisms by which two putative proteases, MamE and MamO, regulate the earliest stages of biomineralization. Through biochemical, structural and phylogenetic analyses we show that MamO is not a protease and instead participates in biomineralization through a novel metal-binding motif. Additionally, we find that “inactive” proteases are associated with all known magnetosome gene clusters but arose independently multiple times during the evolution of magnetotactic bacteria. Finally, I will highlight some of our work in expanding the universe of model magnetotactic bacteria beyond AMB-1 and MSR-1.

The role of HtrA proteins in magnetosome biomineralization

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It has been understood for several years that homologs belonging to the ubiquitous HtrA family of proteases play a central role in magnetosome formation within magnetotactic bacteria [1]. Specifically, the genes *mamE* and *mamO* are conserved throughout magnetotactic proteo-bacteria and *mamE* is conserved within all sequenced magnetotactic bacteria.

Using a variety of genetic and biochemical approaches we will provide evidence highlighting the distinct roles that *mamE* and *mamO* play in magnetosome biomineralization. In addition to to addressing the non-proteolytic function of MamO we will provide evidence of several targets of MamE proteolysis [2]. These targets have been determined using both a candidate gene approach as well as observing proteomic difference between magnetosomes harvested from either wild-type or mutant strains of *Magnetospirillum magneticum AMB-1.* Finally, we will show the MamE proteolysis is under some degree of control and is not a constitutive process.

[1] Quinlan A, et al. *The HtrA/DegP family protease MamE is a bifunctional protein with roles in magnetosome protein localization and magnetite biomineralization*. **Molecular microbiology**. 2011. 80.4, 1075-1087.

[2] Hershey D., et al. *MamO Is a Repurposed Serine Protease that Promotes Magnetite Biomineralization through Direct Transition Metal Binding in Magnetotactic Bacteria*. **PLoS Biol 2016.** 14.3, e1002402.

Understanding MMS biomineralisation proteins to controlling the precise biomimetic/biokleptic synthesis of magnetic nanoparticles

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Magnetosomes are highly monodispersed intracellular compartments containing magnetite nanoparticles (MNP), with strict conservation of morphology within each strain, but a diversity of shapes across different strains. This indicates a high degree of biological precision and control over the biomineralisation process. Proteomic and genetic studies have identified specific proteins which are responsible for exerting control over crystal formation. Here we focus on Mms6 and MmsF. These proteins can be used *in vitro* to control the formation of precise MNP [1, 2]. We report our research into understanding how these proteins interact with iron ions and control the crystallisation of precise MNP [3]. This understanding is helping to inform our protein mediated chemical precipitation of precise MNP and enabling us to develop “easier to express” mimics of these proteins that can be produced in greater yield. Furthermore these proteins can be used to pattern MNP in arrays on surfaces [4-6]. In addition, we have used a phage display protein library to identify protein sequences and amino acid preferences that specifically bind to MNP [7], and this information is used to complement our understand of how MNP morphology controlling and nucleation proteins function.

[1] Amemiya, Y., Arakaki, A., Staniland S. S., Tanaka, T. and Matsunaga, T. Controlled formation of magnetite by partial oxidation of ferrous hydroxide in the presence of recombinant protein Mms6. **Biomaterials** (2007) **28**, 5381-5389

[2] Rawlings, A. E., Bramble, J. P., Walker, R., Bain, J., Galloway, J. M. and Staniland, S. S. Self-assembled MmsF proteinosomes control magnetite nanoparticle formation in vitro. **PNAS**. (2014) **111**, 16094-16099

[3] Rawlings, A. E., Bramble, J. P., Hounslow, A. M., Williamson, M. P., Monnington, A. E., Cooke, D. J. and Staniland, S. S. Ferrous iron key to Mms6 magnetite biomineralisation: A mechanistic study to understand magnetite formation using pH titration and NMR. **Chemistry: A European Journal.** (2016) **22**, 7885-7894

[4] Bird, S. M., El-Zubir, O., Rawlings, A. E., Leggett, G. J. and Staniland, S. S. A novel design strategy for nanoparticles on nanopatterns: interferometric lithographic patterning of Mms6 biotemplated magnetic nanoparticles. **J. Mater. Chem. C** (2016) **4,** 3948-3955

[5] Bird, S. M., Galloway, J. M., Rawlings, A. E., Bramble, J. P. and Staniland, S. S. Taking a hard line with biotemplating: cobalt-doped magnetite magnetic nanoparticle arrays. **Nanoscale**. (2015) **7**, 7340-7351

[6] Galloway, J. M., Bramble, J. P., Rawlings, A. E., Burnell, G., Evans, S. D. and Staniland, S. S. Biotemplated Magnetic Nanoparticle Arrays. **Small.** (2012) **8**, 204-208

[7] Rawlings, A. E., Bramble, J. P., Tang, A. A. S., Somner, L. A., Monnington, A. E., Cooke, D. J., McPherson, M. J., Tomlinson, D. C. and Staniland, S. S. Phage display selected magnetite interacting Adhirons for shape controlled nanoparticle synthesis. **Chem. Sci.** (2015) **6**, 5586-5594

Unique localization behaviour of mms6 protein during magnetite formation

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Magnetotactic bacteria synthesize nano-sized single crystalline magnetite (Fe_3O_4) within an organelle specialized for magnetite crystal synthesis. The complex mechanism of magnetite crystal formation is precisely regulated by magnetosome proteins in a stepwise manner. In the previous study, we showed that Mms6 plays important role in crystal growth and morphological regulation of cubo-octahedral magnetite [1,2]. However, the detailed function of this protein on the magnetite biomineralization still remains unclear. Here, we demonstrate defect of specific region or substitute single amino acid residues in the Mms6 protein to identify functional region of this protein *in vivo*. We also demonstrated subcellular localization analysis to use GFP-tag. Single amino acid substitutions indicated that the 3 acidic residues in the C-terminal region had a direct impact on magnetite crystal morphology. Subcellular localization analysis revealed that Mms6 localized along magnetosome chain structures under magnetite-forming conditions, but was dispersed in the cell under non-forming conditions. We suggest that a highly organized spatial-regulation mechanism controls magnetosome protein localization during magnetosome formation in magnetotactic bacteria.

[1] Tanaka M., et al. *Mms6 protein regulates crystal morphology during nano-sized magnetite biomineralization in vivo.* **J Biol Chem.** 2011. 286, 6386-6392.
[2] Arakaki A., et al. *Co-ordinated functions of Mms proteins define the surface structure of cubo-octahedral magnetite crystals in magnetotactic bacteria.* **Mol Microbiol.** 2014. 93, 554-567.

The actin-like cytoskeletal protein MamK and its ATPase activity play a role in the positioning of magnetosomes

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Half a century ago, bacterial cells were thought of as a simple 'bag of enzymes'. But currently it has been demonstrated that they have ordered complex macromolecular structures, bacterial organelles and cytoskeletons, similar to their eukaryotic counterparts. In eukaryotic cells, the positioning of the organelles is regulated by cytoskeletal elements. However, the function of cytoskeletal elements in the positioning of bacterial organelles is still an enigma. A well-known example of a bacterial organelle is the magnetosome, which is associated with the cytoskeletal filaments consisted of the actin-like protein MamK.
Protein dynamics, such as cytoskeletons, have been extensively studied in eukaryotic cells. However, in magnetotactic bacteria, the proposed function of MamK was based on the observations of static electron microscopy images. To determine the function of the MamK cytoskeleton in magnetotactic bacteria, we developed a live-cell time-lapse fluorescence imaging technique for examining the subcellular dynamics of magnetosomes in AMB-1. The magnetosomes were fluorescence labelled with MamC-GFP fusion proteins, and were then imaged throughout entire of the cell cycles using highly inclined and laminated optical sheet (HILO) microscopy. We observed and compered the dynamics of magnetosomes in three different types of cells, the wild type cells, the mamK deletion mutant cells, and the ATPase defective MamK mutants expressing cells. We found that the function of the MamK cytoskeleton is to position and to reliably segregate the magnetosomes. Furthermore, our results showed that MamK ATPase activity is necessary for the stable linear positioning of magnetosomes. In this presentation, we will discuss our recent working model describing the function of the MamK cytoskeleton.

A new magnetosome chain positioning determinant in *Magnetospirillum gryphiswaldense*

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Magnetotactic spirilla have evolved sophisticated mechanisms to precisely position their magnetosome chain within the cell and to maintain this positioning throughout the cell cycle. For example, within the spirillum-shaped cell body, the magnetosome chain adopts a continuous and straight line that runs parallel to the cell`s long axis. We recently identified a mutant in which this chain positioning is perturbed, i.e. the magnetosome chain is shifted to ectopic cellular positions resulting in reduced magnetic orientation. We hypothesize that in this mutant we have hit an unrecognized membrane topology sensing structural protein which also accounts for the presence of short magnetosome chains in the *M. gryphiswaldense mamK* mutant. This notion is supported by double deletion mutants, fluorescence microscopy and protein interaction data. Our findings highlight that magnetotactic spirilla use dedicated adaptations not only to assemble magnetosomes into a chain but also to match twisted cell morphology with requirements for optimal magnetic orientation.

Segregation of magnetosomes is driven by MamK filament treadmilling and its transient interaction with MamJ in *Magnetospirillum gryphiswaldense*

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Magnetosomes are organized into a linear chain by the actin-like MamK filament and its interaction with MamJ, a magnetosome-associated protein. During the cell cycle, the magnetosome chain must be split and further inherited into the offspring. Previously, MamK has been hypothesized to be involved in magnetosomes recruitment to the cell division plane. However, the underlying mechanisms of magnetosomes motion and segregation have remained largely unknown. Here, we show that fluorescently labeled magnetosome chains undergo rapid intracellular repositioning from the new poles towards midcell into the newborn daughter cells, motion which is likely provided by the pole-to-midcell treadmilling growth of MamK filaments and its interplay with MamJ. FRAP analysis show that MamK has a turnover ($T_{½}$) of ≈1 min, whereas MamJ exhibits a $T_{½}$ ≈10 s. Further impairment of MamK dynamics by a point mutation that generates rigid filaments ($T_{½}$ ≈10 min) does not affect MamJ dynamics ($T_{½}$ ≈10 s), evidencing a rather transient interaction between MamJ and MamK. Remarkably, we found that equipartitioning of the magnetosome chain occurs with unexpected high accuracy, which is directly dependent on the dynamic of MamK filaments. We propose a novel mechanism for prokaryotic organelle segregation that relies on the action of cytomotive MamK filaments together with the MamJ connector, in which the magnetosome cargo is transported in a fashion reminiscent of eukaryotic actin-organelle transport and segregation mechanisms.

Flagellar nanotubes engineered with MamI and mms6 magnetosome-associated proteins for magnetite binding

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Our goal was to build one-dimensional magnetic nanostructures in vitro by using surface-modified bacterial filaments as templates. The filament part of the bacterial flagellum is built up from thousands of flagellin subunits by a self-assembly process. In this work, we constructed flagellin mutants that contained motifs supposed to bind to magnetite nanocrystals. We chose to use fragments of two magnetosome-associated proteins (MamI and Mms6) and two synthetic sequences as the magnetite-binding motifs. The mutant flagellin variants were produced in large scale by a flagellin-deficient strain of *Salmonella typhimurium* that became capable of growing filaments displaying periodically repeated magnetite-binding sites on their surfaces. A magnetic selection procedure performed with all four mutants identified the variant containing the loop segment from the MamI protein to have the highest binding affinity towards magnetite. Isothermal titration calorimetric measurements also verified the strong binding of magnetite by the MamI-loop filament variant. In contrast, flagellar filaments displaying the C-terminal segment of Mms6 protein were not able to bind magnetite nanoparticles. The MamI-loop-displaying filaments were used as templates to form chains of magnetite nanoparticles along the filaments, by capturing magnetite nanoparticles from suspension. Besides demonstrating a new route for the production of magnetic filaments under ambient conditions, our study confirms the interaction between MamI and magnetite nanocrystals, with implications for the role of the protein in magnetotactic bacteria. Secondary structure prediction of MamI suggested that the short loop consisting of three amino acids (Th35, Glu36, Leu37) does not interact with magnetite but its function may be to bend the magnetosome membrane. The results of our experiments contradict these assumptions by showing that the loop region of MamI has the ability to strongly bind magnetite, suggesting a new, as yet unknown function of the protein.

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Structural studies of magnetosome-associated proteins: a key approach for basic science and biotechnology development

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Magnetic nanoparticles are key components in many technologies and biotechnologies. Yet, it is not easy to modify them and control their shape and size. Natural organisms that perform such control are magnetotactic bacteria. Magnetotactic bacteria navigate along geomagnetic fields by forming magnetosomes chains. Magnetosomes are intracellular membrane-enclosed, nanometer-sized crystals of the magnetic iron mineral magnetite (Fe_3O_4) or gregite (Fe_3S_4). Biomineralization of magnetite within these unique prokaryotic organelles involves the formation of the magnetosome, the transport of iron and the nucleation and controlled growth of magnetite via magnetosome-associated proteins. Here we present the use of biochemical and structural biology approaches to understand the structure-function relationship of magnetosome-associated proteins. We show here few examples of magnetosome-associated proteins, such as MamB, MamM, MamC, MamA and others, that highlight the connection between the protein structure and its function. Furthermore, structure based rational design is a key point for biotechnological application development. We discuss here the use of the data obtained by the field to develop new biotechnological tools.

The polar landmark proteins PopZ and TipN affect cell division and motility in *Magnetospirillum gryphiswaldense*

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MTB have the ability to align and navigate along the earth magnetic field lines and to swim predominantly towards one of the magnetic poles (swimming polarity). However, the molecular mechanisms which govern magnetotaxis are rather poorly understood [1]. Cells of *M. gryphiswaldense* swim by means of two flagella (one at each of the poles) and are apparently symmetrical shaped. Since the magnetic moment of the magnetosome chain has a fixed orientation (magnetic polarity) with respect to the cellular axis, control of swimming polarity might require a cellular asymmetry such as polar placement of motility related proteins. In search for polarity determinants, in the present study we investigated candidate genes coding for putative polar landmark proteins. Polar landmark proteins play important roles in defining cellular polarity in other bacteria such as the related alphaproteobacterial model organism *Caulobacter crescentus* [2]. For example, among others, genes coding for orthologues of the PopZ and TipN polarity factors were identified in *M. gryphiswaldense*. As described previously for *C. crescentus*, deletion of *popZ* resulted in delayed growth, cell elongation and formation of mini-cells. Overproduction of PopZ resulted in a polar PopZ-rich region mostly devoid of ribosomes and chromosomal DNA. However, PopZ exhibited a bipolar localization pattern in *M. gryphiswaldense*, distinct compared to the unipolar to bipolar transition described for *C. crescentus*. A *popZ* deletion mutant was motile, but aerotactic swarm halo formation in semi-solid medium was significantly impaired, whereas deletion of *tipN* had only a minor effect on motility but led to asymmetric cell division and formation of mini-cells. Our results point towards important functions as polar landmark proteins. Deeper knowledge how the cellular polarity axis is defined could help to understand how the orientation of the magnetic chain dipole and swimming polarity are linked and inherited.

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Magnetosomes meet ferrosomes : characterization of an iron-accumulating microbial organelle

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Bacterial cells, much like their eukaryotic counterparts, use subcellular compartmentalization to separate specialized, and potentially toxic, chemical reactions from sensitive cellular components. Due to their simplicity and diverse capabilities, such bacterial compartments, or organelles, have become important platforms for the design of novel biotechnological applications. Despite their relevance in both basic and applied settings, the structure and function of many bacterial organelles remain unknown. Here, I present the discovery and characterization of a novel class of iron-accumulating microbial organelles called ferrosomes (for "iron body"). Ferrosomes were serendipitously discovered by our lab while attempting to visualize the formation of magnetosomes in *Desulfovibrio magneticus* by transmission electron microscopy (TEM) [1]. Whereas magnetosomes are comprised of crystalline magnetite, ferrosomes are composed of non-crystalline iron that is surrounded by a biological membrane [1]. Additionally, ferrosomes are only observed by TEM in *D. magneticus* during transitions from limited iron to low or high iron conditions. I hypothesize that specific proteins form ferrosomes and function to accumulate iron within the compartment. Using liquid chromatography tandem mass spectrometry on isolated ferrosomes, I have identified the Fez proteins that are important for ferrosome formation in *D. magneticus*. Mining microbial genomes uncovered that *fez* genes are arranged in an operon and are common in many diverse microorganisms. Overexpression of the *fez* operon in *D. magneticus* results in constitutive ferrosome formation. In addition to *D. magneticus*, *Rhodopseudomonas palustris*, a non-magnetotactic bacterium, can also form ferrosomes. Deletion of the *fez* operon in *R. palustris* abolishes ferrosome formation, which can be rescued by complementation. Preliminary evidence suggests that ferrosomes may be important for fitness in low-iron environments. Through studying ferrosome formation and structure in many bacteria, I hope to further uncover the function(s) of ferrosomes. Additionally, this work will set the stage for future use of ferrosomes in applications that leverage their metal-accumulating capabilities.

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A novel LysR type transcriptional regulator OxyR-Like impairs magnetosome maturation via controlling the expression of pyruvate dehydrogenase complex

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Magnetotactic bacteria (MTB) synthesis intracellular membrane enveloped magnetite nanoparticles, referred to as magnetosomes. Though the chemical and genetic routes of the biomineralization of magnetosomes have been studied for decades, it still remains obscure. Here, we characterized a novel LysR type transcriptional regulator, OxyR-Like, and analyzed its function in magnetosome formation in *Magnetospirillum gryphiswaldense* MSR-1. The disruption of *oxyR-Like* resulted in low intracellular iron content and decreased ferromagnetism. Further transmission electron microscopy analysis showed that the diameter of magnetosome decreased dramatically, and types of ferric oxide nanoparticles co-existed with magnetite in *oxyR-Like*$^-$ mutant. Detailed biochemical analysis suggested that OxyR-Like can directly regulate the expression of pyruvate dehydrogenase complex. Moreover, it is also confirmed that the expression level of various genes participated in tricarboxylic acid cycle decreased dramatically in *oxyR-Like*$^-$ mutant. In summary, these results firstly provide the possibility that the formation of magnetosome requires the energy and reducing power generated by carbon metabolism, which shed new light on the link between biomineralization and bacterial foundation metabolism.

The reaction pathway to magnetite

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Magnetotactic bacteria are microorganisms that intracellularly form ferrimagnetic nanoparticles. The bacteria produce species-specific minerals (Fe_3O_4 or Fe_3S_4), particle sizes and morphologies. We will first review the case of the most studied species so far, which are α-*Proteobacteria* and form magnetite with thermodynamically favored isometric or prismatic morphologies such as cubo-octahedrons. Such crystallization occurs from ferric oxyhydroxide precursors [1, 2]. However, several species, often from the δ-*Proteobacteria* produce unusual crystal symmetry-breaking particle shapes [3]. The pathway leading to such particles has remained mostly unknown [4]. Here we will also demonstrate the existence of poorly ordered precursors to magnetite in *Desulfovibrio magneticus* RS-1 that produces bullet-shaped nanoparticles. In contrary to α-*Proteobacteria* however, the precursors are ferrous-based and not ferric-based. Our findings suggest that particle-mediated growth from solid-state precursors could be required to produce irregular crystals. Furthermore, the observations support the notion of an oxidative route to magnetite in contrast to reductive reactions in other species, reflecting potential physiological differences between bacterial species.

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Magnetite biomineralization in *Magnetospirillum magneticum* strain AMB-1: An iron isotope study

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Since the discovery of magnetotactic bacteria (MTB), tremendous advances have been made in constraining the biological and chemical pathways leading to MTB magnetite biomineralization, but the precise mechanisms for cellular Fe trafficking and magnetite formation are still a matter of debate. Here, we determined the Fe isotope fractionation by the magnetotactic strain *Magnetospirillum magneticum* AMB-1 to better constrain Fe uptake and magnetite biomineralization in MTB.

AMB-1 cultures were carried out with either Fe(III)-quinate or Fe(II)-ascorbate as Fe sources. Iron sources, bacterial growth media after AMB-1 cultures, bacterial lysates (corresponding to AMB-1 cells devoid of magnetite) and magnetite samples were analyzed for Fe isotope compositions. In the two culture conditions, growth media after AMB-1 cultures were enriched in light Fe isotopes relative to Fe sources. Two distinct bacterial Fe reservoirs were characterized in AMB-1: (1) magnetite enriched in the light Fe isotopes by 1.5 to 2.5‰ relative to Fe sources, and (2) lysate enriched in the heavy Fe isotopes by 0.3 to 0.8‰ relative to Fe sources. More importantly, mass-independent fractionations in odd (^{57}Fe) but not in even isotopes (^{54}Fe, ^{56}Fe and ^{58}Fe) were observed for the first time, highlighting a magnetic isotope effect. Magnetite samples were significantly enriched in ^{57}Fe by 0.23‰ relative to ^{54}Fe, ^{56}Fe and ^{58}Fe. Based on our results, we propose a model for Fe cycling and magnetite biomineralization in AMB-1.

Following the incorporation of new elements into the magnetosome structure

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The high biological control imposed in the synthesis of magnetosomes leads to well-defined properties such as shape, size or magnetic properties, suitable in numerous applications. Their biological coating protects them from oxidation and eases the functionalization of the surface, an interesting point for biotechnological applications.
However, the lack of tunability of species-dependent properties sometimes could be a limitation. Being able to change the stoichiometry of magnetosomes by adding small amounts of some elements into the nanoparticle structure gives rise to a change in their final properties, and therefore an extension of their range of applications. For that purpose, in the last years some authors have been involved in the synthesis of doped magnetosomes [1,2], [3]. These works report the addition of new elements, such as Co, Mn, Ni, Zn and Cu, into the growth media, probing the incorporation of some of them by compositional methods.
The aim of this work is to determine if these elements have been incorporated into the magnetosomes or are stored anywhere else in the bacteria. With this aim we have added small amounts of the already mentioned metals into the growth media of the bacteria *Magnetospirillum gryphiswaldense*. By means of magnetic characterization we can infer whether the dopant is incorporated into the magnetosome by analysing the changes of the magnetic response with respect to the well-known magnetic behavior of magnetite. In addition, we have performed *X-ray absorption spectroscopy* (XAS). This is an element-selective technique that probes the local environment of the absorbing atom. By choosing the absorption edge corresponding to the dopant element we can determine whether the element is inside the magnetite structure. The combination of the magnetic analysis and XAS has led us to conclude that the Co is incorporated into the magnetosome structure while Mn and Cu are taken up by the bacteria but are not incorporated into the magnetosomes. Thus far, no conclusive results have been obtained about Ni and Zn incorporation.

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Insights into the magnetite formation medium in Magnetotactic Bacteria from measurements of trace and minor elements

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In a previous study [1], we have compared trace and minor elements incorporation (34 elements analyzed) in chemically synthesized magnetites with those made by the magnetotactic bacterial strain AMB-1. The measurements were carried out by ICP-MS, after careful elimination of extra-magnetite trace element sources (e.g. adsorption at mineral surface or on organic residuals). As expected, the partitioning of most elements between the external medium and magnetite is lower by two orders of magnitude in the case of bacterial magnetites, i.e. bacterial magnetites contain relatively less minor and trace elements than abiotic ones. The origin of this difference is not clear yet however. A geochemical code (CHESS) has thus been adapted to model these results and to predict trace element contents of magnetite in a large range of biogeochemical contexts. It results from this model that pH and redox potential (Eh) differences are evidenced between the abiotic synthesis and intracellular/intravesicular media. However, even taking into account those pH and Eh effects, the model also shows that most other transition elements are strongly depleted in the intracellular biomineralization medium. Based on these experimental measurements and modeling, a tentative composition is proposed for the growth medium of magnetites in magnetosomes of AMB-1. This approach opens new insights for using magnetites trace element contents as reporters of the mineralization internal medium of magnetotactic bacteria in relation with their geochemical environment and could lead to a better identification of magnetofossils.

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Magnetite magnetosome biomineralization *in situ*

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Understanding magnetosome magnetite biomineralization is of fundamental interest to devising the strategies for bioinspired synthesis of magnetic materials at the nanoscale. We investigated the early stages of magnetosome formation in this work and correlated the size and emergent crystallinity of magnetosome nanoparticles with the changes in chemical environment of iron and oxygen by utilizing advanced analytical electron microscopy techniques [1]. This approach provided spatially resolved structural and chemical information of individual magnetosomes with different particle sizes, attributed to magnetosomes at different stages of biomineralization. Magnetosomes in the early stages of biomineralization with the sizes of 5–10 nm are amorphous, with a majority of iron present as Fe^{3+}, indicative of ferric hydroxide. The magnetosomes with intermediate sizes are partially crystalline, with a majority of iron present as Fe^{3+} and trace amounts of Fe^{2+}, as shown in **Figure 1**. The fully maturated magnetosomes are indexed to magnetite. Future studies will include use of electron holography to elucidate the effect of magnetic interactions between the growing magnetosome particles on their emergent crystalline structure.

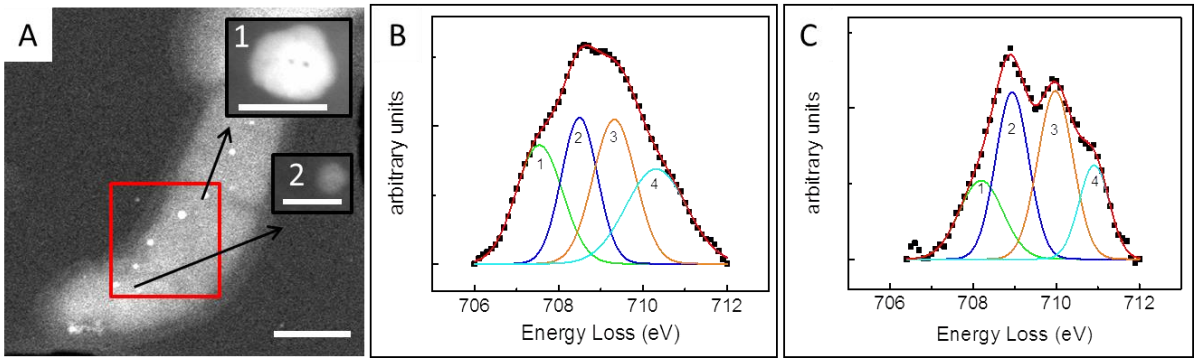

Figure 1. Magnetosomes in bacterial cell, with magnified images of the particles shown in the insets. Fe L3 core EEL spectra of the particles shown in the inset 1 with size of 52 nm and in the inset 2 with size of 10 nm are given in (b) and (c), respectively. The ratio of the areas of peak 1 to peaks (2 + 3), corresponding to Fe^{2+} (octahedral) to Fe^{3+} (tetrahedral + octahedral) ratio, is around 1:2.1 for large particle and 1:3.3 for small particle. Peak 4 is attributed to the FeO(OH). Scale bars: (a): 500 nm, inset 1 and 2: 20 nm.

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Mapping iron close to magnetosome surface using high resolution analytical transmission electron microscopy

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Magnetite crystals from magnetotactic bacteria (MTB) grow inside vesicles (magnetosome membranes) produced by the invagination of the cytoplasmic membrane of the bacteria [1]. Crystals are nucleated and grown using iron transported inside the vesicle by specific proteins. One unaddressed question is: can iron transported inside bacteria to produce magnetite crystals be spatially mapped using electron microscopy? A partial answer was obtained in this work with analytical transmission electron microscopy by studying cultivated and uncultivated bacteria from a brackish water lagoon, near Rio de Janeiro city. A drop of water enriched with MTB was deposited on holey lacey carbon grids, washed with distilled water and air-dried. Two microscopes operating in STEM mode with a 1 nm focused beam were used: i) a JEOL 2200F equipped with a high sensitivity EDSX detector was used to produce X-ray maps and ii) a JEOL 2100F microscope equipped with an EELS detector was used to collect information on electron energy loss spectra obtained from lines adjacent and parallel to crystal faces. EDSX mapping showed small amounts of iron accumulating outside the magnetosome and near the membrane, forming a continuous layer (Fig. 1a). EELS obtained at high spatial resolution (Figs. 1b-1d) confirmed that iron is present in the membrane region. Our results open the question of directional growth of crystal faces. Indeed, it has been shown that different morphologies of crystals are produced by MTB [2], but still the control over the process of crystal growth and shape modulation is not completely understood [3].

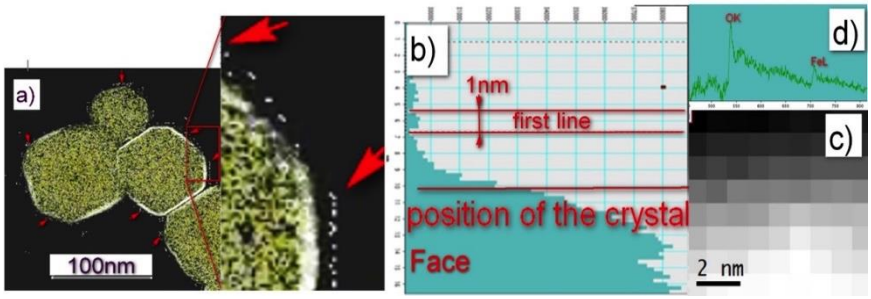

Figure 1. Analysis of iron in the periphery of the magnetosome. a) EDSX map on cultivated MTB; arrows indicate the presence of iron (detail in the insert). b) Intensity profile obtained from dark field image and c) the corresponding line by line analysis from outside to the inside the crystal region. d) EELs spectrum from the first line, with an oxygen near edge structure (ELNES) characteristic of oxygen inside organic molecules and the corresponding iron ELNES.

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Probing the intracellular pH in magnetotactic bacteria

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Biomineralisation of the magnetic particles by magnetotactic bacteria is not only under genetic control but also influenced by environmental factors. The synthesis of magnetite nanoparticles *in vitro* typically requires physical and chemical conditions such as redox potential (E_h ~ -0.2 to -0.4 V) and pH (pH > 8) that are eventually toxic to microorganisms. Therefore, the question of the intracellular conditions within magnetotactic bacteria enabling magnetite formation has remained puzzling.
In order to understand how magnetotactic bacteria biomineralise iron nanoparticles in physiological conditions, we engaged into chemically mapping the cell. We started by measuring the cytoplasmic pH in *Magnetospirillum gryphiswaldense* MSR-1 using ratiometric fluorescence microscopy and fluorescence lifetime imaging microscopy. Ratiometric fluorescence microscopy and fluorescence lifetime imaging microscopy are widely used for studies of chemical, physical and biological systems *in vivo.* They overcome drawbacks due to inhomogeneity of the probe concentration and photobleaching and allow quantitative information. We will show how the cells are able to buffer the cytoplasm (figure 1).

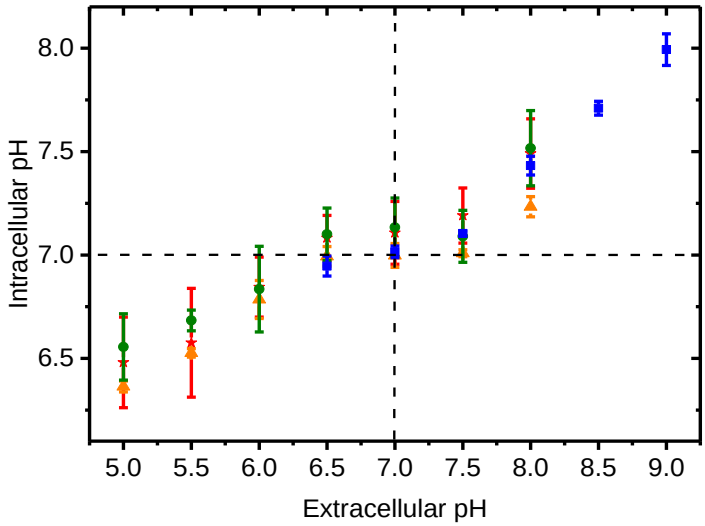

Figure 1: Buffering capacity of *Magnetospirillum gryphiswaldense*

Magnetic characterization of magnetosomes before and after extraction from bacteria

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Magnetite (Fe3O4) or greigite (Fe3S4) nanoparticles that are biomineralized by magnetotactic bacteria (MTB) and called magnetosomes fascinate scientists in a wide range of research fields from biosciences to geosciences. Magnetosomes are single domain well faceted nanocrystals with homogenous size. They are characterized by specific physical, chemical and crystallographic characteristics compared to abiotic magnetite and are aligned in chains within the MTB. In natural environments MTB are preferentially found at the oxic/anoxic zone with specific iron, oxygen and sulfide concentrations that favour their growth. In sediments the preserved magnetosomes known as magnetofossils reflect also specific environmental conditions. Furthermore, MTBs contribute to the natural remanent magnetization of sediments, where present, and with great magnetic stability due to its crystal size, ideal for recording the geomagnetic field.

We investigated the magnetic signatures of magnetosomes before and after extraction from the MTBs using TEM images and rock magnetic parameters (hysteresis parameters, room temperature coercivity analysis, low temperature remanence measurements and first-order reversal curves). *Magnetospirillum gryphiswaldense* strain MSR-1 biomineralizes cuboctahedral magnetosomes of magnetite. It was cultivated in a 70l fermenter and then purified in order to obtain different samples, which may be representative of the evolution of magnetofossils in sediments. The 1st sample is composed of intact MTB with its magnetosomes arranged in chains. The chains of magnetosomes are extracted from the MTBs in the 2nd sample and some aggregation of chains is observed. The third sample is obtained after strong chemical treatment and contains only aggregated magnetosomes without any chain structures.

TEM images and the derived magnetosome crystal size distributions do not show any apparent modification in shape or size between samples, whereas strong magnetic and mineralogical changes are observed. The magnetic properties of sample 1 are similar to previously published data for intact MSR-1 MTB, weak inter-chain interactions resulting in a narrow coercivity distribution and a strong central ridge in the FORC distribution. The magnetosomes are magnetite displaying a sharp Verwey transition at 100 K. No mineralogical changes are observed in sample 2 but having the chains extracted from the MTB leads to strong magnetic interactions that alter the magnetic properties with respect to sample 1. The magnetosomes of sample 3 display strong inter-particle magnetic interactions and its mineralogy is converted from magnetite to maghemite.

Modeling Magnetotactic Bacteria

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Magnetotactic bacteria provide ample opportunities for modeling studies. In particular, the question how the magnetic forces work together with the cellular signaling network is intriguing and requires integration of experimental and theoretical approaches. In the talk, the role of modeling will be illustrated with examples from our recent work on magnetoaerotaxis and cell division in magnetotactic bacteria.
Magnetoaerotaxis can be described with different theoretical approaches depending on the questions that are addressed: The interplay of the magnetic torque with the forces arising from the flagellar rotation can be studied with explicit hydrodynamic simulations of the propulsion mechanism, while coarse-grained models (random walks and active particles models) can be used to study the tactic behavior of individual cells and whole populations in oxygen gradients and magnetic fields.
Cell division provides another rich field of study. Since magnetotactic bacteria divide their magnetsome chain during cell division, they must possess solutions to localize the division septum in the center of the chain, to deal with magnetic attraction when separating the half-chains and to localize newly synthesized cellular components with respect to the magnetic polarity. The latter is particularly important for the motility apparatus in mono-flagellated bacteria.

Taking the temperature of rotating magnetotactic bacteria

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The contribution of non-thermal stochastic noise, or “biological noise”, to the rotational diffusion of bacteria has recently come into focus, in particular because of its possible influence on cells' foraging strategies. Magnetotactic bacteria, whose orientation is linked to a magnetic potential energy, provide an appealing system to explore cell rotational behaviors and the influence of non-thermal noise has on them. Using optical microscopy, and taking into account the magnetic susceptibility anisotropy of magnetite, we have measured the effective temperature of *Magnetospirillum magneticum* cells in two separate ways. We find that their distribution of orientations follows a Boltzman-like distribution with an effective temperature T_{eff} ~ 900 K, while their rotational diffusion coefficient corresponds an effective temperature T_{eff} ~ 1000 K. This system is therefore strongly influenced by non-thermal biological noise, yet following something akin to a fluctuation-dissipation principle.

Elastic properties of magnetosome chains

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Here we investigate the mechanical properties of the magnetosome chain, a cellular 'compass needle' that consists of a string of vesicle-enclosed magnetic nanoparticles aligned on a cytoskeletal filament. We determine the contribution of magnetic interactions to the bending stiffness and the persistence length of the chain. This contribution is comparable to, but typically smaller than the contribution of the semiflexible filament. Moreover, our calculations show that the presence of the cytoskeletal filament stabilizes the chain against ring closure, confirming that such stabilization is one of the roles of this structure in these bacterial cells.

In addition, we investigated the dynamics of magnetosome chain rupture under the influence of an external magnetic field. A magnetic field at a nonzero angle with the chain axis strains the linkage of the magnetosomes to the filament and eventually leads to their rupture. We used Monte Carlo simulations to study the dynamics of rupture and the resulting new configurations. In our simulations, rupture is seen to occur beyond a threshold of the field strength and through two discrete events at different critical values of the angle between field and chain axis. At the first, the two ends of the chain detach from the cytoskeletal filament and tilt away from the chain axis into the direction of the field. At the second, the chain breaks into disconnected smaller pieces oriented in the direction of the field and attached to the filament only in their center. This final state is in agreement with the experimental findings [1].

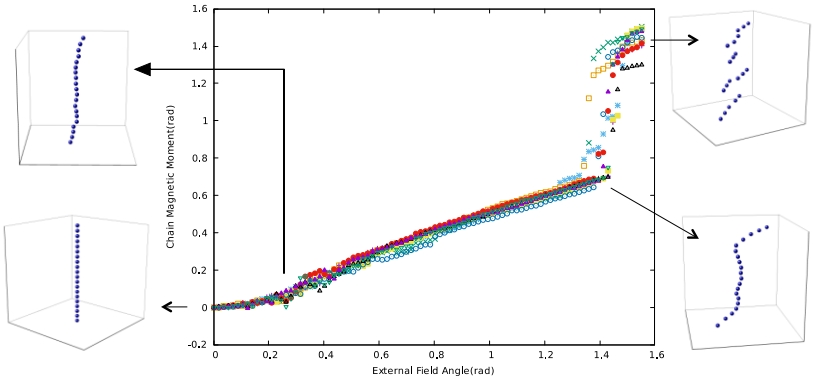

Fig. 1. Direction of magnetization of the magnetosome chain subject to an external field at different field angles

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Directed Evolution of *Magnetospirillum magneticum* (AMB-1) with Magnetic Ratcheting Platform

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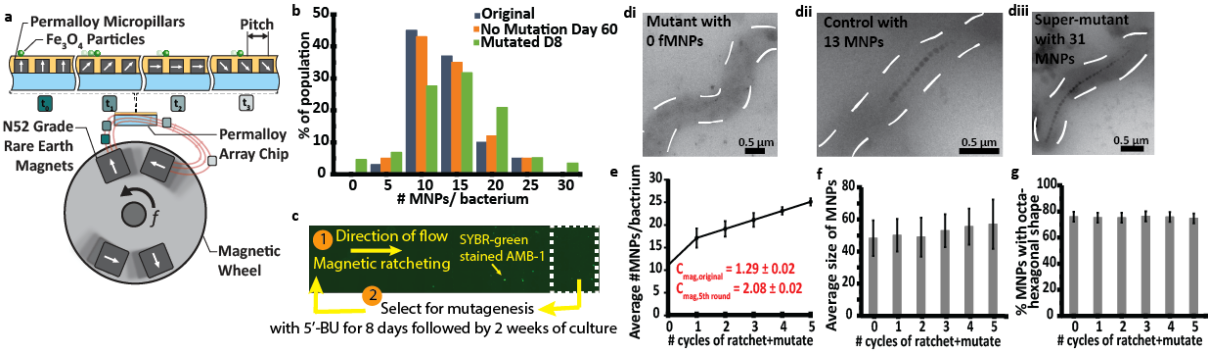
Magnetotactic bacteria (MTB) are being evolved to over-produce magnetic nanoparticles (MNPs) for biomedical applications such as cancer hyperthermia. However, screening of MTB mutants are currently performed using electron microscopy, colour visualisation of colonies or C-mag which are slow, laborious, non-quantitative and subject to users' biases. Here, (**a**) we describe a magnetic ratcheting platform consisting of arrays of permalloy (i.e. alloy of nickel and iron with exceptionally high magnetic permeabilization) micro-pillars [1] with increasing widths in horizontal pitches. Magnetic particles traverse through the pitches until they reach their critical pitch which is determined by the balance between magnetic forces and Stokes' drag (particle size). Using this concept, AMB-1 with similar sizes but greater number of MNPs will hence traverse through pitch with greater width, allowing subsequent selection. We also performed (**b**) random chemical mutagenesis on AMB-1 with 5'-bromouracil (10 µg/mL) to show that this treatment generated AMB-1 mutants that over-produced MNPs. After 5 cycles of mutation and selection (procedure shown with SYBR-green stained AMB-1 in **c**), we generated a library of AMB-1 mutants without MNPs (**di**) and those which produced 2-folds more MNPs (**diii, e**) than control (**dii**). The size (**f**), shape (**g**), elemental composition and magnetic properties of the MNPs produced by the AMB-1 super-mutants were also not significantly different from the control population, demonstrating the utility of our mutation-selection procedure for directed evolution of MTB. Our platform can also be used to screen for AMB-1 mutants without any MNPs generated with targeted genetic manipulations to understand the role of different genes regulating the biomineralization process which MTB use to produce MNPs



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From magnetosome to artificial magnetosomes for biomedical applications

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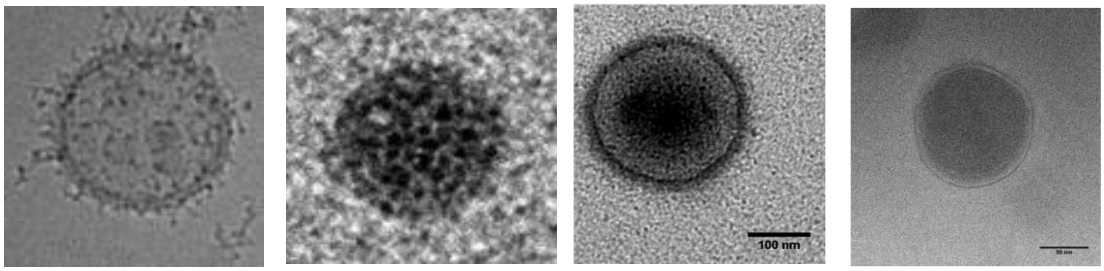
Magnetosomes show great potential for a range of biomedical applications: from diagnostic to therapeutic and combined as theranostic materials. To realise these applications we are currently modifying magnetosomes by changing their magnetic properties with dopants [1, 2] and functionalising their surfaces, then testing their cell uptake, toxicity and activity in cancer cell lines.

Further, magnetosomes themselves may not be the best magnetic nanomaterial for these applications with respect to economy of production and adaptability for different specifications. We have therefore taken inspiration from magnetic bacteria to create artificial magnetosome-like theranostic magnetic nanovesicles *in situ* under mild reaction conditions. Here we describe how iron ions are imported into base filled vesicles to form a range of magnetovesicles, from magnetic nanoparticles embedded in the membrane [3, 4] to magnetic nanoparticle core liposome similar to a native magnetosome. We show how different magnetovesicles vary in their hyperthermic and MRI response and demonstrate how we can control and tune the particle size and number to tailor them for different applications.



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Genetically functionalized magnetosomes used as MRI contrast agent for *in vivo* molecular imaging of brain tumor in U87 mice

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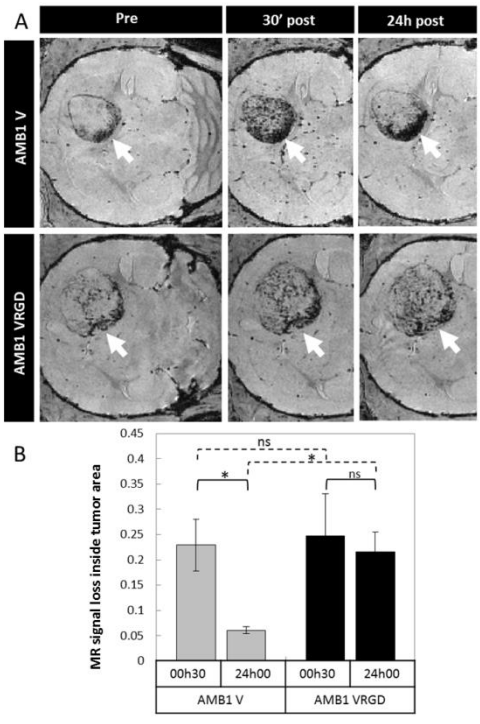
Magnetosomes can be used as very efficient contrast agent for MRI. We investigate here the use of genetically RGD functionalized magnetosomes for MR molecular imaging of U87 glioblastoma. We show *in vivo* that 24 h after intravenous injection, higher magnetosomes retention is observed in tumor with RGD functionalization compared to control. *Magnetospirillum magneticum* AMB-1 was transformed with a plasmid harboring a translational fusion between a chimeric Venus-RGD gene and the gene coding for MamC membrane protein. Functionalized (AMB1 VRGD) and control (AMB1 V) magnetosomes were purified from modified bacteria. Magnetosome suspensions were prepared in HEPES at 54 $mM_{[Fe]}$ and injected intravenously to glioblastoma bearing mouse. MR images were acquired on two groups of injected mice (5 AMB1 VRGD and 4 AMB1 V, both at 200 $\mu mol_{[Fe]}/kg_{BW}$), using 11.7 T MRI scanner. Numerous dark spots are detected on T_2*-weighted FLASH images in the tumor 30 min post injection with both magnetosomes (Fig A), confirming their efficiency to reach tumor vessels. Quantification of MR signal loss in tumor area, computed from T_2* mapping MGE images, is an indirect measure of magnetosomes uptake, which reveals significantly higher accumulation of AMB1 VRGD in tumor after 24 h compared to AMB1 V (Fig B). Furthermore, no significant decrease of AMB1 VRGD uptake is observed between 30 min and 24 h, which indicates a reduced clearance in the first 24 h, while significant clearance of AMB1 V is occurring in the same time frame (Fig B). This work is the first production under biological process of an efficient MR molecular imaging probe, here evidenced on a glioblastoma model.



Bioprocessing Magnetotatic Bacteria and production of magnetosomes: challenges toward industrial applications

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Biologically synthesized magnetic nanoparticles namely magnetosomes, are an attractive alternative to existing commercial magnetic particles because of their ferrimagnetic properties, narrow size distribution and high specific absorption rate.
Numerous efforts have been made over the last few years to improve magnetosome yields using genetic modifications or improvement of culture conditions of magnetotactic bacteria. However, large-scale cultures delivering high-cell densities in combination with efficient magnetosome recovery remains a challenging step toward industrial application. Moreover, magnetosome-membrane proteins can be used as an anchor for the fusion of proteins for a wide range of applications and therefore, its integrity needs to be taken into account upon cell disruption.
Here, we will present a robust platform for the production of magnetosomes in relatively high-cell density cultures of *Magnetospirillum gryphiswaldense* MSR-1 in 5L bioreactors. We have used a simple fermentation strategy employing a pH-stat approach and minimal control of process parameters. Chemical and mechanical methods were tested for magnetsome release followed by magnetic separation. The potential application and benefits of a newly designed 'rotor-stator' type High Gradient Magnetic Fishing (HGMF)[1] for the downstream processing and separation of magnetosomes will be discussed.
Our work represents a significant advance in the bioprocessing of magnetosomes, paving the road to a sustainable and cost-effective biotechnological application.

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Optimal parameters for hyperthermia treatment of magnetosomes

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Magnetosomes become a quality standard with regard to crystallinity, chemical purity, or biocompatibility. On the side of magnetic applications, they hold great potential as heat mediators between alternating magnetic fields (AMF) and biological tissues, as in hyperthermia, but the conditions under which the energy absorption rate is optimized, are still controversial. We hereby focus on this problem with magnetosomes extracted from *Magnetospirillum gryphiswaldense*. AC magnetometric measurements have allowed us to determine directly the power absorption rate (SAR) as a function of frequency and amplitude of the magnetic excitation, both in particles immersed in water and agorose gel. Intrinsic hysteresis losses appear to be the main dissipation mechanism responsable for the heat production. It is found that there exists a magnetic field threshold below which SAR is negligible and above which it increases quite steeply until reaching saturation. We demonstrate that this behavior can be explained in the framework of the Stoner-Wohlfart theory of uniaxial particles subjected to significant inter-particle dipolar interactions, which are quantified in the context of a biaxial anisotropy model [1]. Based on these findings we proposed optimal magnetic field amplitude and frequency to maximize the production of heat while keeping the undesired eddy current effects below safe and tolerable limits. To check the potency for cancer therapy of magnetosomes as heat nanoinductors and explore their impact on cell viability, hyperthermia and cytotoxicity experiments were also conducted in murine macrophages. Our results clearly indicate that AMF cause cell death and inhibition of cell proliferation. Specifically, only a 36% of the treated macrophages remained alive 2 hours after AMF exposure and 24 hour later the percentage fell down to 22%.

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Clinical platform based on Magnetotactic Bacteria

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Although more than 85% of all cancers are localized, delivery systems still rely on systematically circulating passive or active vectors based primarily on physical characteristics and biochemical principles alone which result to only a few percents of the administered dose reaching the tumor cells. Despite the fact that these delivery strategies are far from optimal, they are still pursued due to technological barriers preventing the replacement of these passively-drifting vectors by agents with functionalities such as the ones used in robotics that have proven to be very effective to perform tasks with challenges similar to the ones involved in the transport of therapeutics straight to active tumoral zones (direct targeting). These active oxygen-depleted tumoral areas known as hypoxic zones can be directly targeted by computer-guided magneto-aerotactic bacteria such as *Magnetococcus marinus* strain MC-1 that when combined with nanomedicine [1], leads to natural medical nanorobots with the characteristics and actuation-navigation-sensory capabilities necessary to function as “homing guided therapeutic missiles.” Taking advantage of the capabilities of the MC-1 cells to deliver therapeutics to such hypoxic tumor areas relies on a platform capable of generating an artificial environment that exploits their magneto-aerotactic migration behavior. Although such a platform has already been successfully developed for small animals [2], scaling it to fit a human adult was not a trivial task. Here, the design and the fundamental principles of such a new clinical platform dedicated to cancer therapy will be presented.

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Antitumor effect and translocation of drug-loaded magnetosomes

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In the last decades, cancer nanotherapeutics are attracting an increasing level of interest and have a spectacular development. The fate of nanoparticles within cells is a critical objective in their future biological application. Bacterial magnetosomes (BMs) can be used as magnetic targeted carriers for cancer therapy due to their properties.

In this study, we prepare drug-loaded magnetosomes (DBMs) by binding doxorubicin, epirubicin and daunorubicin to BMs (Dox-BMs, Epi-BMs or Dau-BMs) and investigate their antitumor effect in HepG2 or HL7702. *In vitro* MTT (3- (4, 5- dimethylthiazol – 2 - yl) - 2, 5 – diphenyltetrazolium bromide) assay shows that the inhibition ratio of Dox-BMs and Dau-BMs are higher than free drugs except for Epi-BMs. All these complexes affect cell proliferation in a time-dependent manner. Scanning electron microscope observation shows significant morphological differences between cells treated with DBMs and free drugs, hinting that DBMs affect cell proliferation in different ways from free drugs. Treatment of DBMs leads to down-expression of *c-myc* and up-expression of *p53* gene in cells, respectively, indicating that DBMs inhibit cell proliferation by regulating the expression of oncogenes and tumor suppressor genes. Just only 30% of Dox-BMs transfer to cells under low temperature (4℃) compared to control, suggesting drug-BMs cross cellular barriers through energy-dependent pathways.

Engineering of magnetotactic bacteria for cobalt accumulation

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Since their discovery, magnetotactic bacteria (MTB) have raised interest for biotechnological applications. Their magnetic abilities coupled with their capacity to deal with high concentrations of metals make them useful tools to operate in metal-polluted area. Our project consists of engineering new strains of magnetotactic bacteria to accumulate metal ions for decontamination of polluted effluents.

In order to fulfill those objectives, we chose to heterologously express in MTB three enzymes (CntK, CntL, CntM) known to be involved in the synthesis of staphylopine, a recently discovered wild spectrum metallophore molecule produced in *Staphylococcus aureus* [1]. In this pathogen, staphylopine was shown to contribute in cobalt acquisition. This molecule is homologous to plant nicotianamine, a 303 Da metallophore that can bind a large variety of metal ions with high affinity, for their transport in the different organs of the plant.
We used *E. coli, Magnetospirillum gryphiswaldense* MSR-1 and *Magnetospirillum magneticum* AMB-1 to express CntK, CntL and CntM from *Staphylococcus aureus.* As a result the heterologous expression of genes encoding this enzymatic machinery responsible for staphylopine synthesis increases both the bacterial resistance towards cobalt and the intracellular sequestration of the metal in the three strains. Moreover, co-expression of specific importers of cobalt and nickel with CntK, CntL and CntM resulted in a synergistic effect on cobalt uptake, improving further the accumulation of metals. In addition, ICP-AES quantification of the metal content in the cytoplasmic and magnetosomal fractions coupled with XANES and EXAFS analysis revealed an increased accumulation of cobalt at the magnetosome. These engineered strains represent a highly efficient bio-accumulation system that can be easily recovered from the medium by using a magnet.

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Applications of magnetotactic bacteria under the regulation of magnetic fields to combat *Staphylococcus aureus*

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Staphylococcus aureus (*S. aureus*) is a common pathogen, causing a series of diseases, such as various soft-tissue wound infections. The conventional separation and detection methods are time-consuming and the abuse of antibiotics has led to the development of drug-resistant *S. aureus*. Thus, how to resolve these problems is still a challenge.
Magnetotactic bacteria (MTB) synthesized intracellular iron oxide nanoparticles called magnetosomes which assist them to swim autonomously in the geomagnetic field. The kind of movement provides them with the autonomic target ability. Meanwhile, magnetosomes arranged in chains endow MTB with a big magnetic moment. Basing on these properties, MTB are able to be used widely under the regulation of different magnetic field in biomedical applications, such as combating *S. aureus*. MTB could be constructed as bacterial microrobots by coating MTB with their antibody. When placed in a microfluidic chip, MTB microrobots were able to attach *S. aureus* via the affinity of Protein A to antibody and finally separate *S. aureus* in a micro-sample successfully under the control of uniform magnetic field. MTB could also be served as a heat source in magnetic hyperthermia owing to their magnetosomes. After attached to *S. aureus* MTB killed much more the pathogens under the alternating magnetic field. Moreover, when placed under a swing magnetic field MTB swung due to magnetosome chains and could kill *S. aureus* only when they attached to each other. The mechanical force were possibly created by MTB under the swing magnetic field and may be considered to damage the integrity of *S. aureus* and induce the cell death. According to the comparison and our analysis, we speculated magnetic hyperthermia effect may be a combined effect of temperature (gradient) and mechanical force. Therefore, the control of MTB by various magnetic fields might provide an alternative tool to remedy *S. aureus*-infected disease.

Preparation of the BMs based drug carrier with crossing blood-ocular barrier ability and its therapy of Choroidal melanoma

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Blood-ocular barrier (BOB) and side effect of chemotherapeutics are puzzles in the therapy of ocular tumours. In the present study, the natural ferromagnetic nanoparticles “Bacteria magnetosomes (BMs)”, coating the “transmembrane” signal peptide of “TAT” are used for drug carrier and hoping to cross the BOB in vitro. We have characterized the morphology, surface groups and magnetic properties of the drug carrier, studied its ability of trans-BOB and its therapy of Choroidal melanoma. The TAT-BMs drug carrier have potential ability to overcome the difficulties in the ocular tumours and provide important basis for design and application of the “transmembrane” drug delivery system.

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Generation of multifunctional bacterial nanoparticles by genetic engineering and surface display of peptides and reporter enzymes

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Due to their unprecedented properties, magnetosomes have an enormous potential for biomedical and biotechnological applications. Both their crystal morphologies and the composition of the enveloping membrane can be manipulated by genetic means, allowing a controlled functionalization of the magnetosome surface by genetic engineering. For that purpose, a versatile and diverse genetic "toolkit" for the generation of "smart" multifunctional magnetic nanoparticles with several tailored properties is being created.
Using an optimized expression system [1], several of the most abundant proteins of the magnetosome membrane (MamC/A/F/G) were tested as membrane anchors for the expression of reporter proteins, including eGFP as well as the β-glucuronidase GusA. Magnetosome-bound GusA followed Michaelis-Menten kinetics, and specific activities were comparable to the non-immobilized enzyme and resistant against repeated freeze-thawing steps. Furthermore, GusA could be expressed in tandem, triple or even multiple arrays while maintaining proper folding and catalytic activity.
In addition, shell size and properties could be modified by the expression of artificial peptides. These functional extensions, e.g. repeated units of charged amino acids [2], significantly increased the hydrodynamic diameter of the magnetosome shell and altered the surface charge. As an alternative to our inorganic coatings [3], strategies are investigated to generate organic / polymeric porous shells that are expected to improve biocompatibility and provide tuneable characteristics.

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Clues to culturing freshwater magnetococci using a metagenomic approach

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The discovery of magnetotactic bacteria (MTB) by R.P. Blakemore in 1974 was based on observations of magnetotactic cocci, the most commonly observed morphotype of MTB in aquatic habitats [1]. Although several strains of marine magnetococci have been cultivated, freshwater strains have continually resisted attempts at isolation and cultivation despite the fact that both types are morphologically similar and phylogenetically related. All known cultured and uncultured magnetotactic cocci biomineralize magnetite magnetosomes and belong to the *Magnetococcales* order within the *Alphaproteobacteria* [1]. Marine strains in axenic culture include: *Magnetococcus marinus* strain MC-1, *Magnetofaba australis* strain IT-1 and strain MO-1 [1]. These strains grow chemolithoautotrophically oxidizing reduced sulfur compounds under microaerophilic conditions and the reverse tricarboxylic acid (rTCA) cycle for autotrophy. Here we used a metagenomics approach to predict the metabolism of some freshwater cocci in order to obtain clues to culturing this group of bacteria. Magnetococci studied here were those where they were the only morphotype in freshwater samples examined by light microscopy after magnetic enrichment and purification. Genomic DNA from magnetically purified cells was subjected to whole genome multiple displacement amplification and sequenced. Contigs were assembled using CLC Genomics Workbench. Preliminary analyses suggest that these freshwater magnetococci possess an autotrophic metabolism similar to marine magnetococci based on the presence of rTCA cycle genes. Genes that encode uptake hydrogenases and some involved in the oxidation of reduced sulfur compounds are also present suggesting that hydrogen and sulfur compounds act as electron donors for growth. Genes for the oxidation of other compounds supporting chemolithotrophic growth (e.g., NH_4^+, Fe^{2+} etc.) were not found. Genes for nitrogen fixation (e.g., *nif*QAEBHN) are present supporting the possibility that the freshwater cocci, like their marine counterparts, are capable of this process.

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Culture-independent characterization of magnetotactic bacteria from acidic habitats

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Most described and characterized magnetotactic bacteria (MTB) are found in mesophilic aquatic habitats. However, in the last decade or so, a number of extremophilic, with regard to salinity, pH and temperature, MTB have been isolated and/or characterized. Recognized extremophilic MTB include: alkaliphilic, anaerobic, dissimilatory sulfate-reducing strains belonging to the *Deltaproteobacteria* [1]; a moderately thermophilic vibrio belonging to the *Nitrospirae* [1]; moderately halophilic or strongly halotolerant MTB from hypersaline environments including multicellular magnetotactic prokaryotes (MMPs) and a vibrio (strain ML-1) belonging to the *Deltaproteobacteria*, a cultured rod (strain SS-5) belonging to the *Gammaproteobacteria*; and a group of uncultured psychrophilic, alphaproteobacterial magnetotactic cocci from the Antarctic [2]. There are no published reports of MTB occurring in strongly acidic environments. Here, we used culture independent techniques to characterize MTB from an acidic freshwater lagoon in Brazil. Sediment samples had a pH of 4.4 and contained MTB with several cell morphotypes including rods, spirilla and cocci containing magnetosomes of various morphologies. Intracellular pH measurement showed that cytoplasmic pH in these MTB is close to neutral. Most retrieved 16S rRNA gene sequences were closely related to the genus *Herbaspirillum* within the *Betaproteobacteria*. Using fluorescent *in situ* hybridization, a *Herbaspirillum*-specific probe hybridized with a vibrioid MTB that contained two intracellular granules. No other MTB morphotype was detected using FISH and this probe. Transmission electron microscopy of samples revealed the presence of a population of MTB with the identical morphology of the *Herbaspirillum*-like bacterium. These cells contained two intracellular electron-dense granules and elongated, prismatic magnetite magnetosomes. This appears to be the first report of MTB in acidic environments and the first description of a betaproteobacterial MTB.

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Large diversity and temporal variation of the multicellular magnetotactic prokaryotes in Sanya

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Multicellular magnetotactic prokaryotes (MMPs) exhibit two morphotypes, i.e. spherical mulberry-like MMPs (s-MMPs) and ellipsoidal pineapple-like MMPs (e-MMPs). Regarding to the e-MMPs we have identified total three species from the intertidal zones of Qingdao, Rongcheng and Six-Fours-les-Plages, and Drummond Island. They display a mono-species property at each sampling site. We have also performed systematic sampling in the intertidal zones of Sanya Bay, the South China Sea in March from 2014 to 2016. We purified MMPs using magnetic-micromanipulation, amplified and sequenced the 16s rDNA genes. Unexpectedly, total eight species belonging to four genera of e-MMPs were discovered at the same sampling site. In addition, the numbers of identified species vary according to sampling time; three species in 2014 and four species in both 2015 and 2016. Simultaneously, the annual variation of dominant species was remarkable: the common species SYP14-01 was the dominant species in 2014, and was replaced by species SYP15-05 as the dominant phylotype in 2015. Species SYP15-07 was minor species in 2015 and became dominant in 2016. Not only e-MMPs, s-MMPs also showed large diversity with eight species belonging to three genera at the Sanya Bay sampling site. The reasons that the large diversity of MMPs in Sanya Bay may be relate to the complexity of the environment there, including open sea, irregular tide and violent waves which strongly disturb the sampling site and change the physical and chemical parameters of the sediments. These findings provide new insights into the diversity and distribution of MMPs.

Transcriptional response of *Magnetovibrio blakemorei* MV-1 to different terminal electron acceptors and iron availability

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Magnetovibrio blakemorei strain MV-1 is a marine magnetotactic bacterium that phylogenetically belongs to the *Alphaproteobacteria* and biomineralizes truncated hexa-octahedral magnetosomes [1]. It grows microaerobically with O_2 or anaerobically with nitrous oxide (N_2O) as the terminal electron acceptor [1]. Organization of the genes in the putative magnetosome island of *M. blakemorei* is significantly different than that of many other magnetotactic bacteria and contains some genes in addition to recognized magnetosome genes well as several homologous copies of the same genes. However, there is no data on the involvement of these additional and homologous genes in magnetosome formation. In this study, we use total RNA sequencing and bioinformatics to investigate genes involved in magnetosome biomineralization in *M. blakemorei* by examining changes in gene expression levels when cultured with N_2O versus O_2 as the terminal electron acceptor and at very low and high concentrations of iron (required for magnetosome formation). Moreover, we use computational analysis to identify genes involved in biomineralization. Other goals of this study include determining whether low iron availability in the environment down- or up-regulates expression of magnetosome genes and whether magnetosome genes are co-transcribed as has been shown in other magnetotactic bacteria or are transcribed independently as was previously reported for *M. blakemorei*. Overall, total RNA sequencing allows for a better understanding of the regulatory mechanisms of magnetosome biomineralization in response to different environmental factors.

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Genomic insights into magnetotactic bacteria

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Whole genome sequences are powerful source of data not only to investigate magnetotactic bacteria (MTB) evolution, but also to decipher the genetic basis of magnetosome formation and that of MTB adaptation to their specific ecosystems. These last years, the improvement of genome sequencing technics and DNA purification has enabled to dramatically increase the quality and quantity of MTB genomes, opening prospects for new discoveries. Here, I discuss how data that can be harvested from published and newly sequenced genomes, and how comparative and functional genomics can be used at the whole genome scale to provide new insights into the biomineralization processes or MTB ecology. Among other examples, I will also propose a general scenario of the magnetotaxis evolutionary history among bacteria using both whole genomes and magnetosome genes as markers. The constant increase of genomes and metagenomes sequenced and ensuing analyses shall significantly improve our understanding of this unique group of bacteria and their associated features the next decade.

Metagenomics of magnetotactic bacteria

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Magnetotactic bacteria (MTB) that form intracellular magnetite and/or greigite magnetosomes play important roles in geochemical cycles of iron, sulfur, and carbon. They represent an intriguing model system for the study of magnetoreception, bacterial organelle formation, and microbial biogeography. However, only a few of MTB strains have been isolated and cultivated, which poorly represents their true diversity in nature. Metagenomics access the genomic DNA from complex microbial communities, which circumvents the culture barrier of microorganisms. To comprehensively explore the overall diversity of MTB in nature, we have conducted a high-throughput metagenomic approach to facilitate the targeted discovery of dozens of magnetosome gene cluster-containing contigs directly from naturally occurring MTB communities. Genome reconstruction from metagenomic data results in several high-quality draft genomes of uncultivated MTB belonging to the phylum *Nitrospirae* and the class *Alphaproteobacteria*. These results extend our knowledge on origin, evolution, biomineralization mechanism and metabolic potential of MTB, and highlight the unexpected diversity of MTB still awaiting discovery.

Characterization and genomic analysis of an uncultivated magnetotactic bacterium in the phylum *Nitrospirae*

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Magnetotactic bacteria (MTB) within the phylum *Nitrospirae* are of great interest due to their biomineralization of bullet-shaped magnetite magnetosomes. However, no *Nitrospirae* MTB has been cultured thus far, and only four draft genomes of MTB from this phylum are currently available in GenBank (accession numbers: LACI00000000.1, JMFO00000000.1, JZJI00000000.1, and LNQR00000000.1). Recently, we discovered a novel watermelon-shaped MTB from a freshwater lake in Xianyang, China, which contained ~100 bullet-shaped magnetosomes. Cells of this bacterium had an average length of 3.59 μm and width of 3.12 μm. It formed 4 to 6 bundles of magnetosome chains per cell that were parallel to the long axis of the cell, and the average length and width of magnetosome crystals were about 91 and 36 nm, respectively. 16S rRNA gene sequence-based and fluorescence *in situ* hybridization analyses revealed that this bacterium was affiliated within the *Nitrospirae* phylum and most similar to previously identified LO-1 (>98% nucleotide identity) [1]. Genomic DNA of this bacterium has been extracted and sequenced. Detailed genomic analysis of this novel magnetotactic *Nitrospirae* is underway.

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POSTERS

POSTERS OF THE 2016 MTB MEETING

The Dual Role of MamB in Magnetosome Formation

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Biomineralization of magnetite nanoparticles have been shown to be controlled by a set of ~30 proteins. Most of these proteins are associated with the magnetosome membrane and encoded within a genomic region termed the magnetosome island (MAI). Among the essential genes are the highly conserved *mamB* and *mamM* encoding cation diffusion facilitator (CDF) homologs. Accordingly, MamB is expected to assemble as a dimer and to contain metal-ion-binding sites at its TMD and CTD.

Additionally, previous studies have shown that deletion of *mamB* led to the lack of the intracellular magnetosome membrane (MM), suggesting that MamB participates in two different magnetosome formation stages: first in membrane invagination and second in magnetosome iron accumulation.

In this work we employed a multi-disciplinary approach to investigate MamB structure and function. Adjacent to the structures, we examined *in-vivo* how a mutation in the metal binding site effects the magnetosomes formation. Herein we present the crystal structure of the cytoplasmatic domain (CTD) of MamB in its apo and zinc-bound forms which reveals new CDF-ion binding site position. Altogether, our results provide that MamB is not only involved in magnetosome formation but also participant in iron accumulation.

A protein-protein interaction in magnetosome: MamA oligomer interacts with Mms6 oligomer

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Magnetosomes use a suite of proteins, for their synthesis and maintenance. These proteins have specific individual functions, but some of them can interact with each other to form supramolecular complexes within the magnetosome. However, the detailed protein organization in magnetosome is still unresolved. MamA is one of the most abundant protein in magnetosome, and consists of a tetratricopeptide repeat (TPR) motif used for proteinprotein interactions. It was proposed that MamA is anchored to the surface of magnetosome through protein-protein interactions [1]. In this study, we screened MamA binding proteins from the magnetosome-associated proteins using an affinity chromatography. We identified a magnetosome membrane protein, Mms6, as the binding partner of MamA. The interaction between MamA and Mms6 were further confirmed by pull-down, immunoprecipitation and size-exclusion chromatography assays [2]. In addition to this we found that there are two types of Mms6 associated with magnetosomes, one is 14.5-kDa and the other is 6.0-kDa. This difference led us to try using truncated mutants of Mms6 to examine the MamA binding site in Mms6. Prior to this, Mms6 was assumed to be only involved in magnetite biomineralization, however, these results suggested that Mms6 has an additional responsibility, binding to MamA.

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Ferritin-Mms6 chimera proteins: function-structure studies

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Magnetotactic bacteria (MTB) is a group of microorganisms uniquely possessing an intracellular organelle capable of biomineralization of magnetic magnetite and greigite nanocrystals. The magnetosome assembly and biomineralization processes are currently known to involve ~30 different proteins, one of which being Mms6. Mms6 is believed to be involved in the regulation of crystal morphology via association with the positively charged iron atoms within the magnetosome enclosed environment.
Ferritin is a widely studied protein, prevalent in many living organisms. Its native appearance and functional shape is as a protein nanocage which functions as an iron storage site. The iron within the protein cavity is held in the form of ferrihydrite, a nontoxic biologically available form. Ferritin has been well established as a possible biotemplate for highly regulated biomineralization processes.
In this research, a chimera proteins were constructed using the Mms6 C-terminal iron binding site (M6A) fused to the serval ferritin monomers at the C-terminal, hereby forming Ferritin-M6A proteins. The chimera monomer of mouse H-ferritin (FHM6A) and of mouse L-ferritin (FLM6A) have been shown to form a 24-meric sphere, similar to the one formed by the native mouse H/L-ferritin protein. Our objective is to study and characterize the magnetite biomineralization process within the Ferritin-M6A spherical nanocage.

Electron transfer pathway mediated by MamP for magnetite biomineralization

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Although the molecular mechanism of magnetite biomineralization in magnetotactic bacteria remains unknown, it has been reported that some magnetotactic bacteria-specific proteins are involved with the magnetite crystal synthesis. Among these proteins, MamP was reported as a new type of cytochrome c with iron oxidase activity [1]. On the other hand, MamP is a membrane-bound protein and not localized in the magnetosomes, but in cytoplasmic membrane [2]. This implicates that MamP may play a role in a redox reaction that occurs in cytoplasmic membrane or periplasmic space. In this study, we focused on MamP function in the denitrification pathway because the denitrifying proteins were reported to participate in formation of magnetite crystals [3]. We first constructed the mamP deletion mutant of *Magnetospirillum magneticum* AMB-1 by two step homologous recombination and monitored consumption of nitrate, the first substrate of denitrification, in media during the logarithmic growths. As a result, the ΔmamP cells consumed nitrate more slowly than both the wild type and the rescue strain (ΔmamP +mamP) cells. This result suggests that MamP may be involved in electron transfer reactions to the denitrification pathway.

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Direct observation of actin-like MamK cytoskeletal filaments by HS-AFM

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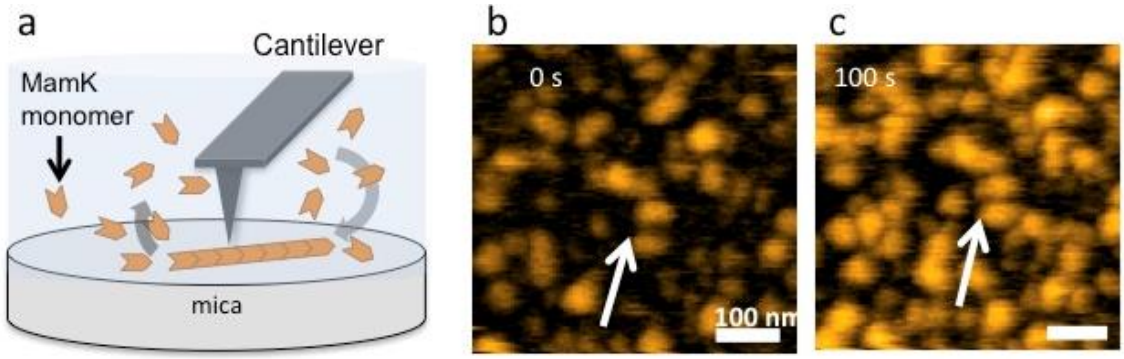
Magnetotactic bacteria have intracellular membranous organelles giving them the capability of sensing earth's magnetic field. In the case of *Magnetospirillum magneticum* AMB-1, the magnetosomes form a single chain in the cell, which acts like a magnetic compass. MamK, an actin-like cytoskeletal filament found in AMB-1, is thought to act as a scaffold, keeping the magnetosomes in their chain-like arrangement. Under the right physiological conditions, individual monomers of MamK polymerize to form a filament. Our goal is to elucidate the molecular mechanism for MamK polymerization in vitro. One of the most effective tools for this is the high-speed atomic force microscope (HS-AFM). The HS-AFM is capable of imaging biological molecules at single nanometer spatial resolution and millisecond temporal resolution, in liquid that mimics the physiological conditions inside the cell. Therefore, HSAFM can visualize the structure and dynamics of a native protein complex. According to HSAFM observations, MamK filaments showed single directional growth. However, the purified MamK sample contained irregular shaped aggregates, which impedes the observation of MamK polymerization. The main focus of our current research is to improve the purification method for preparing monomeric MamK.



(a) HS-AFM generates 3D images by displaying changes in the spatial information of the cantilever tip as the cantilever scans the sample surface. The MamK monomers are in a buffer solution containing ATP and form filaments on the surface of the mica. The cantilever gently rasters over the surface of the mica to generate the image. (b, c) HS-AFM images of MamK polymerization on the mica surface. The arrow shows the unidirectionalgrowth of a single MamK filament.

Functional analysis of a magnetotactic bacterial actin-like cytoskeleton MamK using fluorescence live-cell imaging

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Although the detailed structures of magnetosomes can be observed in high-resolution using electron microscopy or atomic force microscopy, these techniques cannot visualize the intracellular dynamics of organelles in living cells. In this study, we used a live-cell time-lapse fluorescence imaging technique to examine magnetosome dynamics in *Magnetospirillum magneticum* AMB-1. Using this technique, we imaged the dynamic movements of magnetosomes throughout the cell cycle. In the wild type cells, magnetosomes were positioned in a stable, chain-like arrangement along the cell axis throughout the cell cycle. On the other hand, in the mamK deletion mutant, instead of staying in a straight chain, the magnetosomes were scattered throughout of the cell, and moved randomly or formed large aggregates. We also observed the cells expressed two different MamK mutants, MamKE143A and MamKD161A. E143 and D161 are conserved in the actin superfamily, and are essential for ATP hydrolysis activity. According to the results of live-cell imaging, the cells expressed both of ATPase defective MamK mutants showed intermediate phenotypes between wild type and ΔmamK. Most of the magnetosomes were positioned as a chain, but a portion of magnetosomes moved randomly or formed aggregates in the same cell, suggesting that MamK ATPase activity is necessary for magnetosome positioning and to create a stable chain-like arrangement.

Cell cycle related proteins in magnetosome chain segregation of *Magnetospirillum gryphiswaldense*

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During the cell cycle of *Magnetospirillum gryphiswaldense* MSR-1*,* the midcell positioned magnetosome chain has to be properly segregated into the offspring [1]. The actin-like MamK filament plays an essential role in magnetosome chain assembly, motion, positioning and ultimately segregation, processes that depend directly on intact MamK filaments dynamics. The tight coordination of magnetosome chain positioning and partitioning to cell division raises the hypothesis of a putative link with the cell division machinery. However, the mechanism of FtsZ-ring positioning and septum formation has remained poorly understood, as MSR-1 does not possess either the nucleoid occlusion or MinCD equivalent systems. In this study, we identified two genes that have a 46% and 43% identity to MipZ (*mipZ-like1* and *mipZ-like2,* respectively), a gradient-forming protein that couples chromosome segregation to cell division by localizing the FtsZ-ring at midcell in *C. crescentus* [2].
Surprisingly and in contrast to *C. crescentus*, we were able to independently delete both *mipZ-like* genes. The successful generation of the two deletion mutants further allowed investigating the role of the MSR-1 MipZ homologs in both magnetosome chain segregation and cell division. Here, these issues were addressed by means of photokinetic and ultrastructural analysis. Additionally, Δ*mipZ-like1* showed growth deficiencies as to the wild type and seemed to have a cell division defect. We analysed the dynamics of both MSR-1 MipZ–like proteins by FRAP and Photoconversion, and we further examined the MSR-1 Δ*mipZ-like1* and Δ*mipZ-like2* strains by *in vivo* time-lapse microscopy, TEM and Cryo-EM. Although, both homologs seem to play an important but not essential role in the cell cycle of MSR-1, its function in magnetosome chain segregation is currently tested.

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Biochemical properties of the bacterial actin protein MamK from '*Candidatus* Magnetobacterium casensis' in the phylum Nitrospirae

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The bacterial actin protein MamK play an essential role in arranging intracellular magnetosomes in magnetotactic bacteria (MTB). The *in vitro* properties of *Magnetospirillum* MamK, which plays essential roles in the linear arrangement of magnetosomes in magnetotactic bacteria cells belonging to the *Proteobacteria* phylum, have been characterized [1, 2]. However, the biochemical features of MamK in *Nitrospirae* MTB remain largely unknown. Here, we report that the MamK from the uncultivated *Nitrospirae* '*Candidatus* Magnetobacterium casensis' (Mcas) had a high ATP hydrolysis activity and were rapidly assembled into well-ordered filaments with unique architectures and physiochemical characteristics. MamK in Mcas can convert NTP to NDP and NDP to NMP, showing the highest preference to ATP. Based on the analysis of conserved active sites, the sites of Asp12 and Glu151 were conserved in all known ATPase proteins and mutants were constructed to study their effects on MamK. The ATP hydrolysis activities were both decreased in the mutants D12K and E151A. Moreover, the polymerized MamK from Mcas is independent of metal ions and nucleotides except for ATP, and is assembled into well-ordered filamentous bundles consisted of multiple filaments. Our results suggest an *in vitro* biochemical properties and assembly behaviour of MamK from the uncultivated *Nitrospirae* MTB that synthesizes multiple magnetosome chains.

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A RB-Tnseq screen to probe for the essential metabolic and biomineralization gene sets of AMB-1

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Magnetotactic bacteria (MTB) are a diverse group of prokaryotes spanning multiple phyla, remarkable for their ability to biomineralize iron and form subcellular compartments called magnetosomes. In *Magnetospirillum magneticum* AMB-1, the magnetosome gene island (MAI) makes up more than 2% of the genome [1]. Based on work on AMB-1 and *Magnetospirillum gryphiswaldense* MSR-1 a subset of genes within the MAI are thought to be necessary and sufficient for magnetosome formation [2,3]. It stands to reason that a significant amount of energy is required to replicate the MAI and synthesize the proteins needed to form magnetosomes. We have a limited understanding of MTB metabolism since they are only grown under specific conditions in culture. In their natural environment MTB may be exposed to a variety of nutrient and energy sources. The response of MTB to varying environmental conditions is unknown. Examination of the MSR-1 growth and biomineralization in response to alternative nitrogen sources and electron acceptors indicates that specific magnetosome and metabolic genes might be required, depending on the growth condition [4]. A systematic unbiased analysis of the genes involved in metabolism and biomineralization will shed light on the interplay between the environment and magnetosome formation. I plan to use a barcoded transposon mutagenesis screen (RB-TnSeq) in AMB-1, followed by high throughput sequencing to probe for genes that are required for magnetosome formation under varying media conditions. This screen will reveal genes both inside and outside the magnetosome gene island that are essential for biomineralization, leading to a greater understanding of the metabolic capabilities of AMB-1. Additionally, it will provide a set of essential genes in AMB-1 and establish a general methodology to conduct RB-TnSeq experiments in AMB-1.

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A compact cassette for transfer of the entire magnetosome biosynthesis gene cluster

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The recently accomplished stepwise transfer of the entire set of magnetosome genes into *Rhospirillum rubrum* has demonstrated that synthesis of such a highly complex structure as magnetosome can be reconstituted in a foreign non-magnetotactic host [1]. In the same way, transfer of the additional copies of the magnetosome operons into the native host Magnetospirillum gryphiswaldense (MSR-1) resulted in the increased number of magnetosomes produced by the cells [2]. However in these studies the genes were harbored on several vectors that made manipulations and transfer to other hosts cumbersome. Here we present the construction of a single transposable vector harboring all major magnetosome operons from MSR-1, which we combined using Gibson assembly and subsequently cloned into the transposon containing vector to enable chromosomal integration. One-step conjugative transfer of a non-magnetic mutant MSR-1B and wild type *R. rubrum* with the resulting plasmid fully restored the magnetic phenotype in the former and endowed the latter with magnetosome synthesis. Chromosomal insertion of the cassette into a *recA* deficient MSR-1 strain resulted in duplication of all MAI gene clusters and significantly enhanced magnetosome biosynthesis. In future work, transfer of the obtained construct into various foreign bacteria will be tested to analyze their potential to support magnetosome biosynthesis.

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Investigating the genetic basis for magnetosome formation in *Desulfovibrio magneticus* RS-1

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The process of magnetosome formation is tightly controlled by a suite of magnetosome-associated genes (mam) in the alpha-proteobacteria species *Magnetospirillum magneticum* AMB-1 and *Magnetospirillum gryphiswaldense* MSR-1[1,2]. These genetically tractable species produce membrane-enclosed cubooctahedral magnetite crystals and have become the models for magnetosome formation[3]. *Desulfovibrio magneticus* RS-1 (RS-1) is a delta-proteobacteria that biomineralizes tooth-shaped crystals of magnetite and has been isolated in pure culture[4]. In addition to having homologs of the many of the mam genes, RS-1 possesses a suite of genes specific to magnetotactic delta-proteobacteria (mad genes)[5]. In order to investigate the role of the mad genes in RS-1, we have taken a two-fold approach. The first approach is to delete the 11kb genomic region comprising the mad genes using a double homologous recombination approach. RS-1 has proven recalcitrant to traditional double HR deletions, yet our recent efforts have improved this system. Secondly, to elucidate the role of the mad genes in magnetosome formation we are looking at the localization of these proteins using HaloTag technology (Promega). Creating genetic fusions of the mad proteins to the HaloTag protein will allow us to use fluorescent microscopy in fully anaerobic conditions in order to determine which mad genes associate with the magnetosomes in RS-1. Using this combinatorial approach, we hope to determine which mad genes, if any, are essential to magnetosome formation in RS-1, and to determine possible roles for these genes by investigating protein localization patterns.

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Characterization and genomic analysis of the freshwater magnetotactic bacteria *Magnetospirillum moscoviense* BB-1 and *Magnetospirillum marisnigri* SP-1

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Magnetotactic bacteria are remarkable by their ability to synthetize magnetosomes, which are nano-sized magnetic particles enveloped by a belayed lipoprotein membrane. The magnetosome biomineralization is a highly complex process, which is controlled by a set of specific genes, which are lumped together in a single genomic region referred to as Magnetosome Genomic Island (MAI). In this study, we present draft genome sequences of two recently described freshwater magnetotactic species *Magnetospirillum moscoviense* BB-1 and *Magnetospirillum marisnigri SP-1* [1].

The genomes of *M. moscoviense* BB-1 and *M. marisnigri* SP-1 were sequenced using HiSeq1500 from Illumina. A total of 10.569.046 and 10.022.55 reads providing 100-fold genome coverage, were obtained from M. moscoviense BB-1 and M. marisnigri SP-1, respectively. The reads were assembled using SPAdes version 3.1.0 [2]. The assembled sequence was submitted to the National Center for Biotechnology Information (NCBI) Prokaryotic Genome Annotation Pipeline (PGAP) for annotation.

The draft genome sequence of M. moscoviense BB-1 consists of 4,164,497 bp in 207 contigs with an average G+C content of 65.2%. The genome contained 3950 genes, of which 3752 were coding DNA sequences, 6 coded rRNAs (5S, 16S, and 23S), 46 coded tRNAs. The draft genome sequence of Magnetospirillum marisnigri SP-1 consists of 4,619,819 bp in 131 contigs with an average G+C content of 64.7%. The genome contained 4279 genes, of which 4130 were coding DNA sequences, 6 coded rRNAs (5S, 16S, and 23S), 47 coded tRNAs.

This work was funded by the Russian Foundation for Basic Research (RFBR) (16-34-00802 mol_a).

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CRISPR-CAS9 system for genetic enginnering in *Magnetospirillum gryphiswaldense*

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Expanding the tools for genetic engineering in magnetotactic bacteria is crucial for the efforts of studying these organisms, as genetic engineering in magnetotactic bacteria is cumbersome, time consuming and inefficient. Though, there are some tools developed for genetic engineering in *Magnetospirillum gryphiswaldense (MSR-1)* and *Magnetospirillum magneticum (AMB-1)* there is still need of a system to efficiently perform genetic engineering. The CRISPR-Cas9 system is used as a versatile tool for genetic engineering in many eukaryotes and bacteria; however, its utilization in bacteria is not as wide spread as in eukaryotes.
The poster will present the work of implementing the CRISPR/CAS9 system based on *Streptococcus pyogenes* in *M. gryphiswaldense-MSR1*.

Magnetotactic bacteria identification in the geological record: perspectives from iron isotopes

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Magnetotactic bacteria (MTB) generally live within the oxic-anoxic transition zone of aquatic environments. They produce magnetite ($Fe(III)_2Fe(II)O_4$) or greigite ($Fe(III)_2Fe(II)S_4$) crystals within organelles called magnetosomes. Magnetosomes are assembled in chains inside the cell and provide the bacteria with a net magnetic moment, which they presumably use for moving along local magnetic field lines. The magnetic properties of magnetosome chains can be used to infer the presence of MTB fossils (magnetofossils) in sediments. Although MTB may represent some of the oldest biomineralizing organisms on Earth, the identification of magnetofossils in the rock record has remained challenging. For instance, this is due to alteration of the chain structure during sediment burial and possibly metamorphism owing to the degradation of organic compounds assembling magnetosomes in chains. Based on a series of *Magnetospirillum magneticum* strain AMB-1 cultures, we determined Fe isotope fractionation of magnetite relative to growth medium in order to develop new tools for magnetofossils identification. In all experiments, magnetite produced by MTB was depleted in heavy Fe isotopes relative to the growth medium by 1.5 to 2.5‰. More importantly, we observed from multi-isotope analyses the first deviations from mass-dependent fractionations, affecting specifically the odd (^{57}Fe) but not even isotopes (^{54}Fe, ^{56}Fe, ^{58}Fe). This Fe isotope anomaly may constitute a unique biosignature of magnetite produced by MTB and needs to be searched in modern natural environment and ancient sedimentary rocks. In the present work, we target magnetite formed in sediments deposited during the Great Oxidation Event (*i.e.* first oxygen release to atmosphere and ocean) about 2.45 billion years ago. Two different magnetite populations have been identified based on their magnetic properties, and their Fe isotope compositions are now being analyzed.

Interaction of biomolecules with magnetite

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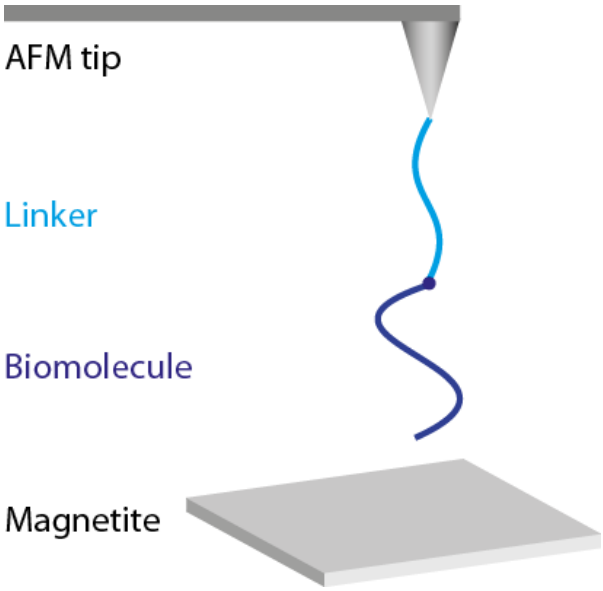
Magnetite crystals in magnetotactic bacteria are monodisperse and uniform in shape. We are specifically interested in investigating the interaction of biomolecules with magnetite with the goal of understanding their putative role in controlling the above-mentioned properties of magnetite crystals.
For investigating these interactions single-molecule force spectroscopy is performed. This approach enables for testing the binding capacities of biomolecules like proteins to magnetite particles, and eventually to specific magnetite surfaces to probe if the interaction is surface-specific.
The outcome of these experiments will improve the knowledge about how several magnetotactic bacteria strains mineralize magnetite crystals and can extend the capabilities regarding green synthetic routes for magnetite particles towards applications in bio- and nanotechnologies.



Schematic drawing of an experimental setup for single-molecule force spectroscopy. A biomolecule, site-specifically attached to an AFM cantilever tip through a linker, is approaching a magnetite surface.

The combined effect of MamC and Mms6 from *Magnetococcus Marinus* MC-1 in magnetite biomineralization *in vitro*

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Magnetite (Fe_3O_4) nanoparticles are interesting in biotechnology since they can be manipulated using an external magnetic field and can be used for anchoring relatively large amounts of molecules. They have been used in various biomedical applications, which require that the particles have strict physicochemical conditions and, also, are biocompatible. Magnetotactic bacteria have the capacity to biomineralize magnetite nanoparticles with properties that make them the ideal single domain particle (SDP) for numerous applications, although to scale up magnetosome production is still challenging. Instead, protein-mediated growth of synthetic magnetite crystals is being explored as an alternative to produce magnetosome-like nanoparticles [1]. In this context, much work has been done by using the magnetosome protein Mms6 from *Magnetospirillum magneticum* AMB-1, although other proteins as MamC from *M. marinus* MC-1 [2] and MmsF from *M. magneticum* AMB-1 [3] have also been proposed as candidates to produce biomimetic magnetite nanoparticles, but all these experiments only used one magnetosome protein at the time. In this work, we present data from magnetite crystal precipitated by using Mms6 and MamC from *M. marinus* MC-1. *In vitro* magnetite precipitation experiments have been carried out in the presence of each one of the proteins individually and mixtures of both at different concentrations. Our results show that, compared to magnetite crystals produced in the absence of any protein and under identical conditions, MamC controls the size of magnetite crystals and produces SDP (most of them > 30 nm) while Mms6 produces better faceted magnetite crystals.

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Swimming behavior of magnetotactic bacteria

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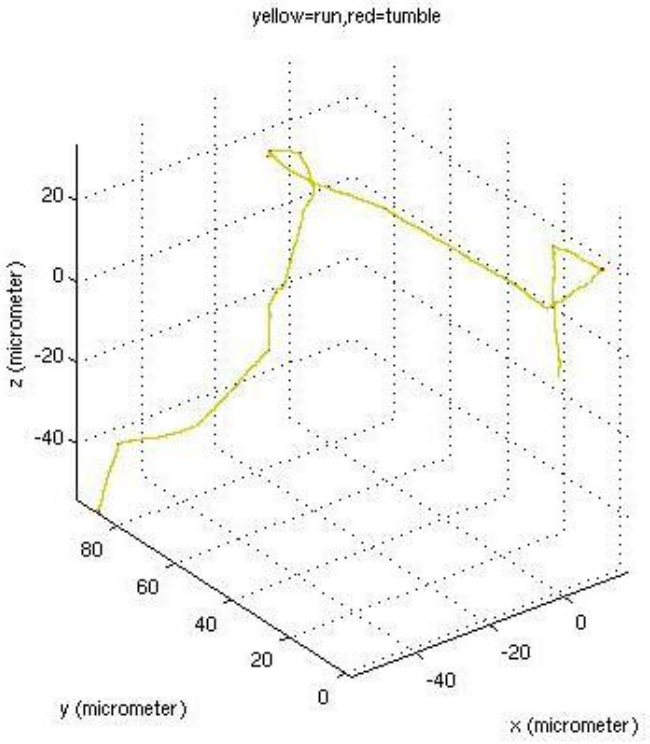
Magnetotactic bacteria swimming behavior is determined by various parameters. First of all, these bacteria produce a chain of magnetosoms that acts like a compass, passively orienting the bacterium in the earth magnetic field. Thus the bacteria actively swim mostly in one dimension along the field lines, with probably run and reverse motion. Second, they perform aerotaxis to reach the oxic-anoxic transition zone, situated at the bottom of lakes and seas. Swimming along the field lines helps them to find the zone easily, since the oxygen gradient is antiparallel to the earth magnetic field lines in our hemisphere. To better understand their swimming behavior we study it through a simulation. The chosen model is general, and can be applied to any swimming behavior. It consist in an active brownian particle model, in which there is a run state and a change of direction. Moreover in this model are included forces and torques due to interaction with external fields (for ex. the magnetic field), and also chemotaxis (for ex. aerotaxis). I will present the general model and show how it can be used to describe E. Coli run and tumble swimming behavior, and how it can be applied to magnetotactic bacteria.



Drawing 1: *E. Coli* run and tumble behavior from simulation.

Magnetic Response of *Magnetospirillum Gryphiswaldense*

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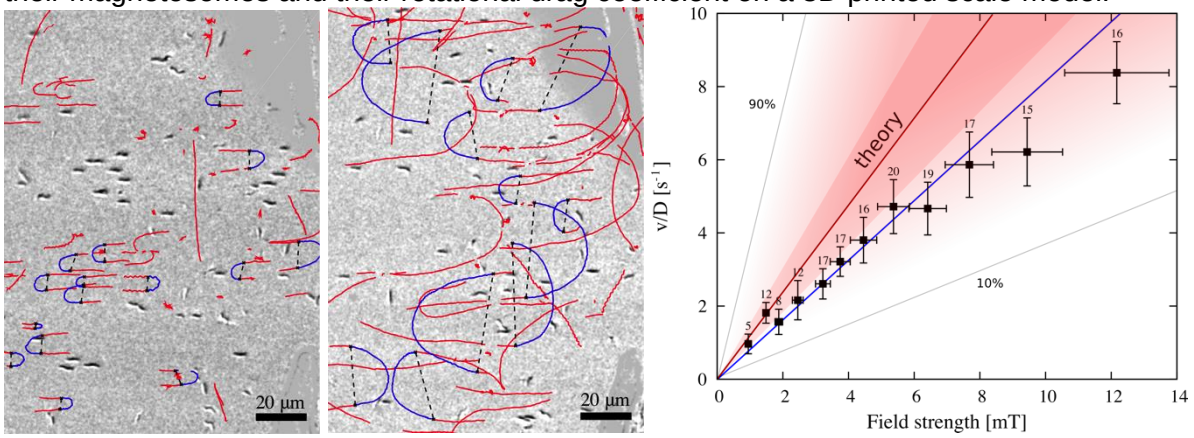
We studied the response of *Magnetospirillum Gryphiswaldense (MRS-1)* to rotation of an external magnetic field inside a 5 µm high microfluidic channel. We developed a detailed magnetic model shows that the torque on the magnetosome is linear with the applied field up to 10 mT, after which the torque starts to saturate. The theoretical analysis of trajectories shows that the bacteria perform a U-turn under 180° rotation of the external field, but not with a constant angular velocity. The average rate of rotation, (velocity over U-turn diameter) for an instantaneously reversing field is linear within 2% with the applied field up to 12 mT.
From the microscale experiments we have found that the bacteria velocity is independent of the applied field in the measured range. The population of MTB has a non-Gaussian distributed velocity with an average of 49(7) µm/s with a standard deviation of 9 µm/s. As predicted, the average rate of rotation is linear with the external magnetic field in the measured range of 1-5 mT. The proportionality factor 0.84(4) rad/mTs is very close to the theoretical prediction of 1.2(3) rad/mTs based on measurements of the size of bacteria and their magnetosomes and their rotational drag coefficient on a 3D printed scale model.



Trajectories of MRS-1 under reversal of a high (left) and low (right) magnetic field. The ratio of bacterium velocity over U-turn radius varies linearly with the field with a slope that is very close to a theoretical model based on rotational drag and magnetic torque.

Polarity of a bacterial nano-compass reversed by magnetic tweezers

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Magnetotactic bacteria synthesize uniform-shaped nano-sized magnetic crystals within unique prokaryotic organelles called magnetosomes. These function as a cellular magnetic nano-compass allowing the cells to navigate along the geomagnetic field. In this study, we observed and analysed detailed swimming behaviour of single cells under the custom-made magnetic tweezers, by which strength and orientation of external magnetic fields are quantitatively applied to specimens in the flow chamber. We observed responses of swimming behaviours of Magnetospirillum magneticum AMB-1 cells to 180-degree reversal of the applied magnetic fields at 8 mT or 37 mT. When the applied magnetic field was reversed at 8 mT, the cells turned their swimming directions. However, when it was reversed at 37 mT, the cells swam straight without changing the swimming direction. Interestingly, we found that the reversal the applied magnetic field at 37 mT caused reversal of the cellular magnetic polarity. The cellular magnetic polarity was reversed depending on the strength of the applied magnetic field. At 21 mT, the cellular magnetic polarities of 50% cells were reversed. The reversals of the cellular magnetic polarity were observed using tethered cells on a grass surface and chemically fixed cells by glutaraldehyde. Here, we show a novel technique for manipulating of magnetotactic bacterial "taxis" by the operation of external magnetic field with magnetic tweezers.

Visualization of flagellar rotation during magnetotactic motility using *Magnetospirillum magneticum* AMB-1

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Magnetospirillum magneticum AMB-1 is an amphitrichous flagellated bacterium, which has a single flagellum on each end of the cell. In the oxic-anoxic transition zone, AMB-1 cells repeatedly reverse their swimming direction by performing an 'l' shaped turn to stay within their most favorable microaerobic environment. In this study, we aimed to visualize of the individual polar flagellar rotations of swimming AMB-1 cells to clarify the mechanism of magneto-aerotactic motility using the amphitrichous flagella. For fluorescence imaging of flagellar rotation, we labeled flagella by using Qdot nano beads. AMB-1 cells were harvested from the early stationary growth phase culture and the flagella were biotinylated and labeled using Qdot streptavidin conjugates. We placed the cells in a chamber slide and observed flagellar motility by using a HILO (highly inclined and laminated optical sheet) fluorescence microscopy. During swimming, the lagging flagellum showed counterclockwise (CCW) rotation. On the other hand, the leading flagellum showed clockwise (CW) rotation as proposed previously by Murat et al. [1]. This is the first direct observation of the direction of the leading flagellum rotation in AMB-1. The leading flagellum was rotated at the side of the cell body, whereas the lagging flagellum was rotated at the posterior end of the cell body. In this poster, we discuss about the regulation of flagellar rotations during the magneto-aerotactic motility.

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Magnetic Traps that provide analysis of individual Living MTB cells

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For bacteria living in the natural environment it is imperative that they have the ability to quickly sense stimuli and navigate within their surroundings. Magnetotactic bacteria (MTB) have unique organelles called magnetosomes that give them the ability to sense a magnetic field, which in turn helps them to navigate to an environmentally preferred location. This behavior is know as magnetoaerotaxis because the bacteria use a chain of magnets in the magnetosome organelle to align themselves with earth's geomagnetic field, and in combination with chemicals signals (e.g. oxygen concentration) from the environment, trigger the cells to use their flagella to swim towards or away from the stimulant. Examining the behavior of MTB under different environmental conditions is import to fully understand the mechanisms underlying magnetoaerotaxis. We have developed a technique to trap and manipulate single MTB cells by exploiting MTBs' sensitivity to a magnetic field. Our apparatus consists of two principle parts, a 3-axis electromagnet that is placed on a microscope stage and a microscope "slide" containing a pattern of bar magnet-like structures that produce large magnetic field gradients that can guide and even trap the bacteria onto them. Even though the bacteria are trapped, their flagella and bodies are completely free and we can manipulate their trajectories. Once the bacteria are trapped, we can study them in that exact location for as long as they stay active. This means we can do different experiments such as manipulate their angle of trajectory and study their hydrodynamics, or we can study the movement of flagella and the cell body, while altering the surrounding environment. Using this new method to examine the different responses of MTB to various stimulants will improve our understanding of magnetoaerotaxis.

Controlled cobalt doping in the spinel structure of magnetosome magnetite: new evidences from element- and site-specific X-ray magnetic circular dichroism analyse

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The biomineralization of magnetite nanocrystals – magnetosomes - by magnetotactic bacteria (MTB) has attracted intense interest in biology, geology and materials science due to the precise morphology of the particles, the chain-like assembly and their unique magnetic properties. Great efforts have been recently made in producing transition metal-doped magnetosomes with modified magnetic properties for a range of applications. Despite some successful outcomes, the coordination chemistry and magnetism of such metal-doped magnetosomes still remain largely unknown.

Here, we present new evidences from X-ray magnetic circular dichroism (XMCD) for element- and site-specific magnetic analyses that cobalt is incorporated in the spinel structure of the magnetosomes within *Magnetospirillum magneticum* AMB-1 through the replacement of Fe2+ ions by Co2+ ions in octahedral (Oh) sites of magnetite. Both XMCD at Fe and Co L2,3 edges, and energy-dispersive X-ray spectroscopy on transmission electron microscopy analyses reveal a heterogeneous distribution of cobalt occurring either in different particles or inside individual particles.

Compared with nondoped one, cobalt-doped magnetosome sample has lower Verwey transition temperature and larger magnetic coercivity, related to the amount of doped cobalt. This study also demonstrates that the addition of trace cobalt in the growth medium can significantly improve both the cell growth and the magnetosome formation within M. magneticum AMB-1. Together with the cobalt occupancy within the spinel structure of magnetosomes, this study indicates that MTB may provide a promising biomimetic system for producing chains of metal-doped single-domain magnetite with an appropriate tuning of the magnetic properties for technological and biomedical applications.

Genetically functionalized Magnetosomes for Biotechnological applications

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The comprehensive analyses of *Magnetospirillum magneticum* strain AMB-1 including genome, transcriptome and proteome analyses enabled us to extract molecular components for magnetosome formation [1]. The researches on protein function analysis in magnetotactic bacteria provided not only the information of magnetosome formation mechanism but also the nano-materials for tremendous biotechnological applications. Functionalization onto magnetosome surface was attained by protein display techniques enabling introductions of enzymes, antibodies, and receptors, and the functionalized magnetosomes have also been utilized in various biosensors and bio-separation processes [2,3]. The precise regulation of magnetite crystal size was achieved by controlling gene expression of *mms* genes, and this technique will provide new opportunities for the *in vivo* synthesis of magnetite materials with controlled size, shape, and magnetic properties [4]. We present recent attempts for the preparation of functionalized magnetosomes and the applications.

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In vitro multimodal imaging characterization of RGD functionalized magnetosomes accumulation in U87 glioblastoma cells

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Magnetosomes produced by magnetotactic bacteria exhibit high MRI contrasting properties. Using both fluorescence imaging and MRI, we study *in vitro* the incorporation kinetics of RGD functionalized magnetosomes by U87 human glioblastoma cells, due to specific targeting of $\alpha_v\beta_3$ integrins overexpressed at cells membrane. U87 cells were incubated with two doses of AMB1VRGD magnetosomes (250 and 1000 μg_{Fe}/L) during 4, 24 or 48 hours. Immunostaining with specific antibody against AMB1 confirms AMB1VRGD accumulation in U87 cells along time (Fig a), whereas YFP signal (Venus fluorophore) decreases after 24h. Interestingly, specific antibody fluorescence signal is also relatively weak after 48h of incubation, and does not match in brightfield image or Pearl's staining image to spots revealing presence of magnetosomes iron-oxide core (Fig b). MRI results confirm iron accumulation in U87 cells observed on microscopy images, and especially a 3.5 times higher iron uptake is quantified for the 1000 μg_{Fe}/L dose compared to 250 μg_{Fe}/L at 48h (Fig c). The next step will be to optimize *in vivo* the administration of functionalized magnetosomes to U87 tumor bearing mice, for confirming iron accumulation in tumor cells along time.

Fig. a

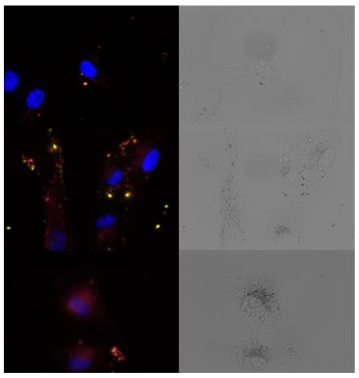


Fig. b

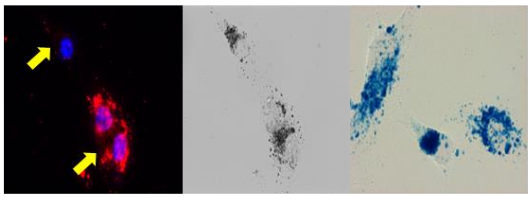
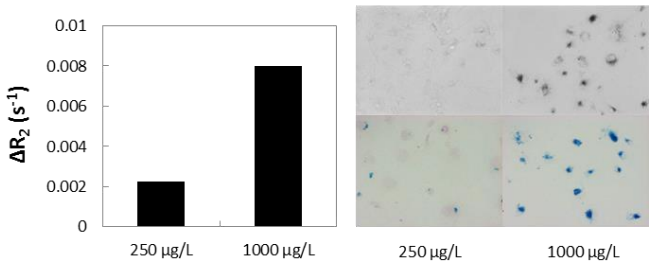


Fig. c



a/ Microscopy images (x63) of fixed U87 cells after 4, 24 or 48h of incubation with media containing AMB1VRGD (250 μg_{Fe}/L). Merge images (*left*) show cell nuclei (DAPI), Venus fluorophore at magnetosome membrane (YFP) and magnetosome membranes stained with a dedicated anti-AMB1 antibody revealed in red. Brightfield images (*right*) show the iron-oxide core clusters.
b/ Microscopy images (x63) of fixed U87 cells after 48h of incubation with media containing AMB1VRGD (1000 μg_{Fe}/L). Yellow arrows show the lack of detection of magnetosomes by specific anti-AMB1 antibody (*left*), whereas brightfield image (*middle*) and Perl's staining image (*right*) show the large amount of iron in U87 cells.
c/ Iron uptake by U87 cells incubated during 48h with RGD magnetosomes at two different doses (250 versus 1000 μg_{Fe}/L) is measured with quantitative MRI (*left*): ΔR_2 is direclty proportional to iron uptake. Microscopy images (x20) of these U87 cells (brightfield and Perl's staining images, *right*) show the higher uptake of iron at high dose compared to low dose.

Using the magnetosome to model gene-based iron contrast for magnetic resonance imaging

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There is a critical need to noninvasively image molecular activities that define early stages of disease progression. For this, the magnetosome is an ideal structure by which reporter gene expression for molecular magnetic resonance imaging (MRI) may be refined [1].
Hypothesis: The optimal expression of magnetosome-like particles in mammalian cells requires a subset of magnetotactic bacterial genes and may be tailored to specific magnetic resonance (MR) signatures that reflect biomineral localization, size, shape and composition.
To improve the formation of magnetosome-like particles for mammalian cell tracking, we are examining genes deemed essential for magnetosome vesicle formation. In bacteria, this step is not only critical to compartmentalizing the iron biomineral and protecting the cell from iron toxicity but may also confer a regulatory role in magnetosome formation [2]. We are expressing *mamI*, *mamL* and *mamB* from AMB-1 in MDA-MB-435 melanoma cells using vectors with fluorescent protein tags. Fluorescence microscopy of the resulting fusion proteins identifies sub/cellular location and potential for spontaneous co-localization. Transverse relaxation rates, measured at 3 Tesla in transfected cells mounted in a gelatin phantom [3], indicate the influence on MR contrast and role of extracellular iron supplementation (250 μM $Fe(NO_3)_3$/medium).
The formation of magnetosome-like particles for mammalian cell tracking may benefit from a subset of magnetotactic bacterial genes as well as clarify the role that individual magnetosome genes play in the regulation of iron biomineralization.

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Light irradiation help magnetotactic bacteria to eliminate intracellular reactive oxygen species

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Magnetotactic bacteria show phototactic behaviour, but the mechanism is not well understood. Magnetosomes were reported possess intrinsic enzyme mimetic activity similar to that found in horseradish peroxidase (HRP) and can scavenge reactive oxygen species (ROS) depending on peroxidase activity [1]. Our previous work has demonstrated that extracted magnetosomes from *Magnetospirillum magneticum* strain AMB-1 can enhance peroxidase-like activity under illumination [2]. In this work, the effects of light irradiation on the growth of AMB-1 were investigated and the intracellular ROS level was detected. The results showed that light irradiation did not affect the cell growth of AMB-1, but increased the synthesis of magnetosomes significantly, and reduced the intracellular ROS level. Total RNA was extracted from samples for quantitative RT-PCR (light treatment/dark control) of the stress-related genes recA (recombinase), oxyR (oxidative stress transcriptional regulator), SOD (superoxide dismutase), amb0664 and amb2684 (peroxiredoxins). We found light induced up-regulation in non-magnetic cells but down-regulation of recA, oxyR, SOD, amb0664, and amb2684 in magnetic cells under light exposure. Our results suggested that light irradiation may enhance the ability of magnetotactic bacteria to eliminate intracellular ROS and help them adapt to the light living environments.

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Isolation and characterization of five kinds of marine magnetotactic bacteria

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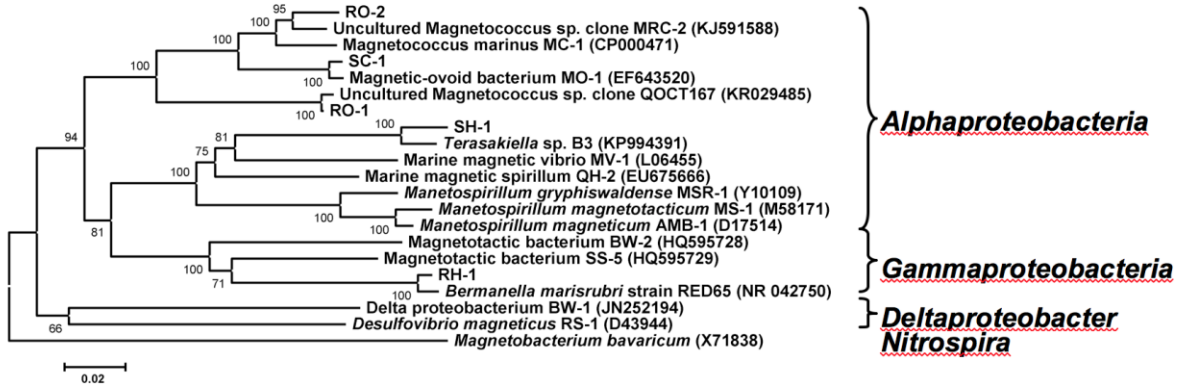
Five marine magnetotactic bacteria were isolated from intertidal sediments. Three of them are magnetococci (RO-1, RO-2 and SC-1), and two of them are manetospirilla (RH-1 and SH-1). Strain RO-1, RO-2 and RH-1 were from Lake Yuehu, Rongcheng. Strain SC-1 and SH-1 were from Sanya. Magnetosomes arrange in a disorganized cluster in RO-1, and in one chain in others. All the magnetosome crystals are prismatic magnetites.
Phylogenetic analysis reveals that four strains (RO-1, RO-2, SC-1 and SH-1) belong to the *Alphaproteobacteria* (Figure 1). Strain RH-1, whose morphology is different from BW-2 and SS-5 (rod-shaped), belongs to the *Gammaproteobacteria.* RO-1, RO-2, RH-1 and SH-1 are novel cultured magnetotactic bacteria. Strain RO-1 shows a maximum sequence identity with uncultured Magnetococcus clone QOCT167 (KR029485) (99.5%). Strain RO-2 shows a maximum sequence identity with uncultured Magnetococcus clone MRC-2 (KJ591588) (98.0%), and is 96.9% identical to MC-1. Strain RH-1 is 99.6% identical to *Oceanospirillales* sp. Y40. Strain SH-1 is 96.9% identical to *Terasakiella* sp. B3. Strain SC-1 is 98.6% identical to MO-1.



Phylogenetic tree showing the relationship between five strains and related magnetotactic bacteria

Diversity of magnetotactic bacteria in freshwater sediments from distinct aquatic habitats

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Magnetotactic bacteria (MTB) inhabit different aquatic biocoenoses. It was previously demonstrated that in the majority of the investigated communities the dominant types of MTB were cocci. Despite they are rather cosmopolitan, the prevalent phylotypes vary in different habitats. Moreover, novel phylogenetic groups of MTB are described in the majority of recent studies.

In this study we report the diversity of magnetotactic bacteria collected from four distinct freshwater habitats: the Moskva river, site near district Ilinskoe, Moscow (55.74768°N, 37.2479°E), the Moskva river, site near district Strogino, Moscow (55.4840°N 37.2505°E), Mschensky cold spring, Tver region (58.0377°N, 33.7400°W); and the Uda river, Ulan-Ude (51.8229°N, 107.6199°E). The collected sediments and water were used to make model ecosystems which were stored in the dark under room temperature. MTB were separated from the other bacterial community using “race track” method, and investigated by microscopy (light and TEM) and through 16S rRNA genes clone library analysis.

Microscopy observations of all studied samples revealed the presence of different morphotypes of MTB. Magnetotactic cocci dominated in all microcosms, rods and spirilla represented minor components. The combined library comprised 369 16S rRNA sequences which divided into 21 OTUs. The majority of sequences was affiliated with the order *Magnetococcales.* As minor components sequences with the highest identities (93-97%) to the representatives of the genus *Magnetospirillum* were identified. None of OTUs were shared among all testes habitats. Only two OTUs were found in three of the investigated ecosystems (Strogino, Mschensky, Ilinskoe). 18 OTUs included phylotypes described previously, 6 OTUs were endemic. *S*everal novel phylotypes of magnetotactic cocci and MTB distantly related to Magnetospirillum were identified.

This study provides useful insight about the structure of MTB community in freshwater aquatic ecosystems.

A novel culture device to cultivate microaerophilic magnetotactic bacteria

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Magnetotactic Bacteria are fastidious organisms regarding their growth. Although numerous microaerophilic strains have recently been isolated in pure culture, most of their phenotypic descriptions are only based on genomic analyses by identifying the presence/absence of genes potentially involved in metabolic pathways. One major obstacle to the growth of these O_2-gradient-loving bacteria is their specific oxygen requirements. Here we describe a novel culture device, named Micro-Oxygenated Culture Device (MOCD), specifically designed for routine liquid cultures of microaerophiles including microaerophilic magnetotactic bacteria. This system enables both continuous micro-oxygenation of the culture and promotes oxygen-gradient development. Major advantages for such device are I) the ease of implementation, II) the possibility for multiple simultaneous cultures and III) the adaptability depending on the strain oxygen requirement. The efficiency of the MOCD was demonstrated with two magnetotactic strains, characterized by different oxygen-tolerance patterns: the freshwater *Magnetospirillum gryphiswaldense* strain MSR-1 [1] and the marine *Magnetospira sp*. strain QH-2 [2]. This novel device has been successful in improving the growth of the two strains.

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Effects of the O_2 partial pressure on growth and on magnetosome synthesis in *Magnetospira* sp. strain QH-2

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Marine magnetotactic spirillum *Magnetospira sp*. strain QH-2 was isolated from intertidal zone sediments in China [1] and has been found to be phylogenetically closely related to the American obligate microaerophile *Magnetospira thiophila* strain MMS-1 [2]. So far, these two strains have only been grown in semi-solid or liquid (in flasks) cultures. Hence, no precise study has been established regarding QH-2 oxygen-tolerance. In this work we used specific oxygen-controlled bioreactor in order to clarify the effect of oxygen tension (pO_2) on growth and magnetosome synthesis in QH-2. Results showed that QH-2 similarly to MMS-1, was an obligate microaerophile, whose optimal pO_2 to growth was inferior to 5%*. Moreover, under our experimental conditions and with pO_2s ranged between 1 and 15%*, QH-2 magnetosome synthesis was surprisingly not affected.

*Air composition, i.e. 21% oxygen, represented 100% on the saturation scale and corresponded to 224µM dissolved O_2 in presence of 23 $g.L^{-1}$ of NaCl

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Comparative genomics of *Magnetospirillum* related species give insight into the origin and diversification of magnetotaxis

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Species of the *Magnetopirillum* genus, such as strains MSR-1 and AMB-1, are the most extensively characterized magnetotactic bacteria (MTB), for which genetic tools have been developed in the past ten years to decipher the molecular basis of magnetotaxis. Since the cultivation of *M. magnetotacticum* strain MS-1 in 1979, many species were isolated and their genome sequenced, which makes the *Magnetospirillum* group the perfect model to infer the evolutionary history of magnetotaxis along with MTB diversification. In this study, we build a genomic database of sequenced magnetotactic and non-magnetotactic genomes closely related to the *Magnetopirillum* genus, that we enriched with genomes of newly isolated diversity. Genomes and particularly the magnetotactic islands (MAI) were annotated using the MaGe platform, then core and accessory genomes were determined. Population and functional genomics analysis were performed to reveal variation patterns between and within species. Among other things, this approach allowed us to (i) infer the evolutionary relationships between *Magnetospirillum* relatives, (ii) define new taxonomic and ecological boundaries to each species/species complex and (iii), compare the evolution of the MAI genes in relation with the *Magnetospirillum* diversification. We not only identified new MTB specific genes, but we could propose a scenario about the emergence and evolution of MAI within the *Magnetospirillum* group. These results constitute a solid framework for future studies aiming at deciphering the genetic basis of magnetosomes formation and their role in MTB.

Greigite or magnetite : environmental and genetic determinants controlling biomineralization in magnetotactic bacteria

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Magnetotactic bacteria represent a phylogenetically and ecologically diverse group of prokaryotes able to biomineralize magnetic nanocrystals of magnetite [an iron oxide ($Fe(II)Fe(III)_2O_4$)] or greigite [an iron sulfide ($Fe(II)Fe(III)_2S_4$)] in their organelles called magnetosomes aligned in chain for a navigation along the Earth's magnetic field lines.
Until recently, only magnetite-building strains were available in pure culture. Thus, only the magnetite biomineralization has been studied. Recently, a new bacterium able to form both magnetite and greigite, *Candidatus Desulfamplus magnetomortis* strain BW-1, was isolated in culture at the LBC.
In this thesis, we propose to use an integrated and multidisciplinary approach to understand the mechanisms of greigite biomineralization in BW-1 strain. The first step will be to determine the environmental and biological conditions favoring magnetite and greigite formation. The bacteria and its metabolism will also be characterized. Secondly, we will use global and targeted transcriptomic approaches to evaluate the transcription level of the genes involved in magnetosomes (magnetite *vs*. greigite) formation under various growth conditions.
Our results will bring a fundamental understanding of *in vivo* biomineralization, particularly for greigite production in magnetotactic bacteria. Moreover, iron nanoparticles have been of an increased interest for biotechnological applications especially in medical field. These data will bring new efficient ways for *in vitro* or *in vivo* custom nanoparticles production.

Observation of magnetotactic bacteria on three volcanic lakes of "Siete Luminarias", Guanajuato, Mexico

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The magnetotactic bacteria are a heterogeneous group prokaryotes capable of navigating along with a magnetic field, a phenomenon known as magnetotaxis. Here we present the preliminary results of a search for magnetotaxis behaviour on the microorganisms from three crater lakes (*Cíntora*, *La Joya* and *Parangueo*) located in the "*Siete Luminarias*" site, Guanajuato state, Mexico. The three lakes are maars formed by runoff waters: *Parangueo* is alkaline (pH > 9) and hypersaline, *Cíntora* is also alkaline but for most of the year it is dry, and *La Joya* is an eutrophic lake, with pH near neutral values, artificially maintained due to its importance to migratory birds. While the colonies from *Cíntora* and *La Joya* are formed by rod-shaped individual cells, the ones from *Parangueo* lake form clusters of cells; all of them are being subjected to an isolation process.

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Sunday		Monday *Genes and proteins involved in magnetosome synthesis*			Tuesday *MTB and magnetosomes: physico-chemical characterizations*			Wednesday *MTB and magnetosomes: applications*			Thursday *Biodiversity and ecophysiology*		
		08:45-09:15	Chair: D. Pignol & C.T. Lefèvre	**D. Schüler**	08:45-09:15	Chair: S. Staniland & S. Klumpp	**D. Faivre**	08:45-09:15	Chair: R. Zarivach & N. Ginet	**S. Staniland**	08:45-09:15	Chair: D. Schüller & A. Taoka	**D. Bazylinski**
		09:15-09:35		**S. Li**	09:15-09:35		**M. Amor**	09:15-09:35		**M. Boucher**	09:15-09:35		**U. Lins**
		09:35-09:55		**S. Barber-Zucker**	09:35-09:55		**A. Muela**	09:35-09:55		**A. Fernández-Castané**	09:35-09:55		**Y Chen**
		09:55-10:15		**R. Uebe**	09:55-10:15		**F. Guyot**	09:55-10:15		**M. L. Fdez-Gubieda**	09:55-10:15		**D. Trubitsyn**
		10:15-10:35		**H. Nudelman**	10:15-10:35		Coffee Break	10:15-10:35		**S. Martel**	10:15-10:35		Coffee Break
		10:35-10:55		Coffee Break	10:35-11:05		**T. Prozorov**	10:35-10:55		Coffee Break	10:35-11:05		**C. Lefèvre**
		10:55-11:25		**A. Komeili**	11:05-11:25		**J. Werckmann**	10:55-11h15		**Y. Geng**	11:05-11:25		**W. Lin**
		11:25-11:45		**P. Browne**	11:25-11:45		**E. Günther**	11:15-11:35		**J-B Abbé**	11:25-11:45		**W. Zhang**
		11:45-12:05		**A. E. Rawlings**	11:45-12:05		**R. Le Fevre**	11:35-11:55		**T. Song**	11:45-12:15		Final words
		12:05-12:25		**A. Arakaki**			Photo	11:55-12:15		**L. Qi**			
								12:15-12:35		**F. Mickoleit**			
		12:30-14:00		Lunch	12:30-14:00		Lunch	12:35-14:00		Lunch	12:30-14:00		Lunch
		14:00-16:00		Free-time: Swimming-pool, private beach, volley, pétanque…	14:00-16:00		Free-time: Swimming-pool, private beach, volley, pétanque…						
14:00-18:30	Shuttles from airport or train station and Check-In	16:00-16:30	Chair: A. Komeili & D. Bazylinski	**A. Taoka**	16:00-16:30	Chair: D. Faivre	**S. Klumpp**				14:00-17:00		Check-out and Shuttle to airport / train station
		16:30-16:50		**F. Müller**	16:30-16:50		**C. Fradin**						
		16:50-17:10		**M. Toro-Nahuelpan**	16:50-17:10		**B. Kiani**	14:00-20:00		Visit of Marseille			
		17:10-17:30		**É. Bereczk-Tompa**	17:10-17:30		**A. Tay**						
		17:30-17:50		Coffee Break	17:30-19:00		**Coffee Break with Poster Session**						
		17:50-18:20		**R. Zarivach**									
18:30-19:00	**Welcome Speech**	18:20-18:40		**D. Pfeiffer**									
18:30-19:00	**Richard Frankel**	18:40-19:00		**C. Grant**									
		19:00-19:20		**Y. Zhang**									
19:30-20:00	Aperitif	19:30-20:00		**First look to the posters**	19:00-20:00		French wine degustation						
20:00-21:30	Dinner	20:00-21:30		Dinner	20:00-21:30		Dinner	20:00-21:30		Barbecue Party			
21:30	Bar is open	*21:30*		**Poster session with a beer at the Bar**	*21:30*		**Poster session with a beer at the Bar**	*21:30*		Bar still open			