6th International Meeting on Magnetotactic Bacteria (MTB 2018 meeting)

The Kanazawa Chamber of Commerce & Industry,
Kanazawa, Japan
September 10-14, 2018

Program

September 10	Reception and Registration
September 11	Opening, Oral presentation, Poster presentation 1
September 12	Oral presentation, Poster presentation 2
September 13	Oral presentation, Conference dinner (KKR Hotel Kanazawa)
September 14	Excursion

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MTB 2018 meeting

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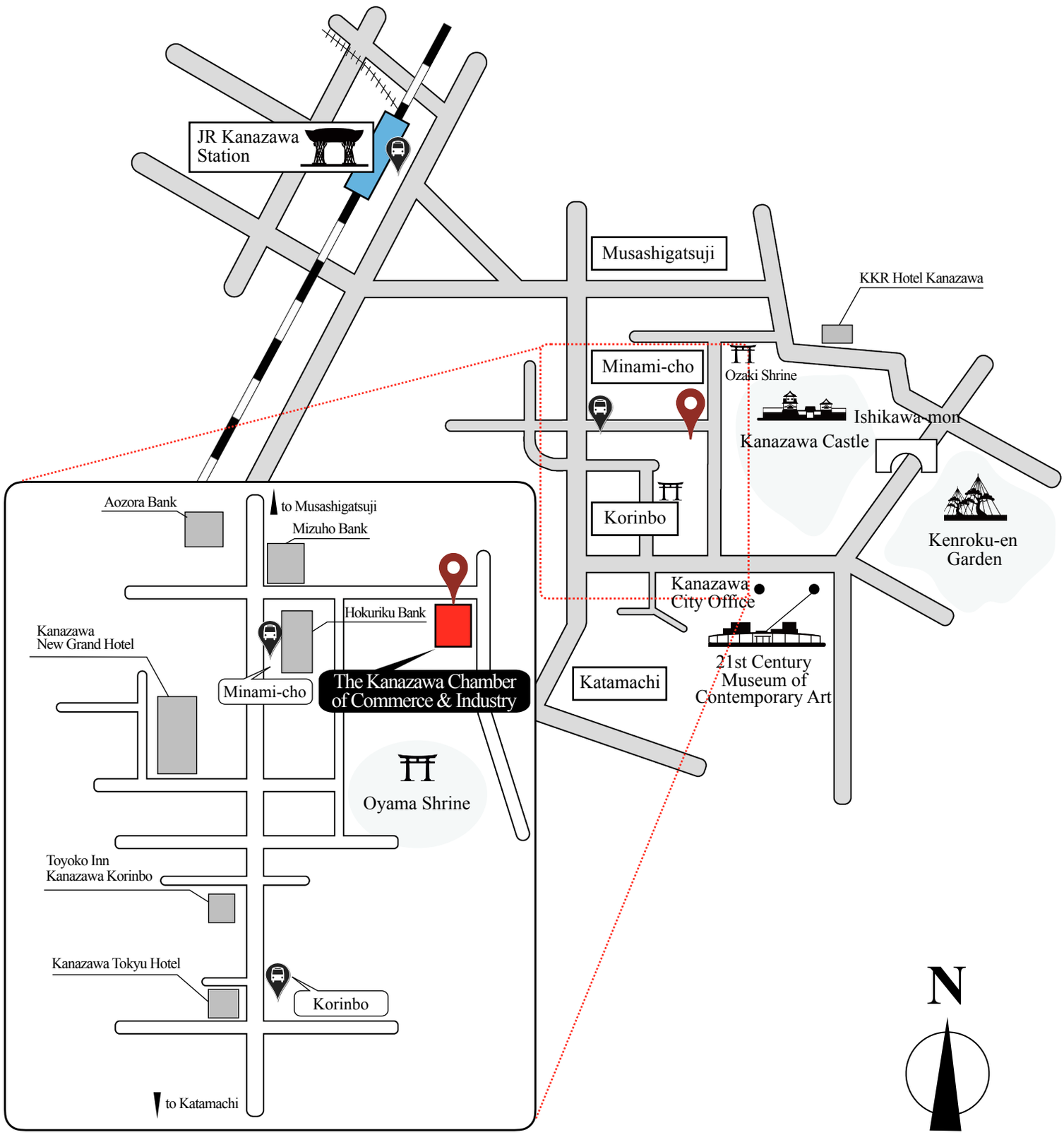
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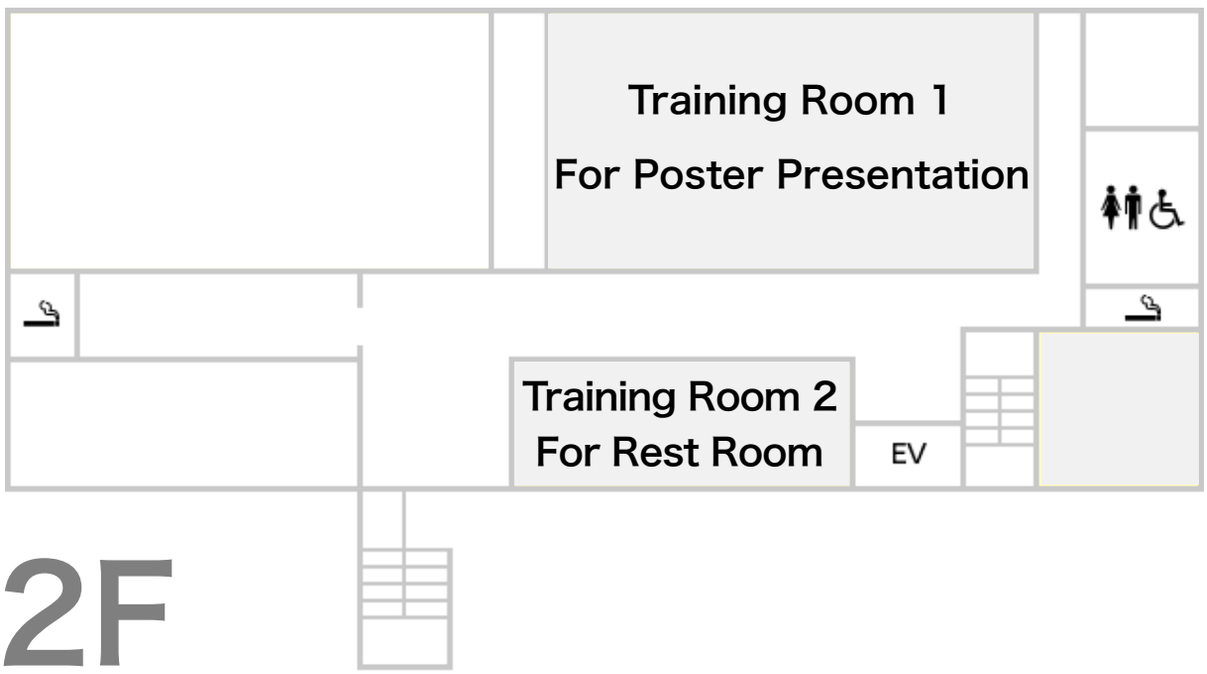

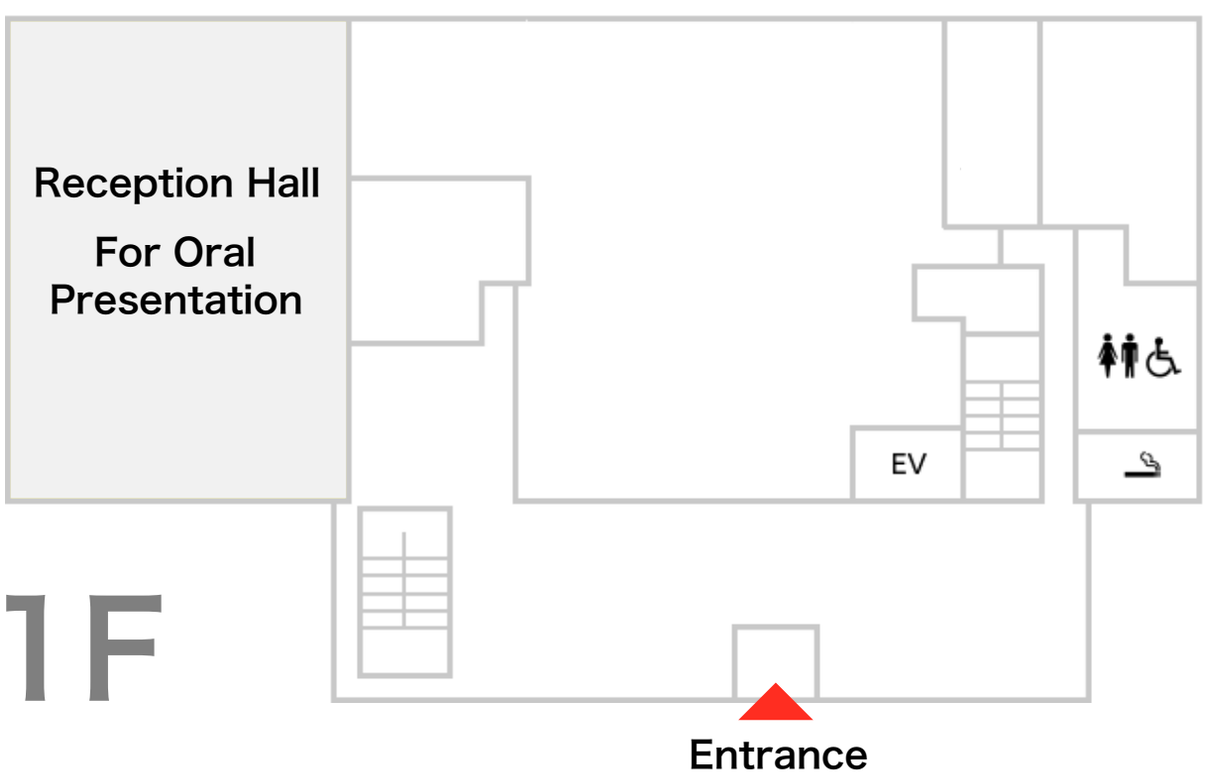

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Scientific program of MTB 2018 meeting

September 10th (Monday)		
15:00-20:00	Reception and registration	
September 11th (Tuesday)		
09:00-09:10	Y. Fukumori/T. Matsunaga	Opening remark
09:10-09:35	D. Schüler	The multipart magnetoskeleton of *Magnetospirillum gryphiswaldense*
09:35-09:53	M. Dziuba	Transcriptional organization of magnetosome operons in *Magnetospirillum gryphiswaldense*: a new look
09:53-10:11	M. Schüler	Genome-wide identification of essential and auxiliary genes for magnetosome biosynthesis in *Magnetospirillum gryphiswaldense*
10:11-10:29	R.P. Awal	Functional expression of foreign magnetosome genes in the *Alphaproteobacterium Magnetospirillum gryphiswaldense*
10:29-10:50	Coffee break	
10:50-11:15	Y. Fukumori	Structure and function of molecular machinery for magnetotaxis
11:15-11:33	A. Taoka	Measuring magnetosomal pH in living magnetotactic bacterial cell
11:33-11:51	E. Günther	The *in vivo* mechanics of the magnetosome chain by FLIM-FRET and STED microscopy
11:51-12:09	T. Prozorov	Magnetotactic bacteria as a platform for liquid phase electron microscopy live cell imaging
12:09-12:27	N. Karen-Khadmy	The dual role of MamB in magnetosome membrane assembly and magnetite biomineralization
12:27-14:30	Lunch	
14:30-14:55	A. Komeili	The past, present, and future of genetic analysis in magnetotactic bacteria
14:55-15:13	C. Grant	An iron-accumulating organelle—the ferrosome—is formed via a small operon in diverse bacteria and archaea
15:13-15:31	J. Wan	Size determination of magnetosome membrane by a protease-mediated switch
15:31-15:49	Q. Sun	Interaction of essential magnetosome genes in mammalian cells
15:49-16:07	M. Tanaka	Enhanced tubulation of liposome containing cardiolipin by MamY protein from *Magnetospirillum magneticum* AMB-1

16:07-16:25	D.S. Grouzdev	Increased knowledge of magnetotactic bacteria diversity through the analysis of metagenomic data
16:25:16:43	M. Uzun	Novel approaches of magnetotactic bacteria investigation revealed new taxonomic groups
16:43-17:01	C.L. Monteil	Retracing the evolutionary history of magnetotaxis
17:01-18:30	Poster session 1	
19:00-21:00	Dinner 1	
September 12th (Wednesday)		
08:45-09:10	R. Zarivach	Structural studies of magnetosome-associated proteins
09:10-09:28	A. Pohl	Magnetite-binding proteins from *Deltaproteobacteria*: A new route towards anisotropic magnetite nanoparticles?
09:28-09:46	M. Amor	Single-cell determination of iron content in magnetotactic bacteria
09:46-10:04	R. Uebe	Analysis of the iron uptake in *Magnetospirillum gryphiswaldense*
10:04-10:22	J. Werckmann	Detection of low amounts of iron in the outer membrane of magnetotactic bacteria
10:22-10:45	Coffee break	
10:45-11:10	A. Arakaki	Reconstruction of magnetosome formation ability in non-magnetic mutant of *Magnetospirillum magneticum* AMB-1
11:10-11:28	T. Yoshino	Bioengineering of magnetic nanoparticles produced by *Magnetospirillum magneticum* AMB-1 for extensive applications
11:28-11:46	A. Fernandez-Castane	Study of physiological responses to changing environmental conditions of *Magnetospirillum gryphiswaldense* MSR-1 in flask and bioreactor cultures
11:46-12:04	E. Duprat	Magnetotactic coccus from ferruginous Lake Pavin: a new model for intracellular sequestration of phosphorus
12:04-12:22	H. Hamamura	Diversity of microbial metalloid redox transformation pathways associated with the contaminated environment
12:22-14:30	Lunch	
14:30-14:55	D. Faivre	A cooperative flagellar movement explains *Magnetococcus marinus* swimming speed and movement
14:55-15:13	S. Klumpp	Modelling magnetotactic bacteria
15:13-15:31	A. Codutti	Influence of magnetic fields on chemotaxis in magnetotactic bacteria

15:31-15:49	L. Abelmann	Magnetic response of *Magnetospirillum gryphiswaldense* observed inside a microfluidic channel
15:49-16:07	M.L. Fdez-Gubieda	On the magnetosomes chain configuration
16:07-16:25	M. Charilaou	Quantitative magnetic analysis of magnetotactic bacteria by means of ferromagnetic resonance spectroscopy
16:25:16:43	A.R. Muxworthy	Numerical calculations of the effect of magnetic interactions on magnetotaxis efficiency
16:43-17:01	A. Garcia-Prieto	Magnetic study of Co-doped magnetosome chains
17:01-18:30	Poster session 2	
19:00-21:00	Dinner 2	
September 13th (Thursday)		
08:45-09:10	J. Kirschvink	Scaling up the Kalmijn-Blakemore pulse remagnetization experiment from magnetotactic bacteria to the human head
09:10-09:28	V. Busigny	Iron isotope perspectives for magnetotactic bacteria identification in the geological record
09:28-09:46	R.J. Harrison	Micromagnetic simulation of magnetofossils with realistic size and shape distributions: Linking rock magnetism with microscopic observations and implications for magnetofossil identification
09:46-10:04	A.P. Roberts	Widespread occurrence of magnetofossils in the geological record and implications for living environments of magnetotactic bacteria
10:04-10:22	P. Liu	Pylogenetic and morphological identification of magnetotactic cocci using coupled FISH-SEM method
10:22-10:45	Coffee break	
10:45-11:03	K. He	Spatio-temporal distribution of magnetotactic bacteria in freshwater sediments
11:03-11:21	K. Suthindhiran	Diversity of uncultured *Magnetospirillum* sp. from the sedimentary basin of southern India
11:21-11:39	J. Liu	Seasonal changes in abundance of two multicellular magnetotactic prokaryotes in sediment layers of Lake Yuehu
11:39-11:57	X. Qian	Description of the first *Nitrospira* phylum magnetotactic bacteria isolated from ocean
11:57-12:15	P. Leão	Diversity of magnetotactic bacteria in a coastal lagoon complex in Rio de Janeiro state, Brazil
12:15-12:30	Group photo	
12:30-14:30	Lunch	

14:30-14:55	D. Kisailus	Biological synthesis and structural developments in ultrahard teeth of chiton
14:55-15:13	J. Li	Phylogenetic and biomineralogical study of uncultured magnetotactic bacteria at single-cell level
15:13-15:31	W. Lin	Genomic insights into the origin and evolution of magnetotactic bacteria
15:31-15:49	W. Zhang	Matagenomic analysis of magnetotactic bacteria from saline lake in Inner Mongolia
15:49-16:07	Y. Konishi	Microbial biomineralization and the catalytic activity of platinum group metal nanoparticles obtained with metal-reducing microorganism
16:07-16:20	Break	
16:20-16:38	S. Staniland	Magnetosomes for the masses: scaling-up magnetic nanoparticle formation with biomimetic additives, inspired by MTB
16:38-16:56	J. Wang	Mms6 protein from *M. magneticum* AMB-1 and magnetite biomimetic synthesis
16:56-17:14	J. Tian	Engineered magnetosome, a green synthetic and cheap composite nanomaterial can be used as a nano-sized immunomagnetic beads
17:14-17:32	D. Trubitsyn	Functionalization of bacterial magnetic nano-particles for specific binding to human cells
17:32-17:50	E. Alphandery	Development of non-pyrogenic magnetosome minerals coated with poly-l-lysine leading to full disappearance of intracranial U87-Luc glioblastoma in 100% of treated mice using magnetic hyperthermia
17:50-18:08	M. Muthana	Breast cancer immunotherapy using magnetised oncolytic virus
18:08-18:25	R.B. Frankel	Business meeting
18:25-18:30	Y. Fukumori	Closing
19:00-21:00	Banquet	
September 13th (Thursday)		
08:30-17:00	Excursion	

Oral Presentations

The multipart magnetoskeleton of *Magnetospirillum gryphiswaldense*

Dirk Schüler

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To serve efficiently as a magnetic field sensor, magnetosomes of magnetotactic spirilla have to be aligned into coherent chains and centered at midcell opposite to incipent septum. Over the last years, it became evident that all steps of magnetosome organization are under strict genetically control. Here, I will summarize some of our recent findings on the determinants and functions of the cytoskeletal structures governing magnetosome chain assembly and positioning in *M. gryphiswaldense* and present some novel structural features uncovered by genetic and microscopic approaches.
We found hat magnetosome chains undergo rapid intracellular repositioning from the new poles towards midcell into the newborn daughter cells, which is driven by the pole-to-midcell treadmilling growth of actin-like MamK filaments connected to the magnetosome particles via the acidic MamJ. We further discovered that splitting and equipartitioning of magnetosome chains occurs with highest possible accuracy, which also depends on the dynamics of MamK. A further important, but so far mostly unappreciated question is how the straight chain of magnetosomes essentially akin a magnetic rod is properly accomodated within the helical, higly curved cell. We found that the transmembrane MamY protein forms a novel organelle-scaffolding cytoskeletal structure able to maintain support linear chains in the absence of MamK. This reconciles previously observed and partly conflicting phenotypes of *mamJ* and *mamK*. We also show that MamY localizes as a cell-spanning polymeric filament that identifies the cellular geodetic axis *in vivo,* likely by curvature sensing. We propose that MamY's function is to enable proper positioning of the straight magnetosome chain into the spiral cell body, thereby perfectly aligning the cell's magnetic dipole parallel to its axis of locomotion to ensure most efficient magnetotaxis. Finally, I will higlight an extended model of a multipartite, interactive "magnetoskeleton" which controls the assembly one of the most sophisticated type of organelles found in a prokaryotic cell.

Transcriptional Organization of Magnetosome Operons in *Magnetospirillum gryphiswaldense*: a New Look

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Magnetosomes of magnetotactic bacteria represent complex prokaryotic organelles, which are synthesized under control of >30 genes. In the alphaproteobacterium *Magnetospirillum gryphiswaldense* magnetosome genes are organized in 5 polycistronic operons: *mamABop*, *mamGFDC*, *mms6op*, *mamYXZ* and *feoAB1*, which were predicted to be constitutively transcribed from a single promoter located upstream of the first gene in each operon (Schübbe et al., 2006). However, it has remained unknown whether there are multiple promoters within the operons, whether any of magnetosome genes are regulated by environmental conditions, as well as if the transcript ratio of magnetosome operons is important for the magnetosome formation. Understanding this would shed light on the regulation of magnetosome biogenesis at the transcriptional level, and provide additional clues for the engineering of the process.

The recently obtained transcriptomic data verified the known promoter regions and predicted several inter- and intragenic promoters within the magnetosome operons.To test their activity, we developed the bacterial luciferase reporter assay for the application in *M. gryphiswaldense*. In consistence with the previous observations, P*mamG* (*mamGFDC*) exhibited the strongest activity among all analyzed promoters, whereas P*mamH* (*mamABop*), P*mms6* (*mms6op*) and P*mamY* (*mamYXZ*) were substantially weaker. Some previously predicted promoters were corrected with regard to length (P*mamH*) or position (P*mms6*). Analysis of the intergenic regions from magnetosome operons revealed the presence of an active promoter in the 403 bp sequence between *mmsF* and *mms36* in *mms6op*, suggesting the possible independent expression of the downstream genes *mms36* and *mms48.* No significant activity was detected for the other intergenic regions. The intragenic promoters were predicted by transcriptomic data predominantly in the long (>16 kb) *mamABop*. The luciferase reporter assay confirmed the presence of *bona fide* promoters in genes *mamH*, *mamE* and *mamL.* All these results suggest that the architecture of magnetosome operons is more complex than it was assumed before, and there are deeper layers of transcriptional regulation of magnetosome biosynthesis to be discovered.

Genome-wide identification of essential and auxiliary genes for magnetosome biosynthesis in *Magnetospirillum gryphiswaldense*

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Biosynthesis of bacterial magnetosomes is an intricate process, comparable in structural and genetic complexity to other microbial processes like sporulation and photosynthesis. Previously, it has been assumed that a genomic region of about 120 kbp, the so-called magnetosome island (MAI), harbors all genetic determinants, some 30 genes, necessary for magnetosome generation. However, recent evidence from several laboratories indicates that in addition to these MAI genes there must be other, auxiliary genes that in the first place enable an organism to produce magnetosomes. For a complete understanding of the complex process of magnetosome biosynthesis, identification of a comprehensive set of such auxiliary genes is of great interest. We pursued this objective in *M. gryphiswaldense* MSR-1 by a systematic genome-wide transposon mutagenesis study. A Tn5-insertion library of 80,000 clones was generated and screened for loss of dark-brown colony color to identify mutants with mild (weakly magnetic, Wmag) to severe (non-magnetic, Nmag) impairment of magnetosome biosynthesis. We obtained 202 clones with essentially WT-like growth behavior but reduced C_{mag} values. Wmag cells typically showed deviant chain formation/positioning and/or magnetite crystals with partly aberrant morphology. About 50% of Tn5 insertion sites were found to map within the MAI (mostly Nmag phenotype), and in several Wmag clones insertion affected genes outside MAI whose products have been implicated in magnetosome formation before (e.g. nitrite reductase, periplasmic nitrate reductase). These results validated our screening approach. Moreover, since a large number of the identified Wmag clones exhibited Tn5 insertion in previously unsuspected genes, our work considerably extends the candidate set of subsidiary determinants for magnetosome biosynthesis. Many of these genes are of yet unknown function while others seem to be involved in electron transport and redox state balance, periplasmic oxidative protein folding, cell wall biosynthesis/modification, and nitrogen metabolism. Several gene clusters encoding these pathways turned out to be hotspots for Tn5 insertion. These are currently investigated further by markless deletion mutant characterization and biochemical methods.

Functional expression of foreign magnetosome genes in the *Alphaproteobacterium Magnetospirillum gryphiswaldense*

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Magnetotactic bacteria (MTB) produce spectacular varieties of magnetosomes in terms of shapes, sizes, and compositions. Biosynthesis of magnetosomes is controlled by specific large gene clusters in the genomic magnetosome island (MAI), which despite being conserved in MTB are diverse with respect to their sequences, gene contents and orders. However, only few of those gene clusters could be analysed in well-characterised laboratory model MTBs so far. To elucidate their functions in magnetosome biosynthesis, we attempt to express selected genes in the *Alphaproteobacterium Magnetospirillum gryphiswaldense*, a well-studied tractable model organism. As a first approach, orthologous genes influential in the biosynthesis of magnetosomes from *Magnetospirillum magneticum*, *Magnetovibrio blakemorei* and *Desulfamplus magnetovallimortis* were transferred into the corresponding gene-deleted mutant strains of *M. gryphiswaldense* by Tn5 (tnpA) transposition and chromosomal recombination and studied with respect to their effects on magnetosome formation. Our preliminary results show that single foreign genes were expressed in the corresponding *M. gryphiswaldense* deletion mutant strain. In addition, they complemented the mutants by restoration of magnetosomes and chain formation. Furthermore, a transfer of the entire mamAB operon from *M. magneticum* into the corresponding *M. gryphiswaldense* deletion mutant strain restored magnetosome formation. In summary, we show that genes from closely related MTB can be expressed in *M. gryphiswaldense* and are functionally equivalent. Moreover, we further plan to transfer and characterize single/multiple genes or entire biosynthetic clusters from foreign MTB that produces diverse magnetosomes in *M. gryphiswaldense.*

Structure and function of molecular machinery for magnetotaxis

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Magnetotactic bacteria have a unique bacterial organelle, termed the magnetosome, which functions as a cellular magnetic sensor. The integration of this cellular magnetic sensor into their motility allows magnetotactic bacteria to swim along the geomagnetic field in order to locate and maintain within a favorable microaerobic habitat. Here, we introduce progresses in studies on structures and functions of magnetosome and magnetosome-associated proteins using microscopic techniques, including atomic force microscopy (AFM) and live-cell fluorescence imaging. Our AFM studies provided detailed structural model of magnetosome. The magnetite crystal was wrapped around by the organic layer, ~7 nm thickness, and magnetosome-associated protein MamA was localized at the surface of the organic layer. Furthermore, we conducted live-cell time-lapse fluorescence imaging to observe dynamic localization of magnetosomes in growing cells during the entire cell cycle. Using this live-cell imaging technique, we found that MamK prevents simple diffusion of magnetosomes within the cell. MamK cytoskeleton tethers the magnetosomes into a static chain-like arrangement, which generates a magnetic dipole and functions as an effective magnetic sensor. Further study is currently in progress to elucidate molecular mechanism of magnetosome positioning by MamK cytoskeleton. The MamK cytoskeleton and the magnetosome serve as an excellent model to facilitate future studies for understanding how bacteria use the cytoskeleton to maintain and to make functional their organelles.

Measuring magnetosomal pH in living magnetotactic bacterial cell

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Little is known about the nature of magnetosome within living cell because of their small size making direct imaging using light microscopy difficult. The live-cell fluorescence imaging technique is a powerful tool to revealed localization, dynamics, compositions, and environments of magnetosomes in living cells. We conducted live-cell fluorescence imaging analyses employing a highly inclined and laminated optical sheet (HILO) microscopy to know the nature of magnetosomes. Using this live-cell imaging technique, we found that MamK cytoskeleton prevents diffusion or aggregation of magnetosomes by physical disturbances with in the cell. Furthermore, we developed a method for measuring the pH in magnetosome vesicle in a single living *M. magneticum* AMB-1 cell using a pH-sensitive fluorescent protein E_2GFP. We estimated pH in the magnetosome lumen and cytoplasmic surface using fusion proteins of E_2GFP and magnetosome-associated proteins. The internal environment of the magnetosome should be well-controlled for the synthesis of a magnetite crystal. Understanding of magnetosome pH homeostasis provides a clue to reveal molecular mechanisms of magnetite biomineralization in the bacterial organelle.

The *in vivo* mechanics of the magnetosome chain by FLIM-FRET and STED microscopy

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The bacterial magnetosome chain is build up by two molecular players. The actin-like MamK protein forms the cytoskeletal filament to which the magnetosomes are connected by the MamJ protein. The magnetosome chain functions as a magnetic actuator and allows the bacteria to passively align and navigate along the Earth's magnetic field to find their favoured environment. Hence, precise chain arrangement and mechanical stability of MamJ and MamK are essential for the orientation of the bacteria in an external magnetic field. Fluorescence microscopy is one of the most versatile and powerful tools to study structural as well as functional aspects of cellular processes at qualitative and quantitative levels. However, the main drawback of conventional fluorescence microscopy is the limitation of the spatial resolution. Here, we present the development a new pair of fluorescent proteins that combine for the first time Förster resonance energy transfer based on fluorescence lifetime imaging microscopy (FLIM-FRET) and stimulated emission depletion (STED) microscopy simultaneously, *in situ* and *in vivo* at the nanoscale level. This combination allowed us to study the mechanics of the magnetosome filament protein MamK and its interaction with the magnetosome connector MamJ while applying a magnetic torque. We demonstrate that a magnetic torque can be used to directly assess structural properties of biological players *in vivo.* FLIM-FRET reveals the stability of the MamJ-MamK interaction, whereas STED microscopy allows the characterization of the mechanical properties of the MamK filament.

Magnetotactic bacteria as a platform for liquid phase electron microscopy live cell imaging

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Magnetotactic bacteria have been established as one of the best model systems for investigating the molecular mechanisms of biomineralization. Understanding biomineralization in these organisms in terms of crystal nucleation and growth, as well as the involvement of biological macromolecules and cellular processes, is a topic of great interest for nanotechnology, functional materials, and astrobiology.

Magnetite magnetosome biomineralization in these bacteria is a complex dynamic process involving a number of steps including cellular uptake and reduction of ferric ions, complexation of the iron with membrane proteins, and nucleation and growth of mature magnetosomes. The variety of processes involved in magnetosome formation, ranging from the bacterial uptake of iron to nucleation and growth of nanoscale magnetite, necessitates the use of various modes of analysis and continuous development of experimental technique enabling following these steps at high spatial and temporal resolution, while mitigating the electron beam damage to the cells.

Microscopy aided in many important discoveries in life science by providing structural information about cellular compartments. Recently, we have developed the correlative electron microscopy- fluorescent microscopy approach to imaging of intact, fully hydrated cells of various biological specimens in their natural liquid environment with nanometer resolution. Our early work on was focused on a correlative scanning TEM (STEM) and fluorescence microscopy technique for imaging viable cells of *Magnetospirillum magneticum* strain AMB-1 in liquid phase using an *in situ* fluid cell TEM holder. Our current *in situ* liquid cell electron microscopy effort is focused on establishing the tolerable electron radiation dose for imaging biological materials. The findings obtaining with using magnetotactic bacteria as model system lend themselves naturally to the investigation of many real-world samples. The systems of interest span from probing the interactions of living cells with engineered nanomaterials to imaging and monitoring the biofilm formation and plants.

I will outline the latest development in liquid phase electron microscopy of magnetotactic bacteria *in situ* and discuss the ways to minimize the electron beam damage to the specimen, towards the live cell imaging.

The dual role of MamB in magnetosome membrane assembly and magnetite biomineralization

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Magnetospirillum gryphiswaldense MSR-1 synthesizes membrane-enclosed magnetite (Fe_3O_4) nanoparticles, magnetosomes, for magnetotaxis. Formation of these organelles involves a complex process comprising key steps which are governed by specific magnetosome-associated proteins. MamB, a cation diffusion facilitator (CDF) family member has been implicated in magnetosome-directed iron transport. However, deletion mutagenesis studies revealed that MamB is essential for the formation of magnetosome membrane vesicles, but its precise role remains elusive. In this study, we employed a multi-disciplinary approach to define the role of MamB during magnetosome formation. Using site-directed mutagenesis complemented by structural analyses, fluorescence microscopy and cryo-electron tomography, we show that MamB is most likely an active magnetosome-directed transporter serving two distinct, yet essential functions. First, MamB initiates magnetosome vesicle formation in a transport-independent process, probably by serving as a landmark protein. Second, MamB transport activity is required for magnetite nucleation. Furthermore, by determining the crystal structure of the MamB cytosolic C-terminal domain, we also provide mechanistic insight into transport regulation. Additionally, we present evidence that magnetosome vesicle growth and chain formation are independent of magnetite nucleation and magnetic interactions, respectively. Together, our data provide novel insight into the role of the key bifunctional magnetosome protein MamB, and the early steps of magnetosome formation.

The past, present, and future of genetic analysis in magnetotactic bacteria

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Genetic analysis of magnetotactic bacteria has been a cornerstone of understanding the molecular mechanisms of magnetosome formation. Combined with proteomics and genome sequencing, genetics allowed for the identification of magnetosome gene island in model cultured lab strains. This information was instrumental in identifying magnetosome formation genes in uncultured magnetotactic bacteria, thus providing a unique insight into the evolution of magnetotaxis. In this presentation, I will provide a historical perspective on the methodological advances in using genetics to study magnetotactic bacteria. I will also introduce current work from my group on the complex genetic interactions between the magnetosome island and magnetosome islet genes of *Magnetospirillum magneticum* AMB-1. Finally, I will discuss the future role of genetics in uncovering the mechanistic basis of magnetosome formation in diverse organisms.

An iron-accumulating organelle—the ferrosome—is formed via a small operon in diverse bacteria and archaea

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Like eukaryotic cells, bacteria are highly organized and often contain subcellular membrane-bounded structures, or organelles. Magnetosomes of magnetotactic bacteria (MTB) are the best studied examples of lipid-bounded organelles in bacteria and serve as model systems for studying bacterial cell biology, biomineralization, vesicle formation, and global iron cycling. Magnetosome biogenesis is primarily studied in Alphaproteobacteria *Magnetospirillum* spp., which form cubooctahedral-shaped magnetite crystals within a lipid membrane. However, chemically and structurally distinct magnetic particles have also been found in physiologically and phylogenetically diverse bacteria. *Desulfovibrio magneticus* RS-1 is an anaerobic sulfate-reducing Deltaproteobacterium that forms irregular bullet-shaped magnetite crystals via a conserved magnetosome gene island. In addition to forming magnetosomes, *D. magneticus* forms amorphous iron-containing granules that are enclosed by a lipid membrane—termed "ferrosomes", for "iron body". In order to understand the mechanistic basis of ferrosome formation, I used LC-MS/MS to identify a set of "Fez" proteins that are associated with ferrosomes isolated from *D. magneticus*. Mining microbial genomes shows that "*fez*" genes are arranged in an operon and are common in phylogenetically diverse bacteria and archaea. To test if the *fez* genes are required for ferrosome formation, I used a reverse genetic method we developed for *D. magneticus* to target two *fez* genes for mutagenesis. Importantly, the *fez* mutant is unable to form ferrosomes but still forms magnetosomes. Expression of the *fez* operon in both the *fez* mutant and wild type *D. magneticus* results in constitutive ferrosome formation—a phenotype that does not interfere with magnetosome formation. In addition to *D. magneticus*, non-MTB *Rhodopseudomonas palustris* and *Shewanella putrefaciens* also form ferrosomes. Deletion of the *fez* operon in both *R. palustris* and *S. putrefaciens* abolishes ferrosome formation, a defect that can be rescued by complementation. Furthermore, *Escherichia coli* forms ferrosomes upon heterologous expression of the *S. putrefaciens fez* operon. Overall, these results suggest that the *fez* operon is both essential and sufficient for bacteria to form ferrosomes. Moreover, the reverse genetic method presented here is a crucial step in developing *D. magneticus* as a model for the study of anaerobic sulfate reduction, ferrosome formation, and diverse mechanisms of magnetosome formation by MTB.

Size determination of magnetosome membrane by a protease-mediated switch

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Magnetotactic bacteria produce one or multiple chains of magnetosomes, which are membrane-bound organelles that contain magnetite (Fe_3O_4) and/or greigite (Fe_3S_4). Magnetosome membranes are not uniform in size, and can grow in a biomineralization-dependent manner [1]. However, the underlying mechanisms of magnetosome membrane growth are still unknown. Here, by using cryo-electron tomography, we found that the protease domain of MamE is required for magnetosome membrane growth. Consistent with this finding, the MamE protease activator MamO is also necessary for magnetosome membrane growth. On the other hand, we found that MamN is needed for restricting magnetosome membrane growth before magnetite nucleation. Epistasis analysis showed that MamN functions at the downstream of MamE protease. We are currently testing if MamE can cleave MamN, and also searching for other proteins that may contribute to the magnetosome membrane growth process.

[1] Cornejo E, Subramanian P, Li Z, Jensen GJ, Komeili A (2016). Dynamic Remodeling of the Magnetosome Membrane Is Triggered by the Initiation of Biomineralization. MBio 7(1):e01898-15

Interaction of Essential Magnetosome Genes in Mammalian Cells

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Introduction: The magnetosome is an ideal model for gene-based iron contrast for magnetic resonance imaging (MRI) [1]. To improve the MRI signal provided by single expression systems like MagA or Mms6, *mamI, mamL, mamB* and *mamE* were selected to form a rudimentary magnetosome-like structure in mammalian cells. While *mamI, mamL,* and *mamB* may have a role in designating the magnetosome vesicle [2], they may also provide docking site(s) for additional proteins, like *mamE,* that facilitate biomineralization [3,4]. In addition to improving MR contrast, multiple magnetosome genes may also provide distinct MR signatures, reflecting structure and subcellular location of a magnetosome-like nanoparticle.

Hypothesis: Essential magnetosome proteins colocalize in the intracellular compartment of mammalian cells to form rudimentary magnetosome-like structures.

Methods: MTB genes *mamI, mamL,* and *mamE* were cloned into fluorescent protein vectors (peGFP and ptdTomato) to create Mam fusion proteins. The human MDA-MB-435 melanoma cell line was transfected with each hybrid construct to develop stable expression systems. Synthesis of fluorescent Mam fusion proteins was verified by Western blot. Subcellular location was examined by epifluorescence and confocal microscopy. Magnetosome protein interactions were analyzed *in vivo* and *in vitro* using correlation spectroscopy (XCS).

Results: When expressed alone, eGFP-MamI and eGFP-MamE both displayed intracellular fluorescence in a mammalian cell model, with no apparent localization at the plasma membrane. While expression of tdTomato-MamL was also intracellular, its red fluorescence was punctate and mobile. Co-expression of eGFP-MamI and tdTomato-MamL revealed colocalization of these fusion proteins without interrupting MamL mobility. Analysis by XCS suggests that both tdTomato-MamL and eGFP-MamI are part of supramolecular structures.

Conclusions: Stable overexpression of MamI, MamL, and MamE is compatible with mammalian cell culture. Each of these membrane proteins localizes to the intracellular compartment and co-expression of MamI and MamL indicates a protein-protein interaction.

References:

[1] Goldhawk *et al* (2017) Design and Applications of Nanoparticles in Biomedical Imaging, pp 187-203 **[2]** Nudelman and Zarivach (2014) Front Microbiol 5, 1 **[3]** Quinlan *et al* (2011) Mol Microbiol 80, 1075 **[4]** Uebe and Schuler (2016) Nat Rev Microbiol 14, 621

Enhanced tubulation of liposome containing cardiolipin by MamY protein from *Magnetospirillum magneticum* AMB-1

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MamY protein has been previously identified in *Magnetospirillum magneticum* AMB-1 as the magnetosome protein responsible for magnetosome vesicle formation and stabilization. Furthermore, MamY has been shown *in vitro* liposome tubulation activity through the direct interaction with spherical liposome. In this study, the interaction of MamY and phospholipids was investigated by using a lipids-immobilized membrane strip and a peptide array. The binding of MamY to the anionic phospholipid, cardiolipin, was found and enhanced liposome tubulation efficiency. We propose the interaction is responsible for recruiting and locating cardiolipin to elongate liposome *in vitro*. We also suggest a similar mechanism for the invagination site in magnetosomes vesicle formation, where the lipid itself contributes further to increasing the curvature. These findings are highly important to develop an effective biomimetic synthesis technique of lipid tubules and to elucidate the unique prokaryotic organelle formation in magnetotactic bacteria.

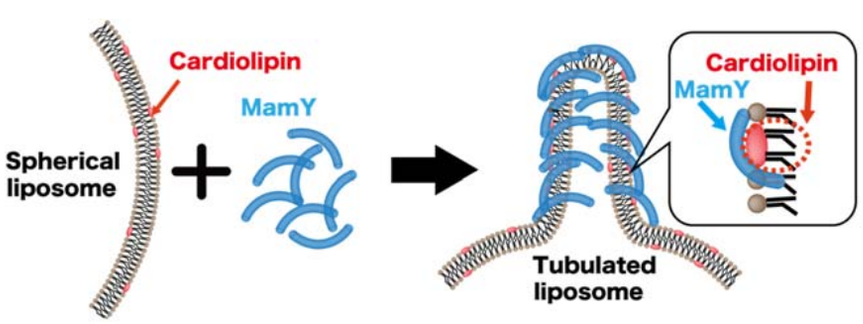

Fig. Schematic illustration of liposome tubulation mechanism by MamY

Increased knowledge of magnetotactic bacteria diversity through the analysis of metagenomic data

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For the last half-century of studying magnetotactic bacteria, our knowledge of their diversity still remains negligible and only about 60 genomes of MTB are known. With the advent of the post-genomic era, the number of information is constantly increasing which mankind is not in time to analyse it. For instance, there are approximately 20 thousand metagenome data in IMG database by the July 2018. This information takes nearly 15 trillion no. of bases. Actually, the vast majority of metagenomic data are not assembled into complete genomes. Therefore, tremendous amount of information has not been processed yet. The question of the MTB presence in open databases is relevant for increasing knowledge of the magnetotactic bacteria diversity.

In the present study 61410 bacterial genomes and 10587 metagenome data from aquatic, terrestrial, host-associated and engineering ecosystems from IMG database were analysed for the presence of magnetotactic bacteria. For the first time, such a large-scale metagenomic survey was used for the analysis of magnetotactic bacteria diversity.

As a marker protein for BLAST analysis was used MamK. As a result, 222 MamK proteins were obtained from 139 metagenomic DNA sequences. The phylogenetic affiliation of bacteria containing *mamK* was determined and new phyla were found. This research greatly increases our knowledge about the diversity of magnetotactic bacteria. Due to collected bacteria database, it becomes possible to reconstruct the ways of evolution of magnetotaxis among magnetotactic bacteria.

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Novel approaches of magnetotactic bacteria investigation revealed new taxonomic groups

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In this research, the diversity of MTB from Lake Beloe Bordukovskoe (Moscow region) was studied. Samples of water and sediment layers were collected from the lake and a microcosm was formed. The diversity of MTB in obtained microcosm was studied with help of a new separation method based on the use of MACS® Columns. This novel method is not limited by speed or motility of MTB and shows great results of MTB enrichment.

Due to the fact that the ability to synthesize magnetosomes is not a taxonomic feature, it is a challenge to identifying MTB by the sequences of 16S rRNA genes. This occures because MAI genes could be transferred horizontally, therefore, inside one taxon there could be found MTB and non-MTB organisms. To avoid this difficulty, the primer system based on *mamK* genes, which is specific for all of MTB, was developed. After cell separation by MACS® columns the amplification, cloning and sequencing of 16S rRNA and *mamK* genes were performed. A total of 576 clones of 16S rRNA and *mamK* genes were obtained and analysed. Phylogenetic analysis of 16S rRNA gene sequences revealed 7 OTUs related to MTB. Among received OTUs the majority was represented by phylum *Nitrospirae*. They had similarity with *Ca.* Magnetominusculus xianensis HCH-1 (99%), *Ca.* Thermomagnetovibrio paiutensis HSMV-1 (94%) and the dominant group had similarity with *Ca.* Magnetobacterium bavaricum TM-1 (92%). Also, it was indicated 1 OTU closely related to Alphaproteobacterium LM-1 (99% 16S rRNA sequence identity) and 1 more - to uncultured *Magnetococcus* sp. clone OTU2 (99%). Moreover, for the first time the putative representatives of the phylum *Acidobacteria* (85% identity with *Luteitalea pratensis* HEG_-6_39) among MTB were discovered. For the correlation of the genomic data with the morphology of MTB FISH-TEM analysis was performed. Furthermore, with dominant *Nitrospirae* group of MTB the whole genome sequencing was performed and genome was assembled. Analysis of the data obtained by the usage of developed primer system based on *mamK* gene revealed the similar results to the 16S rRNA gene sequence.

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Retracing the evolutionary history of magnetotaxis

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Seeking for new diversity and resolving the evolution of magnetotactic organisms is a prerequisite to understand the environmental and biological conditions underlying the emergence, the divergence and the genetic determinism of bacterial behaviors as complex as magnetotaxis. This task has been facilitated lately by the massive amount of data generated by the next generation sequencing technologies, but is still greatly limited by the lack of cultivated organisms. At some point, culture is unavoidable to properly investigate the link between their physiology, morphology and genetic contents, and to elucidate the molecular mechanisms responsible for biomineralization. Such ambition requires the improvement of protocols to separate MTB from complex communities, to purify and grow them in axenic culture. In parallel to metagenomic investigations led by other groups (Lin *et al*., 2018), we are performing such an extensive effort using cultured strains belonging to new bacterial groups within *Proteobacteria* (for example, Monteil *et al*., 2018). By inferring species and genes phylogenetic trees, and comparing genetic contents with phenotypes, we retraced the evolutionary history of magnetotaxis in this group and showed the multiple ancestry of *mam* genes. We provided evidences that the genetic determinants responsible for magnetotaxis are mobile in some genetic lineages, and identified some functions putatively linked to biomineralizing bacteria. We discussed how this approach progressively help to better understand the biology and ecology of these organisms in their ecosystem.

References

- Lin, W., Zhang, W., Zhao, X., Roberts, A.P., Paterson, G.A., Bazylinski, D.A., and Pan, Y. (2018) Genomic expansion of magnetotactic bacteria reveals an early common origin of magnetotaxis with lineage-specific evolution. *ISME J*. 1.
- Monteil, C.L., Perrière, G., Menguy, N., Ginet, N., Alonso, B., Waisbord, N., et al. (2018) Genomic study of a novel magnetotactic *Alphaproteobacteria* uncovers the multiple ancestry of magnetotaxis. *Environ. Microbiol*. 0.

Structural studies of magnetosome-associated proteins

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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 (Fe3O4) or greigite (Fe3S4). Biomineralization of magnetite within these unique prokaryotic organelles involves the formation of the magnetosome membrane, the transport of iron, the iron nucleation and the controlled growth of magnetite via magnetosome-associated proteins (MAPs). To understand the role and mechanism of MAPs, we focused on three different MAPs, MamC, Mms6, and Mms7, that are involved in iron nucleation and affect magnetite size and shape. By using NMR and X-ray crystallography, we studied their individual iron-binding sequences and differentiated their recognition modes based on ion specificity, affinity, and binding residues. The critical residues in the different MAPs were evaluated for their significance through mutation and their effect on magnetite through iron co-precipitation assay. Our results shed light on how different MAPs affect magnetite synthesis and provide new insights on the relation of sequence-structure-function relationships.

Magnetite-binding proteins from *Deltaproteobacteria*: A new route towards anisotropic magnetite nanoparticles?

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Magnetite nanoparticles mineralized by magnetotactic bacteria of the *Deltaproteobacteria* class show anisotropic growth and exhibit morphologies that defy crystallographic rules. Biomineralization of these crystals is genetically controlled, but proteins involved in this nanoparticle formation remain to be identified and characterized. We are investigating several protein candidates that are putatively responsible for controlling the growth of anisotropic magnetite crystals with the goal of understanding the molecular processes determining nanoparticle morphologies.

Here, we introduce one highly conserved protein that is unique to bacteria that mineralize anisotropic magnetite crystals. We recombinantly express this protein in the *E. coli* cytoplasm and purify it *via* affinity chromatography. Using a quartz-crystal microbalance with dissipation, we show that this protein binds to magnetite almost irreversibly. For a more detailed characterization of the protein-magnetite interaction we perform single-molecule force spectroscopy (SMFS), using an atomic force microscope setup. For this purpose, we concentrate on a peptide that constitutes the protein's most conserved region. SMFS shows that the observed strong binding is the result of a slow off-rate combined with a fast on-rate, originating from the high concentration of binding sites on the magnetite surface. The protein is currently tested as an additive in magnetite nanoparticle synthesis to investigate its role in mineralization.

The results provide new insights into the molecular mechanisms that determine biomineralization processes. The magnetite nanoparticles synthesized *via* a green synthetic route can find applications in bio- and nanotechnologies.

Single-cell determination of iron content in magnetotactic bacteria

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Magnetotactic bacteria (MTB) synthesize intracellular magnetite [$Fe(III)_2Fe(II)O_4$] or greigite [$Fe(III)_2Fe(II)S_4$] nanoparticles in a genetically controlled way. MTB have an exceptional capacity of iron assimilation. Assessment of their iron content has only been carried out from electron microscopy observations, mainly because of technical limitations. The number and size of nanoparticles were measured, allowing estimation of the iron mass contained in magnetite. However, recent iron isotope studies have demonstrated that MTB can accumulate a significant iron pool in a reservoir distinct from magnetite. Furthermore, the porosity of magnetite has never been assessed. Direct measurement of iron at the cellular level is thus needed to determine the mass of iron contained in MTB cells. In this study, we present time-resolved inductively coupled plasma – mass spectrometry analyses to determine the mass of iron contained in *Magnetospirillum magneticum* strain AMB-1 cells cultivated with Fe(III)-citrate at different concentrations ($10 \leq [Fe] \leq 500$ μM). Single-cell measurements of 10^5 bacteria were performed in each AMB-1 population corresponding to a specific culture condition. Cellular iron content ranged between ~3 10^{-7} ng/cell ([Fe] = 10 μM) and ~10^{-6} ng/cell ([Fe] = 500 μM). The maximal mass of iron per cell was reached at an iron concentration in the external medium of 200 μM. The variability of cellular iron content in AMB-1 populations also increased at higher iron concentrations in the external medium: the difference between the minimal and the maximal mass of iron ranged between ~5 10^{-7} ng/cell ([Fe] = 10 μM) and 2 10^{-6} ng/cell ([Fe] = 500 μM). Our work has important implications, as it will help to constrain the contribution of MTB to the iron biogeochemical cycle. Single-cell inductively coupled plasma – mass spectrometry will also allow the characterization of iron uptake and iron content in mutant AMB-1 strains to better constrain the function of the genes involved in magnetite biomineralization.

Analysis of the iron uptake in *Magnetospirillum gryphiswaldense*

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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. However, despite its importance for the understanding of iron biomineralization the iron metabolism of MTB is still relatively poorly characterized. 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.*

Deletion of putative iron-responsive regulators caused no effects on magnetite biomineralization, suggesting that magnetite biomineralization is only poorly integrated into cellular iron homeostasis. Interestingly, also the deletion of most iron uptake systems caused no or only minor effects on magnetite biomineralization. A major exception was the feoAB1 deletion which encodes a ferrous iron transport system. The *feoAB1* mutant produced magnetite crystals that were 25% smaller than those of the wildtype. Fluorescence microscopic analyses revealed a cytoplasmic membrane localization although further co-deletion studies suggest that FeoAB1 specifically supplies iron for magnetosome formation but not for the general iron metabolism. Additionally, co-deletion of several iron transport systems revealed two distinct intracellular magnetosomal iron uptake routes and suggests the existence of a third yet unidentified pathway. In conclusion we could show that iron uptake for magnetite biomineralization proceeds through multiple pathways.

Detection of low amounts of iron in the outer membrane of magnetotactic bacteria

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Magnetotactic bacteria take the necessary iron ions to form nanocrystals of magnetite or greigite in the liquid medium where they live. Many advances have been made on the role of specific proteins in the mechanism of nucleation growth and morphology of the nanocrystals [1]. Recently, it has been proposed that a macromolecular structure will act as a "sponge" and will accumulate iron ions before they are transferred to the magnetosome in formation [2]. The present study was carried out using Analytical Scanning Transmission Microscopy (ASTEM) consisting of dark annular dark field imaging (HAADF), analysis of X-ray energy dispersive spectroscopy (EDSX) and electron energy loss spectroscopy (EELS). We applied these methods for the mapping of the iron that passes through the outer shell and membranes of the bacterium and of the magnetosome, to possibly identify specific regions for the entry of iron ions into the cytoplasm. A number of technical difficulties have to be overcome to gain access to an exploitable iron signal with regard to the unfavorable signal-to-noise ratio, linked to the low quantity of iron to be detected, and to the need of the accumulation of numerous observations that must be processed manually. From examples obtained on thin sections of bacteria prepared by high pressure freezing cryo techniques we show that it is possible locally in the outer membranes to extract the iron signal from the high noise information.

[1] Uebe, R. & Schüler, D. Magnetosome biogenesis in magnetotactic bacteria. Nat. Rev. Microbiol. 14, 621-37 (2016)

[2] Jacques Werckmann, Jefferson Cypriano, Christopher T. Lefèvre, Kassiogé Dembelé, Ovidiu Ersen, Dennis A. Bazylinski, Ulysses Lins and Marcos Farina. Scientific Reports | *7: 8291* | DOI:10.1038/s41598-017-08994-9

Reconstruction of magnetosome formation ability in non-magnetic mutant of *Magnetospirillum magneticum* AMB-1

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Magnetosome island (MAI) of *Magnetospirillum magneticum* AMB-1 consists of several operons playing main roles for magnetosome formation. *mamAB* operon contains genes for vesicle formation, magnetosome alignment, iron transport, and crystal nucleation. *mamGFDC* and *mms6* operons encode proteins for magnetite crystal growth involving size and shape controls. In addition, the cells spontaneously lose MAI from the chromosome, and generate a non-magnetic mutant. In this study, we demonstrated reconstruction of magnetosome formation ability in the non-magnetic MAI deletion mutant by introducing replicable plasmid carrying *mamAB*, *mamGFDC* and *mms6* operons. Formation of magnetosomes enveloped within membranous structures in the cells was confirmed by TEM. Genome sequencing of the complemented strain revealed chromosomal integration of the plasmid with magnetosome genes through a transposon-like mechanism. To utilize this gene integration mechanism, magnetotactic bacterial strains containing various sets of magnetosome genes were constructed. The chromosomal integration mechanism is useful to create genetically modified cells producing magnetosomes with regulated crystal morphology and enhanced crystal numbers.

Bioengineering of Magnetic Nanoparticles Produced by *Magnetospirillum magneticum* AMB-1 for Extensive Applications

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Magnetic nanoparticles are currently one of the most important materials with extensive biomedical applications produced in industries. We have developed functional magnetic nanoparticles with a magnetotactic bacterium, *Magnetospirillum magneticum* AMB-1, within a stable lipid bilayer also known as a magnetosome. Since the bioengineering methodology for magnetotactic bacteria was first established, expressions of a wide range of functional proteins onto magnetic nanoparticles have been successfully performed. In these expression, native proteins in the lipid membrane Mms13, which efficiently transport the target to the magnetic particles, can serve as anchors. The expression system—which we refer to as the "magnetosome-display system" here—has enabled us to reduce the cost of production of protein-magnetic particle complexes. To date, various receptors, enzymes, and antibodies, including artificial peptides, have been successfully expressed[1-3]. Here we provide an overview of the developmental status of magnetic nanoparticles for bioassays. Furthermore, we summarize the magnetosome display system including novel expression strategies—"*in vitro* or *in vivo* docking"—in magnetotactic bacteria and discuss their potential applications in the medical and environmental sciences. Having shown considerable promise in improving the expression efficiency of difficult-to-express proteins, this system is expected to advance the development of functional magnetic nanoparticles for use in biotechnology.

1) T. Yoshino et al. "Biosynthesis of Thermoresponsive Magnetic Nanoparticles by Magnetosome Display System" *Bioconjugate Chem.*, 29, 1756 (2018)
2) T. Honda et al. "Stoichiometrically Controlled Immobilization of Multiple Enzymes on Magnetic Nanoparticles by the Magnetosome Display System for Efficient Cellulose Hydrolysis" *Biomacromolecules*, 16, 3863 (2015)
3) T Honda et al. "Functional Expression of Full-Length TrkA in the Prokaryotic Host *Magnetospirillum magneticum* AMB-1 by Using a Magnetosome Display System" *Appl. Environ. Microbiol.*, 81, 1472 (2015)

Study of physiological responses to changing environmental conditions of *Magnetospirillum gryphiswaldense* MSR-1 in flask and bioreactor cultures

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Magnetosomes are nanoscale magnetic organelles with biotechnological applications synthesized by magnetotactic bacteria. Current applications of magnetosomes are hindered by the relatively low cell densities achieved during fermentative production, and poor yields of subsequent recovery and purification operations. Hence, there is a need to explore the factors that affect bacterial growth and magnetosome production in order to develop more robust and efficient bioprocesses. In this work, the development of a simple pH-stat fermentation strategy for the production of *Magnetospirillum gryphiswaldense* MSR-1 and magnetosomes is described. We exploited flow cytometry as a powerful analytical tool enabling rapid monitoring of cell morphology, physiology and polyhydroxyalkanoate production in flask cultures and pH-stat bioreactor cultures. The flow cytometry methodology was employed and optimized in flask cultures. The pH-stat growth strategy was developed by varying the concentrations of the carbon source (lactic acid) and the alternative electron acceptor (sodium nitrate) in the feed. Growth conditions were optimized with respect to basis of biomass concentration, cellular magnetism (indicative of magnetosome production), and intracellular iron concentration. The highest biomass concentration and cellular iron content achieved were respectively an optical density at 565 nm of 15.5 (equivalent to 4.2 g DCW·L^{-1}) and 33.1 mg iron·g^{-1} DCW. This study demonstrates the importance of analyzing bacterial physiology during fermentation development, and will potentially aid the industrial production of magnetosomes, which can be used in a wide range of biotechnology and healthcare applications.

Magnetotactic coccus from ferruginous Lake Pavin: a new model for intracellular sequestration of phosphorus

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Microorganisms have been shown to be major actors in modern and past geochemical cycles of phosphorus (P) either as reservoirs and/or catalysts of processes exchanging P between different reservoirs [1,2]. The recognition of their role has led to the design of bioremediation processes such as those for the treatment of P-rich wastewater [3] or long-term sequestration of heavy metals such as U by P mineral precipitation [4]. However, the extent to which they control the geochemical cycle of P over abiotic processes remains poorly determined.

Few microbial models highly sequestering P are known (e.g. marine sulfoxidizers [5]), most of which have been identified at water-sediment interfaces in oxic-anoxic transition zones (OATZ). Magnetotactic bacteria (MTB) may be of particular interest in this context: most of them are microaerophilic, they can be easily sorted by a magnetic field, and large P-rich inclusions were previously described in MTB from environmental samples [6-8].

By combining correlative x-ray, electron and light microscopies and spectroscopies, we recently evidenced that MTB of the *Magnetococcaceae* family strongly accumulate intracellular polyphosphates at the OATZ located within the water column of the ferruginous Lake Pavin. MTB cells appear as P hotspots in the >0.2 µm particulate fraction at this depth [9]. This high accumulation may be characteristic of this family and may also relate to the specific chemical conditions prevailing at this depth in the lake. As a result, these cocci can be considered as new models playing a potentially important role in the P geochemical cycle, similar to sulfur oxidising bacteria such as *Thiomargarita* and *Beggiatoa* but thriving in a ferruginous, poorly sulphidic environment. We will present here their identification in the water column of Lake Pavin and current project aiming to delineate the relative importance of biological from environmental parameters on P-hyperaccumulation by MTB.

[1] Cosmidis et al. (2013) *Chem. Geol.* 359, 10. [2] Diaz et al. (2008) *Science* 320, 652. [3] Tarayre et al. (2016) *Sensors*. [4] Newsome et al. (2014) *Chem. Geol.* 363, 164. [5] Schulz & Schulz (2005) *Science* 307, 416. [6] Oestreicher et al. (2011) *Microsc. Microanal.* 17(2), 140. [7] Cox et al. (2002) *Geomicrobiol. J.* 19, 387. [8] Chen et al. (2015) *J. Nanomaterials* 902121. [9] Rivas-Lamelo et al. (2017) *Geochem. Persp. Let.* 5, 35.

Diversity of microbial metalloid redox transformation pathways associated with the contaminated environment

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Arsenic (As) and antimony (Sb) are both naturally occurring toxic elements and are considered to be priority pollutants of interest by the USEPA. Although the concentrations of these toxic metalloids in natural systems are generally low, the elevated levels of As and Sb have been released via natural processes and human activities. Arsenic and antimony mainly exist in two oxidation states, trivalent (III) and pentavalent (V), in natural systems. The trivalent forms, As(III) and Sb(III), are highly reactive with thiol-containing proteins and are considered more toxic to biota than As(V) and Sb(V). Despite their toxicity, microorganisms have developed mechanisms to tolerate and catalyze redox transformation of As and Sb. In this study, we characterized microbial metalloid redox transformation pathways associated with As and Sb-impacted environments using combined geochemical, physiological and molecular biological approaches. Soils from an old stibnite mine tailing area in Ehime prefecture, Japan, were characterized geochemically. Total concentrations of Sb and As were higher in the surface soil (0-3 cm: 2280 and 1240 mg kg^{-1}, respectively) and decreased with depth (9-12 cm: 330 and 130 mg^{-1} kg). After the enrichment culturing, pure cultures of Sb(III)-oxidizing *Pseudomonas*- and *Stenotrophomonas*-related isolates, along with As(III)-oxidizing *Sinorhizobium* strain were obtained. Furthermore, *Mesorhizobium*-related isolate capable of anaerobic and aerobic Sb(III) oxidation was also obtained. Interestingly, these Sb(III)-oxidizing isolates did not exhibit As(III) oxidation activity, suggesting the involvement of different mechanisms for Sb and As oxidation. Anaerobic enrichment cultures capable of reducing Sb(V) were also obtained, in which the precipitation of antimonite as antimony trioxide was observed. These resutls demonstrate that indigenous microorganisms associated with stibnite mine soils are capable of Sb and As transformations, indicating the potential importance of biological processes in regulating mobility and toxicity of toxic metalloids in contaminated environment.

A cooperative flagellar movement explains *Magnetococcus marinus* swimming speed and movement

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Bacteria propel and change direction by rotating long, helical filaments, called flagella. The number of flagella, their arrangement on the cell body and their sense of rotation determine the locomotion characteristics of a species as shown for several model organisms at moderate swimming and reorientation speeds. However, the movements of microorganisms at the upper speed limit remain poorly understood since the speed of the involved processes is at the limit of classical observation techniques.

In the world of magnetotactic bacteria, *Magnetococcus marinus* MC-1 is one of such extremely rapid microorganism. The specie is characterized by two flagella bundles on one pole, and its swimming speed indeed exceeds 400 μm s^{-1} or more than 200 body lengths per second, making them one of the fastest natural microswimmers. Using 3D tracking at high frame rates, we show that cells reorient an order of magnitude faster than reported so far for any other bacteria.

Finally, we observe a double helical swimming track with high-speed dark-field microscopy. Combining these experimental results with hydrodynamic modeling, we explain the reported characteristics of the swimming by a so-far ignored flagellar movement. We anticipate this movement will find application for future microswimmer designs.

Modelling magnetotactic bacteria

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Magnetotactic bacteria provide ample opportunity for modelling studies, from the interplay of magnetic forces with biological processes in the cytoplasm to the magneto-aerotactic motion. The talk will give an overview on our recent theoretical work. One focus will be on the physical properties of magnetosome chains: the self-organization of magnetic particles with and without binding to a cytoskeletal filament; and the bending stiffness of a chain of magnetic particles, which provides an important functional constraint for the design of the magnetotactic apparatus. We may also discuss how structural properties of magnetosome chains can be studied by combining FMR (ferromagnetic resonance) spectroscopy with theoretical modelling.

In addition, we will address different modelling approaches to the swimming of magnetotactic bacteria and to magneto-aerotaxis.

Influence of Magnetic Fields on Chemotaxis in Magnetotactic Bacteria

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Chemotaxis refers to the bacterial ability to bias their motion to reach the preferred concentration of a molecular species. In particular, aerotaxis is the ability of sensing external oxygen gradients and reaching the optimal oxygen level. Magnetotactic bacteria couple aerotaxis to the interaction with external magnetic fields, which supposedly results in an advantage compared to non-magnetic cells. Here, we quantify this advantage from a microscopic point of view, considering the single cell behavior as well as the collective motion in the band formation assay. We propose simulations based on a modified Active Brownian Particle model, which describes active swimming, active changes of directions (tumbles or reversals), chemotaxis/aerotaxis (axial or polar), and passive alignment with external magnetic fields. Through comparison with experiments, the model sheds light on the modes of aerotaxis seen in magnetotactic bacteria. We explore in detail the axial and polar strategies in the capillary experiments, where axial bacteria robustly form a band with any magnetic field configuration, while a strong field-orientation dependency is observed for polar bacteria. As main results, we show that the coupling to magnetic fields imposes a constraint on which change-of-direction strategy the magnetotactic bacteria can employ: run-and-tumble compromises chemotaxis when magnetic fields are presents, while run-and-reverse enhances the chemotactic velocity towards the preferred concentration, thus providing a fitness advantage, for inclinations of the magnetic field up to 60˚ with respect to the chemical gradient. We also show that chemotaxis remains functional even at inclination of 90˚ as it is the case for magnetotactic bacteria at the Earth's Equator.

Magnetic response of *Magnetospirillum gryphiswaldense* observed inside a microfluidic channel

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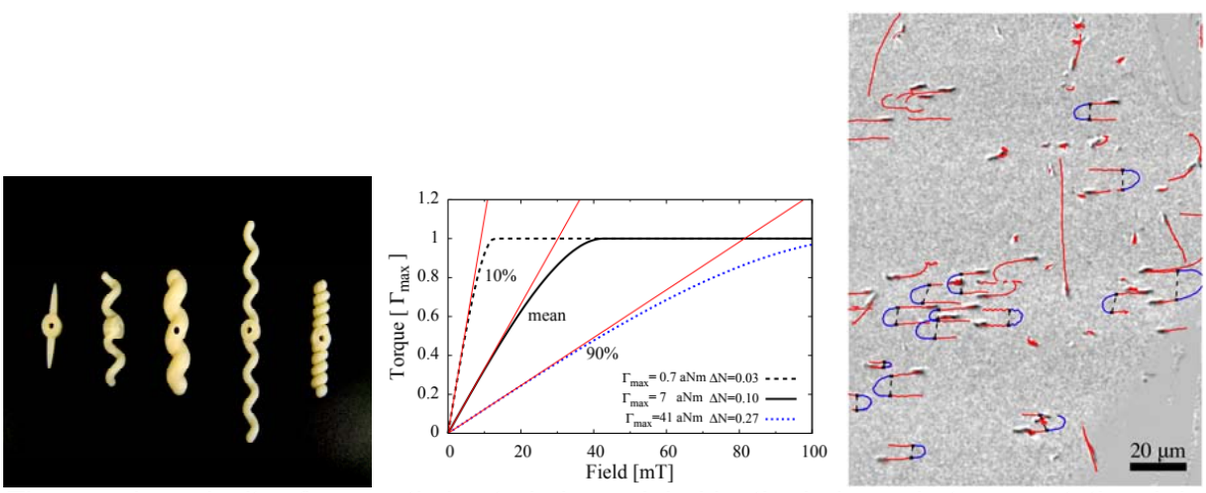
We modelled and measured the U-turn trajectories of individual magnetotactic bacteria under the application of rotating magnetic fields, ranging in amplitude from 1 to 12 mT. The model is based on the balance between rotational drag and magnetic torque. The magnetic model takes saturation into account and the rotational drag was measured on macroscale 3D printed models. For accurate verification of this model, bacteria were observed inside 5 µm tall microfluidic channels, so that they remained in focus during the entire trajectory. From the analysis of hundreds of trajectories and accurate measurements of bacteria and magnetosome chain dimensions, we confirmed that the model is correct within measurement error. The resulting average rate of rotation of MSR-1 is 0.74±0.03 rad/mTs.



The angular velocity of magnetic bacteria is modeled by the balance between rotational drag (left) and magnetic torque (center) and is observed inside a microfluidic chip (right).

[1] M.Pichel, T. Hageman, I. Khalil, A Manz, L Abelmann "Magnetic response of *Magnetospirillum gryphiswaldense* observed inside a microfluidic channel" *Journ. Magn. Magn. Mat.* (2018) 460, p340.

On the magnetosomes chain configuration

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Magnetospirillum gryphiswaldense contains a variable number of magnetosomes arranged in a chain. Since magnetosomes are single magnetic domains, the chain behaves like a large single permanent magnetic dipole able to passively orient the whole bacteria along external magnetic field lines. Rather than straight lines, magnetosome chains are slightly bent, as evidenced by electron cryotomography (ECT). The present work is devoted to shed light on the underlying mechanisms that determine the arrangement of the magnetosomes and consequently the geometry of the chain. For that reason, we have explored the direction of the magnetic moment using state-of-the-art techniques carried out on a set of different bacterial arrangements: i) small angle neutron/x-ray scattering (SANS/SAXS) on a bacterial colloid, ii) macroscopic magnetometry on 3D and 2D fixed arrangements of randomly distributed and aligned bacteria, and iii) x-ray photoemission electron microscopy (XPEEM) on an individual chain of magnetosomes extracted from bacteria. Our experimental and theoretical findings[1] indicate that the effective magnetic moment of individual magnetosomes is tilted out of the chain axis ([111] crystallographic easy axis of magnetite) about 20º. This tilt does not affect the direction of the chain net magnetic moment, which remains along the chain axis, but turns out to be the key to understand the arrangement of magnetosomes in helical-shaped chains. In fact, by considering a interplay between the magnetic dipolar interactions between magnetosomes and a lipid/protein-based mechanism, modelled as an elastic recovery force exerted on the magnetosomes, we were able to reproduce the experimental chain geometry imaged by ECT, see figure 1.

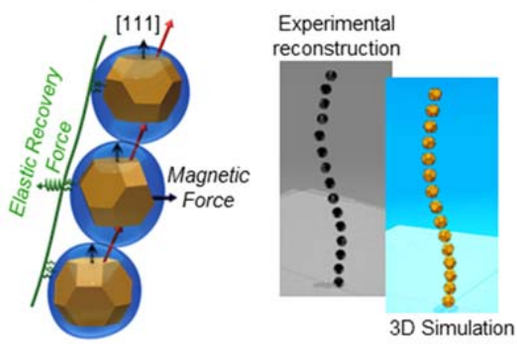

Fig.1: Left) Schematic representation of the force involved in construction of the chain; Right) Experimental reconstruction from ECT images and 3D simulation of the chain

1. I. Orue *et al*; Nanoscale **10** (2018) 7407, DOI: 10.1039/c7nr08493e

Quantitative magnetic analysis of magnetotactic bacteria by means of ferromagnetic resonance spectroscopy

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Magnetotactic bacteria (MTB) produce magnetosomes in linear assemblies, thus producing a permanent magnetic dipole which aligns with the Earth's magnetic field and enables the navigation of the MTB towards favorable habitats (magnetotaxis). The efficiency of magnetotaxis depends on the properties of the permanent dipole moment, and this in turn depends on the configuration of magnetosomes in the chain, which is genetically driven and varies from strain to strain. I will present a robust method for the investigation of the configuration of magnetosomes in MTB by a precise and quantitative measurement of the magnetic anisotropy in MTB based on ferromagnetic resonance (FMR) spectroscopy and modeling. By fitting FMR spectra of MTB, we are able to determine the anisotropy fields in MTB in absolute units, and with that we can extract valuable information about the configuration of magnetosomes in the chain [1,2]. This method is a magnetic tool to identify different cultured MTB strains and MTB in natural systems and it can further be used to detect magnetofossils in geological deposits.

[1] M. Charilaou, M. Winklhofer, A. U. Gehring: Simulation of ferromagnetic resonance spectra of linear chains of magnetite nanocrystals. *J. Appl. Phys*. 109, 093903 (2011)

[2] M. Charilaou: Ferromagnetic resonance of biogenic nanoparticle-chains. *J. Appl. Phys*. 122, 063903 (2017)

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Numerical calculations of the effect of magnetic interactions on magnetotaxis efficiency

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We present numerical micromagnetic models that examine the effect of magnetic interactions on the magnetic structures found in magnetosomes, and their contribution to magnetotaxis efficiency. The critical size for stable single domain (SD) behaviour has been calculated as a function of grain elongation for magnetite grains using a numerical micromagnetic algorithm. Importantly we consider the contribution of inter-grain magnetostatic interactions on the SD/multidomain (MD) critical size (d_0). For individual grains our numerical estimates for d_0 for elongated grains are lower than that determined by previous analytical and numerical calculations. Nevertheless, the inclusion of magnetostatic interactions into the model was found to increase d_0 to values significantly higher than any previously published estimates of d0 for individual grains. Therefore, the model calculations show that there is a relatively wide range of grain sizes within which depending on the degree of magnetostatic interactions and elongation, a grain can be either SD or MD. The model results are compared to observations of magnetosomes found in magnetotactic bacteria. The newly calculated upper d_0 limit for the interacting grains now accommodates the largest magnetosomes reported in the literature. These large magnetosomes were previously thought to be MD, suggesting that evolutionary processes are highly efficient at optimizing magnetosome grain-size and spatial distribution.
We have also calculated the effect of magnetic interactions on the blocking volume for SD magnetic states. The inclusion of magnetic interactions was found to decrease the blocking volume, increasing the range of stable SD behaviour. Overall it is seen that magnetic interactions significantly increase the stable SD range. It is argued that chains of interacting magnetosomes found in magnetotactic bacteria have utilized this effect to improve magnetotaxis.

Magnetic study of Co-doped magnetosome chains

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Magnetotactic bacteria impose a high genetic control over the synthesis of magnetosomes. This implies that the size, shape and composition of the magnetosomes are species-specific. Despite the genetic limitations, the structural and magnetic properties of magnetosomes can be slightly tuned by adding different metals to the growth medium [1,2]. In this work, *Magnetospirillum gryphiswaldense* MSR-1 has been grown in a Co-rich medium aimed to produce Co-doped magnetosome chains, a model system where to study basic magnetism.

We have used a range of complementary techniques to determine the structure and magnetic properties of the Co-doped magnetosome chains and compare them with their undoped counterparts. By means of energy-dispersive x-ray spectroscopy (EDS), x-ray absorption near edge structure (XANES), and x-ray magnetic circular dichroism (XMCD), we have determined the incorporation of 1 at.% Co in the magnetite crystal structure in substitution of Fe^{2+} ions located in octahedral sites. Despite the low Co incorporation achieved, DC magnetic measurements reveal significant changes in coercivity and remanence over a temperature range from 300 K to 5 K. In the framework of the Stoner–Wohlfarth model, we have analyzed the evolution of the hysteresis loops, identifying the different magnetic anisotropy contributions and their evolution with temperature. We find that, in contrast with the control, whose effective anisotropy is uniaxial in the whole temperature range from 300 K to 5 K, the effective anisotropy of Co-doped magnetosome chains changes appreciably with temperature, from uniaxial down to 150 K, through biaxial down to 100 K, to triaxial below 100 K [3].

[1] S. Staniland *et al*., *Nat. Nanotech*. **3**, 158 (2008)
[2] M. Tanaka *et al*., *J. Mater. Chem*. **22**, 11919 (2012)
[3] L. Marcano *et al*., *J. Phys. Chem*. C **122**, 7541 (2018)

Scaling up the Kalmijn-Blakemore Pulse Remagnetization Experiment from Magnetotactic Bacteria to the Human Head

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Shortly after Blakemore's (1975) publication of the magnetotactic bacteria, and the TEM observation of iron-rich particles later identified as magnetite, Kalmijn and Blakemore (1978) reported the results of an intriguing experiment on them using a small capacitive – inductor oscillator (LC) circuit. This was configured with a diode to prevent oscillations, and to generate only one precisely configured magnetic pulse. It was designed to test the prediction that the magnetite crystals were single magnetic domains, for which strong but brief magnetic pulses are able to flip the direction of the magnetic moment of the particles relative to the minimum anisotropy direction of the crystals. The pulse produces a permanent flip in magnetization direction, the same way information is coded on magnetic tape. Strong pulses (up to ~ 100 mT), if aligned antiparallel to the local magnetic field guiding the bacteria, did indeed cause them to passively roll over and migrate to the South, rather than the North-seeking preference in those populations. Coupled with subsequent biophysical analyses showing the quantity of magnetite present was enough to align them in the geomagnetic field relative to thermal disruption (Frankel et al., 1980), the pulse experiment is now taken as definitive evidence for the role of ferromagnetic materials in the magnetotactic response of living organisms. The pulse technique has even been used to select populations of magnetotactic bacteria for higher coercivity, over several generations (Diaz-Ricci & Kirschvink, 1991).

Observations of geomagnetic sensitivity by migratory and homing animals have puzzled biophysicists for over 70 years. Widely dismissed as biophysically implausible due to the lack of physiological ferromagnetic materials [Griffin, 1945], clear and reproducible responses to earth-strength magnetic fields is now firmly established in Eukaryotes ranging from Protists through Animals from numerous phyla, including Mollusks, Arthropods, and the Chordates. Behavior demands sensory transduction, as external stimuli only 'get into the nervous system' through sensory cells specialized to transduce the physical stimulus into a modulated stream of action potentials in neurons.

Three basic physical mechanisms could plausibly explain the transduction of geomagnetic cues, including electrical induction, hyperfine magnetic field effects on photo-activated free radicals (the 'Quantum Compass'), or receptor cells containing ferromagnetic crystals. The definitive test of a ferromagnetic receptor is a modification of this classic bacterial pulse-remagnetization experiment, in which the pulse is slowed down to prevent direct stimulation of sensory nerve endings but is rapid enough to prevent the magnetite crystals from physically rotating in phase with the field as it is ramping up. A brief, uni-directional magnetic pulse of about 0.5 mS in duration is adequate for this. There are now over 16 peer-reviewed papers in which this experiment has been applied to animals, all of which show clear and long-lasting effects of the pulse. Such a pulse would have no lasting effect on a quantum compass or from electrically-induced artifacts. Initial experiments ramping up the magnetic pulse intensity to 70 mT on a Human Participant converted a CCW-downwards brainwave response into a CW-upwards response, indicating that the transduction mechanism in humans is indeed ferromagnetic, and that the coercivity of the crystals involved is < 70 mT. Further experiments are planned to refine this estimate.

Iron isotope perspectives for magnetotactic bacteria identification in the geological record

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Magnetotactic bacteria (MTB) may represent some of the oldest microbial organisms on Earth, as suggested by genomic and phylogenetic analyses and putative MTB fossils (magnetofossils) found in ancient sedimentary rocks. Magnetofossils identification was proposed based essentially on morphological, crystallographic and magnetic criteria. However, magnetite properties can be lost during sediment burial and metamorphism and cannot be firmly used to demonstrate or refute a possible biological origin of magnetite. In contrast, geochemical signatures, such as Fe isotope composition, could potentially survive crystal aging and be used as a MTB tracer in the geological record. We characterized Fe isotope fractionation associated with magnetite biomineralization in the strain AMB-1. Magnetite was depleted in heavy Fe isotopes by 1.5 to 2.5‰ (δ^{56}Fe) relative to the growth medium. These light Fe isotope signatures may represent a specific feature of MTB, since abiotic precipitation of magnetite from aqueous Fe(II) produces a fractionation in the opposite direction. More importantly, we observed for the first time a deviation from mass dependent relationships, affecting specifically the odd isotope (^{57}Fe) but not the even isotopes (^{54}Fe, ^{56}Fe, ^{58}Fe) (Amor et al., 2016). These preliminary results are very promising but the experiments were performed only with a single bacterial strain, and no measurements were performed on natural samples. Moreover, our results differ from those of a pioneer work (Mandernack et al., 1999), where no Fe isotope fractionation was observed during biomineralization in the strains MS-1 and MV-1. The origin of this discrepancy may result from differences between the strains and/or culture conditions. Thus Fe isotope fractionation by MTB needs to be investigated further in well-controlled laboratory cultures and its potential applicability must now be tested in natural environments.

References: Amor M. et al. (2016) *Science* 352, 705-708 ; Mandernack et al. (1999) *Science* 285, 1892-1896.

Micromagnetic simulation of magnetofossils with realistic size and shape distributions: Linking rock magnetism with microscopic observations and implications for magnetofossil identification

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We build three-dimensional micromagnetic models to investigate the magnetic properties of biogenic magnetite – a common type of magnetic mineral that is responsible for recording a wide range of biological, geophysical and geological processes on earth. The particle geometry in our model is based on realistic size and shape distributions from direct microscopic observations. Systematic changes in biogenic magnetite chain microstructures are built and their magnetic properties are calculated. Our modelling results indicate large variations in magnetic properties with wide distributions of coercivity (Bc = ~10-60 mT), coercivity of remanence (Bcr = ~14-81 mT), dispersion parameter (DP = ~0.1-0.5), and skewness values (S = ~0.7-1.1), although the same particle size and shape distributions are used in all calculations. Previously, the commonly observed “biogenic soft” and “biogenic hard” components in natural samples are thought to reflect crystal morphologies of individual biogenic magnetite crystals, and that the small DP values of coercivity distributions observed on whole-cell magnetotactic bacteria and magnetofossil-bearing samples are an indication of narrow particle size distributions. Our simulations suggest that these speculations are not always the case and that microstructures of magnetosome chains exert dominant control over their magnetic properties. Our micromagnetic modelling results provide new theoretical perspective on the magnetic properties of biogenic magnetite, which is important for understanding magnetic proxy signals from magnetofossils in a wide range of geological and environmental settings, and for the search for biogenic magnetite in terrestrial rocks and in extra-terrestrial materials.

Widespread occurrence of magnetofossils in the geological record and implications for living environments of magnetotactic bacteria

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While magnetotactic bacteria (MTB) occur widely in aquatic environments and their post-mortem remains have been speculated to be important carriers of paleomagnetic signals in sedimentary rocks, only a small handful of pre-Quaternary magnetofossil occurrences were reported before 2008. Widespread use of diagnostic methods for magnetofossil identification has fundamentally changed this situation in recent years and magnetofossils are now considered to be abundant in the geological record. This raises other questions about the living habitats of MTB. It is emphasized in the literature that MTB are gradient organisms that thrive around chemical redox gradients. However, such environments are not conducive to geological preservation of magnetite magnetofossils because magnetite dissolves under sulphidic conditions, so when it is buried below the redox gradient in which the MTB thrived, biogenic magnetite will dissolve completely. With such poor preservation potential, why are magnetite magnetofossils preserved so widely in the geological record? Recent studies indicate that extensive magnetofossil preservation has occurred in sediments that remained oxic throughout their long geological history. This suggests that redox gradients cannot be important for the MTB species whose magnetofossils are preserved geologically. Widespread geological preservation of magnetofossils suggests that an adjustment is needed in representing the living environments of MTB even though MTB may generally be gradient organisms. Also, in contrast to the fate of biogenic magnetite during burial below the redox gradients in which the respective MTB lived, the greigite produced by greigite-mineralizing MTB should be preserved. Few records of geologically preserved greigite magnetosomes exist, which is a future frontier for understanding geological records produced by MTB.

Pylogenetic and morphological identification of magnetotactic cocci using coupled FISH-SEM method

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Magnetotactic bacteria (MTB) are morphologically and phylogenetically diverse prokaryotes. They can form intracellular chain-structured nanocrystals of magnetite (Fe_3O_4) or greigite (Fe_3S_4) each surrounded by a lipid bilayer membrane called a magnetosome. Magnetotactic cocci have been found to be the most abundant morphotypes of MTB in various environments. However, knowledge on magnetosome biomineralization within magnetotactic cocci remains elusive due to small number of strains that have been cultured. In this paper, we phylogenetically and structurally identified 12 strains of magnetotactic cocci from freshwater, brackish and marine environments in China by using a coordinated fluorescence and scanning electron microscopy method (so-called coupled FISH-SEM) at the single-cell level. Phylogenetic analyses besed on 16S rRNA gene sequences revealed that most represents novel MTB species of the *Alphaproteobacteria*. The cellular sizes and the physical properties of magnetosomes such as the chain configuration, the crystal number, morphology and size within these 12 MTB strains were systematically studied. The experimental results provide evidence for the relationship between magnetosome biomineralization and MTB phylogenies, but also lay a solid foundation for further studying the process and mechanisms of magnetosome biomineralization within uncultured magnetotactic cocci.

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Spatio-temporal distribution of magnetotactic bacteria in freshwater sediments

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Magnetotactic bacteria (MTB) synthesize membrane-enveloped ferrimagnetic crystals that contribute to the remanent magnetization in sediments, yet knowledge of how different morphotypes of MTB populations vary in sediments over long-periods of time remains limited. MTB bearing sediments were taken from a freshwater pond near Munich, Germany and put into two aquaria. We counted three dominant MTB morphotypes at 36 discrete points in replicate aquaria every ~30 days over 198 days. We found that the spatial distribution of population centres changed over time and that the two most abundant morphotypes (cocci and spirilla) occupied distinctly different niches displaying significant anti-correlation.

To investigate the seasonal variation of MTB in a natural environment, we report abundances of three MTB morphotypes (cocci, spirilla and rods) from nine sites collected and measured monthly over a two-year period from the uppermost 1 cm sediments of the pond mentioned above. Morphotype populations underwent coherent temporal trends among the nine sites—especially at proximal sites with similar water depths. MTB abundances varied independently of bottom water oxygen concentrations or temperature over the observation period, except for magnetotactic spirilla, which flourished during the summer at deeper-water area. Magnetic properties of the uppermost 1 cm sediments did not reflect living MTB populations in 2016, but instead varied with water depth. Deeper sites, which were also lower in total organic carbon, total nitrogen, and bottom water oxygen concentrations than shallower sites, had higher saturation magnetizations and were richer in single-domain magnetic particles.

Diversity of Uncultured *Magnetospirillum* sp. from the Sedimentary Basin of Southern India

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Studies on the geographic distribution of Magnetotactic bacteria (MTB) revealed the ubiquitous presence in diverse habitats on all continents. However, little is known about MTB inhabiting in the Indian coastal ecosystem. Here we investigate the diversity of *Magnetospirillum* sp. from the iron mineral sediments deposit of the South Kerala sedimentary basin, India using culture independent methods. The collected sediment samples were analyzed for the presence of nitrate (zinc reduction), sulfide (cline method), Fe^{2+}, total iron (ferrozine assay) and iron minerals (XRD analysis). Based on the geochemical measurements the sediment possesses major factors such as nutrients, pH, temperature and chemical gradients in metabolic accessible form for MTB. The cubo – octahedral crystals of the magnetosome are also evident from the TEM micrographs of magnetically enriched sediment. CARD-FISH analysis showed the presence of *Magnetospirillum* in all the six samples analyzed. Phylogenetic analysis based on 16S rRNA gene library showed the clones belong the class Alphaproteobacteria and members of the genus *Magnetospirillum*. The results of the metagenomic study are consistent with CARD-FISH analysis and the identified uncultured *Magnetospirillum* were morphologically and phylogenetically similar to isolates from diverse habitat. *The identification of Magnetospirillum from* Indian coast supports the hypothesis of wide geographic distribution of the bacteria.

Seasonal changes in abundance of two multicellular magnetotactic prokaryotes in sediment layers of Lake Yuehu

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There are two genetically distinct morphological types of multicellular magnetotactic prokaryotes (MMPs), ellipsoidal MMPs (eMMPs) and spherical MMPs (sMMPs). Both of them are gram-negative cell aggregates and show synchronized cell division and complex movements under the microscope. To understand the ecological behaviour of the uncultivated organisms, we carried on a research on the vertical distribution of eMMPs and sMMPs in sediment of Lake Yuehu, China. We sampled at least once a month from October, 2014 to October, 2015. The distribution depths of both types of MMPs ranged from 1 to 34 cm, depending on the seasons. The eMMPs were observed at depths of 2-34 cm during spring, 1-11 cm during summer, 2-21 cm during autumn, and 9-32 cm during winter. *Candidatus* Magnetananas rongchenensis with magnetite magnetosomes dominated among eMMP species at all depths. These results suggested that *Ca*. M. rongchenensis migrated vertically during four seasons. During spring, sMMPs were present at depths of 3 to 27 cm; during summer, 1 to 18 cm; during autumn, 1 to 21 cm; and during winter, 10 to 30 cm. The dominant species of sMMPs varied during four seasons. These results suggested that there were seasonal species replacement in sMMP population. The vertical distribution of redox in Lake Yuehu changed seasonally. The changes coincided with the seasonal distribution of MMPs, suggesting that redox affected the vertical distribution of MMPs. The microcosm experiments in lab suggested that the original redox gradient of sediment was vital to MMPs' survival. Both types of MMPs preferred low concentration of inorganic nutrients. In addition, high concentrations of ammonium and silicate might interfere with the vertical migration of MMPs. Therefore, the seasonal vertical distribution of MMPs was related to the seasonal variation of environmental factors, which represented the unique adaption of MMPs.

Description of the first Nitrospira phylum magnetotactic bacteria isolated from ocean

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Magnetotactic bacteria (MTB) can synthesize magnetosomes in order to swim along the magnetic field lines. MTB display various morphologies and great taxonomic diversity. Unlike large number of MTB affiliated in Proteobacteria phylum, only eleven MTB species belonging to phylum Nitrospirae were discovered. All of which were found in freshwater or low salinity aquatic environment. In this work, we report a new member of the phylum Nitrospirae group, isolated from the Mediterranean sea and named as *Canidatus* Nitrospirae marinus clone MBB-1.

Morphologically, MBB-1 is an ovoid-shaped bacterium with average length of 5.0 μm and width of 4.1 μm. The cells were surrounded by small particles at the outer layer and emitted autofluorescence when illuminated with various wavelengths. They exhibited a north-seeking magnetotaxis with an average velocity of 148.9±28.0 μm/s. Phylogenetically, the 16S rRNA gene sequences of MBB-1 is affiliated to the phylum *Nitrospirae* sharing 93% and 94% identities with the two closest relatives of LO-1 and MWB-1, respectively.

To inspect magnetosome biomineralization in a defined species, correlative FISH-SEM analysis was conducted firstly by identified the MTB cells with FISH followed with observation of magnetosomes within the same bacteria with SEM. As a result, five to ten bundles of magnetosome chains were found aligning in parallel to the long axis of the MBB-1 cells. STEM-HAADF analysis revealed the magnetite nature of magnetosome.

This is the first marine Nitrospirae species of MTB that has been reported, which expands the diversity and wide distribution of Nitrospirae MTB.

Diversity of Magnetotactic Bacteria in a Coastal Lagoon Complex in Rio de Janeiro State, Brazil

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Magnetotactic bacteria (MTB) are a diverse group of prokaryotes that biomineralize magnetic crystals by a genetically controlled process. For almost 40 years, these organisms have been collected and studied from different habitats worldwide. Although several cultured and uncultured species have been described, the true metabolic and phylogenetic diversity of this group of bacteria is still considered underestimated. Furthermore, many environmental parameters related to phylogenetic diversity of MTB are not well understood although salinity and magnetic field strength have been shown to influence MTB diversity in the Northern Hemisphere (Lin et al., 2012; Lin et al., 2013).

In this work, we examined the phylogenetic diversity of MTB in 11 lagoons of the Jurubatiba Restinga National Park (Rio de Janeiro, Brazil). Each lagoon had a specific physical-chemical composition; varying in salinity, pH, and O_2 concentration ([O_2]) profile in the water column and sediment. We collected sediment and water periodically for the past four years. MTB were magnetically concentrated from samples from each lagoon and used to determine MTB morphotypes by light microscopy, to characterize magnetosomes and their organization within cells by transmission electron microscopy and to determine the phylogenetic diversity of MTB based on 16S rRNA gene sequences. Physical-chemical analysis of water was done *in situ* (pH, salinity, temperature) or in the laboratory just after sampling ([O_2] profile, carbon, phosphorus, nitrogen, iron). Preliminary data showed the presence of MTB in 7 of the 11 lagoons which included fresh-, saline and brackish water environments. Interestingly, the highest diversity of species was found in the Comprida Lagoon, which is an acidic freshwater lagoon. Four different morphotypes of MTB were identified in this lagoon including spirilla, cocci, rods and vibrios. The Piripiri II Lagoon is a non-permanent, brackish-saline lagoon in which three different morphotypes of MTB including spirilla, cocci and multicellular forms, were occasionally observed. Phylogenetic analysis of these and other MTB from these lagoons and correlation between the presence of different, specific MTB with environmental factors in the lagoons are in progress.

References:

Lin, W., Wang, Y., Li, B., & Pan, Y. (2012). A biogeographic distribution of magnetotactic bacteria influenced by salinity. *The ISME Journal*, *6*(2), 475.

Lin, W., Wang, Y., Gorby, Y., Nealson, K., & Pan, Y. (2013). Integrating niche-based process and spatial process in biogeography of magnetotactic bacteria. *Scientific Reports*, *3*, 1643.

Biological Synthesis and Structural Developments in Ultrahard Teeth of Chiton

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There is an increasing need for the development of multifunctional lightweight materials with high strength and toughness. Natural systems have evolved efficient strategies, exemplified in the biological tissues of numerous animal and plant species, to synthesize and construct composites from a limited selection of available starting materials that often exhibit exceptional mechanical properties that are similar, and frequently superior to, mechanical properties exhibited by many engineering materials. These biological systems have accomplished this feat by establishing controlled synthesis and hierarchical assembly of nano- to micro-scaled building blocks. This controlled synthesis and assembly require organic that is used to transport mineral precursors to organic scaffolds, which not only precisely guide the formation and phase development of minerals, but also significantly improve the mechanical performance of otherwise brittle materials.

Here, we investigate an organism that have taken advantage of hundreds of millions of years of evolutionary changes to derive structures, which are not only strong and tough, but also demonstrate abrasion resistance. All of this is controlled by the underlying organic-inorganic components. Specifically, we discuss the formation of heavily crystallized radular teeth the chitons[1-4], a group of elongated mollusks that graze on hard substrates for algae. From the investigation of synthesis-structure-property relationships in these unique organisms, we are now developing and fabricating multifunctional engineering materials for energy conversion and storage. We discuss the crystallization of these materials and their subsequent impact on performance.

[1]. “Competing mechanism in the wear resistance behavior of biomineralized rod-like microstructures,” E. Escobar de Obaldia et al., Journal of the Mechanics and Physics of Solids, 96 (2016) 511-534.
[2]. "Stress and Damage Mitigation from Oriented Nanostructures within the Radular Teeth of Cryptochiton stelleri," L.K. Grunenfelder et al., *Adv. Funct. Mater.,* 24 (2014) 6093-6104.
[3]. Phase transformations and structural developments in the radular teeth of *Cryptochiton stelleri,*” Q. Wang, M. Nemoto, et al., *Adv. Funct. Mater.,* **23** (2013) 2908–2917.
[4]. “Analysis of an ultrahard magnetic biomineral in chiton radular teeth,” J. Weaver, QQ. Wang, et al., *Materials Today*, **13** (2010) 42-52.

Phylogenetic and biomineralogical study of uncultured magnetotactic bacteria at single-cell level

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Magnetotactic bacteria (MTB) are phylogenetically diverse, which can biomineralize diverse magnetic nanocrystals of magnetite or greigite, termed magnetosomes. Their remains within sediments or sedimentary rocks, i.e. magnetofossils, have been increasingly used to retrieve paleomagnetic and paleoenvironmental information of deposition time, as well as to trace the origin and evolution of life on Earth. Therefore, a precise identification of magnetofossils heavily depends on knowledge of phylogenetic diversity and magnetosomal biomineralization within natural MTB. In this paper, we will present a novel method which can rapidly characterize both the phylogenetic and biomineralogical properties of uncultured MTB at the single-cell level by coupling fluorescence and electron microscopy. Through this so-called coupled FISH-SEM/TEM method, MTB cells from nature environments can be phylogenetically and structurally identified, and subsequently the crystal growth, chain assembly and even magnetic properties of magnetosomes within these phylogenetically-defined, uncultured bacteria could be systematically investigated with various advanced TEM approaches. By using this new method, we have successfully identified several uncultured MTB strains from natural environments. These MTB are phylogenetically affiliated with the *Alphaproteobacteria*, *Deltaproteobacteria*, *Gammaproteobacteria* and *Nitrospirae* phylum, and form octahedral, cuboctahedral, prismatic, tooth-like and bullet-shaped magnetite magnetosomes, which indicate magnetosome morphology and bacterial phylogenetics on each MTB strain having a species/strain-specific magnetosome biomineralization. The FISH-SEM/TEM method is promising for better understanding the relationship between magnetosome mineral habits and MTB phylogenies, and useful for unambiguously identifying magnetofossils and their environmental significance.

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Genomic insights into the origin and evolution of magnetotactic bacteria

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Magnetotactic bacteria (MTB) biomineralize intracellular, nano-sized magnetic mineral crystals of Fe_3O_4 and/or Fe_3S_4 magnetosomes. Magnetosomes impart a permanent magnetic dipole moment to the cell causing it to align along magnetic field lines as it swims, a behavior called magnetotaxis. We have reconstructed draft genomes from uncultivated MTB through genome-resolved metagenomics from both northern and southern hemispheres. These novel genomes expand the coverage of MTB in the domain *Bacteria*, indicating that the diversity of MTB is much greater than is generally appreciated. Phylogenetic and comparative genomic analyses of all available MTB genomes have suggested that magnetotaxis is an ancient trait that has a single common origin with lineage-specific evolution.

Matagenomic analysis of magnetotactic bacteria from saline lake in Inner Mongolia

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Magnetotactic bacteria (MTB) are aquatic microorganisms that synthesize intracellular magnetic nanoparticles composed of magnetite and/or greigite. MTB have thus far been identified in the phyla of *Proteobacteria*, *Nitrospirae*, *Omnitrophica*, *Latescibacteria* and *Planctomycetes*. Most of these organisms have been detected from freshwater and marine environments, while relatively little attention has been paid to the diversity and ecology of MTB in extreme environments. Previously, MTB have been identified in deep-sea sediments, seamounts, hot springs and saline lakes (Bazylinski and Lefèvre, 2013; Lin *et al*., 2017). However, due to the lack of genomic analysis, the physiological ecology and metabolic mechanism of MTB remains very limited.

Recently, we have conducted a survey of MTB in saline-alkaline lakes around Inner Mongolia in China. MTB were detected in sediments with pH > 9 and sanity > 100 ppt. Variety of coccoid, rod, vibrionic, spirillar MTB cells from 12 saline-alkaline lakes have been characterized by transmission electron microscope. Magnetotactic bacteria were magnetically enriched through 'MTB trap' and metagenomic DNA was extracted from enriched MTB cells. DNA was sequenced on Illumina Hi-Seq 2500 platform, resulting in a total of 36 Gbp raw data. These sequences were assembled and several scaffolds containing magnetosome gene clusters have been identified. The following analysis and binning of the metagenomic assemblies will provide novel insights into the ecophysiology, evolution and biomineralization of MTB in extreme environments.

Reference:

1. Bazylinski DA and Lefèvre CT, (2013). Magnetotactic Bacteria from Extreme Environments. Life 3: 295–307.
2. Lin W, Pan Y, Bazylinski DA, (2017). Diversity and ecology of and biomineralization by magnetotactic bacteria. Environmental Microbiology Reports 9(4): 345–356.

Microbial biomineralization and the catalytic activity of platinum group metal nanoparticles obtained with metal-reducing microorganism

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Platinum group metal (PGM) nanoparticles can be applied to a wide range of functions such as catalysts, optics, and biosensing. Although conventional chemical and physical methods have been extensively developed, biological methods now provide an attractive and eco-friendly alternative strategy in which microorganisms are used to prepare PGM nanoparticles via the reductive deposition of soluble metal ions, which is a process called biomineralization.

Our research successfully focused on using the metal ion-reducing bacterium, *Shewanella algae* (ATCC 51181), to synthesize PGM (palladium and platinum) nanoparticles. Resting cells of *S. algae* were able to reduce soluble PGM ions (Pd(II) and Pt(VI)) to metallic nanoparticles at room temperature and neutral pH within 30 min when formate was provided as the electron donor. The biogenic PGM nanoparticles had a mean diameter of 5 nm and were localized in the periplasmic space between the outer and inner membranes. Considering the position of the formed nanoparticles, bio-substances that contributed to the reductive deposition of PGM were likely to reside in the periplasmic space, where is a part of the cell surface that provides easy access to the soluble substrate. The initial concentrations of soluble PGM and formate in the starting solutions strongly influenced the particle size of biogenic PGM nanoparticles.

We expected the biogenic PGM nanoparticles to have high catalytic activity, assuming that the outer membrane of the bacterial cells did not pose a significant barrier to substrate access. We examined the catalytic activity of biogenic PGM nanoparticles for the methylene blue decolorization reaction as a model reaction. Based on the model chemical reaction, the biogenic PGM nanoparticles exhibited excellent catalytic activity owing to their large specific surface area, compared with commercially available PGM catalysts. We believe that our microbial route to PGM nanoparticle catalysts is potentially attractive as an eco-friendly method because the biological systems involve low energy consumption and are environmentally safe.

Magnetosomes for the Masses: Scaling-up magnetic nanoparticle formation with biomimetic additives, inspired by MTB

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Over the last 10 years I have been researching how magnetosome membrane specific (Mms (Mam)) proteins control the formation of magnetite within magnetosomes, specifically their size and shape. We have specifically been looking at Mms6[1] and MmsF[2] and found certain characteristics that seem responsible for either nucleation of regulating growth.[1][2] Both of these proteins can be expressed and purified and added to a chemical precipitation of magnetite to control the formation yielding enhanced and more monodispersed particles.[3] However, using these native proteins as additives in chemical synthesis is not scaleable due to the fact that these are membrane proteins and thus not trivial to obtain. Here we present our most recent work to solve this problem through various strategies. 1. We will report a versatile biopanning strategy to find new shape controlling proteins[4]; 2. How we can mimic the Mms proteins by displaying the active regions on more expressible protein scaffolds; and 3. We report how we can take the understanding from Mms proteins to identify known natural additive/design new economical organic molecular additives with the same functionality. Finally, we consider how we can uses these in artificial magnetosomes.[5]

[1] 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

Staniland, S. S., Rawlings, A. E., Crystallizing the function of the magnetosome membrane mineralization protein Mms6 **Biochemical Society Transactions** (2016) **44 (3)** 883-890

[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] 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

[4] 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

[5] Kaushik Bakhshi, P., Bain, J., Gul, M. O., Stride, E., Edirisinghe, M., and Staniland, S. S., Manufacturing Man-made Magnetosomes: High-Throughput in situ synthesis of biomimetic magnetite loaded nanovesicles. **Macromolecular Bioscience** (2016) **16(11),** 1555-1561

Mms6 Protein from M. Magneticum AMB-1 and Magnetite Biomimetic Synthesis

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The magnetosome-chain within magnetotactic bacteria functions as a "biological compass" for magnetotactic bacteria to migrate along the geomagnetic field. Although many studies have been carried out and many proteins involved in have been identified, how magnetites within magnetosomes form and how proteins regulate the biomineralization process remain interesting but challenging questions. For M. magneticum AMB-1, the magnetosome-associated Mms6 protein is important for regulating the biomineralization within magnetosome [1,2]. Using high resolution NMR technique, we studied the Mms6 protein conformation, as well as its interaction to magnetosome magnetite crystal. The DEEVE residues within Mms6 C-terminal domain involve directly in the crystal surface binding and recognition. the hydrophobic packing by the N terminus is of great significance for the formation of a correct stacking arrangement for specific crystal surface recognition [3]. The binding of Mms6 protein to Fe^{2+} and its role in nucleation process will be discussed. Moreover, the biomimetic synthesis of magnetite can be achieved in a reverse microencapsulation system containing Mms6 protein.

1. Tanaka, M., et al., J Biol Chem, 2011. **286**(8): p. 6386-92.
2. Arakaki, A., et al., Mol Microbiol, 2014. **93**(3): p. 554-67.
3. Ma, K., et al., Journal of Materials Chemistry B, 2017. **5**(16): 2888-2895

Engineered Magnetosome, a Green Synthetic and Cheap Composite Nanomaterial Can be Used as a Nano-sized Immunomagnetic Beads

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Magnetosomes (BMP) are biomembrane-coated magnetic nanoparticles synthesized by magnetotactic bacteria (MTB). The engineered BMPs in association with protein A (termed as ΔF-BMP-FA here) have the ability to bind antibodies automatically. In this study, we evaluated the practicability and characters of ΔF-BMP-FA: developed and optimized the cultivation methods of its host strain *Magnetospirillum gryphiswaldense* ΔF-FA, extraction conditions of ΔF-BMP-FA, the antibody conjugation conditions on ΔF-BMP-FA surface, and procedures for detecting antigens with ΔF-BMP-FA and antibody assembles (BMP-A-Ab). After 36 hour fed-batch culture in a 42L fermentor, up to 2.26 g/L cells of strain ΔF-FA (dry weight) and 62 mg/L ΔF-BMP-FA (dry weight) were obtain. The cost of ΔF-BMP-FA was estimated as low as 0.07 dollar per mg. The fusion of the Protein A with the BMPs leads to the ordered arrangement of antibodies on the BMP surface. An inspiring conjugation efficiency of 962 μg antibody per mg of ΔF-BMP-FAs was achieved in this work. The BMP-A-Ab was then used in the detection of bacterial surface antigen of *Vibrio parahaemolyticus* (Vp) and hapten gentamycin sulfate. The maximum capture rate of Vp by BMP-A-Ab was of 90%, which was higher than the commercial magnetic beads. The detection sensitivity was 13 cfu/mL. ΔF-BMP-FA can also bind antibodies from crude mouse ascites to form the complex BMP-A-Ab. The detection line of gentamycin sulfate was up to 0.01 ng/ml, 400 times lower than double anti-sandwich ELISA, and the recovered gentamycin sulfate by BMP-A-Ab was 93.2%. This implied the strong adsorption ability for conjugating antibodies and exhibited the powerful tool for the diagnosis of the trace pathogens with low concentration antibiotics. The engineered BMPs could be alternative candidates for commercial immunomagnetic beads.

Functionalization of bacterial magnetic nano-particles for specific binding to human cells

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Magnetosomes, bacterially produced nano-particles, have a number of proteins naturally tightly anchored to the magnetic crystal and the surrounding lipid membrane. This feature allows for designing of various genetic fusions, where a foreign protein can be expressed on the surface of magnetosomes thus adding a novel function. This work focuses on genetically engineering of the magnetosomes for specific binding to the parts of human cells. For selective binding to eukaryotic cells, one may choose to target one of many different proteins localized on the surface of the cytoplasmic membrane or, alternatively, to bind to the DNA sequences unique to this type of cells.

In the present work we report the use of MamC anchoring protein in *Magnetospirillum gryphiswaldense* MSR-1 to create protein fusions with parts of the cell recognition receptors from human cells. These receptor molecules are capable of specifically binding to ligand proteins that have been shown to be overexpressed on the surfaces of many types of cancer cells. Such functionalized magnetosomes can be used for cancerous cell identification and sorting due to expression of a fluorescent protein. They can be also used for visualization of tumors *in vivo* and for selective killing of cancerous cells relying on the magnetic responsiveness of the functionalized magnetosomes.

Finally, we use MamC anchor to create a fusion protein that specifically binds to the repeats found in human DNA. The ability to bind to human DNA will help to develop novel methods of DNA extraction, sorting, modification and help with development of methods aimed at localizing magnetosomes in the specific organelles of human cells such as nucleus. The presented genetic modifications will add to the emerging field of magnetosome-based biomedical applications.

Development of non-pyrogenic magnetosome minerals coated with poly-l-lysine leading to full disappearance of intracranial U87-Luc glioblastoma in 100% of treated mice using magnetic hyperthermia

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Magnetic hyperthermia was reported to increase the survival of patients with recurrent glioblastoma by 7 months. This promising result may potentially be further improved by using iron oxide nanoparticles, called magnetosomes, which are synthesized by magnetotactic bacteria, extracted from these bacteria, purified to remove most endotoxins and organic material, and then coated with poly-l-lysine to yield a stable and non-pyrogenic nanoparticle suspension. Due to their ferrimagnetic behavior, high crystallinity and chain arrangement, these magnetosomes coated with poly-l-lysine (M-PLL) are characterized by a higher heating power than their chemically synthesized counterparts currently used in clinical trials. M-PLL-enhanced antitumor efficacy was demonstrated by administering 500-700 µg in iron of M-PLL to intracranial U87-Luc tumors of 1.5 mm3 and by exposing mice to 27 magnetic sessions each lasting 30 min, during which an alternating magnetic field of 202 kHz and 27 mT was applied. Treatment conditions were adjusted to reach a typical hyperthermia temperature of 42 °C during the first magnetic session. In 100% of treated mice, bioluminescence due to living glioblastoma cells fully disappeared 68 days following tumor cell implantation (D68). These mice were all still alive at D350. Histological analysis of their brain tissues revealed an absence of tumor cells, suggesting that they were fully cured. In comparison, antitumor efficacy was less pronounced in mice treated by the administration of IONP followed by 23 magnetic sessions, leading to full tumor bioluminescence disappearance in only 20% of the treated mice.

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Title: Breast cancer immunotherapy using magnetised oncolytic virus

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Background: Oncolytic viruses (OV) are encouraging new immunotherapies for cancer. OVs, replicate in cancer cells inducing immunogenic cell death (ICD) and activating antitumour immunity. To date, clinical use has focused on intratumoural delivery due to concerns over inadequate tumour targeting following systemic administration. We hypothesise that magnetising OVs and magnetic guidance strategies will improve systemic delivery by protecting the viruses from inactivating immune mechanisms and non-specific adsorption.

Methods: To investigate this, we synthesised and characterised complexes of magnetised oncolytic herpes simplex virus (HSV1716) co-assembled with biocompatible magnetic nanoparticles (MAG) derived from magnetotactic bacteria (AMB-1) to give MAG-HSV1716 complexes. Characterization of the physical, chemical and oncolytic potential of our MAG-HSV1716 was performed. The safety and efficacy of our magnetic viral complexes in combination with magnetic guidance strategies were also assessed mammary mouse models.

Results: Stable MAG-HSV1716 complexes of ~90nm diameter successfully infected human and murine breast cancer cells in a dose-dependant manner, and induced tumour oncolysis. Following MAG-HSV1716 infection a significant increase in viral replication (ICP0, gB, ICP8), ICD (HMGB1, CALR, ATP) and apoptotic (CASP 3, CASP8, FASL) signals were detected. Intravenous delivery of MAG-HSV1716 resulted in reduced tumour burden in the presence of magnetic guidance (MAG/OV 1071mm³ vs. OV 1435.7mm³; $p \leq 0.05$) and an increase in tumour-infiltrating T-cells, NK cells and neutrophils. Furthermore, MAG-HSV1716 complexes were protective in the presence of neutralizing HSV antibodies.

Conclusion: This study indicates that magnetizing HSV1716 results in viral protection from neutralising antibodies and in combination with magnetic guidance reduces tumour burden and induces antitumour immunity.

Poster Presentations

Poster presentations of MTB 2018 meeting

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Poster #3	V. Raguraman	Comparative studies on functionalization of magnetosomes for drug delivery
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Poster #7	C. Wagner	Magnetofossil, magnetic particle, and microfossil assemblages in a subtropical coastal environment: environmental change across the Paleocene-Eocene Thermal Maximum
Poster #8	H. Trujillo	Dissecting the physiological relevance and mechanism of ferrosome formation in *Rhodopseudomonas palustris* CGA009
Poster #9	S. Nakano	The discovery and comparative genomics of magnetosome-bearing bacteria from a deep-sea metal sulfide chimney
Poster #10	H. Pan	Comparison of the diversity of magnetotactic bacteria between two seamounts
Poster #11	T. Xiao	Isolation, cultivation and genomic analysis of a novel spirillum marine magnetotactic bacteria belonging to *Methylocystaceae*
Poster #12	W. Zhang	Diversity and characterization of multicellular magnetotactic prokaryotes from coral reef habitats of the Paracel Islands, south China sea
Poster #13	C. Bickley	Temporal localization dynamics of important magnetosome proteins

Poster #14	V.V. Russell	Identifying the genes responsible for the irregular bullet shaped magnetite crystals synthesized in *Desulfovibrio magneticus* RS-1
Poster #15	T. Song	Effect of amb0994 gene on the magnetotactic behavior in *Magnetospirillum magneticum* AMB-1
Poster #16	J. Wang	Biomimetic synthesis of magnetosome based on magnetotactic protein Mms6
Poster #17	L. Abelmann	Long term observation of a single magneto-tactic bacteria inside a microfluidic channel
Poster #18	N. Korkmaz	Real time monitoring of magnetic properties of magnetotactic bacteria during culture growth
Poster #19	N. Korkmaz	Surface functionalization of magnetotactic bacteria with streptavidin coated microparticles
Poster #20	X. Murgia	Exploring the potential of MC-1 magneto-tactic bacteria as carriers for mucosal drug delivery
Poster #21	L. Yan	Diversity of magnetotactic bacteria in surface sediments from Wudalianchi volcanic barrier lakes
Poster #22	S. Kolusheva	The role of magnetite-associated protein in magnetite formation
Poster #23	F. Mathon	Trapping magnetotactic bacteria from natural environments
Poster #24	S. Staniland	Biomimetic and biokleptic synthesis of magnetic nanoparticles and arrays inspired by magnetic bacteria
Poster #25	B. Ryu	Investigation of motility and thermotaxis of *Magnetococcus marinus* (MC-1) as a function of environmental stimuli
Poster #26	S. Iwata	Artificial polarity-reversal of bacterial magnetic compass
Poster #27	D. Matsunaga	Clustering of magnetotactic bacteria in a microchannel
Poster #28	L.M. Alekseeva	The influence of physicochemical factors on magnetotactic bacteria affiliated with phylum *Nitrospirae* from different aquatic environments
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Poster #32	S. Preveral	Targeted thermal therapy with genetically engineered magnetite magnetosomes@RGD: Photothermia is far more efficient than magnetic hyperthermia
Poster #33	G. Adryanczyk-Perrier	Optimization of lysis of magnetotactic bacteria by ultrasound in order to automate purification of magnetosomes

Poster #34	Y. Eguchi	pH imaging in living prokaryotic cells and organelles using pH-sensitive fluorescent protein
Poster #35	Y. Ichinaka	Imaging of living bacterial cell surface structures using high-speed atomic force microscopy
Poster #36	Y. Kikuchi	Characterization of MamK polymerization using high-speed atomic force microscopy
Poster #37	M. Omura	Subcellular localization of MamQ in *Magnetospirillum magneticum* AMB-1
Poster #38	Y. Takaoka	Live-cell imaging of flagellar rotation during magnetotactic motility
Poster #39	I. Yamazaki	Functional analyses of MamJ which is cytoskeleton associating protein for magnetosome positioning
Poster #40	K. Fujimoto	Modification of phospholipid composition of magnetosomes by employing phosphatidylcholine synthase in magnetotactic bacteria
Poster #41	S. Tayama	Functional expression of transmembrane receptors on magnetic nanoparticles through a magnetosome-display system
Poster #42	T. Yoda	Iron uptake and magnetosome formation abilities in MAI deletion mutant and genetically modified strain carrying multiple sets of MAI genes
Poster #43	K. Arai	Adsorption study of Mms6 and Mms7 to iron oxide magnetic nanoparticles

DEVELOPMENT OF MAGNETOSOME BASED BIOSENSOR FOR THE DETECTION OF O-POLYSACCHARIDE IN *ESCHERICHIA COLI* (O55: B5)

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Abstract

The rise in cases of food poisoning and traveller's diarrhea through *E.coli* infection is a global concern. Magnetosome based biosensors offers simple and rapid detection of microbial pathogens from various sources. Magnetosome are biogenic magnetic nanoparticles extracted from magnetotactic bacteria. The current study deals with the development of a prototype magnetosome based biosensor for the detection of antigenic O-polysaccharide in *E.coli*. It is achieved through designing an interdigitated array microelectrode based impedance biosensor coupled with magnetosome – antibody complex. An interdigitated array microelectrode connected to an impedance analyser has been used as a sensor. The magnetosome was extracted from *Magnetospirillum sp.* RJS1 and characterized by microscopic analysis. The magnetosome (1mg/ml, 2mg/ml) were conjugated with antibody (0.08µg/ml - 200µg/ml) and confirmed through spectroscopy. Lipopolysaccharide was added with magnetosome –antibody complex and allowed to bind. The mixture (magnetosome-antibody-lipopolysaccharide) was separated using the external magnetic field and the amount of lipopolysaccharide present was determined by spectroscopy. The least concentration of antibody, magnetosome and lipopolysaccharide needed was optimised in ELISA assay. The concentrated magnetosome- antibody complex (1.6µg/ml) efficiently detected the lipopolysaccharide compared to the antibody (2.6µg/ml) alone. The magnetosome-antibody complex also detected the minimum concentration of lipopolysaccharide (1µg/ml) compared to traditional ELISA method (5µg/ml). Magnetosome use in ELISA assay proved to be cost-effective approach as the amount of antibody was reduced to 39% and 80% more sensitive. Further, magnetosome- antibody complex was applied on screen-printed carbon electrode to detect the lipopolysaccharide using EIS. The increase in resistance was observed in carbon electrode when lipopolysaccharide. Overnight *E.coli* cells were also incubated with modified SPCE and analysed through EIS and SEM. The positive charge transfer of electrons in the electrode and SEM images revealed the interaction of *E.coli* with magnetosome- antibody complex. The prototype model could directly detect the least amount of lipopolysaccharide as well as the *E.coli* cells. The developed magnetosome based biosensor could be implemented on various food, water and other sources to detect *E.coli* and other food pathogens.

Keywords: *E.coli*, lipopolysaccharide from *E.coli*, *Magnetospirillum sp*. RJS1, magnetosome, ELISA, biosensor.

The study of physiological responses to changing environmental conditions in *Magnetospirillum gryphiswaldense* MSR-1 facilitates the establishment of a magnetosome biomanufacturing platform

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Magnetotactic bacteria (MTB) are a phylogenetically diverse group of bacteria that are able to synthesize magnetosomes; sub-cellular nanoscale organelles that comprise a crystal of magnetite or greigite (depending upon the species of MTB) coated in a biological phospholipid membrane containing membrane proteins. Magnetosomes represent an attractive alternative to existing commercially available chemically synthesised magnetic nanoparticles. However, large-scale cultures delivering high-cell densities in combination with efficient magnetosome recovery remains a challenging step toward industrial application. Here, we present flow cytometry methods that our group has recently developed to evaluate a range of physiological and stress parameters in MTB – cell morphology, aspects of metabolism, and the accumulation of intracellular polyhydroxyalkanoate (PHA) – and we demonstrate how these methods are useful to characterise *Magnetospirillum gryphiswaldense* MSR-1 strain in flask and 5 L bioreactors cultures. We believe that flow cytometry is an essential tool that will help to fully understand the bacterial physiology and metabolism of MTB so that they might be grown industrially at large scale to high cell densities with high magnetosome content, allowing development of magnetosome-related applications.

COMPARATIVE STUDIES ON FUNCTIONALIZATION OF MAGNETOSOMES FOR DRUG DELIVERY

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Abstract

Bacterial magnetosomes are biogenic iron oxide nanoparticle surrounded by a bilayer lipid membrane. They have attained great attention as an excellent drug carrier, especially in targeted drug delivery. In this study, magnetosomes-drug (crizotinib) conjugates were developed using crosslinkers such as glutaraldehyde, 3- aminopropyltriethoxysilane (APTES) and by direct adsorption for the targeted delivery of anticancer drugs. The characteristics of the magnetosomes extracted from magnetotactic bacterial strain MSR-1 were examined by High-Resolution Transmission Electron Microscope (HRTEM), Scanning Electron Microscope (SEM), Energy Dispersive X-ray Spectroscopy (EDX), Fourier Transform Infrared (FTIR), and X-Ray Diffraction (XRD). TEM and SEM analysis reveals the octahedral shape and presence of the lipid membrane of magnetosomes. XRD, EDX and FTIR confirm the presence of iron oxide, element composition. FTIR spectra of CM-conjugates confirms the binding of crizotinib to the magnetosome surface. The drug loading efficiency of CM-conjugates developed by direct adsorption, glutaraldehyde and APTES was found to be 94.81, 91.7 and 83.4%, respectively. Drug loading capacity was 670 µg/ml and cytotoxicity of 34.2% for CM conjugate by direct adsorption. A long-term slow release of crizotinib was observed for CM-conjugates by direct adsorption. These results indicate that CM-conjugates developed by direct adsorption shows better coupling with higher loading efficiency and long-term slow drug release.

Keywords: Magnetosomes, Crizotinib, CM-conjugates, Drug delivery, Glutaraldehyde, APTES.

Glycosylate and move: Maf is a glycosyltransferase involved in the glycosylation of the flagellin of *Magnetospirillum magneticum* AMB-1

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The flagella of various Gram-negative bacteria are decorated with diverse glycan structures, amongst them nonulosonic acids related to the sialic acid family. Although nonulosonic sugar biosynthesis pathways have been dissected in various pathogens, the enzymes transferring the sugars onto flagellin are still uncharacterized. The deletion of genes coding for motility associated factors (Mafs) found in many pathogenic strains systematically gives rise to non flagellated bacteria lacking specific nonulosonic sugars on the flagellins, therefore, relating Maf function to flagellin glycosylation and bacterial motility.

A sugars analysis of the flagellin of *Magnetospirillum magneticum* AMB-1 revealed the presence of an analogue of pseudaminic acid, a nonulosonic acid. By genomic analysis, we found a gene (*amb0685*) encoding a homologue of Maf near the only functional flagellin gene, *amb0684*, of AMB-1 (1). So, we investigated the role of Amb0685 from AMB-1, in the glycosylation and formation of the flagellum. Thus, deletion of *amb0685* produced a non flagellated bacterium where the flagellin was still produced but no longer glycosylated. A functional flagellar filament and bacterial motility could be restored by complementation of the deletion mutant. We solved the structure of Amb0685, which is organised into three domains: a N-terminal Rossman-like domain of unknown function, a central domain exhibiting striking structural similarity with sialyltransferases and a C-terminal α-helical bundle with intriguing structural reminiscence of flagellar chaperons. By mutation studies we could identify residues crucial for the glycosyltransferase activity of Maf (2).

1. **Murat D, Hérissé M, Espinosa L, Bossa A, Alberto F, Wu LF**. 2015. Opposite and Coordinated Rotation of Amphitrichous Flagella Governs Oriented Swimming and Reversals in a Magnetotactic Spirillum. J Bacteriol **197**:3275.
2. **Sulzenbacher G, Roig-Zamboni V, Lebrun R, Guerardel Y, Murat D, Mansuelle P, Yamakawa N, Qian XX, Vincentelli R, Bourne Y, Wu LF, Alberto F**. 2018. Glycosylate and move! The glycosyltransferase Maf is involved in bacterial flagella formation. Environ Microbiol **20**:228-240.

Global analysis of genes required for biomineralization in *Magnetospirillum magneticum* AMB-1

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The genes required for magnetosome formation are encoded by the magnetosome gene island (MAI). In *Magnetospirillum magneticum* AMB-1 the MAI is composed of 106 annotated genes representing approximately two percent of the AMB-1 genome.[1] However, the functions of the majority of genes in the MAI are still unknown. Due to the large size and the metabolic cost required to replicate the MAI, it is likely that the unknown genes in the MAI have important roles to play in magnetosome formation. Tightly controlled laboratory conditions may obscure the functions of genes[2,3,4]—both in the MAI and other genomic regions—that would be required for magnetosome formation in a natural environment. In order to investigate the essential set of magnetosome genes, we conducted a random barcoded transposon mutagenesis screen (RB-TnSeq) in AMB-1. We created a library of ~100,000 unique mutants in both wild-type and ΔMAI AMB-1 strains, and mapped the location of each mutation. This analysis will reveal a panel of putative essential genes in the presence, and absence, of magnetosome formation. Additionally, the wild-type mutant library will be subjected to magnetic selection in order to compile a full list of magnetosome formation genes needed under various growth conditions.

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[1] Murat D, Quinlan, Vali H, & Komeili A (2010) Comprehensive genetic dissection of the magnetosome gene island reveals the step-wise assembly of a prokaryotic organelle.
[2] Li Y, Katzmann E, Borg S, Schüler D (2012) The periplasmic nitrate reductase nap is required for anaerobic growth involved in redox control of magnetite biomineralization in Magnetospirillum gryphiswaldense. J Baceteriol 194(18):4847-56
[3] Raschdorf, O Müller FD, Pósfai M, Plitzko JM, Schüler D. 2013. The magnetosome proteins MamX, MamZ, and MamH are involved in redox control of magnetite biomineralization in Magnetospirillum gryphiswaldense. Molecular Microbiology 89(5): 872-86.
[4] Müller FD, Raschdorf O, Nudelman H, Messerer M, Katzmann E, Plitzko JM, Zarivach R, Schüler D. 2014. The FtsZ-like protein FtsZm of Magnetospirillum gryphiswaldense likely interacts with its generic homolog and is required for biomineralization under nitrate deprivation. Journal of Bacteriology 196(3): 650-59.

Magnetotactic bacteria as hyperthermia agents

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In the last years, one of the most interesting approaches for cancer therapy is devising nano-robots capable of targeting and destroying cancer cells. In this work we want to prove the capabilities of *Magnetospirillum gryphiswaldense* bacteria as self-propelled biorobots for cancer treatment, evaluating the magnetic hyperthermia response. We compare these results with isolated magnetosomes.

Figure 1(a, b) shows the Specific Absorption Rate normalized by the frequency, SAR/f, as a function of the magnetic field. In both cases, bacteria and magnetosomes were dispersed in water and in 2% agarose gel with a magnetite concentration of 0.15 mg/ml. While with magnetosomes dispersed in water we reach values of SAR / f close to 5 $Wg^{-1}kHz^{-1}$, with magnetotactic bacteria we can reach values of around 8 $Wg^{-1}kHz^{-1}$, which is originated by the automatic alignment of the bacteria with the applied AC magnetic field, giving rise to a higher squareness of the hysteresis loops (Figure 1c).

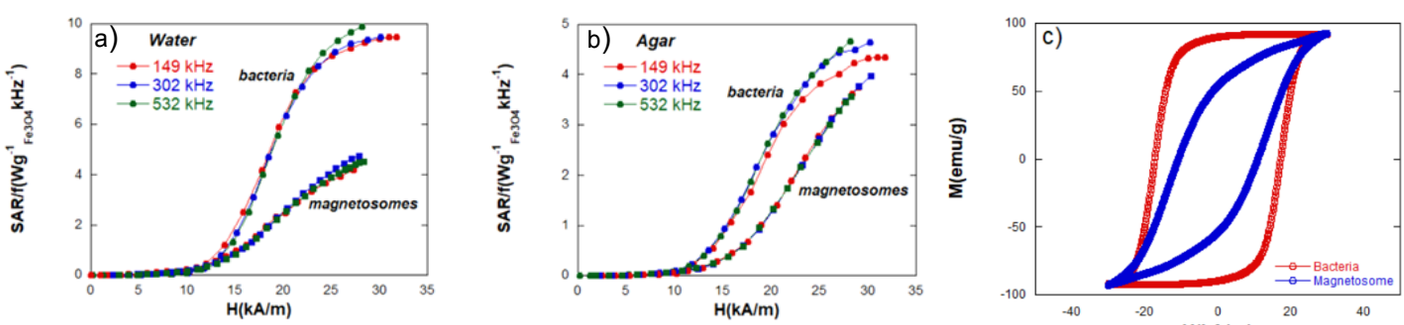

Figure 1. SAR/f values as a function of the applied magnetic field for magnetosomes and bacteria dispersed in wàter **(a)** and in agar **(b). (c)** Hysteresis loops measured at 302 kHz for bacteria and magnetosomes dispersed in wàter.

Magnetofossil, Magnetic Particle, and Microfossil Assemblages in a Subtropical Coastal Environment: Environmental Change across the Paleocene-Eocene Thermal Maximum

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Continental shelf sediments along the NE margin of North America contain a near-pristine magnetofossil-rich interval that is coincident with the Paleocene-Eocene Thermal Maximum (PETM), an abrupt global warming event that occurred ~56 Ma. The source of these magnetofossils has been, and is here, interpreted to be an assemblage of near-shore magnetotactic bacteria (MTB). We present component-specific room-temperature and cryogenic magnetic measurements of samples from the Wilson Lake-A core from New Jersey that span the PETM. We use these data— which can discriminate the distribution of sizes, shapes, and compositions of iron-rich particles in bulk sediments— to assess the relative contribution of biotic *vs.* abiotic magnetite, and to estimate changes in magnetofossil abundance and (potentially) biodiversity during the PETM. High-resolution, first-order reversal curves with distinct central ridges are consistent with published magnetic coercivity and electron microscopy data that suggest that conventional prokaryotic magnetofossils are abundant across the onset of and throughout the PETM. Distinctive high-coercivity components may correspond to 'gigantic' magnetofossils previously characterized by electron microscopy. Low-temperature (LT) magnetic measurements of the same samples indicate that one or more previously unrecognized Fe-bearing mineral phases partially or completely mask distinctive features of the magnetite Verwey transition expected of biogenic magnetite. We present additional magnetic grain size and composition calculations to test whether this masking is caused by diagenesis or additional primary magnetic components in these coastal sediments. Finally, we compare these magnetic measurements with benthic and planktonic foraminiferal isotopic and assemblage data from the same core to test what biogeochemical factors associated with rapid and sustained warming modulated MTB and foraminiferal diversity in this shelf environment.

Dissecting the physiological relevance and mechanism of ferrosome formation in *Rhodopseudomonas palustris* CGA009

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The cell is saturated with biomolecules and cellular activities that need to be organized and regulated to maintain life. To bring order to the biological activity, eukaryotes employ lipid bounded organelles to localize tasks to defined areas. Bacteria have also adapted ways of compartmentalizing activity that are distinct from well-characterized eukaryotic organelles. Our group has recently discovered a new class of bacterial organelles called the ferrosome. Since its discovery, the *fez* operon has been found to be required for ferrosome formation. Phylogenetic analyses of the *fez* operon suggest that this organelle is a cosmopolitan compartment in bacteria. However, given the widespread nature of the *fez* operon, only one gene has a known function, and the physiological function and mechanism of ferrosome formation remain unknown. To shed light on these questions, I will use genetic and cell biological techniques to assess *fez* protein localization during ferrosome formation. I will also perform comprehensive competition analyses between wild type and Δ*fez* genotypes to find conditions where the loss of the *fez* operon leads to a decrease in fitness.

The discovery and comparative genomics of magnetosome-bearing bacteria from a deep-sea metal sulfide chimney

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Magentotactic bacteria (MTB) are known to orient cells along the geomagnetic field with magnetosomes aligned into chains for strong dipole moment. In this study, a metal sulfide chimney formed on the seafloor in the southern Mariana Trough was investigated for the occurrence of MTB. During magnetic separation from non-magnetic material, magnetic particles mainly composed of vivianite [$Fe_3(PO_4)_2 \cdot 8H_2O$] attracted magnetic cells. After further separation of magnetic cells from vivianite particles, the visualization of microbial cells by scanning and transmission electron microscopies unveiled the occurrence of metabolically active bacteria associated with magnetosomes from the metal sulfide chimney. Teardrop shaped magnetosomes and dominant 16S rRNA gene sequences related to *Nitrospirae* MTB (such as *Candidatus* Magnetobacterium bavaricum) in the metal sulfide chimney suggest the magnetosome-bearing cells might belong to *Nitrospirae*. However, the lack of magnetosome chains in the observed cells is distinct from those observed in the known *Nitrospirae* MTB. A near-complete genome that is representative of the dominant chimney *Nitrospirae* was reconstructed from the metagenome of the sulfide chimney. In contrast to the fact that previously known MTB have magnetosome-related genes bundled within magnetosome island, magnetosome-related genes were sparsely positioned in the reconstructed *Nitrospirae* genome. As homologues genes involved in the formation of magnetosome chains were deficient in the *Nitrospirae* genome, it is likely that the *Nitrospirae* genome is derived from microbial cells associated with magnetosomes without chains. The ecological function of unaligned magnetosomes is speculated to attach magnetic vivianite particles for phosphate uptake, because phosphate is generally scarce in deep-sea environments. The further ecological and genomic investigations will shed light on the antiquity and evolutionary history of MTB.

Comparison of the diversity of magnetotactic bacteria between two seamounts

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Seamounts are undersea mountains rising abruptly from the sea floor and interacting dynamically with underwater currents. They represent unique biological habitats with various microbial community structures. Our previous study has shown population structure and the occurrence of magnetotactic bacteria (MTB) that synthesize intracellular iron oxide nanocrystals in sediments of a seamount in the Mariana volcanic arc. And some magnetotactic cocci possess the most complex flagellar apparatus yet reported; 19 flagella are arranged in a 3:4:5:4:3 array within a flagellar bundle. In this study, we also found that high diversity of MTB occurs in another seamount from Caroline. The metagenomic results showed there are 41 MTB OTUs affiliated with phyla Proteobacteria and Nitrospira. Eleven MTB OTUs were shared between two seamounts. Different shapes of MTB were also observed by transmission electron microscope (TEM). Most magnetotactic cocci contain two chains of octahedral magnetosomes, while spiral and rod MTB contain a few magnetosomes with irregular shape. Interestingly, many cells have long flagella, which are typically five to ten times longer than the bodies. Preliminary comparison results present that the diversity and characterization of MTB between two seamounts seem to be different.

Isolation, cultivation and genomic analysis of a novel spirillum marine magnetotactic bacteria belonging to *Methylocystaceae*

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A novel marine magnetic spirillum axenic culture, designated strain SH-1, was isolated from an intertidal zone of Sanya. It was able to grow in 2216E medium and its optimal pH, salinity and temperature were 7.7, 40‰ and 26 ˚C, respectively. Most of SH-1 produced a single chain of prismatic magnetite magnetosomes. Phylogenetic (16S rDNA) analysis revealed that this strain was 96.9% identical to *Terasakiella* sp. B3 belonging to *Methylocystaceae*, which is different from most of other magnetic spirillum (QH-2, 88.4%). The genome of the SH-1 comprises a 3.8 Mb of circular chromosome having a G + C content of 47.5% (1 contig). The chromosome contains 3633 predicted coding sequences (CDS), which corresponds to 90.12% of the genome being coding sequences. The pan/core genome analysis (80% aa identity / 80% align. coverage) showed that the number of core gene shared by SH-1 and QH-2 was 87, accounting for 2.4% of whole genome of SH-1. The number of core gene shared by SH-1 and *Terasakiella pusilla* DSM 6293 was 1080, accounting for 30% of whole genome of SH-1. The magnetosome island of SH-1 is a 34.9 Kb region divided into two parts by a 17.2 kb region. There is a special long inverted repeat sequence appearing in its magnetosome island, which may suggest a recent acquisition by HGT that is also supported by the G + C content bias between the array of magnetosome island (52%) and the average G + C content of the genome. Furthermore, 3 potable prophage related regions (11-27 Kb) were observed in this genome. According to the analysis on other genomes of MTB, the appearance of prophage sequence may be a common phenomenon. Now further studies are being carried out.

Diversity and Characterization of Multicellular Magnetotactic Prokaryotes from Coral Reef Habitats of the Paracel Islands, South China Sea

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Among all the secondary marine ecosystems, the diversity of multicellular magnetotactic prokaryotes (MMPs) in sediments of coral reef ecosystems has rarely been reported. In this study we investigated the diversity and characteristics of MMPs in sediments at 11 stations in coral reef habitats of the Paracel Islands. The results showed that MMPs were present at 9 stations, with spherical mulberry-like MMPs (s-MMPs) found at all stations and ellipsoidal pineapple-like MMPs (e-MMPs) found at 7 stations. The maximum abundance of MMPs was 6 × 103 ind./dm3. Phylogenetic analysis revealed the presence of 5 s-MMP species and one e-MMP species in the coral reef sediments, including 2 new s-MMP species belonging to a new genus. The results indicate that coral reef habitats of the Paracel Islands have a high diversity of MMPs. These findings provide new insights into the diversity of MMPs in general, and expand knowledge of the occurrence of MMPs in coral reef habitats.

Temporal localization dynamics of important magnetosome proteins

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Recently, increased understanding of the complexity of bacteria has challenged the traditional view of a bacterial cell as an unstructured “bag of enzymes”. For instance, similar to eukaryotic cells, many bacterial species compartmentalize their cell contents within membrane-bounded organelles [1]. Magnetotactic bacteria (MTB) build a compartment known as a magnetosome that has been used as a model for bacterial organelle biosynthesis. A subset of genes in the magnetosome island (MAI) in *Magnetospirillum magneticum* AMB-1 and *Magnetospirillum gryphiswaldense* MSR-1 have been shown to be necessary and sufficient for magnetosome formation [2][3]. Though some proteins involved in magnetosome construction have been identified, their temporal dynamics, and the order in which they localize to the membrane or the crystals, remain poorly understood. Using the magnetotactic strain AMB-1, we propose to use time-lapse super resolution microscopy to shed further light on the dynamics of magnetosome formation and the general mechanisms of bacterial organelle biosynthesis.

[1] Cornejo, Elias, Nicole Abreu, and Arash Komeili. “Compartmentalization and Organelle Formation in Bacteria.” *Current opinion in cell biology* 26 (2014): 132–138.
[2] Murat, Dorothée et al. “Comprehensive Genetic Dissection of the Magnetosome Gene Island Reveals the Step-Wise Assembly of a Prokaryotic Organelle.” *Proceedings of the National Academy of Sciences of the United States of America* 107.12 (2010): 5593–5598.
[3] Kolinko I, Lohße A, Borg S, Raschdorf O, Jogler C, Tu Q, Pósfai M, Tompa E, Plitzko JM, Brachmann A, Wanner G, Müller R, Zhang Y, Schüler D. Biosynthesis of magnetic nanostructures in a foreign organism by transfer of bacterial magnetosome gene clusters. *Nat Nanotechnol.* (2014): 193-197.

Identifying the genes responsible for the irregular bullet shaped magnetite crystals synthesized in Desulfovibrio magneticus RS-1

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Desulfovibrio magneticus RS-1, a gram negative, anaerobic, sulfate-reducing magnetotatic bacterium (MTB), synthesizes irregular bullet-shaped magnetite magnetosomes. The biosynthetic pathway for the production of magnetosomes in RS-1 and other delta-proteobacterial MTB is different from alpha-proteobacterial MTB. RS-1 contains a *mam*AB-like gene cluster, which contains orthologs to *mam*A, *mam*B, *mam*E, *mam*K, *mam*M, *mam*O, and *mam*Q. Significantly similar genes for *mam*P and *mam*T are located in an adjacent region. RS-1 is missing a number of genes conserved in alpha-proteobacteria MTB, but it also contains a number of gene only found in delta-proteobacterial MTB named *mad* genes. There are 25 *mad* genes in RS-1 that have homologs to other delta-proteobacteria MTB. However, to date the majority of the *mad* genes still have unknown function and characterization. I hypothesize that some of these *mad* genes are responsible for the unique irregular bullet shaped magnetite magnetosome found in RS-1 and other delta-proteobacterial MTB. Thus, I propose to use targeted mutagenesis and protein localization of *mad* genes and other hypothetical gene found in the magnetosome gene island (MAI) of *Desulfovibrio magneticus* RS-1. I plan to delete candidate genes from the genome of RS-1 and observe any change in phenotype of the bullet-shaped magnetite or change in their behavior in the presence of a magnetic field. I am also planning on tagging these candidate proteins to observe their localization within the cell to assess their role in synthesizing the irregular bullet-shaped magnetite in RS-1 and other delta-proteobacteria.

Effect of *amb0994* gene on the magnetotactic behavior in *Magnetospirillum magneticum* AMB-1

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Magnetotactic bacteria (MTB) are a diverse group of microorganisms capable of using geomagnetic fields for navigation. This magnetotactic behavior can help microorganisms move towards favorable habitats for optimal growth and reproduction. Amb0994 is proposed as a special MCP-like protein in *M. magneticum* AMB-1 which interacts with MamK and plays a key role in magnetotaxis. However, the construction of an in-frame deletion mutant of amb0994 has not succeeded in providing direct evidence to the involvement of Amb0994 in the active response to changes in the magnetic field. In this study, we adapted an engineered CRISPR-Cas9 system for efficient inactivation of genes in a widely used magnetotactic bacteria model strain, *Magnetospirillum magneticum* AMB-1. We successfully constructed an in-frame deletion mutant of *amb0994*. This mutant produces normal magnetosomes; however, its response to abrupt magnetic field reversals is faster than wild-type strain. This behavioral difference is probably a consequence of altered flagella function, as suggested with our dynamics simulation study by modeling *M. magneticum* AMB-1 cell as an ellipsoid. These data indicate that, Amb0994 is involved in the cellular response to magnetic torque changes via controlling flagella. These further enhance our understanding of the active sensing mechanism of magnetotaxis.

Biomimetic Synthesis of Magnetosome Based on Magnetotactic Protein Mms6

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The magnetosome is a special biomineralized product of Magnetotactic bacteria (MTB). It is characterized by a layer of biofilm encapsulated on the outside and internal ferrosoferric oxide (Fe_3O_4) nanocrystals with high Uniformities in size and morphology. Magnetotactic bacteria membrane protein Mms6 plays a crucial role in regulating the morphology of magnetosomes. Magnetosomes from magnetotactic bacteria have various advantages in comparison with the current industrialized magnetic nanomaterials in terms of stability, biocompatibility, and photo-electromagnetism. The biological magnetic nanomaterials like magnetosomes are expected to have broad applications in the future, however, factors such as the extremely complicated artificial cultivation of magnetotactic bacteria, long growth cycle and low yield, et al., have limited the magnetosome application. To synthesize high-quality, high-biocompatible and high-yield magnetosome-like product, we have optimized the existing thermal decomposition [1-2] methods combined with biomimetic factors like Mms6 proteins. The results show that the magnetosomes-like product we obtain not only have a uniform morphology and size (ca 9nm), which is extremely Similar to magnetosome, but also have excellent magnetic properties (Saturation magnetization up to 100emu/g Fe). We further apply this material to contrast agents, which have higher values of T1 (3.65$Mm^{-1}s^{-1}$) and T2 (191.7$mM^{-1}s^{-1}$).

1. Zhichuan Xu, etc. Chem. Mater.,2009,21,1778–1780
2. Tao Luo, etc. Chem. Commun., 2014,50,15952

Long term observation of a single magneto-tactic bacteria inside a microfluidic channel

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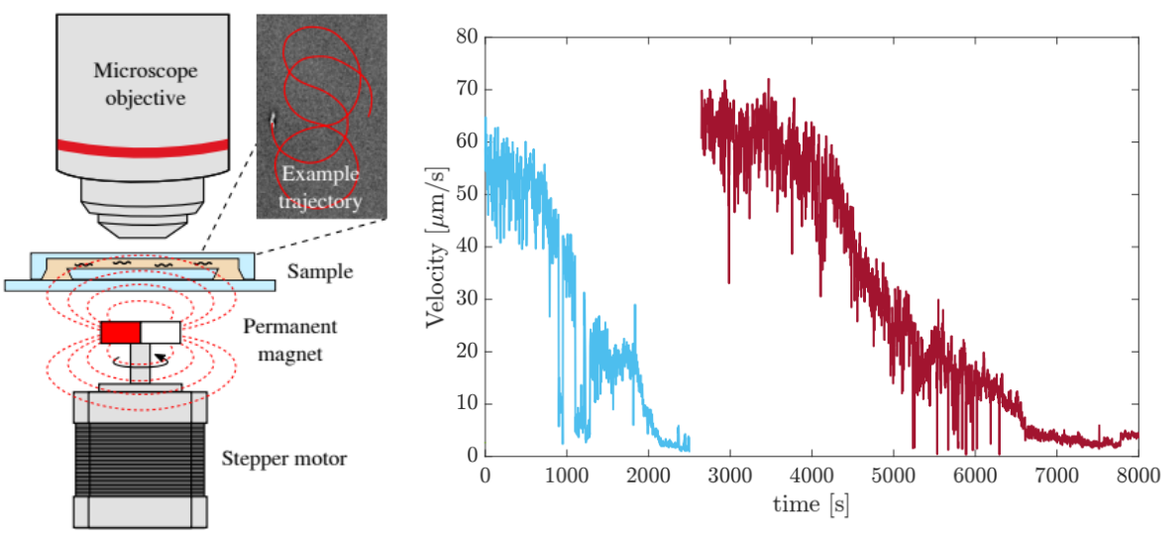
We measured the velocity of individual bacteria of type *Magnetospirillum gryphiswaldense* inside a microfluidic channel, and observed consistently that the bacteria under observation lose their velocity after about 10 minutes, to stop completely after 45 minutes. The observation was made possible by using microfluidic channels of only 5 µm height, so that the bacteria stay in the field of focus, and by making the bacteria swim in an eight-shaped pattern, with occasional manual correction. After coming to a stand still, the bacteria do not recommence their movement in the next hour. Within the period of a day, there were always new bacteria that could be observed. We speculate the decrease in velocity and halting is caused by photon damage to the bacteria, caused by to the high intensity of the microscope illumination. The experiment shows that MSR-1 can survive inside a microfluidic system for very long periods of time, but cannot be observed continuously for longer than 10 minutes.



Left: Microfluidic setup for long term observation of single MSR-1 bacteria. Right, velocity as a function of time for two bacteria. After approximately 10 minutes the bacteria velocity start to decrease, to stop completely after about 45 minutes.

Real time monitoring of magnetic properties of magnetotactic bacteria during culture growth

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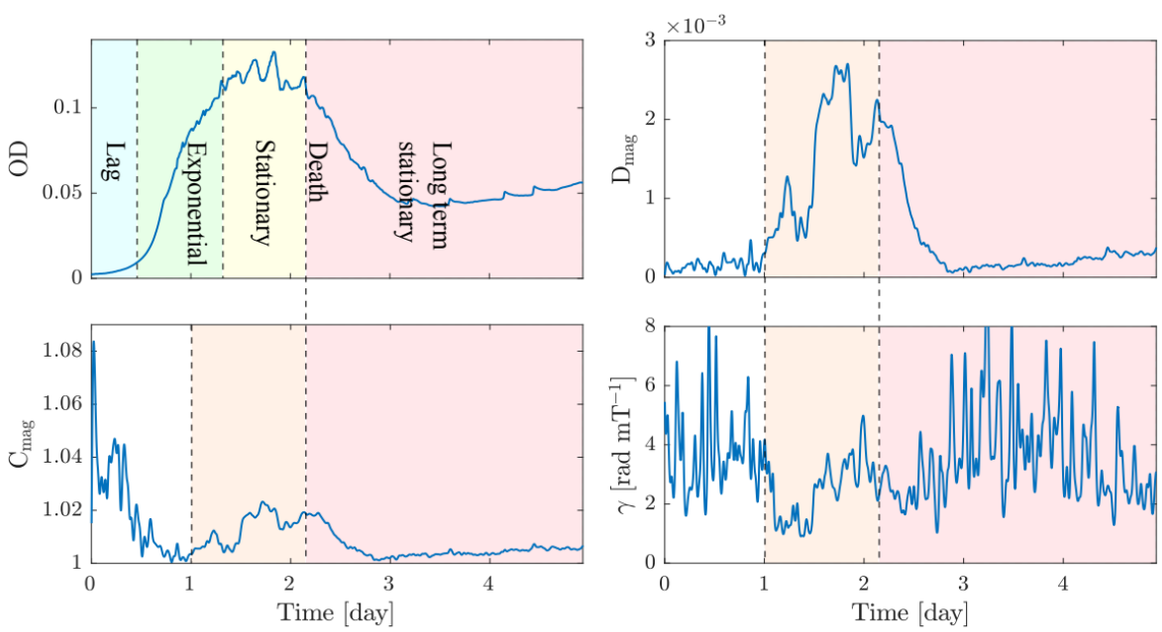
We monitored the real time growth behaviour and magnetic response of *Magnetospirillum Gryphiswaldense* (MSR-1) cells throughout cultivation over six days, in a novel automated, high speed optical density (OD) meter equipped with three sets of magnetic Helmholtz coils. The high data acquisition rate (20 Hz) allows us to monitor the real time rotation of the bacteria. The OD measurement reveals the bacterial growth phases of lag, exponential, stationary, death and long term stationary periods. Magnetic properties of the growing bacteria were detected in terms of C_{mag}, and the novel parameters D_{mag} (absolute OD of magnetic bacteria only) and γ (ratio between magnetic moment of the magnetosome chain and the rotational drag coefficient of the bacteria body).



Real time growth curve of MSR-1 cells showing the OD and different growth phases, ratio of magnetic to non-magnetic bacteria (C_{mag}), absolute value of magnetic bacteria (D_{mag}) and ratio between magnetic moment and rotational drag coefficient (□).

Surface functionalization of magnetotactic bacteria with streptavidin coated microparticles

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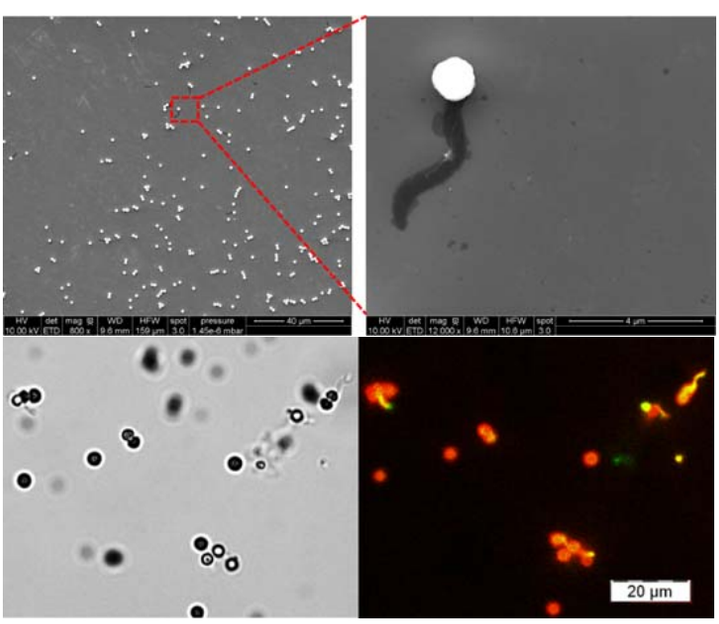
Magnetotactic bacteria which can be steered by an external magnetic field have attracted much interest as self-propelling drug carriers. In this study, *Magnetospirillum Gryphiswaldense* strain MSR-1 cells were first functionalized by biotinylation and then tagged with streptavidin coated magnetic micro particles of 1 µm in diameter. Magnetic particles attached to the bacteria via biotin-streptavidin interaction were collected with a magnet. Unbound bacteria were removed after the washing step and microparticle attached bacteria were resuspended in PBS. Cell viability tests were performed at each step of the coupling reaction in order to observe the effect of used agents on cell viability. Fluorescence microscopy and Scanning electron microscopy (SEM) analyses have demonstrated MSR-1 cells functionalized with microparticles.



Top: SEM images of biotinylated MSR-1 cells after attachment of streptavidin coated 1µm magnetic microparticles. Bottom: Fluorescence microscope images (bright field and green-red overlay) of MSR-1 cells after live/dead (SYTO9-green/Propidium iodide-red) staining following the coupling process (Scale bar = 20µm).

Exploring the Potential of MC-1 Magneto-tactic Bacteria as Carriers for Mucosal Drug Delivery

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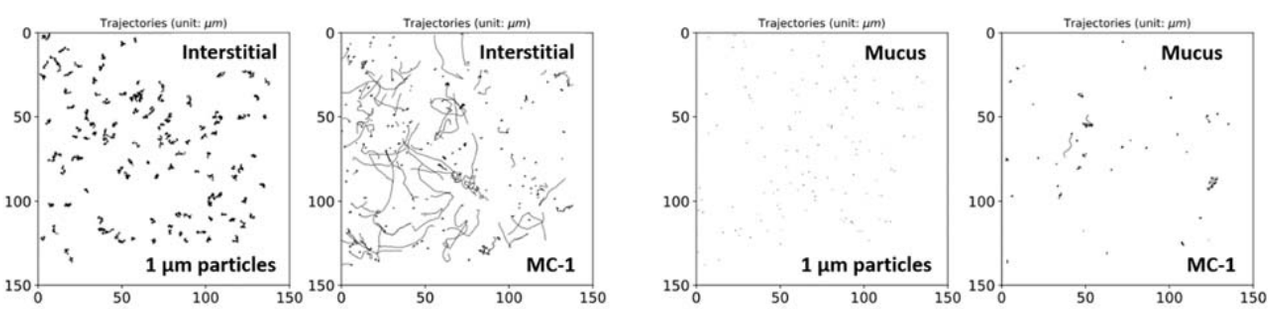
Mucus is a hydrogel with a three-dimensional network structure riddled with pores, which significantly limits the penetration of nanoparticle-based drug delivery systems. In this work, we wanted to elucidate whether the active motion and high velocity of the polar magneto-tactic bacterial strain of *Magnetococcus Marinus* MC-1 could be used to penetrate human tracheal mucus and serve for mucosal drug delivery purposes.

MC-1 were grown in agarose-containing medium for 5-6 days. Native pulmonary mucus was obtained from the tracheal tube of patients undergoing elective surgery and was characterized by classic rheology. To determine the viscosity of the fluid that fills mucus' pores (interstitial fluid), mucus samples were centrifuged at 21,000g for 1 hour and the supernatant was collected. One µm polystyrene spheres were then dispersed in the interstitial fluid and their Brownian motion was tracked by video-microscopy. The Stokes-Einstein equation was applied to determine the viscosity of the fluid. Lastly, independent MC-1 aliquots were mechanically dispersed either in interstitial fluid or in native mucus and tracked with video microscopy. The motion of the MC-1 was compared to the motion of polystyrene particles of an equivalent diameter (1 µm).

The viscosity of the interstitial mucus fluid at room temperature was 1.5±0.4 mPas. In this fluid, MC-1 bacteria showed unhindered trajectories and speeds up to 35 µm/sec (figure, Interstitial MC-1). If MC-1 were dispersed in native tracheal mucus, however, most bacteria appeared strongly hindered, with just few bacteria showing certain motility within mucus (figure, Mucus MC-1). Still, the motion of MC-1 in native mucus was superior compared to the size-matched polystyrene particles, which were completely immobilized (figure, Mucus 1 µm particles). Our preliminary results indicate that MC-1 can diffuse rather freely through the interstitial mucus fluid and deserve attention as potential self-propelling drug carriers.



Diversity of magnetotactic bacteria in surface sediments from Wudalianchi volcanic barrier lakes

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Magnetotactic bacteria (MTB), the members of sediment microorganisms, play an important role in geochemical cycles of iron and sulphur element. Here, we selected 8 volcanic lakes in Wudalianchi volcano, Northeast China. The physicochemical parameters and the MTB phylogenetic diversity of the surface sediments were analysed. The relationship between the MTB diversity and sediment environmental factors was investigated to reveal the environmental significance of MTB in sediments. Results indicated that the Proteobacteria phylum was dominant in all lakes, while the members of genus were significant differences in each lake. *Crenothrix* genus were dominant in YYP and YQH lakes, and their proportion were 14.83% and 20.62% in YYP and YQH lakes, respectively. *Nitrospira* (16.21%), *Magnetospirillum* (31.28%) were found to be dominant in WB and YC lakes, respectively. The dominant genus in EC and FC lakes were *Alphaproteobacteria*, and theirs abundance were 4.27% and 25.56%, respectively. *Staphylococcus* (19.33%) and *Methylophilaceae* (63.71%) were dominant in SC and WC lakes, respectively. The physicochemical analysis showed that total nitrogen, SO_4^{2-}, Fe^{2+}, salinity and nitrate nitrogen were significantly different in 8 volcanic lakes. PCA analysis shed light that total phosphorus, ammonia nitrogen were the main driven factor for MTB distribution. These data will provide more knowledge for MTB molecular geography in specific niche and the contribution of MTB in cycling of iron and sulphur in volcanic environments.

Keywords: Volcanic barrier lake; Wudalianchi volcanos; Magnetotactic bacteria; Phylogenetic diversity

The role of magnetite-associated protein in magnetite formation

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Biomineralization is a process which can be found in all kingdoms of life. This process usually helps the organisms to harden soft tissues by creating inorganic structures for their biological functions. It was shown that biominerals are under highly biological control that involves proteins in initiating nucleation and/or acting as structural skeletons. Magnetotactic bacteria (MTB) use iron biomineralization to create nano-magnetic particles in a specialized organelle, the magnetosome, to navigate through the geomagnetic field. Magnetosome formation can be divided into three major steps, membrane invagination, iron uptake, and magnetic particle biomineralization, which all involve a unique set of proteins. There is a specific set of magnetite associated proteins (MAPs) which are involved in regulating magnetite nucleation, and size and shape. These MAPs are all predicted to be small membrane proteins that contain particular sequences, between 17 to 22 amino acids long, involved in magnetite formation. To understand the mechanism, we focused on three different MAPs, MamC, Mms6 and Mms7 and studied their individual iron-binding sequences. The differences in the size and shape and the presences of peptides in the samples can have an effect on the width and the small variety in the g values (ESR measurements) and XRD-patterns. Our results constitute a complete picture of how different MAPs affect magnetite synthesis.

Trapping magnetotactic bacteria from natural environments

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Magnetotactic bacteria (MTB) represent a group of aquatic microorganisms that probably emerged billions years ago, as suggested by phylogenetic reconstructions or genetic studies (*e.g.* Lefèvre and Bazylinski, 2013; Lin *et al.*, 2017). MTB have organelles called magnetosomes that contain nanocrystals of magnetite, which may be preserved in the sedimentary record. In order to establish the timing of emergence of these bacteria and to distinguish their magnetites from abiotic ones, biosignatures of their magnetosomes have to be determined. Following the recent work by Amor *et al.* (2016), experiments are now led in the laboratory on another cultivated strain (*Magnetovibrio blakemorei* strain MV-1) to determine their Fe isotope signature by mass spectrometry. We also aim at extending the results to magnetite produced by environmental MTB. The main challenge is the recovery of hundreds of micrograms of magnetite nanocrystals to be able to analyze their compositions. In the present work, Lake Pavin, a meromictic lake in the French Massif Central, was selected as a natural sampling site. It is characterized by permanent anoxic and ferruginous deep water topped by oxic water, and can thus be regarded as a modern analog of Archean ocean. Lake Pavin hosts a high concentration and large diversity of MTB around the chemocline in the water column and in the sediments. We tested several MTB trap devices based on magneto or chemo-taxis properties of these bacteria. The recovery of MTB from sediments consists in two steps: (1) global magnetic concentration followed by (2) MTB separation from other bacteria and magnetic solid phases. Our study will aim at harvesting high concentrations of MTB, with high specificity of the traps while preserving MTB alive, dealing with large volumes in a short time, and avoiding iron oxidation and precipitation during the purification process.

References: Amor *et al.* (2016) *Science* 352, 705-708; Lefèvre and Bazylinski (2013) *Microbiology and Molecular Biology Reviews* 77, 497-526; Lin *et al.* (2017) *PNAS* 114, 2171-2176.

Biomimetic and Biokleptic Synthesis of Magnetic Nanoparticles and Arrays Inspired by Magnetic Bacteria

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The precise and consistent control over the size and shape of magnetic nanoparticles (MNPs) is critical for their reliable use in biomedical and nanotechnological applications. Furthermore, the ability to tailor these requirements under ambient environmentally friendly synthetic production is a key goal.

Magnetotactic bacteria produce highly monodispersed magnetosomes, with strict morphology conservation within each strain but a variety of shapes shown across different strains, showing a high degree of biological precision offered by protein control over the process. Here we report our research into understanding how these proteins control the nucleation and crystallisation of magnetite MNP. This understanding helps to inform our protein mediated chemical precipitation of precise MNP. Furthermore we have produced a range of mimics that are easier to express and purified. Thus, we are building up a "toolbox" of protein additives for tailored magnetite MNP synthesis. This has been extended to patterning these proteins on a surface to form precise MNPs on patterned arrays. Finally we have mimicked the formation of magnetosomes using liposomes and polymersomes and can now work towards combining these approaches to create an artificial magnetosome.

Investigation of Motility and Thermotaxis of *Magnetococcus marinus* (MC-1) as a Function of Environmental Stimuli

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Magnetotatic Bacteria (MTB), MC-1 exhibits great potential for targeted drug delivery as an alternative to existing drug delivery technologies, which show certain limitations to deliver efficient doses of chemotherapeutic drugs to the tumoral lesions.[1] As a polar MTB, MC-1 swims persistently in one direction along the magnetic field, ensuring higher swimming speed with an average velocity of approximately 200 μm/s (possibly max. speed of 300 μm/s).[2] For the optimum application of MC-1 in the context of targeted drug delivery, appropriate analysis of motility and swimming speed of MC-1 is essential. The swimming speed and behaviour of bacteria mainly result from the effect of temperature, which is the driving mechanism of bacterial thermotaxis.[3],[4] In this work, the motility and thermotaxis of MC-1 are investigated as a function of different environmental stimuli like temperature and media viscosity. To estimate the swimming speed and behaviour characteristics of MC-1 under the different environmental stimuli, a specific OD-meter and microfluidic system are developed and optimized. The effect of temperature on motility and swimming speed of MC-1 is specifically characterized under various physico-chemical environmental conditions. This information will provide essential information to the optimize the application of MC-1 for certain targeted drug delivery systems.

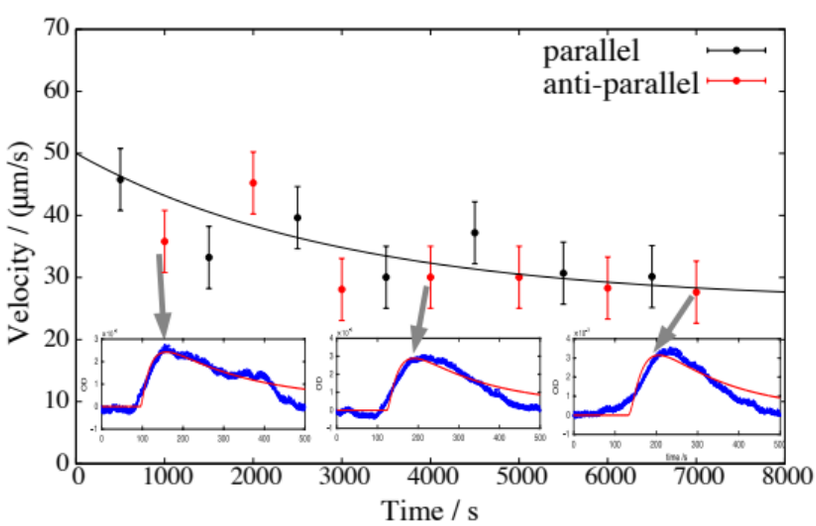

Figure: The most appearing velocity decreases from about 50 to 30 μm/s with increasing time

-The black points are MC-1 swimming along, the red opposite to the applied field.

-The bottom three graphs show the OD meter time traces for the 500 s experiments leading to the calculated data points, including time delayed log-normal fits.

Reference

[1] O. Felfoul, S. Martel; *Nature Nanotechnology* **2016**, 11, 941–947.
[2] S. Martel; *Experimental Robotics*, ISER 2010, December 18-21, **2010**, New Delhi, India, 775-784.
[3] K. Maeda, F. Ooswa; *Journal of Bacteriology* **1976**, 1039-1046.
[4] M. Demir, H. Salman; *Biophysical Journal Volume* **2012**, 103, 1683–169.

Artificial polarity-reversal of bacterial magnetic compass

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Magnetotactic bacteria synthesize nano-sized magnetic crystals, magnetosome, which function as tiny compasses allowing the microbes to navigate using the earth's geomagnetic field. Here, we applied the external magnetic fields to *Magnetospirillum magneticum* AMB-1 under an optical microscope, and quantitatively controlled their swimming direction at the single-cell level with magnetic tweezers. Additionally, the polarity of their swimming was artificially reversed by applying the quick reversal of the large magnetic field, indicating the polarity-reversal of their nano-compasses. We establish the novel technique to manipulate bacterial "taxis" independent from swimming motility, which can be applied to other bacterial motility with the labelling of isolated magnetosome.

Clustering of Magnetotactic Bacteria in a Microchannel

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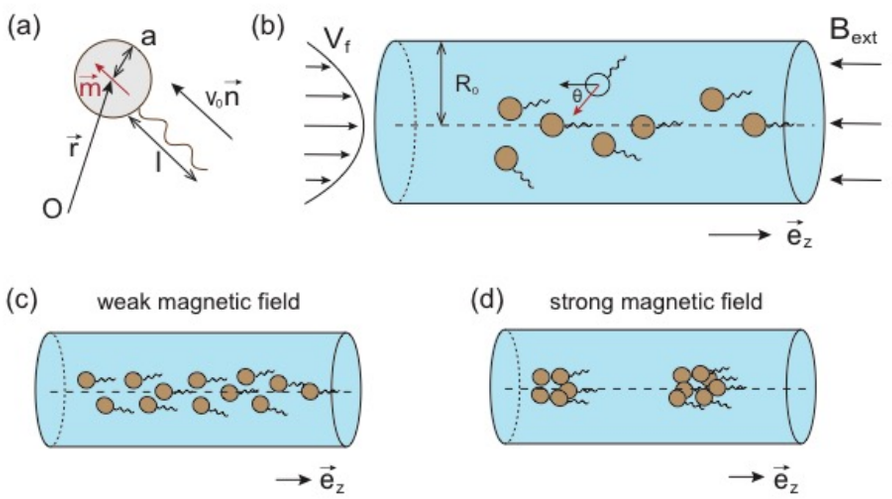

We report the collective behavior of magnetic swimmers, which are suspended in a Poiseuille flow and placed under an external magnetic field, using analytical techniques and Brownian dynamics simulations. We find that the interplay between intrinsic activity, external alignment, and magnetic dipole-dipole interactions leads to longitudinal structure formation [1]. Our work sheds light on a recent experimental observation of a clustering instability in this system [2].

[1] “Clustering of Magnetic Swimmers in a Poiseuille Flow”, F. Meng, D. Matsunaga, R. Golestanian, Physical Review Letters, volume 120 (18), 188101 (2018).
[2] “Destabilization of a flow focused suspension of magnetotactic bacteria”, N. Waisbord, C. T. Lefevre, L. Bocquet, C. Ybert, C. Cottin-Bizonne, Physical Review Fluids, volume 1, 053203 (2016).

The influence of physicochemical factors on magnetotactic bacteria affiliated with phylum *Nitrospirae* from different aquatic environments

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The objects of this research are 20 freshwater and saline aquatic ecosystems from different places in Europe and Asia. During this study the samples of water and sediments were collected and the microcosms were formed. After a few months incubation of microcosms, sediments and water samples were collected, physicochemical analysis and total genomic DNA extraction were conducted.

Using Illumina technology 2.1 million sequences of the V3-V4 region of 16S rRNA gene were obtained with length of 250 bp. Then, chimeric sequences and readings with poor quality were removed. In result 1.2 million sequences were obtained. After combining this readings with a similarity level of > 97% 20407 OTU were obtained.

Further, the reference database of currently known 16S rRNA gene sequences of MTB was created. In total, 1109 sequences were obtained from 52 literary sources. Comparison of 20407 OTU with a collected reference database with a cut-off of 5% and with help of additional BLAST check helped to obtain 112 OTU potentially belonging to MTB.

The majority of the MTB in freshwater ecosystems was represented by the bacteria of the phylum *Nitrospirae.* Correlation analysis was performed and, then, the factors that significantly affect the amount of MTB of phylum *Nitrospirae* were found.

The work was carried out using the scientific equipment of Core Research Facility “Bioengineering” with the support of Russian Federal Agency of Scientific Organizations (FASO) through grant 0104-2014-0203.

Freshwater magnetotactic coccus UR-1 from Uda river

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Magnetotactic cocci are the most observed morphotypes of MTB in aquatic habitats. In contrast to the marine cocci that had been cultivated, freshwater cocci have not yet been obtained in axenic culture. In this study, we were describing uncultured magnetotactic coccus UR-1. Samples were collected from freshwater sediments and MTB were magnetically enriched and purified. The diversity of MTB on this sample was investigated through the clonal library of 16S rRNA sequences. Phylogenetic analysis based on 16S rRNA gene showed that 87% of library formed dominant OTU and was affiliated with order *Magnetococcales*.

Transmission electron microscopy (TEM) observations had revealed that the majority of magnetotactic cocci had disorganized magnetosomes consisting of magnetite. Cell size was about 1 μm in diameter. Magnetosome crystals were 80±15 nm in length and 50±6 nm in width. The number of magnetosomes per cell ranged from 30 to 38.

Total genomic DNA was sequenced and genome of this coccus was reconstructed. The genes that encode proteins of magnetosome biomineralization and key metabolic pathways were identified.

The work was carried out using the scientific equipment of Core Research Facility “Bioengineering” with the support of Program of the Russian Academy of Sciences "Nanostructures: Physics, Chemistry, Biology, Technology Basics" (0104-2018-0058) and Russian Federal Agency of Scientific Organizations (FASO) through grant 0104-2014-0203.

Magnetospirillum kuznetsovii LBB-42 is novel magnetotactic spirillum.

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Magnetotactic spirillum was isolated from freshwater sediments of Lake Beloe Bordukovskoe (Moscow region). Strain LBB-42 biomineralizes the cuboctahedral magnetite magnetosomes 38 nm in size. Phylogenetic analysis of 16S rRNA gene revealed that new strain has a maximum identity with cultivated spirillum *Magnetospirillum marisnigr*i SP-1^T (97.1%). The genome of newly isolated bacteria was sequenced, it consists of 4411256 bp in 69 contigs with average G+C content of 63.4%. Average nucleotide identity (ANI) and digital DNA-DNA hybridization (dDDH) with closely related genomes of *Magnetospirillum* strain SP-1 were computed and identity appeared to be 83.12% and 25.2% respectively. The major fatty acids for the novel strain analysed were $C_{16:1}$ώ7c,$C_{16:0}$ and$C_{18:1}$ώ9. For this strain pH optimum was 5.8–6.6 and T optimum was 30 °C. LBB-42 demonstrated low tolerance of NaCl (<0.1 %). Strain LBB-42 grew under microaerophilic conditions with an oxygen concentration up to 16 % in the gas phase. Strain utilized short chain carboxylic acids: acetate, succinate, malate and lactate. On the basis of this evidences the newly isolated strain was designated as novel species of *Magnetospirillum* genus for which was proposed the name *Magnetospirillum kuznetsovii* LBB-42.

The work was carried out using the scientific equipment of Core Research Facility “Bioengineering” with the support of Program of the Russian Academy of Sciences "Nanostructures: Physics, Chemistry, Biology, Technology Basics" (0104-2018-0058)

Ecophysiology and biomineralization of magnetotactic bacteria as revealed by NanoSIMS and single-cell genomics

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Magnetotactic bacteria (MTB) biomineralize intracellular nanoscaled magnetic crystals (called magnetosomes) and use the geomagnetic field as a navigation system. Our knowledge about the ecophysiology and biomineralization of MTB remains limited due to the lack of axenic cultures. Nano-scale Secondary Ion Mass Spectrometry (NanoSIMS) is an ion probe technique combining high resolution microscopy with isotope analysis (Musat *et al*., 2012). It has been used to analyze the information of elements and isotopes composition of samples at submicron and nanometer scales. Single-cell genomics aims at obtaining the genome of a single cell from complex environmental samples. A combination of NanoSIMS and sing-cell genomics would provide a powerful tool towards investigation of uncultivated MTB. We have applied NanoSIMS to investigate the dynamics of carbon, nitrogen and iron assimilations in several uncultivated MTB populations at single-cell level. Our preliminary results confirmed the metabolic potential of these bacteria proposed by metagenomic analysis. Single-cell genomic analysis of these MTB are underway.

Reference:

Musat N, Foster R, Vagner T, (2012). Detecting metabolic activities in single cells, with emphasis on nanoSIMS. Fems Microbiology Reviews 36(2):486-511.

Targeted thermal therapy with genetically engineered magnetite magnetosomes@RGD: Photothermia is far more efficient than magnetic hyperthermia

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Providing appropriate means for heat generation by low intratumoral nanoparticle concentrations is a major challenge for cancer nanotherapy. Here we propose RGD-tagged magnetosomes (magnetosomes@RGD) as a biogenic, genetically engineered, inorganic platform for multivalent thermal cancer treatment. Magnetosomes@RGD are biomagnetite nanoparticles synthesized by genetically modified magnetotactic bacteria thanks to a translational fusion of the RGD peptide with the magnetosomal protein MamC. Magnetosomes@RGD thus combine the high crystallinity of their magnetite core with efficient surface functionalization. The specific affinity of RGD was first quantified by single-cell magnetophoresis with a variety of cell types, including immune, muscle, endothelial, stem and cancer cells. The highest affinity and cellular uptake was observed with PC3 prostatic and HeLa uterine cancer cells. The efficiency of photothermia and magnetic hyperthermia was then compared on PC3 cells. Unexpectedly, photothermia was far more efficient than magnetic hyperthermia, which was almost totally inhibited by the cellular environment. RGD targeting was then assessed in vivo at tumor site, in mice bearing PC3 tumors. As a result, we demonstrate that targeted magnetic nanoparticles could generate heat on a therapeutic level after systemic administration, but only under laser excitation, and successfully inhibit tumor progression.

Optimization of lysis of magnetotactic bacteria by ultrasound in order to automate purification of magnetosomes

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Magnetotactic bacteria synthesize nanoparticles surrounded by a biological membrane called magnetosomes. The biotechnological potential of these nanoparticles rivals that of magnetic nanoparticles produced chemically: we have indeed demonstrated their use as targeting contrast agents for MRI diagnostic[1] [2] and theranostics[3] . Such applications require the production and purification of large amount of functionalized magnetosomes from bioreactor cultures of genetically modified *M. magneticum* (AMB-1). The extraction and purification of these magnetosomes remain however tedious since they require a succession of manual steps including cellular lysis by French press and washes.

In this context, we designed a device allowing the automatization of the extraction and purification of these bacterial nanoparticles based on the ultrasonic lysis of bacteria associated with a magnetic magnetosome recovery system. The development of this system required many technical adjustments, such as the automatization of the washing and magnetic purification. We determined that a sonication at 160 watts during five minutes with pulses every five seconds is optimal for the lysis of 3 g of bacteria suspended in 30 mL of lysing buffer. In addition, we optimized the washing step in the magnetosome purification protocol with an additional light sonication step in bath at each wash.

The validation of this new process was performed with an approved quality control[2] [3]:

(1) The protein integrity and their functionalization were verified by western blot, SDS-pages and spectrometric techniques.
(2) The lipid composition of membrane was controlled by High-Performance Thin-Layer Chromatography (HPTLC).
(3) The iron dosage was carried out with Inductively Coupled Plasma- Optical Emission Spectrometry (ICP-OES).
(4) The magnetosome crystal structure was observed by Transmission Electron Microscopy (TEM).

Our automatic device appears to be more reproducible and less time consuming than commonly used manual methods.

1. Mériaux, S. *et al.* Magnetosomes, biogenic magnetic nanomaterials for brain molecular imaging with 17.2 T MRI scanner. *Adv. Healthc. Mater.* **4,** 1076–1083 (2015).
2. Boucher, M. *et al.* Genetically tailored magnetosomes used as MRI probe for molecular imaging of brain tumor. *Biomaterials* **121,** 167–178 (2017).
3. Plan Sangnier, A. *et al.* Targeted thermal therapy with genetically engineered magnetite magnetosomes@RGD: Photothermia is far more efficient than magnetic hyperthermia. *J. Controlled Release* **279,** 271–281 (2018).

pH imaging in living prokaryotic cells and organelles using pH-sensitive fluorescent protein

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Magnetotactic bacteria synthesize a uniform-sized and regularly shaped magnetite crystal in a bacterial organelle termed a magnetosome. The milieu of the magnetosome lumen must be regulated to mineralize magnetite crystals. Here, we established a method for measuring pH in the bacterial magnetosome organelle within a living *Magnetospirillum magneticum*AMB-1 cell using the pH-sensitive fluorescent protein E^2GFP. To estimate the pH of a single living cell, emission spectra excited at 488 nm were obtained from E^2GFP-fused-proteins expressed cells using a highly inclined and laminated optical sheet microscope equipped with a grating spectrometer. Cytosolic and periplasmic pH were estimated using E^2GFP and NapA$^{1\text{-}34}$-E^2GFP, respectively. According to the microscopic analyses, the pH values of the cytoplasm and periplasm were estimated to be 7.6 and 7.2, respectively. Moreover, we used Mms6-E^2GFP and MamC-E^2GFP to estimate pH in the magnetosome lumen and cytoplasmic surface of magnetosome, respectively. The pH in the magnetosome lumen differed depending on the growth phase, whereas the growth stage had no effect on that at the magnetosome surface. The pH in the magnetosome lumen showed significant increase during the exponential growth phase when magnetosome formation is highly activated.

Imaging of living bacterial cell surface structures using high-speed atomic force microscopy

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High-speed atomic force microscope (HS-AFM) is a useful technique for studying on molecular dynamics of bacterial cell surface in liquid media and buffers. The HS-AFM is capable of imaging structures of biological molecules with sub-millisecond temporal resolution and subnanometer spatial resolution. In the previous study, we observed dynamic diffusion of proteins in the outer membrane of living magnetotactic bacterial cells [1]. Here we applied this technique to observe biological phenomena occurring outer surface of bacterial cells. Recently, microbial ecological studies have highlighted the importance of the membrane vesicles (MVs) as a carrier that mediates bacterial cell-to-cell communication by transporting cellular components, including chemical signal molecules, protein and DNA. MVs are released from cells to extracellular milieu and are thought to directly fuse on a recipient cell surface. However, the fusion process of MV to the recipient cell surface never have been imaged, yet. We imaged the MV binding processes on *Paracoccus denitrificans* living cell surface. In this poster, we will introduce the advantage of HS-AFM for studies on molecular dynamics in the living bacterial cell surface.

[1] H. Yamashita, A. Taoka, T. Uchihashi, T. Asano, T. Ando, Y. Fukumori, Single-molecule imaging on living bacterial cell surface by high-speed AFM. **J. Mol. Biol.** 422:300-309, 2012.

Characterization of MamK polymerization using high-speed atomic force microscopy

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The intracellular positioning of magnetosome is important for magnetotactic bacteria to sense the geomagnetic field. In *Magnetospirillum magneticum* AMB-1 cells, magnetosomes are aligned linearly along the long axis of the cell by cytoskeletal filaments consist of MamK actin-like protein. Toro-Nahuelpan et al. reported that FRAP analysis revealed that MamK in cell have dynamics depend on the MamK-ATPase activity [1]. Moreover, live cell imaging clarified the MamK-ATPase activity is required for anchoring magnetosomes [2]. In this study, we observed dynamic MamK polymerization process using high-speed atomic force microscope (HS-AFM) *in vitro.* The HS-AFM is capable of imaging structural dynamics of proteins with nano-meter spatial resolution in buffer conditions. Thus, HS-AFM is useful technique to characterize dynamic process of MamK polymerization at single molecular resolution. Polymerization of MamK filament had a polarity. The elongation speed of the plus end was faster than the minus end. Furthermore, we found that the MamK filament performs a sliding movement called a treadmilling.

[1] M. Toro-Nahuelpan, F. D. Müller, S. Klumpp, J. M. Plitzko, M. Bramkamp, D. Schüler, Segregation of prokaryotic magnetosomes organelles is driven by treadmilling of a dynamic actin-like MamK filament. **BMC Biol.** 14:88, 2016.

[2] A. Taoka, A. Kiyokawa, C. Uesugi, Y. Kikuchi, Z. Oestreicher, K. Morii, Y. Eguchi, Y. Fukumori, Tethered magnets are the key to magnetotaxis: direct observations of *magnetospirillum magneticum* AMB-1 show that MamK distributes magnetosome organelles equally to doughter cells. **mBio** 8:e00679-17, 2017.

Subcellular localization of MamQ in *Magnetospirillum magneticum* AMB-1

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Magnetosome vesicle forms by invagination of cytoplasmic membrane. According to genetic experiments, the several membrane proteins, e.g. MamQ, MamI, MamL, MamE, and MamB, are reported as essential proteins for magnetosome vesicle formation. However, the molecular basis of magnetosome vesicle formation remains elusive due to lack of information about characteristics of these proteins, including subcellular localization, biochemical properties and protein-protein interaction. Here we focused on MamQ protein. We constructed *Magntospirillum magneticum* AMB-1 cells expressing each of MamQ-GFP, MamI-GFP, and MamC-GFP proteins and compared the cellular localizations of these membrane proteins using live-cell fluorescence imaging. The localization patterns of GFP-fused MamQ, MamI, and MamC proteins were differed. MamI-GFP and MamC-GFP showed magnetosome localizations as linear fluorescence patters along long axis of the cells, while MamQ-GFP fluorescence signal was detected from the entire of cell body. We fractionated cellular compartments of MamQ-GFP expressing cells into magnetosome, nonmagnetic membrane, and soluble fractions. According Immunoblotting of cellular components showed that MamQ-GFP localized in both of magnetosome and nonmagnetic membrane fractions. The nonmagnetic membrane localization of MamQ suggest that MamQ related the initial step of membrane invagination for magnetosome formation.

Live-cell imaging of flagellar rotation during magnetotactic motility

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Magnetotactic bacteria possess a prokaryotic organelle called magnetosome to swim along the geomagnetic field for facilitating their search for microaerobic environment. The detailed flagellar rotation in magnetotactic bacterial cell during magneto-aerotaxis is not revealed. *Magnetospirillum magneticum* AMB-1 is an amphitrichous bacterium which has a single flagellum on each end of the cell. In this study, we imaged individual polar flagellar rotations of swimming AMB-1 cells in microaerobic environment. For fluorescence imaging of flagellar rotation, we labelled both flagella using Alexa Fluor 488 or Qdot. We placed the cells in a chamber slide and observed flagellar motility by highly inclined and laminated optical sheet (HILO) fluorescence microscopy to clarify the mechanism of magnetotactic motility. The lagging flagellum showed counter clockwise (CCW) rotation during swimming, while the leading flagellum showed clockwise (CW) rotation. During observation, we found a portion of swimming cells using single flagella. The averaged swimming speed of these cells, which used either the leading or the lagging flagellum, was about two-fifth of that of cells using both flagella, indicating that both of leading flagellum and lagging flagellum generate the power of propulsion. These results implied that both of polar flagella propel the cell forward in a coordinated manner. Furthermore, we will image swimming cells using high-speed camera to examine the rotation speed and the switching frequency of both leading and lagging flagella during magneto-aerotactic motility.

Functional analyses of MamJ which is cytoskeleton associating protein for magnetosome positioning

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Magnetosomes are aligned in the center of the cell along the MamK cytoskeleton. MamK is an actin-like cytoskeletal protein, which is required for maintaining the linear arrangement of magnetosomes. On the other hand, MamJ is also involved in magnetosome chain formation, and is known as MamK-binding protein. *In vivo* studies suggested that the dynamics of MamK filament, such as treadmilling, is necessary for the arrangement of magnetosomes. In addition, it was reported that MamJ is necessary for dynamics of MamK cytoskeleton in the cells. However, the biochemical function of MamJ for MamK cytoskeleton has not been elucidated. In this study, we investigated the protein-protein interaction between MamJ and MamK *in vitro.* First, we purified recombinant MamJ and MamK from *Escherichia coli*. Second, we tested the interaction of MamJ protein and polymerized MamK filaments by pelleting assay. As a result, MamJ co-precipitated with MamK filament which were polymerized in an ATP-dependent manner. We demonstrated, for the first time, MamJ interacts with the polymerized MamK filaments *in vitro*. Currently, we are investigating the effects of MamJ on ATPase activity and dynamics of MamK filaments.

Modification of Phospholipid Composition of Magnetosomes by Employing Phosphatidylcholine Synthase in Magnetotactic bacteria

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Magnetospirillum magneticum AMB-1 synthesizes magnetic nanoparticles (magnetosomes) covered with a phospholipid bilayer, which contains a number of proteins. We have developed techniques for functionalization of proteins on magnetosomes via gene fusion techniques; this approach is referred to as the “magnetosome-display system.” Surface modification of magnetosomes through protein engineering contributed to efficient bioassay. Here, we have newly developed a novel technique for *in vivo* modification of phospholipid composition of magnetosomes by controlling the lipid metabolic pathway. The membrane phospholipid composition was modified by introducing phosphatidylcholine (PC), which is abundant in human cells but is not contained in *M. magneticum* synthases genes. In this study, we utilized two enzymes—a PC synthesis gene (*pcs*) derived from *Legionella pneumophila* and a phospholipid N-methyltransferase gene (*pmt*) derived from *Azospirillum brasilense*—for PC synthesis via two different pathways. Subsequently, the membrane lipid composition of the *pcs* or *pmt* introduced transformant strains, which were analyzed through thin layer chromatography (LC-MS). The findings revealed that PC was detected from both whole cells and magnetosome membrane in both transformants. Moreover, the transformants harboring the plasmid with *pcs* produced a higher level of PC in the cells. We have successfully established a technique for modification of phospholipid composition of magnetosomes *in vivo* that will be useful for constructing various phospholipid composition magnetosomes for a range of bioassays.

Functional Expression of Transmembrane Receptors on Magnetic Nanoparticles Through a Magnetosome-display System

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Magnetic nanoparticles produced by *Magnetospirillum magneticum* AMB-1 are covered with a lipid membrane. It is possible to display various proteins on the surface of the magnetosomes via gene recombination technology. In this technology, proteins can be expressed on magnetosomes by introducing a vector containing the genes of the target protein and an anchor protein. By introducing the tetracycline expression induction system into the vector, it is also possible to express proteins that are difficult to express such as transmembrane receptors. We call this approach a “magnetosome-display system.” Through the application of this system, a complex of proteins and magnetosomes can be easily obtained, so its use in drug screening and disease diagnosing is expected. In this study, human thyroid stimulating hormone receptor (TSHR)—one of the transmembrane receptors belonging to the G-protein coupled receptor (GPCR) superfamily—was fused with the magnetosome-localized protein Mms13. Full-length, transmembrane TSHR was successfully overexpressed on lipid-bilayer surfaces of *M. Magneticum* AMB-1 magnetosome using a tetracycline-inducible system. The ligand (TSH) and autoantibody (M22) binding activity was obtained even though the affinity was somewhat lower than for TSHR expressed in human cell membrane. We also investigated TSHR expression on modified lipid-bilayer surfaces of magnetosome using transformants by introducing phosphatidylcholine (PC), which is abundant in human cells but is not contained in *M. magneticum* AMB-1 synthases genes. We then compared the ligand binding activity depending on various phospholipid compositions. Complexes of TSHR and magnetosomes have potential applications not only as diagnostic tools but also in functional analyses of ligand or autoantibody-receptor interactions.

Iron uptake and magnetosome formation abilities in MAI deletion mutant and genetically modified strain carrying multiple sets of MAI genes

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Magnetotactic bacteria contains a common genomic region, magnetosome island (MAI), which consists of several gene operons, such as *mms6* operon, *mamGFDC* operon, and *mamAB* operon. We obtained MAI-deletion mutant strain (ΔMAI), and a genetically modified strain carrying two sets of *mamAB*, *mamGFDC* and *mms6* operons (2×G6A) of *Magnetospirillum magneticum* AMB-1. In this study, iron uptake and magnetosome formation abilities of these strains were analyzed and compared with the wild type of strain AMB-1. ΔMAI strain produced no magnetosomes as reported previously. 2×G6A strain produced approximately 3 times of magnetosomes compared with the wild type strain. On the other hand, iron measurement in media revealed that ΔMAI, 2×G6A and wild type strains uptake approximately same amounts of iron. These observations indicate that the genes in MAI mainly contributes for the magnetite synthesis, but not for the iron uptake ability of the cells.

Adsorption study of Mms6 and Mms7 to iron oxide magnetic nanoparticles

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Mms6 and Mms7 proteins were isolated from the surface of magnetic nanoparticles from *Magnetospirillum magneticum* AMB-1. These proteins were shown to be responsible for morphological control of the magnetic nanoparticles. In previous studies, magnetic nanoparticles synthesized with Mms6 suggested direct interaction of Mms6 with the surface of the magnetic nanoparticles. In this study, in order to evaluate the direct interaction of Mms6 and Mms7 with magnetic nanoparticles, we performed an *in vitro* adsorption study using their recombinant proteins. Non-magnetosomal proteins were also employed for comparison. Mms6 and Mms7 adsorbed to magnetic nanoparticles, while other proteins did not adsorb to the particles. The result indicates a presence of direct interaction of Mms6 and Mms7 with magnetic nanoparticles.

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Time table of MTB 2018 meeting

10-Sep		11-Sep		12-Sep		13-Sep		14-Sep	
Monday		Tuesday		Wednesday		Thursday		Friday	
		Molecular analysis		Physico-chemical characterization		Ecology and application			
				8:45	R. Zarivach	8:45	J. Kirschvink	8:30	Excursion
		9:00	Opening remark	9:10	A. Pohl	9:10	V. Busigny		
		9:10	D. Schüler	9:28	M. Amor	9:28	R.J. Harrison		
		9:35	M. Dziuba	9:46	R. Uebe	9:46	A.P. Roberts		
		9:53	M. Schüler	10:04	J. Werckmann	10:04	P. Liu		
		10:11	R.P. Awal	10:22	Coffee break	10:22	Coffee break		
		10:29	Coffee break	10:45	A. Arakaki	10:45	K. He		
		10:50	Y. Fukumori	11:10	T. Yoshino	11:03	K. Suthindhiran		
		11:15	A. Taoka	11:28	A. Fernandez-Castane	11:21	J. Liu		
		11:33	E. Günther	11:46	E. Duprat	11:39	X. Qian		
		11:51	T. Prozorov	12:04	H. Hamamura	11:57	P. Leão		
		12:09	N. Karen-Khadmy	12:22	Lunch	12:15	Group photo		
		12:27	Lunch			12:30	Lunch		
		14:30	A. Komeili	14:30	D. Faivre	14:30	D. Kisailus		
		14:55	C. Grant	14:55	S. Klumpp	14:55	J. Li		
15:00	Reception and registration	15:13	J. Wan	15:13	A. Codutti	15:13	W. Lin		
		15:31	Q. Sun	15:31	L. Abelmann	15:31	W. Zhang		
		15:49	M. Tanaka	15:49	M.L. Fdez-Gubieda	15:49	Y. Konishi		
16:00		16:07	D.S. Grouzdev	16:07	M. Charilaou	16:07	Break		
		16:25	M. Uzun	16:25	A.R. Muxworthy	16:20	S. Staniland		
		16:43	C.L. Monteil	16:43	A. Garcia-Prieto	16:38	J. Wang		
17:00		17:01	Poster session 1	17:01	Poster session 2	16:56	J. Tian		
						17:14	D. Trubitsyn	17:00	
						17:32	E. Alphandery		
						17:50	M. Muthana		
18:00						18:08	Business meeting		
						18:25	Closing		
		18:30		18:30		18:30			
19:00		19:00	Dinner 1	19:00	Dinner 2	19:00	Banquet		
20:00									
21:00		21:00		21:00		21:00			