Magnetotactic bacteria: magnetism beyond magnetosomes

Carlos Marcuello, Lélia Chambel, Mário S. Rodrigues, Liliana P. Ferreira, Maria Margarida Cruz

***Abstract*— Magnetotactic bacteria are a group of organisms deeply studied in the last years due to their interesting magnetic behavior and potential applications in nanometrology, hyperthermia and biosensor devices. One intrinsic common characteristic is the presence, inside the bacteria, of magnetic nanoparticles called magnetosomes. The role of magnetosomes as bacterial tools to orient the bacteria and find new habitats is universally accepted, but the way they develop still is not fully understood. A strain of *Magnetospirillum magnetotacticum* was grown and investigated at the nanoscale using transmission electron microscopy and atomic/magnetic force microscopy techniques. Magnetosomes were observed as well as long filaments with magnetic response that could be associated to the actin-like filaments being crucial to allow the nanoparticles orientation and magnetosomes formation. To the best knowledge of the authors, this study is the first to visualize these reproducible long-range size magnetic crystalline structures.**

***Index Terms*— actin-like filament, AFM, magnetosome, *Magnetospirillum magnetotacticum*, MFM.**

I. Introduction

MAGNETOTACTIC bacteria (MTB) have intracellular chains of nanometer-sized membrane-enclosed magnetic particles called magnetosomes, composed by magnetite (Fe_3O_4) or greigite (Fe_3S_4) crystals [1-4]. They orient and move along the earth geomagnetic field lines searching the deepest areas of aqueous reservoirs where the magnetic field helps MTB to locate at the oxic-anoxic interface within chemical gradients. Internal redox state of MTB plays a crucial role in the bacteria movement. In deficit of electrons, MTB allocate at the bottom part to compensate the inner redox state. In the opposite condition, MTB move towards higher oxygen concentrations indicating a magneto-aeorotaxis movement [3,5-8]. The orientation and organization of the magnetosomes in chain(s) is determinant to associate a magnetic dipole to the bacteria and thus, promoting magnetotaxis.

Crystalline properties of magnetosomes make them very attractive for industrial applications such as contrast agents in magnetic resonance imaging or nano-heaters in hyperthermia treatments [9]. In the latter case, heating efficiency displayed by magnetosomes is reported to be higher than the one characterizing the nanoparticles prepared by chemical synthesis protocols [10,11]. Since the competitive chemical procedures yield wide-range of nanoparticle distributions [12,13] and smaller heating efficiencies, it became appealing to use the MTB as bioreactors to produce high quality magnetic nanoparticles for medical purposes.

In the last decades, considerable volume of research has been carried out to investigate the morphology and function of magnetosomes in MTB and how they organize and divide [6,14-19]. The knowledge of the molecular mechanisms of membrane remodeling, protein sorting, cytoskeletal organization, iron transport, and biomineralization that lead to the formation of a functional magnetosome organelle is also of extreme importance [1,20-22]. Regarding magnetosome chain formation some studies revealed strands of equivalent dimensions to actin-like filaments beside the magnetosome chain [15,23,24]. One of the genes (*mamK*) in the magnetosome gene island encodes a member of the bacterial actin-like family of proteins [25-27] and MamK may act as a structural scaffold for the organization of the magnetosome chain as a magnetosome-associated cytoskeletal filaments [24]. The actin-like MamK protein was reported to have a significant role being required for the suitable magnetosome chain positioning and segregation [15,22].

These are times of intensive pursuing and understanding the unique MTB mechanisms. Studies of MTB magnetic behavior have been reported [11,28] but there is still lack of information at the nanometer scale. Only few reports are focused on single molecule level studies using techniques such as Transmission Electron Microscopy (TEM), optical magnetic imaging [29] or Scanning Electron Microscopy (SEM) [30]. Atomic Force Microscopy (AFM) is an excellent technique to acquire morphological information at nanometer scale [31]. Sample external surface is scanned with a sharp oscillating tip mounted on a flexible cantilever and depending on AFM tip properties different information can be gathered. Using Magnetic Force Microscopy (MFM) mode new insights about

Manuscript received August 29, 2018; accepted October 8, 2018. This work was supported by Portuguese FCT foundation through projects UID/MULTI/04046/2013 and and Integrative Sciences (BioISI) under P0d project. *(Corresponding author: Carlos Marcuello).*

C. Marcuello was with Biosystems and Integrative Sciences (BioISI), Faculdade Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa. Portugal. He is now with Institute National de la Recherche Agronomique (INRA). 51100, Reims France (e-mail: cmangles@fc.ul.pt).

L. Chambel, M. S. Rodrigues and M. M. Cruz are with Biosystems and Integrative Sciences (BioISI), Faculdade Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa. Portugal (e-mail: lmchambel@fc.ul.pt; mmrodrigues@fc.ul.pt; mmcruz@fc.ul.pt).

L. P. Ferreira is with with Biosystems and Integrative Sciences (BioISI), Faculdade Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa. Portugal and Department of Physics. University of Coimbra. 3004-516. Coimbra. Portugal (e-mail: lmferreira@fc.ul.pt).

Digital Object Identifier TNB-00138-2018.

This article has been accepted for publication in a future issue of this journal, but has not been fully edited. Content may change prior to final publication. Citation information: DOI 10.1109/TNB.2018.2878085, IEEE Transactions on NanoBioscience

magnetosomes were obtained [16,30,32]. In this work, the magnetic properties of magnetosomes and structures that may correspond to MamK filaments in the magnetotactic bacteria *Magnetospirillum magnetotacticum* (DSMZ 3856^T) are interrogated using AFM/MFM together with information from TEM technique. To our knowledge it is the first time that the magnetic filaments are directly observed.

II. MATHERIAL AND METHODS

A. Bacterial growth and magnetosome isolation

Bacterial strain. *Magnetospirillum magnetotacticum* strain DSMZ 3856^T was purchased from DSMZ (Deutsche Sammlung von Mikro-organismen und Zellkulturen, Brunswick, Germany).

Culture medium and growth conditions. The culture medium was prepared according to DSMZ (380 *Magnetospirillum* Medium) and distributed in glass flasks (65 mL in 160 mL total volume). To generate microaerophilic conditions, flasks were sealed before autoclaving with butyl-rubber stoppers under a microaerophilic gas mixture containing 1% O_2 in 99% N_2. An inoculum of 10% of the culture volume was inoculated by injection through the stopper. Cultures were incubated at 30ºC for *ca* three months in static conditions. After incubation three different samples were obtained: a bacterial population sample (S.MTB); an enriched sample of bacteria with magnetosomes (ES.MTB) and a sample of magnetosomes recovered after cells lysis and magnetosomes wash (M.MTB).

Bacterial population sample (S.MTB). A small volume used for microscopy analysis was harvest directly from the incubation vessel or as in most experiments the volume of several flasks was combined and cells were concentrated by centrifugation at 21 600 x g for 10 min.

Enriched sample of bacteria with magnetosomes (ES.MTB). After incubation and cells concentration by centrifugation, a neodymium magnet (Nd-Fe-B) was placed in contact with an outer side wall of the flask until a visible number of cells was obtained close to the magnet. The medium and bacteria not attracted to the magnet were eliminated. The magnet was removed and bacteria were collected in a small volume of buffer.

Magnetosomes recovery from bacterial cells (M.MTB). The cells were harvested by centrifugation at 21 600 x g for 10 min. The supernatant was removed and the cells were re-suspended in 3 mL of Tris–HCl buffer, pH 7.4. The cellular suspension was sonicated for 20 min at 30 W in order to lyse the cells and collect the magnetosomes. After sonication, the suspension containing the magnetosomes was separated from the cellular debris using a neodymium magnet (Nd-Fe-B) placed in contact with an outer side wall of the tube. Almost immediately a dark mass is visible and the supernatant containing the cellular debris and other organic molecules was eliminated. The magnetic material remains in the tube. Magnetosomes were washed 10–20 times in deionized water and re-suspended in deionized water.

B. Transmission Electron Microscopy

The different bacterial samples (S.MTB and ES.MTB) and magnetosomes (M.MTB) were characterized by Transmission Electron Microscopy (TEM) using a Hitachi H8100 with digital image acquisition microscope. The magnetosome size distributions were determined statistically from the size measurements of more than 100 nanoparticles using the ImageJ software.

C. Atomic Force Microscopy/ Magnetic Force Microscopy

The bacterial samples (ES.MTB) and magnetosomes (M.MTB) were analyzed by Atomic Force Microscopy (AFM) and Magnetic Force Microscopy (MFM). For AFM imaging the bacteria immobilization in the flat mica was carried out in two different ways: 1) the liquid media containing the bacteria was allowed to dry in the laboratory ambient conditions or 2) the drying process was done under an applied magnetic field parallel to the mica surface. To perform AFM measurements without dragging biomaterial molecules with the tip, sample-nanoflat surface immobilization is a vital step [33]. This requirement was achieved using cleaved silica wafers providing atomically flat surfaces, obtained from mica disks, purchased from Agar Scientific, on top of which 10 µL of sample were deposited, left to incubate for 30 min at room temperature, rinsed with ultrapure water and dried with Nitrogen flow. AFM images were acquired in dynamic mode, using an Agilent 5500 AFM setup, equipped with AC picoLE Molecular Imaging mode controller. Amplitude modulation AFM excites the lever with constant amplitude at the resonance frequency. To ensure sample homogeneity several images were acquired in different regions with different scan sizes. Raw data images were analyzed using Gwyddion software [34]. For MFM measurements, AFM tips with external cobalt-chromium (Co-Cr) coating and nominal radius of 60 nm, with resonance frequencies of 75 kHz and spring nominal constants of 2.8 N/m, were used. Both AFM/MFM probes were purchased by MikroMasch (NanoandMore). Lift heights from 60 nm to 300 nm were tested for each relevant feature. Cross section profiles with sub-nanometer accuracy resolution allowed achieving complementary height profiles.

III. EXPERIMENTAL RESULTS

Transmission Electron Microscopy and Atomic Force Microscopy imaging techniques were used to characterize magnetotactic bacteria. In Fig. 1 TEM images of *Magnetospirillum magnetotacticum* (DSMZ 3856^T) with the corresponding flagella and one bacterium with a magnetosome chain are shown. Since flagella are a fragile part of the bacteria, a considerable number of them breaks during sample preparation and frequently bacteria are visualized by TEM and AFM images with lack of this organelle. Also based in the analysis of several TEM images it was possible to conclude that only 10% of the bacteria from sample S.MTB include magnetosome chains. In the enriched sample of bacteria with magnetosomes (ES.MTB) they were observed in most of the cells. From TEM and AFM images DSMZ 3856^T bacteria size

This article has been accepted for publication in a future issue of this journal, but has not been fully edited. Content may change prior to final publication. Citation information: DOI 10.1109/TNB.2018.2878085, IEEE Transactions on NanoBioscience

was estimated between 1 and 2 µm long ranging from 500 to 700 nm wide (Fig. 2).

AFM imaging analysis of the two different bacteria attachment procedures allowed us to turn evident the structures of the cells with magnetic behavior. Only magnetic structures exhibited differences regarding bacteria immobilization method. To further investigation of the nature, morphology and magnetic response from magnetic components of *M. magnetotacticum* DMSZ 3856^T, AFM/MFM experiments were carried out after bacteria lysis (M.MTB) and dried under an external magnetic field. Sonication is a physical step where ultrasonic frequencies are exerted leading energy in order to promote the breakage of the cells. Magnetosomes clusters and long rod-shape nanometer size filaments were observed by AFM (Fig. 3A). These long structures are compatible with actin filaments in terms of length and diameter. These long rod-shape structures are aligned parallel adopting a preferable orientation in the magnetic field indicating their magnetic nature. Under amplification (Fig. 3C) the structures are found to be overlays of well-packaged narrow rods with thickness around 42 nm, corresponding to 24 nm after correction for tip size convolution effect [35]. They are extremely flat in consonance with an arrangement of crystalline structures having 1.7 nm as the smallest height observed. The long structures share similar morphology with the actin filaments observed in magnetic bacteria [15,18].

MFM images for the same sample show the existence of small nanoparticles with heights ranging from 1.5 to 9.0 nm (Fig. 4A) that display a magnetic signal for lift heights of 75 nm (Fig. 4B) and that the long structures (Fig. 4C) are not the result of randomly aggregation of simple nanoparticles. This data is in consonance with wild-type *Magnetospirillum* cells where filament bundles have approximately 6 nm in width. The width values (~1.5 nm) are compatible with the thickness reported in our study. After application of an external magnetic field, small filaments orient towards magnetic lines packaging among others and thus, enlarging their lateral dimensions. These structures present a magnetic signal at a lift height of 60 nm, along most of their length (Fig. 4D). To rule out the fact that the observed structures may be due to any compound present in the bacteria growth medium, AFM and MFM tests with non-inoculated medium were carried out and no features similar to those presented in Fig. 3 and Fig. 4 were observed.

In Fig. 5 the AFM and MFM images show that magnetosomes are clearly seen as chains of nanoparticles with average height of 20 nm and lengths above 800 nm (Figs. 5A and 5C) and very different from the long strands observed and described before. For the magnetosomes the different crystalline blocks that constitute the chains are clearly identified. The images are similar to previously reported results for magnetosomes immobilized on silicon wafer substrates [30]. MFM revealed a weak contrast working with a lift height which ensures only magnetic response discharging non-desirable AFM tip-sample van der Waals interactions (Figs. 5B and 5D). Magnetosomes from DMSZ 3856^T exhibit a weak magnetic signal explained by the biological membrane presence and its effect in the surface magnetic moment orientation.

IV. DISCUSSION AND CONCLUSIONS

Magnetospirillum magnetotacticum strain DSMZ 3856^T was studied from the point of view of the integrated magnetic structures. In intact bacterial cells magnetosomes are evident (Fig. 1). After bacterial lysis in conditions that most of the magnetosomes biological membrane do not break, retaining their organization in chains, clustering is observed due to the magnetic interaction (Figs. 5A and 5C). Also individual nanoparticles from broken chains can be observed in AFM images most probably due to the fact that in some chains the membrane as well as the interaction with the actin filament that works as a cytoskeletal-like structure was destroyed with cell lysis. (Figs. 3A, 3B, 4A and 4C). Magnetosome chains are also clearly identified in AFM and MFM images (Fig. 5). The weak magnetic signal detected for the magnetosomes is attributed to the attenuation of the magnetic signal recorded by the AFM coated tip due to the organic nature of the membrane phospholipids and fatty acids and to the interference of the membrane in the magnetic moment orientation. This last effect was also visualized in other biological systems as ferritin which works as scaffold for non-magnetic ferrihydrite nanoparticles storage [36].

Besides the well-known magnetosomes, AFM and MFM results indicate that other magnetic features exist in the magnetotactic bacteria. After bacteria lysis and recovery of the magnetic constituents using a magnet, long and flat stranded structures featuring a magnetic signal were observed. These strands have dimensions compatible with crystalline structures and they are aligned along an applied magnetic field. MFM images show that these structures are magnetic along most of their length and do not result from loose aggregated nanoparticles. The dimensions of these long magnetic structures could be compatible with the actin-like MamK protein filaments observed in magnetotactic bacteria. Such protein forms the cytoskeletal magnetosome filament and seems to have an essential role in concatenating and intracellular positioning of the magnetosome [15]. These protein filaments were never reported as magnetic, but the results of this work indicate that the single crystalline-like magnetic filaments found are compatible with the actin filaments providing a mechanism for the formation of magnetic nanoparticles with the same aligned orientation in each magnetosome chain.

The observation of these strands with magnetic behavior is unprecedented. Their small size could have application in nanoscale magnetic sensor devices.

ACKNOWLEDGMENTS

We thank Prof. Rogério Tenreiro and Prof. Margarida Godinho for enlightening discussions during this work.

REFERENCES

[1] D. Faivre, and D. Schüler, "Magnetotactic Bacteria and Magnetosomes," *Chem. Rev*., vol. 108, pp. 4875-4898, 2008.

[2] C. Lefèvre, A. Bernadac, K. Yu-Zhang, N. Pradel, and L. F. Wu, "Isolation and characterization of a magnetotactic bacterial culture from the Mediterranean Sea," *Environ. Microbiol*., vol. 11, no. 7, pp. 1646-1657, 2009.

[3] B. H Lower, and D. A. Bazylinski, "The Bacterial Magnetosome: A Unique Prokaryotic Organelle," *J. Mol. Microbiol. Biotechnol*., vol. 23, no. 1-2, pp. 63-80, 2013.

[4] J. Jacob, and K. Suthindhiran, "Magnetotactic bacteria and magnetosomes – Scope and challenges," *Mater. Sci. Eng. C Mater. Biol. Appl*., vol. 68, pp. 919-928, 2016.

[5] R. Frankel, and D. Bazylinski, "Magnetosomes and Magneto-Aerotaxis," *Contrib. Microbiol.*, vol. 16, pp. 182-193, 2009.

[6] D. Kong, W. Lin, Y. Pan, and K. Zhang, "Swimming motion of rod-shaped magnetotactic bacteria: The effects of shape and growing magnetic moment," *Front. Microbiol*., vol. 5, no. 8, 2014

[7] M. J. Smith, P. E. Sheehan, L. L. Perry, K. O'Connor, L. N. Csonka, B. M. Applegate, and L. J. Whitman, "Quantifying the Magnetic Advantage in Magnetotaxis," *Biophys. J*., vol. 91, no. 3, pp. 1098-1107, 2006.

[8] C. Lefèvre, and L. F. Wu, "Evolution of the bacterial organelle responsible for magnetotaxis," *Trends Microbiol*., vol. 21, no. 10, pp. 534-543, 2013.

[9] E. Alphandéry, F. Guyot, and I. Chebbi, "Preparation of chains of magnetosomes, isolated from Magnetospirillum magneticum strain AMB-1 magnetotactic bacteria, yielding efficient treatment of tumors using magnetic hyperthermia," *Int. J. Pharm*., vol. 434, no. 1-2, pp. 444-452, 2012.

[10] E. Alphandéry, "Applications of Magnetosomes Synthesized by Magnetotactic Bacteria in Medicine," *Front. Bioeng. Biotechnol*., vol. 2, no. 5, 2014.

[11] R. Hergt, R. Hiergeist, M. Zeisberger, D. Schüler, U. Heyen, I. Hilger, and W. A. Kaiser, "Magnetic properties of bacterial magnetosomes as potential diagnostic and therapeutic tools," *J. Magn. Magn. Mater*., vol. 293, no. 1, pp. 80-86, 2005.

[12] S. A. Kahani, and Z. A. Yagini, "Comparison between Chemical Synthesis Magnetite Nanoparticles and Biosynthesis Magnetite," *Bioinorg. Chem. Appl.*, vol. 2014, no. 7, 2014.

[13] K. Nishio, M. Ikeda, N. Gokon, S. Tsubouchi , H. Narimatsu, Y. Mochizuki, S. Sakamoto, A. Sandhu, M. Abe, and H. Handa, "Preparation of size-controlled (30–100nm) magnetite nanoparticles for biomedical applications," *J. Magn. Magn. Mater*., vol. 310, pp. 2408-2410, 2007.

[14] N. Zeytuni, S. Cronin, C. T. Lefèvre, P. Arnoux, D. Baran, Z. Shtein, G. Davidov, and R. Zarivach, "Correction: MamA as a Model Protein for Structure-Based Insight into the Evolutionary Origins of Magnetotactic Bacteria," *Plos One*, vol. 10, no. 7, pp. 013556, 2015.

[15] E. Katzmann, F-D. Müller, C. Lang, M. Messerer, M. Winklhofer, J. Plitzko and D. Schüler, "Magnetosome chains are recruited to cellular division sites and split by asymmetric septation," *Mol. Microbiol*., vol. 82, no. 6, pp. 1316-1329, 2011.

[16] Z. Oestreicher, C. Valverde-Tercedor, L. Chen, C. Jimenez-Lopez, D. A. Bazylinski, N. N. Casillas-Ituarte, S.K. Lower, and B. H. Lower, "Magnetosomes and magnetite crystals produced by magnetotactic bacteria as resolved by atomic force microscopy and transmission electron microscopy," *Micron*, vol. 43, pp. 1331-1335, 2012.

[17] L. Yan, S. Zhang, P. Chen, H. Liu, H. Yin, and H. Li, "Magnetotactic bacteria, magnetosomes and their application," *Microbiol. Res*., vol. 167, no. 9, pp. 507-519, 2012.

[18] E. Ozyamak, J. Kollman, D. A. Agard, and A. Komeili. The bacterial actin MamK: In vitro assembly behavior and filament architecture. *J. Biol. Chem*., vol. 288, no. 6, pp. 4265-4277, 2012.

[19] A. Taoka, J. Kondo, Z. Oestreicher, and Y. Fukumori, "Characterization of uncultured giant rod-shaped magnetotactic Gammaproteobacteria from a freshwater pond in Kanazawa, Japan," *Microbiology*, vol. 160, no. 10, pp. 2226-2234, 2014.

[20] D. Schüler, and E. Bäuerlein, "Dynamics of Iron Uptake and Fe3O4 Biomineralization during Aerobic and Microaerobic Growth of Magnetospirillum gryphiswaldense," *J. Bacteriol*., vol. 180, no. 1, pp. 159-162, 1998.

[21] D. Bazylinski, and R. Frankel, "Magnetosome formation in procayotes," *Nat. Rev. Microbiol*., vol. 2 no. 3, pp. 217-230, 2004.

[22] S. Sakaguchi, A. Taoka, and Y. Fukumori, "Analysis of Magnetotactic Behavior by Swimming Assay," *Biosci. Biotechnol. Biochem*., vol. 77, pp. 940-947, 2013.

[23] A. Komeili, "Molecular Mechanisms of Compartmentalization and Biomineralization in Magnetotactic Bacteria," *FEMS Microbiol. Rev.*, vol. 36, no. 1, pp. 232-255, 2011.

[24] A. Komeili, Z. Li, D. K. Newman, and G. Jensen, "Magnetosomes Are Cell Membrane Invaginations Organized by the Actin-Like Protein MamK," *Science*, vol. 311, no. 5758, pp. 242-245, 2006.

[25] K. Grünberg, C. Wawer, B. Tebo, and D. Schüler, "A Large Gene Cluster Encoding Several Magnetosome Proteins Is Conserved in Different Species of Magnetotactic Bacteria," *Appl. Environ. Microbiol*., vol. 67, no. 10, pp. 4573-4582, 2001.

[26] K. Grünberg, E-C. Müller, A. Otto, R. Reszka, D. Linder, M. Kube, R. Reinhardt, and D. Schüler, "Biochemical and Proteomic Analysis of the Magnetosome Membrane in Magnetospirillum gryphiswaldense," *Appl. Environ. Microbiol*., vol. 70, no. 2, pp. 1040-1050, 2004.

[27] P. Arnoux, M. Siponen, C. Lefèvre, N. Ginet, and D. Pignol, "Structure and evolution of the magnetochrome domains: No longer alone," *Front. Microbiol*., vol. 5, no. 117, 2014.

[28] E. Alphandery, A. T. Ngo, C. Lefèvre, I. Lisiecki, L. F. Wu, and M. P. Pileni, "Difference between the Magnetic Properties of the Magnetotactic Bacteria and Those of the Extracted Magnetosomes: Influence of the Distance between

the Chains of Magnetosomes," *J. Phys. Chem. C*, vol. 112, no. 32, pp. 12304-12309, 2008.
[29] D. Le Sage, K. Arai, D. Glenn, S. J. DeVience, L. Pham, L. Rahn-Lee, M. D. Lukin, A. Yacoby, A. Komeili, and R. L. Walsworth, "Optical magnetic imaging of living cells," *Nature*, vol. 496, pp. 486-489, 2013.
[30] H. Gojzewski, M. Makowski, A. Hashim, P. Kopčanský, Z. Tomori, and M. Timko, "Magnetosomes on surface: An imaging study approach," *Scanning*, vol. 34, no. 3, pp 159-169, 2012.
[31] G. Binnig, C. F. Quate, and C. Gerber, "Atomic Force Microscope," *Phys. Rev. Lett*., vol. 56, pp. 930-933, 1986.
[32] D. Yamamoto, A. Taoka, T. Uchihashi, H. Sasaki, H. Watanabe, T. Ando , and Y. Fukumori, "Visualization and structural analysis of the bacterial magnetic organelle magnetosome using atomic force microscopy," *Proc. Natl. Acad. Sci. USA*, vol. 107, no. 20, pp. 9382-9387, 2010.
[33] L. S Wong, J. Thirlway, and J. Micklefield, "Direct Site-Selective Covalent Protein Immobilization Catalyzed by a Phosphopantetheinyl Transferase," *J. Am. Chem. Soc*., vol. 130, no. 37, pp. 12456-12464, 2008.
[34] D. Nečas, and P. Klapetek, "Gwyddion: An open-source software for SPM data analysis," *Cent. Eur. J. Phys*., vol. 10, no. 1, pp. 181-188, 2011.
[35] K. Meinander, T. Jensen, S. Simonsen, S. Helveg, and J. V. Lauritsen, "Quantification of tip-broadening in non-contact atomic force microscopy with carbon nanotube tips," *Nanotechnology*, vol. 23, no. 40, pp. 405705, 2012.
[36] M. Martinez-Perez, R. de Miguel, C. Carbonera, M. Martínez-Júlvez, A. Lostao, C. Piquer, C. Gómez-Moreno, J. Bartolomé, and F. Luis, "Size-Dependent Properties of Magnetoferritin," *Nanotechnology*, vol. 21, no. 46, pp. 465707, 2010.

C. Marcuello was born in Zaragoza, Spain in 1986. He received the B.S. and M.S. degrees in cellular molecular biology in 2010 and the PhD certificate in nanotechnogy at University of Zaragoza in 2014.

He worked as postdoctoral researcher with Biosystems and Integrative Sciences (BioISI), Lisbon. From 2017 he is carrying out his research with Institute National de la Recherche Agronomique (INRA), Reims. His topics of interest include the use of SPM techniques to gain knowledge in terms of physico-chemical properties from biological systems.

L. Chambel was born in Alentejo, Portugal, in 1964. She received the B.S. degree in Biology, in 1989, and the Ph.D. degree in Microbiology in 2001, from Universidade de Lisboa.
Since 2001 she is Assistant Professor in the Plant Biology Department of Faculdade de Ciências da Universidade de Lisboa. She integrates the BioSystems and Integrative Sciences Institute (BioISI) da Faculdade de Ciências Universidade de Lisboa and is the author of more than 20 scientific papers. Hers research interests are diverse but always related with microbiology, mainly microbial taxonomy, the study of viable but not culturable bacterial cells and food pathogens.

M. S. Rodrigues was born in Portugal, 1979. He graduated in Physical Engineering in 2004 and in 2009 he obtained his PhD degree in physics, from Université Joseph Fourier after his research as PhD student at the European Synchrotron Radiation Facility, Grenoble, France. He has been assistant professor in the Physics Department of Faculdade de Ciências da Universidade de Lisboa since 2013 and from 2015 he integrates the BioSystems and Integrative Sciences Institute (BioISI) at the same faculty. His research interests include condensation and properties of water at very small confinement, friction, protein aggregation, protein-protein interactions and cellular mechanics.

L. P. Ferreira was born in 1960, in Angola, a Portuguese colony at that time. In 1993, she got a Ph.D. in Physics from Universidade de Coimbra and since then she is Assistant Professor at the Physics Department of that university. Her main research interests include the study of nanostructured magnetic systems for applications in biomedicine and transition metal complexes for applications in catalysis and magnetic sensors. All the scientific research activity is being developed in the BioSystems and Integrative Sciences Institute (BioISI), from the Faculty of Sciences of Universidade de Lisboa.

M. M. Cruz was born in Lisbon, Portugal, in 1960. She received the B.S., M.S. degrees in Physics, in 1985 and the Ph.D. degree in Physics in 1989, from Universidade de Lisboa.

From 1989 to 2010, she was Assistant Professor with the Physics Department of Faculdade de Ciências da Universidade de Lisboa and from 2013 she is Associate Professor with the same Department. She integrates the BioSystems and Integrative Sciences Institute (BioISI) da Faculdade de Ciências Universidade de Lisboa and is the author of more than 70 scientific papers. Hers research interests includes the experimental study of magnetic and transport properties in nanostructured systems, namely magnetic nanoparticles for hyperthermia applications.

This article has been accepted for publication in a future issue of this journal, but has not been fully edited. Content may change prior to final publication. Citation information: DOI 10.1109/TNB.2018.2878085, IEEE Transactions on NanoBioscience

FIGURES

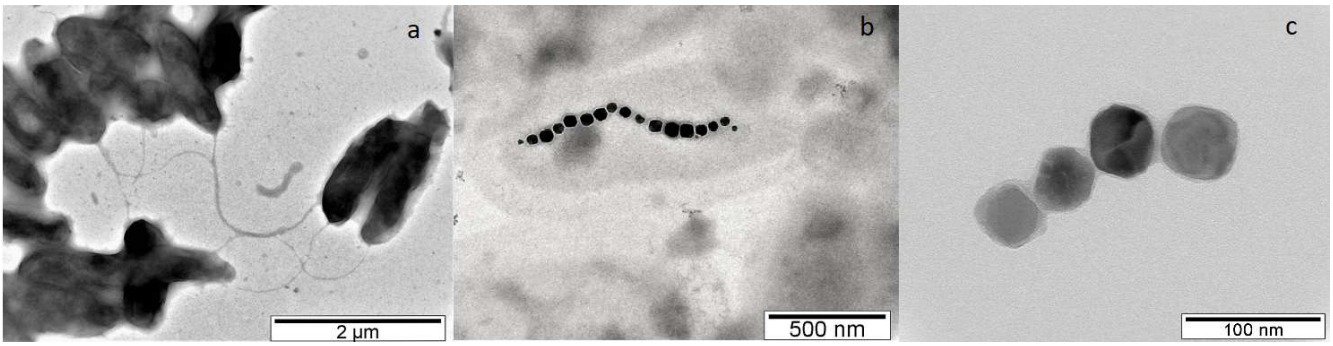

Fig. 1. TEM images of (a) ensemble of *M. magnetotacticum* bacteria showing apical flagella; (b) bacterium with magnetosome chain and (c) magnetosome extracted from bacterium.

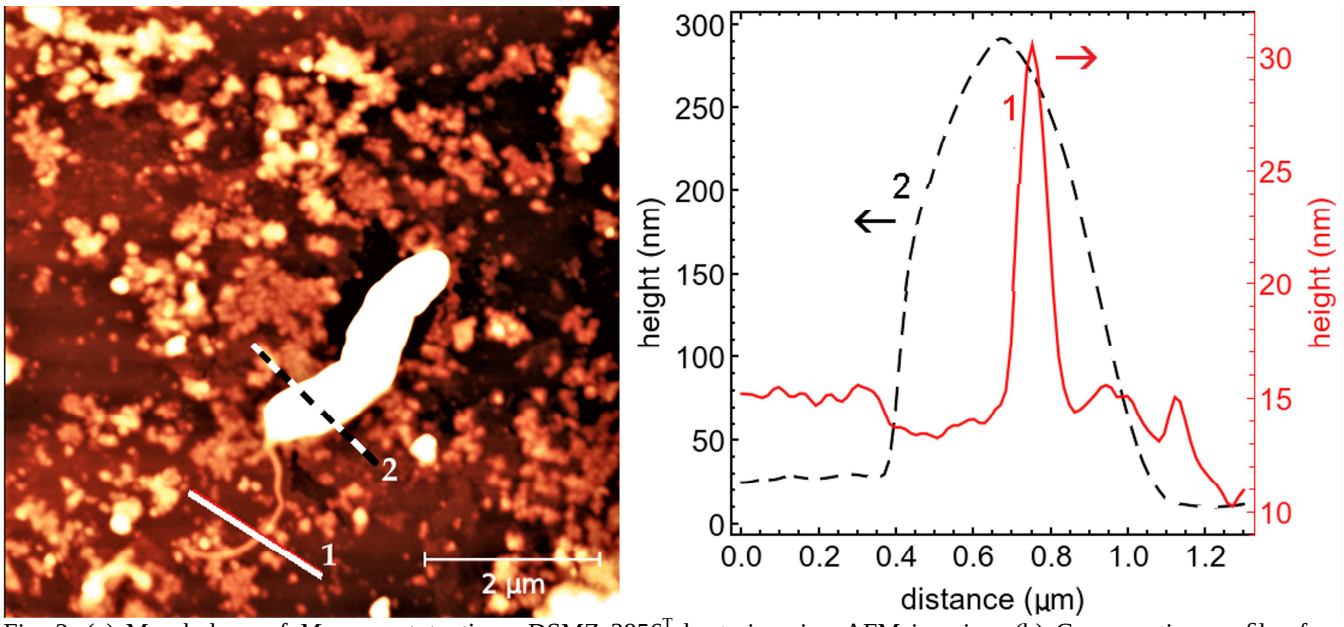

Fig. 2. (a) Morphology of *M. magnetotacticum* DSMZ 3856^{T} bacteria using AFM imaging. (b) Cross section profiles from bacteria in (a). Profiles 1 and 2 are represented as full and dashed lines, respectively. Scale bar indicated is 2.0 µm.

This article has been accepted for publication in a future issue of this journal, but has not been fully edited. Content may change prior to final publication. Citation information: DOI 10.1109/TNB.2018.2878085, IEEE Transactions on NanoBioscience

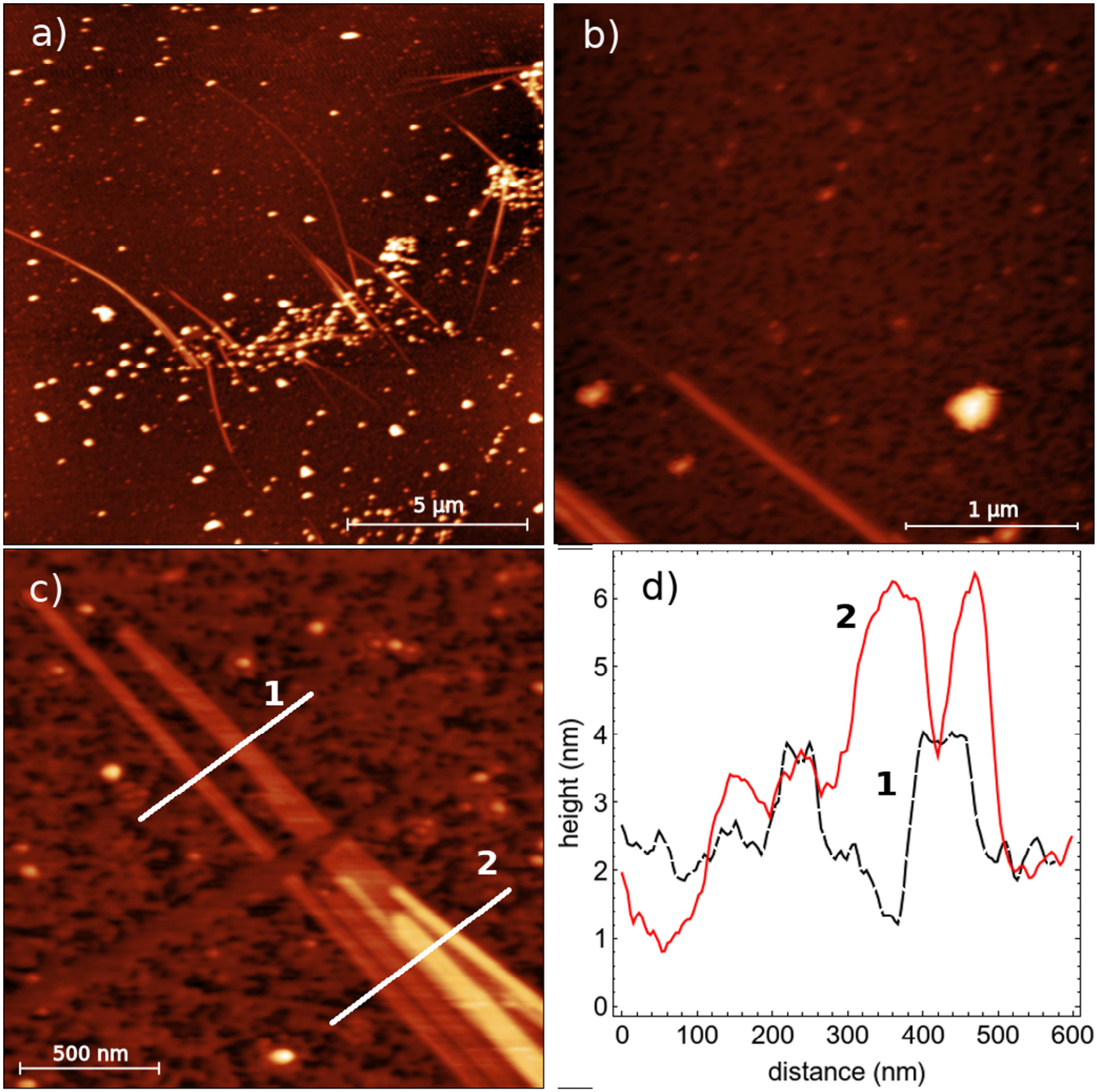

Fig. 3. Atomic Force Microscopy topography images of M.MTB sample of *M. magnetotacticum* DSMZ 3856^{T}. (a) 15 µm x 15 µm scan evidencing the presence of long strands. (b) Scan 5 µm x 5 µm showing the sharp ending of these strands. (c) and (d) Image of the structures and cross section plot of profile 1 (dashed line) and profile 2 (full line) marked in (c) showing that the observed structures are flat at the atomic level. Scale bars are 5 µm, 1 µm and 500 nm, respectively in (a), (b) and (c).

This article has been accepted for publication in a future issue of this journal, but has not been fully edited. Content may change prior to final publication. Citation information: DOI 10.1109/TNB.2018.2878085, IEEE Transactions on NanoBioscience

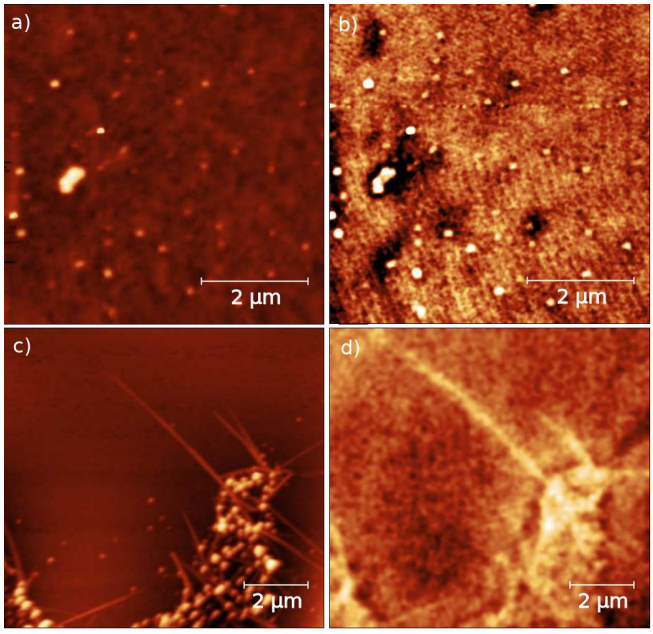

Fig. 4. Atomic Force Microscopy and Magnetic Force Microscopy data from of M.MTB sample of *M. magnetotacticum* DSMZ 3856^{T}. (a) Topographic image and (b) phase lift for the same scanned area showing several magnetic nanoparticles (lift height was 75 nm, tip was pulled away 250 nm with a delay of 300 ms for each pixel scan). (c) Topographic image of an area with several long strands and (d) phase lift for the same scanned area (lift height was 60 nm with the same pulled away and delay parameter values as in (b). In both cases, lift distances are far away from the sample surface to avoid Van der Waals interactions. Scale bars are 2 µm.

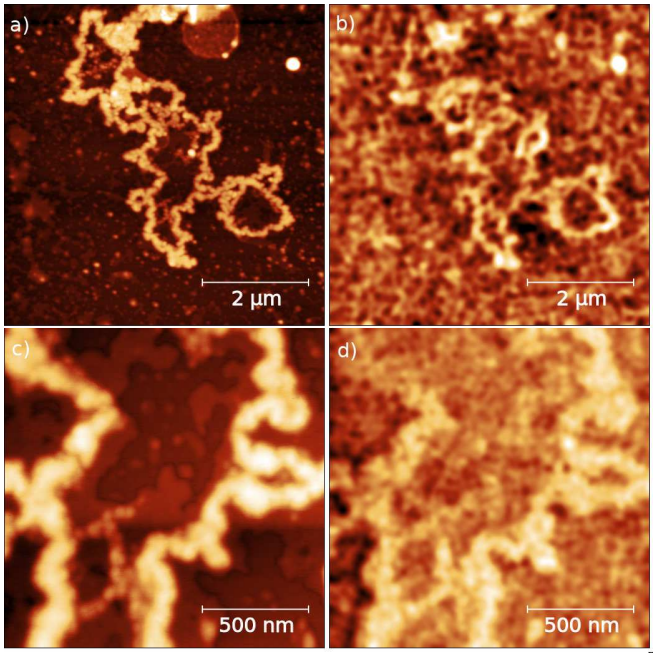

Fig. 5. Magnetic Force Microscopy data for DMSZ 3856^{T} bacteria evidencing the presence of magnetosomes. (a) and (c) Topographic images. (b) and (d) Phase lift for the corresponding scanned areas. Scale bars are 2 µm for the top images and 500 nm for the down images.