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1 **Magnetic and structural properties of biogenic magnetic nanoparticles along their**  
2 **production process for use in magnetic hyperthermia**

3

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16 **Abstract**

17 Iron-oxide nanoparticles synthesized by magnetotactic bacteria can be used in cancer  
18 treatment by means of magnetic hyperthermia. In order to study the structural properties of  
19 samples through the successive steps of their production from cultivated magnetotactic  
20 bacteria to coated magnetosomes, and their effect on magnetic properties, we performed a  
21 combination of (cryo-)Transmission Electron Microscopy observations and magnetic  
22 hysteresis as well as First-Order Reversal Curve diagrams at low temperature. The anisotropy  
23 of magnetic interactions was investigated by recording field-cooled FORC diagrams with a  
24 measuring field either parallel or perpendicular to the field-cooling direction. The results from  
25 the various techniques are in very good agreement. We found that magnetosomes in  
26 magnetotactic bacteria are arranged in chains and have magnetic properties typical of stable  
27 single-domain aligned particles. Extracted magnetosomes form clusters of interacting  
28 maghemite particles with some remaining chains, while purified magnetosomes are solely  
29 arranged in clusters. Finally, magnetosomes coated with 1,2-dioleoyl-sn-glycero-3-  
30 phosphocholine (DOPC) are still organized in clusters but with less magnetostatic interactions  
31 than in purified magnetosomes, while magnetosomes coated with citric acid tend to recover a  
32 chained structure forming loops. The main features observed on the FORC diagrams are  
33 consistent with the various arrangement observed. Lastly, hyperthermia properties were  
34 measured for the two coated samples and we found that magnetosomes coated with citric acid  
35 display a higher Specific Absorption Rate and therefore hold a better potential for biomedical  
36 applications.

37

38

39 **Index terms**

- 40 FORC diagram, Magnetic hyperthermia, Magnetosomes, Magnetotactic bacteria,
- 41 Transmission Electron Microscopy

42

## 1- Introduction

43 The production and use of iron oxide nanoparticles (IONPs) in cancer treatment has been  
44 studied for the last twenty years with the goal of targeting tumors more accurately, thus  
45 avoiding damage to healthy cells of patients [1]. Treatment of cancer with IONPs can be  
46 achieved by magnetic hyperthermia by heating nanoparticles through the application of an  
47 alternating magnetic field to eradicate a tumor through localized moderate heating. IONPs are  
48 already commercialized for the treatment of iron deficiency anemia diseases [2] and several  
49 types of cancers [1]. In terms of toxicity, IONPs are particularly attractive mostly because  
50 liver and kidneys easily eliminate them after completion of injection and treatment [3].

51 Magnetosomes, which are synthesized by magnetotactic bacteria (MTB), appear to be at the  
52 most advanced stage of development among the different types of bio-synthesized IONPs (see  
53 [3] for a review). In nature, MTB use magnetosomes as a "magnetic compass" guiding them  
54 in the direction of the Earth's magnetic field [4]. This capacity enables bacteria to orientate  
55 and find mediums in water that are best adapted to their needs. Magnetosomes present several  
56 interesting characteristics: particles have a homogeneous size distribution and uniform  
57 morphology [5], they are composed of high crystallinity magnetite and/or maghemite [5], and  
58 their magnetic moment is stable at room temperature [6]. Moreover, biocompatible iron oxide  
59 magnetosomes are free of elements that are not compatible with medical treatment and  
60 injection in the body [7], and they can be produced in large quantities by cultivation in non-  
61 toxic growth medium [8]. The chain structure of the magnetosomes, which cannot be  
62 controlled accurately when using chemically synthesized IONPs, results in unique structural  
63 and magnetic properties of magnetosomes.

64 In this work, we characterize the magnetic and structural properties of MTB-derived  
65 IONPs at various stages of their production, i.e., from whole bacteria (Sample 1), extracted  
66 magnetosomes (Sample 2), purified magnetosomes (Sample 3), to coated magnetosomes with

67 two different coating agents (Samples 4 and 5), as potential candidates for hyperthermia. The  
68 MTB used in this study are MSR-1 *Magnetospirillum gryphiswaldense* (referred to as MSR-  
69 1). The coating for the last two samples is necessary to make the magnetosomes  
70 biocompatible for cancer treatment [9, 10]. Magnetosomes extracted from MSR-1 have been  
71 shown to be less toxic and more efficient than chemically synthesized nanoparticles coated  
72 with amino-silane used in nanomedicine [11] and are bio-compatible [7, 12, 13]. Here, the  
73 coating agents were chosen based on their different effects on the arrangement of  
74 magnetosomes.

75 Transmission Electron Microscopy (TEM) and cryo-TEM observations were  
76 conducted to determine the distribution of magnetosome sizes and the structural organization  
77 of magnetosomes. Measurements of hysteresis curves and First-Order Reversal Curve  
78 (FORC) diagrams were performed at low temperature to identify the magnetic domain state of  
79 the magnetosomes, to provide insight into particle size distribution and spatial organization  
80 [14], [15], and to determine coercivity and magnetic interaction distribution, for which FORC  
81 diagrams are an ideal tool (see [16] for a review). Finally, measurements of the Specific  
82 Absorption Rate (SAR) were performed for both coated magnetosome samples, in order to  
83 assess their heating capacities under the application of an alternating magnetic field.

84 Because we carry out (cryo-)TEM observations and FORC diagram measurements  
85 together, our study provides insight about the effect of magnetosome arrangement, which  
86 depends on the sample type, on the distribution of magnetic properties measured with FORC  
87 diagrams, in particular the marginal and vertical distributions. Finally, because FORC  
88 diagrams were measured at low temperature, we can also assess the effect of field-  
89 cooled/zero-field-cooled treatment on the FORC diagrams and obtain information about the  
90 anisotropy of the two distributions derived from FORC diagrams. Such low-temperature  
91 FORC measurements on samples in solution are seldom carried out.

92

93

## 2- Experimental method

### 94 2.1.1. Sample synthesis

95 Five samples were studied in the present work, all of them produced by the Nanobacterie  
96 Company. They were collected at the five different steps of the typical production process for  
97 cancer treatment applications of magnetosomes, each sample corresponding to a different  
98 step. The five samples are as follows:

99 - Sample 1: MSR-1 bacteria, in their culture medium, at a concentration of  $4.10^9$   
100 cells/mL.

101 - Sample 2: magnetosomes extracted from bacteria through lysis, by placing bacteria in  
102 a basic medium composed of KOH and heating to 90 °C. Magnetosomes were  
103 subsequently collected with a magnet and put in water. The concentration was 50 mg  
104 of Fe/mL.

105 - Sample 3: purified magnetosomes, heated in air to 380 °C. Most biological material is  
106 thus removed from the magnetosomes, though there could be some phosphate  
107 residues. The concentration was also 50 mg of Fe/mL.

108 - Sample 4: magnetosomes coated with 1,2-dioleoyl-sn-glycero-3-phosphocholine  
109 (DOPC), which is a bitailed phospholipid. It is a bio-compatible molecule and  
110 therefore bio-applied. The concentration was 25 mg of Fe/mL.

111 - Sample 5: magnetosomes coated with and 2-Hydroxypropane-1,2,3-tricarboxylic acid  
112 (citric acid, CA). The concentration was also 25 mg of Fe/mL.

113 Culture of MTB in 7.5L fermenter, extraction, and purification of magnetosomes were  
114 performed as described in [17]. Iron concentrations were determined with a colorimetric  
115 dosage with a 5% precision [18]. The coating for the last two samples is a necessary  
116 condition, both to stabilize magnetosomes in water and to make them compatible with

117 injection in a patient's body for cancer treatment. Coating in citric acid (CA) favors the  
118 formation of chains because it is a triacid that enables the complexation of Fe with several  
119 magnetosomes, leading to a chain-like structure [18], [19]. On the contrary, 1,2-dioleoyl-sn-  
120 glycerol-3-phosphocholine (DOPC) coating favors interactions between phosphate groups of  
121 the coating and Fe oxide, leading to a nano-micelle type structure [20]. Moreover, the  
122 proportion of DOPC at the surface of purified magnetosomes is not sufficient to induce  
123 hydrophobicity in the nanoparticle suspension. Therefore, these coated magnetosomes are not  
124 soluble but behave as a stable suspension. Sample 1 was stored at -80°C in a freezer while the  
125 other four samples were stored at 4°C in a cold room.

126

## 127 *2.2. TEM and cryo-TEM observations*

128 TEM and cryo-TEM observations were carried out at IMPMC in order to characterize  
129 magnetosomes and their organization within the different samples. Cryo-TEM in particular  
130 allows preserving the integrity of bacteria as well as the organization of nanoparticles, since it  
131 does not require drying the sample on a TEM grid. Standard TEM observations were realized  
132 with a Jeol 2100F equipped with a Schotky FEG gun, and cryo-TEM observations with a Jeol  
133 2100, equipped with a LaB6 gun.

134 For Sample 1 preparation observed with standard TEM, the sample was diluted 400 times, the  
135 solution was centrifuged 10 min at 14500 rpm. The supernatant was removed, replaced by  
136 deionized water, and centrifugation and change of supernatant was re-iterated a second time.  
137 This was done to remove the culture medium which can alter the observation on the TEM-  
138 grid once dried. 2.5 µL of the obtained solution was deposited on an ionized TEM grid, and  
139 dried for 24 hours at room temperature. For Samples 2 and 3, diluted solutions at a concentra-  
140 tion of 100 µg of Fe/mL were deposited on a TEM-grid and dried for 24 hours at room tem-  
141 perature.



142 For cryo-TEM images, a 5  $\mu\text{L}$  drop of the initial sample solution was deposited on  
143 “Quantifoil”<sup>®</sup> (Quantifoil Micro Tools GmbH, Germany) carbon membrane grids. The ex-  
144 cess of liquid on the grid was absorbed with a filter paper and the grid was quench-frozen  
145 quickly in liquid ethane to form a thin vitreous ice film using an homemade mechanical cryo  
146 plunger. Once placed in a Gatan 626 cryo-holder cooled with liquid nitrogen, the samples  
147 were transferred in the microscope and observed at low temperature (-180 °C). Cryo-TEM  
148 images were recorded on ultrascan 1000, 2k x 2k pixels CCD camera (Gatan, USA), using a  
149 LaB<sub>6</sub> JEOL JEM2100 (JEOL, Japan) cryo microscope operating at 200kV with a JEOL low  
150 dose system (Minimum Dose System, MDS, JEOL, Japan) to protect the thin ice film from  
151 any irradiation before imaging and reduce the irradiation during the image capture.

### 152 *2.3. Magnetic measurements*

153 **Hysteresis loops-** Hysteresis loops were measured with a Physical Properties Measurement  
154 System (PPMS) coupled with a Vibrating Sample Magnetometer (VSM) at the Low-  
155 temperature Physical Measurements Platform of Sorbonne Université. Hysteresis loops were  
156 measured from -3T to 3T. Samples 4 and 5 were measured in solution, Sample 3 as a powder  
157 because of its tendency to sediment very quickly when dispersed in solution, and Sample 2  
158 both in solution and as a powder. Sample 1 was not measured because its magnetization was  
159 expected to be too weak. Temperature was varied between 5 and 300 K except for Samples 2  
160 and 3 as powders that were measured both at 10 and 300 K. Samples were weighted and  
161 magnetizations were normalized to mass.

162

163 **FORC diagrams** - FORC diagrams allow a qualitative characterization of the magnetic  
164 domain structure and magnetostatic interactions, by measuring a set of partial hysteresis  
165 curves that originate from the descending branch of the hysteresis loop [15], [21]. FORC  
166 diagrams were measured with a magnetometer ( $\mu$ -VSM) equipped with a cryostat from

167 Princeton Measurements Corporation at the IPGP-IMPMC Mineral Magnetism Analytical  
168 Facility. For stable single domain (SSD) particles with weak interactions, the FORC  
169 distribution along the vertical axis can be interpreted as the distribution of the particle  
170 interaction fields, and the marginal FORC distribution, which corresponds to the total  
171 magnetic contribution of all particles with a given switching field, as the coercivity  
172 distribution of the system [22].

173 FORC diagrams were each calculated from 130 individual FORCs measured with a field step  
174 of 2 mT, at a temperature of 50 K, and with an averaging time of 0.2 s. The measurement time  
175 for each FORC diagram was about one hour. The temperature of 50 K was chosen in order for  
176 the results to be comparable with results from cryo-TEM and hysteresis. They were  
177 subsequently analysed with the Forcinel software [23], with a variable smoothing factor [24].  
178 The variable smoothing considerably reduces the noise levels by applying larger smoothing  
179 factors to the background, while preserving the areas along the axes with relatively small  
180 smoothing factors.

181 For each of the five samples, three FORC diagrams were measured. First, solutions containing  
182 20  $\mu$ L of each magnetosome sample was prepared and enclosed in a polycarbonate gelcap and  
183 placed between the coils. The sample was cooled in zero-field to 50 K, and a first FORC  
184 diagram was measured (ZFC measurement). Then, the sample was re-heated to room  
185 temperature, and cooled in a field of 0.5 T back to 50 K. A second FORC diagram was  
186 measured (FC measurement). Finally, the VSM head, to which the sample is attached, was  
187 turned by 90° and a third FORC diagram was measured (FC90 measurement).

188

#### 189 *2.4. Hyperthermia measurements*

190 Hyperthermia measurements are used to gain information about the heating power of a  
191 sample, i.e. the ability of the sample to release heat when submitted to an alternating magnetic

192 field (AMF). It is an important measurement to evaluate the efficiency of a sample in  
193 potential cancer treatment. Temperature is acquired as a function of time, during the  
194 application of an AMF, until an equilibrium temperature is reached (about 15 min). The SAR  
195 is then calculated using the slope of the curve T(t) at the origin, as:

$$SAR = \frac{c_{water}}{\chi_{Fe}} \times \frac{\Delta T}{\delta t}$$

196 with  $\chi_{Fe}$  the iron concentration of the sample and  $c_{water}$  the heat capacity of water in  $J.L^{-1}.K^{-1}$ ,  
197 and SAR expressed in W/g.

198 The AMF was set at 31 mT with a frequency of 198 kHz. Hyperthermia was only measured  
199 on the two coated samples (Samples 4 and 5) potentially used for cancer treatment, and for  
200 three different concentrations of each sample (2.5, 5 and 10 mg(Fe)/mL) in order to track a  
201 possible concentration dependence of SAR. The temperature measurements within the sample  
202 were conducted in triplicates. The median and standard deviation for each point of the T(t)  
203 curves was used to obtain minimum, median and maximum SAR values.

204

205

### 3- Results

#### 206 *3.1. Structural organization of magnetosomes from TEM and cryo-TEM*

207 Samples 1, 4 and 5 were successfully observed with cryo-TEM, while Samples 2 and 3 were  
208 observed with standard TEM, as extracted uncoated magnetosomes tend to aggregate too  
209 rapidly during cryo-TEM grid preparation, and the resulting large clusters of particles are not  
210 suitable to the scope of studying their self-organization by cryo-TEM. Sample 1 was also  
211 observed with standard TEM.

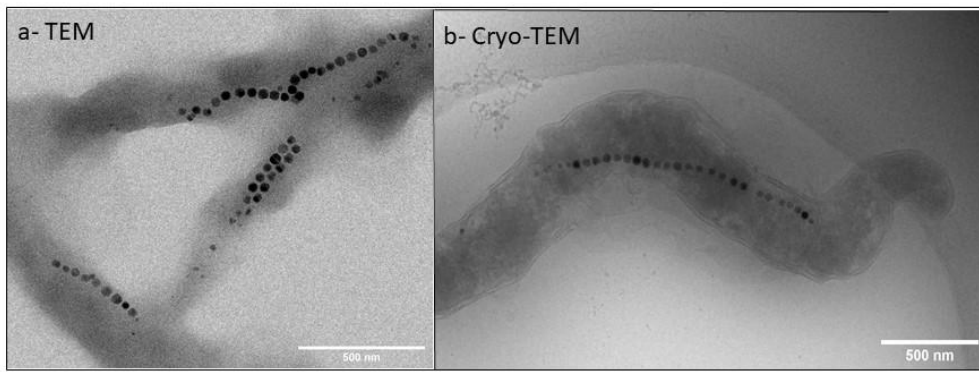
212 Chains of magnetosomes are clearly visible in Sample 1 under standard TEM (Fig. 1a) and  
213 cryo-TEM (Fig. 1b). However, bacteria tend to aggregate more on standard TEM grids, and

214 their boundaries are difficult to identify with the image contrast. Double chains were  
215 sometimes observed within individual bacteria. The length of chains ranges from 122 nm to  
216 1.5  $\mu\text{m}$ , with a mean length of 624 nm. They are composed on average of 14 magnetosomes  
217 (Fig. A-1a, f, g). Magnetosome shapes vary from rounded to cubic and cuboctahedral.  
218 Following a rounded shape approximation for all particles, magnetosomes have a mean  
219 diameter of 34.6 nm, with a standard deviation of 6.9 nm. The interparticle distance is  $11.2 \pm$   
220 4.2 nm.

221 Sample 2 consists of extracted magnetosomes, arranged in chains and agglomerates of  
222 particles (Fig. 2a, b). Chains are attached to clusters of particles, at least at one end. The  
223 length of chains ranges from 200 nm to 1.6  $\mu\text{m}$ , with an average length of 678 nm, and are  
224 composed of 18.4 magnetosomes on average (Fig. A-1b, h). Individual magnetosomes have a  
225 mean diameter of 35.1 nm (standard deviation of 5.6 nm).

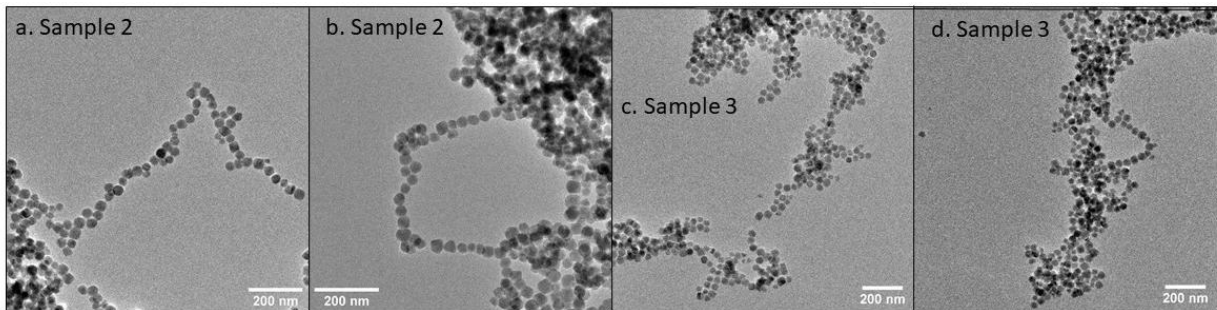
226 Sample 3 is composed of purified magnetosomes, i.e. magnetosomes removed from their  
227 original organic membrane. Magnetosomes in this sample have a mean particle size of 35.1  
228 nm (standard deviation of 5.4 nm). This particle size is similar to that in Sample 2, although  
229 there are more particles in the 34-38 nm range in Sample 3 than in Sample 2 (60% vs. 55%)  
230 (Fig. A-1c). TEM images show that particles are more likely to form agglomerates (Fig. 2c,  
231 d), which was expected since magnetic interactions are now dominant and cause the  
232 magnetosomes to be mechanically unstable in solution. We also observe less particles with a  
233 diameter  $<20$  nm in Samples 2 and 3 than in Sample 1: these particles represent 7.8% of  
234 particles in Sample 1, but only 4 % in Sample 2 and 3.9 % in Sample 3.

235



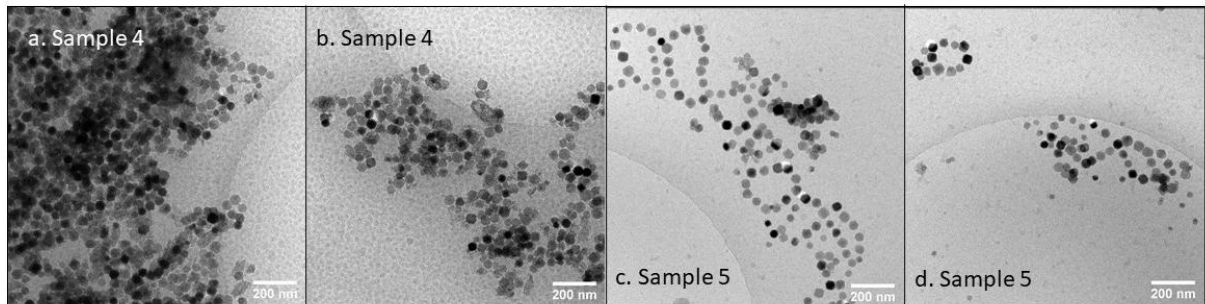
236

237 **Fig. 1:** TEM (panel a) and cryo-TEM (panel b) images of Sample 1.



238

239 **Fig. 2:** TEM images of Sample 2 (panels a, b) and Sample 3 (panels c, d).



240

241 **Fig. 3:** cryo-TEM images of Sample 4 (panels a, b) and Sample 5 (panels c, d).

242

243 Cryo-TEM observations of coated magnetosomes (Samples 4 and 5) show important  
 244 differences depending on the coating agent. The magnetosomes of sample 4 are coated with  
 245 DOPC and form aggregates (Fig. 3a, b), while magnetosomes of Sample 5 are coated with CA  
 246 and tend to form organized rings with increased interparticle distances in comparison to other

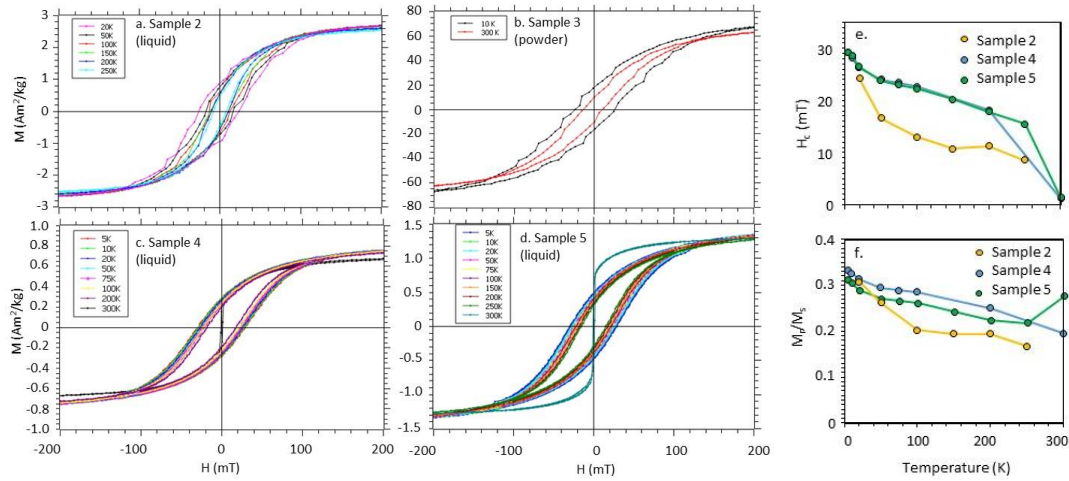
247 samples (Fig. 3c, d). The mean diameter of magnetosomes is similar for both coated samples:  
248 40.9 nm (standard deviation of 8.1 nm) for Sample 4 and 41.5 nm (standard deviation of 7.3  
249 nm) for Sample 5 (Fig. A-1d, e). The interparticle distance is  $13.4 \pm 4.3$  nm for Sample 5.

250

### 251 *3.2. Magnetic hysteresis curves*

252 Hysteresis loops (Fig. 4a-d) show that, regardless of temperature, magnetization is saturated  
253 in an applied magnetic field of 2 T for all samples. Hysteresis loops for Samples 4 and 5 close  
254 progressively when temperature is increased from 5 to 250 K, and close more sharply above  
255 250 K to room temperature. For Sample 2 (Fig. 4a), blocking seems to begin at lower  
256 temperatures than for Samples 4 and 5. The variation of bulk coercivity ( $H_c$ ) and the ratio of  
257 remanent magnetization over saturation magnetization ( $M_r/M_s$ ) reflect this behavior (Fig. 4  
258 e,f): when temperature is increased, both  $H_c$  and  $M_r/M_s$  decrease.  $H_c$  then drops sharply at  
259 room temperature (when measured). There is no evidence of a drop around 120 K, therefore  
260 no evidence of a Verwey transition in any of the samples. The presence of maghemite rather  
261 than magnetite is further confirmed by the value of saturation magnetization of Sample 2 (as a  
262 powder, Fig. SI-2) measured as  $75.87 \text{ Am}^2/\text{kg}$  at room temperature; a value that is close to the  
263 known value of maghemite ( $74.3 \text{ Am}^2/\text{kg}$  [25]). Moreover, heating curves of field-cooled and  
264 zero-field cooled Saturation Isothermal Remanant Magnetization (SIRM) acquired at 10 K in  
265 a 2.5T field on samples prepared identically to Samples 2 and 3 show that the Verwey  
266 transition is not visible in heated extracted magnetosomes as well as in purified  
267 magnetosomes [26] (Fig. A-3).

268



269

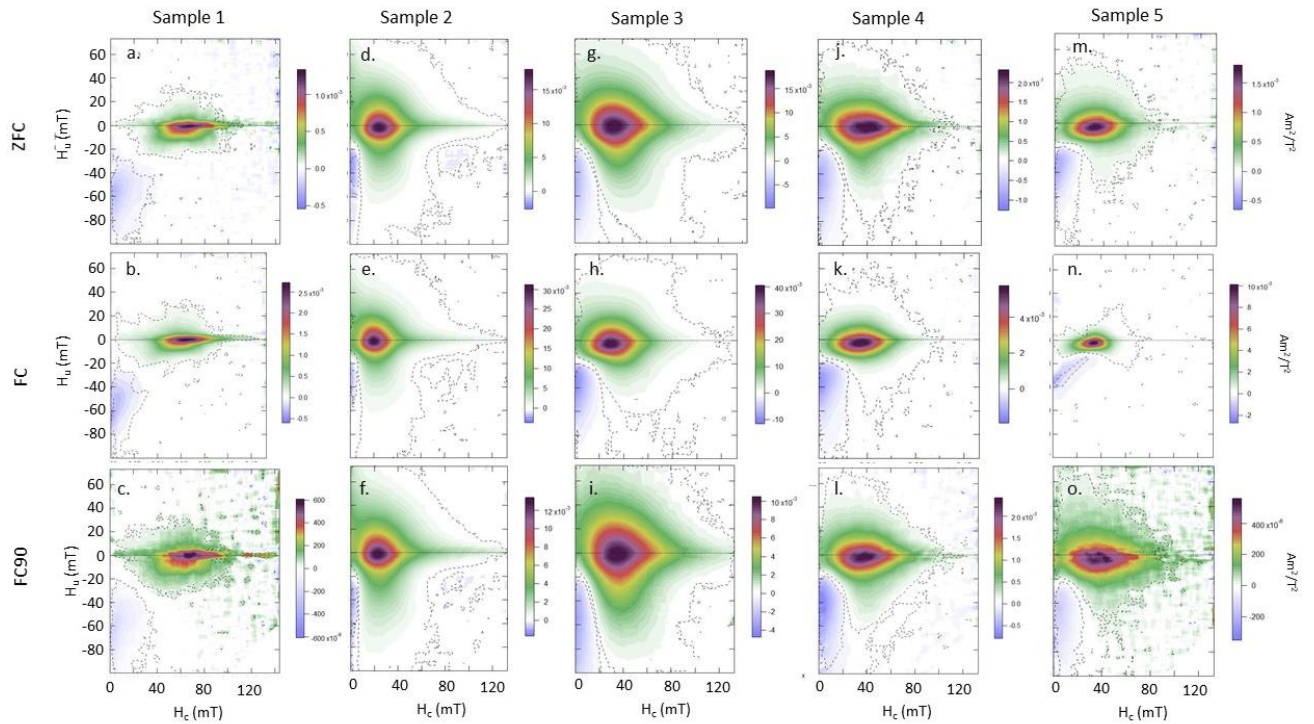
270 **Fig. 4:** Magnetization curves measured for Samples 2 (panel a), 3 (panel b), 4 (panel c) and 5  
 271 (panel d). All samples except Sample 3 are in solution. Variation of hysteresis ratio  $M_r/M_s$   
 272 (panel d) and coercivity  $H_c$  (panel e) as function of temperature, derived from the  
 273 corresponding hysteresis loops.

274

### 275 3.3. Distribution of magnetic properties from FORC diagrams

276 The FORC diagrams measured for the five samples in the three different configurations (ZFC,  
 277 FC and FC90 (see caption of Fig. 5 for the meaning of acronyms)) are shown in Fig. 5. They  
 278 are all plotted on the same ( $H_c$ ,  $H_u$ ) scale and with the same smoothing parameters.

279



280

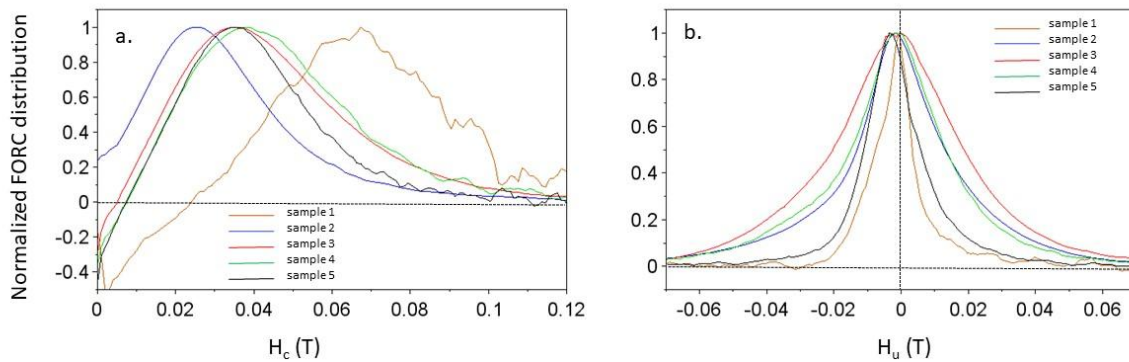
281 **Fig. 5:** FORC diagrams measured for Sample 1 (a, b, c), Sample 2 (d, e, f), Sample 3 (g, h, i);  
 282 Sample 4 (j, k, l), Sample 5 (m, n, o). Panels (a, d, g, j, m) correspond to zero-field cooled  
 283 (ZFC) measurements, panels (b, e, h, k, n) to field-cooled (FC) measurements, and panels (c,  
 284 f, i, l, o) to measurements after FC and turning the sample by  $90^\circ$  (FC90).

285

286 In the ZFC configuration (i.e., the usual configuration when measuring FORC diagrams), the  
 287 FORC diagram measured for Sample 1 (Fig. 5a) is typical of MTB (e.g., [27]). The extended  
 288 coercivity distribution between 40 and 100 mT and the very narrow contours along the  
 289 vertical axis are expected for weakly interacting single domain magnetite particles. Samples 2  
 290 and 3 (Fig. 5d, g) show FORC diagrams with a “teardrop” shape typical of strongly  
 291 interacting systems (e.g., [22], [28]). This shape is still visible on the FORC diagram of  
 292 Sample 4 (Fig. 5j), while contours for Sample 5 have a more elliptical shape intermediate  
 293 between those of Sample 1 and Sample 2 (Fig. 5m). These differences in coercivity  
 294 distribution are reflected on the marginal distribution (Fig. 6a): Sample 1 has the largest



295 coercivity distribution that is also centered on the highest coercivity value, while the  
 296 coercivity distribution for Sample 2 is shifted towards lower values. The vertical profile  
 297 across the maximum of the FORC distribution (Fig. 6b), which is often used as a rough  
 298 estimate of the amount of magnetic interactions, is widest for Samples 3, and narrower for  
 299 Samples 1 and 5.



300  
 301 **Fig. 6:** a. Marginal coercivity distribution for the five samples in ZFC configuration. b.  
 302 Vertical FORC profile through the maximum of the FORC distribution for the five samples in  
 303 ZFC configuration.

304 The FC FORC diagrams show a similar evolution to the ZFC ones when comparing the five  
 305 samples. However, the contours are less spread out than for the ZFC FORC diagrams, both  
 306 along the vertical and horizontal directions. The lobe observed in the lower half of the ZFC  
 307 diagram for Samples 2, 3 and 4 is faint (Samples 2 and 3) or inexistent (Sample 4) in the  
 308 corresponding FC diagram. Finally, the shapes of the FC90 FORC diagrams are rather similar  
 309 to that of the ZFC FORC diagrams. The only notable difference is the higher noise level  
 310 caused by the lower values taken by the FORC distribution themselves.

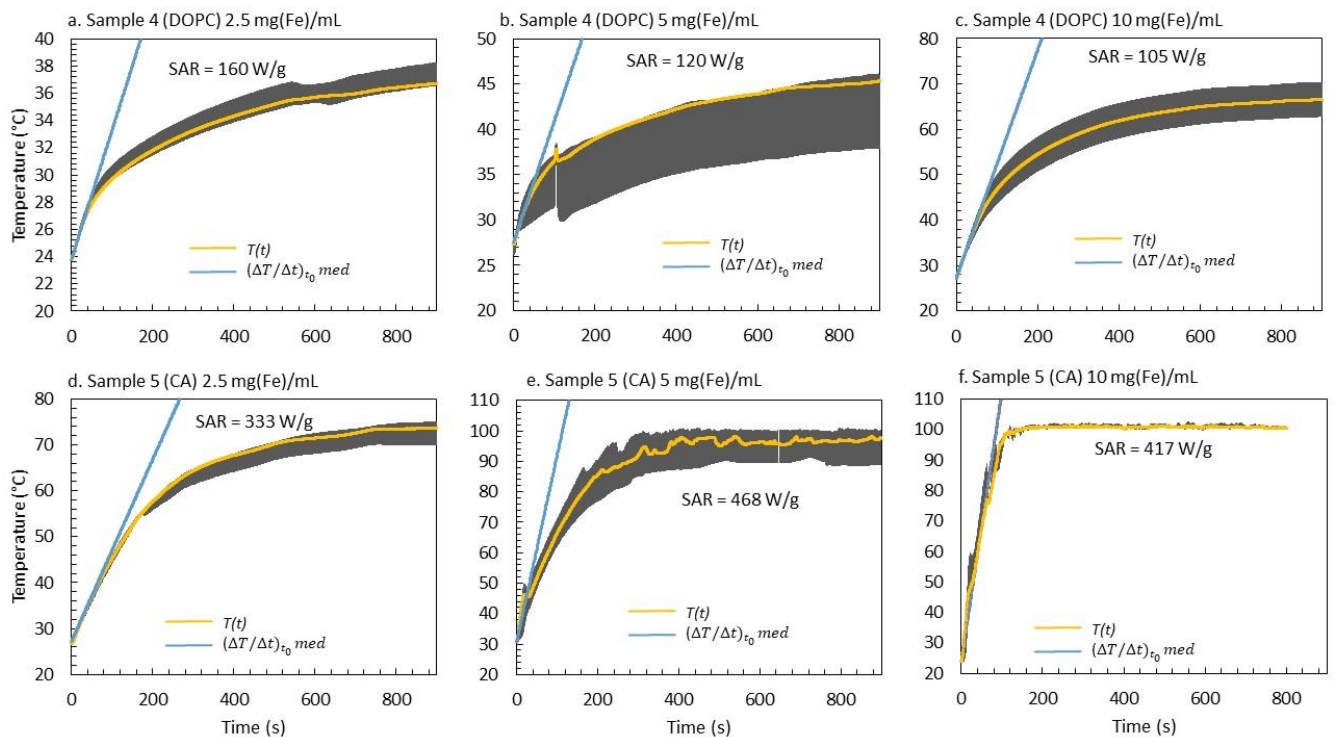
311

### 312 3.4. Hyperthermia measurements

313 Hyperthermia measurements were carried out on three different concentrations of Samples 4  
 314 and 5. The heating temperature after 850 s, for both samples, increases with sample

315 concentration (Fig. 7). For each of the three concentrations, the heating temperature is higher  
 316 for Sample 5 (coated with CA) than for Sample 4 (coated with DOPC). Sample 5 reaches a  
 317 temperature of 74°C for a concentration of 2.5 mg(Fe)/mL, 97°C for 5 mg(Fe)/mL and 100°C  
 318 for 10 mg(Fe)/mL. Sample 4 reaches only 36.4°C for a concentration of 2.5 mg(Fe)/mL, 47.5  
 319 °C for 5 mg(Fe)/mL and 66.5°C for 10 mg(Fe)/mL. Consistently, the calculated SAR values  
 320 are higher for Sample 5 than for Sample 4, regardless of sample concentration. For Sample 4,  
 321 the SAR decreases with increasing concentration, while no similar trend is observed for  
 322 Sample 5, even though the SAR values differ for the different concentrations.

323



324

325 **Fig. 7:** Hyperthermia measurements of temperature (T) as a function of time (t) for Sample 4  
 326 (panels a, b, c) and Sample 5 (panels d, e, f) for various sample concentrations: a, d: 2.5  
 327 mg(Fe)/mL; b, e : 5 mg(Fe)/mL ; c, f : 10 mg(Fe)/mL. The alternating magnetic field (AMF)  
 328 intensity is 31 mT and its frequency is 198 kHz. The yellow lines show the mean values  $T(t)$ ;  
 329 the grey area represents the error bar on T. The SAR value indicated on each figure panel is  
 330 calculated as the average of maximum, minimum and median values.

331  
332

## 4- Discussion

333 The various observations and measurements presented in this study allow to finely  
334 characterize the structural and magnetic properties of magnetosomes through the whole  
335 process of transformation from MSR-1 raw MTB to coated magnetosomes that could be  
336 potentially used for cancer treatment. Because FORC diagrams strongly depend on the  
337 arrangement of individual particles in a sample, we can also observe the effects of these  
338 structural properties on the FORC features. However, it should be kept in mind that samples  
339 observed in cryo-TEM are prepared in a substrate in 2D configuration while for magnetic  
340 measurements the sample is prepared in a 3D configuration.

341

### 342 *4.1. Comparison of structural and magnetic properties between samples*

343 The structural particle organization determined from (cryo-)TEM images agrees well with the  
344 evolution of magnetic properties measured in FORC diagrams. Magnetosomes in Sample 1  
345 are organized in chains, each chain being (in most images) isolated in a bacteria cell. This is  
346 consistent with the small amount of interactions inferred from the narrow FORC vertical  
347 profile (Fig. 6b). The strong shape anisotropy due to the chain organization is responsible for  
348 the high coercivities observed on the marginal coercivity distribution (Fig. 6a).

349 In Sample 2, magnetosomes aggregate in clusters (Fig. 2a, b), which is reflected in the FORC  
350 distribution that widens in the vertical direction due to increased magnetic interactions  
351 between magnetosomes. The whole coercivity distribution is shifted towards lower  
352 coercivities as a result of the partial collapse of the chains. This trend continues for Sample 3  
353 for which clusters are even denser and magnetic interactions are stronger than for Sample 2.

354 Both coated samples (Samples 4 and 5) show less magnetic interactions than extracted  
355 (Sample 2) and purified (Sample 3) magnetosomes. Indeed, Sample 5 is characterized by a  
356 narrow vertical FORC profile, which is consistent with the ring arrangement of

357 magnetosomes that was observed with cryo-TEM (Fig. 3 c,d). However, for Sample 4 (coated  
358 with DOPC) TEM images show highly clustered magnetosomes for which we would expect  
359 strong magnetic interactions, which is not consistent with the small amount of spreading of  
360 the FORC diagram along the vertical axis. A possible interpretation is that the DOPC coating  
361 may help reduce interactions even though magnetosomes are clustered. This result could also  
362 be explained by the relative instability of the DOPC coating with time at room temperature:  
363 although we expected DOPC coated samples to arrange in chains, we did not observe such an  
364 organization on cryo-TEM images, which seems to indicate that the coating and thus the  
365 organization of magnetosomes has been altered when exposed to room temperature. Citric  
366 acid appears to be a more stable coating, which prevents particles to agglomerate. Moreover,  
367 we envision that the organization of CA-coated magnetosomes may depend on the thickness  
368 of coating, which would deserve more investigation in a future work.

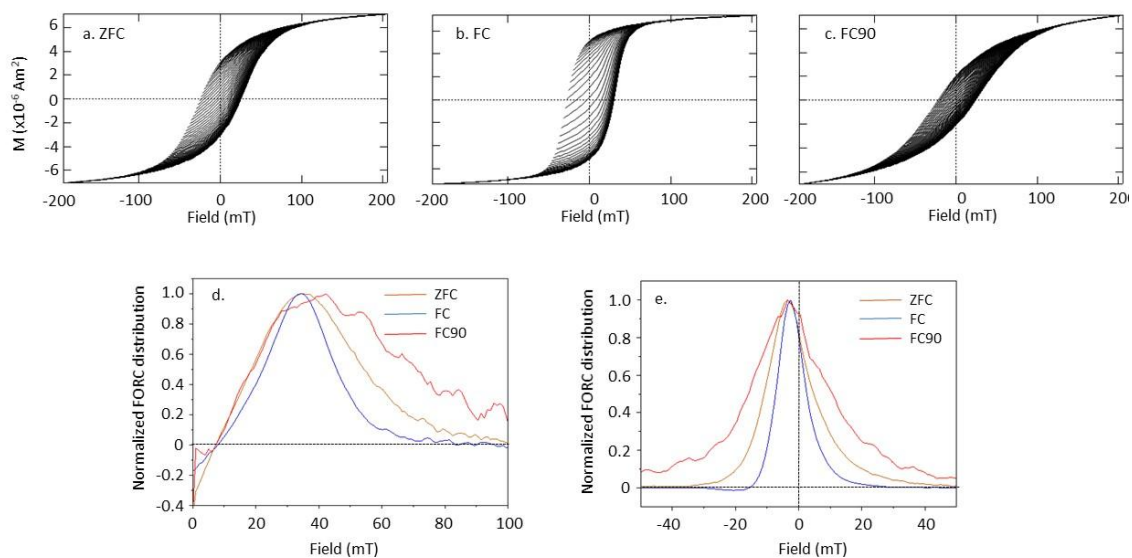
369

#### 370 *4.2. Anisotropy of magnetic interactions*

371 For samples in solution, measurements of informative FORC diagrams can only be performed  
372 at temperatures below the freezing point of water. However, it is possible to orientate the  
373 magnetic moments of particles by applying a magnetic field before the sample is frozen. By  
374 comparing FORC diagrams measured after field cooling, either with a magnetic field kept in  
375 the same initial direction (FC) or in a perpendicular direction to the initial (FC90), the  
376 anisotropy of magnetic interactions, which is connected to the anisotropy of particle  
377 organization, can be further investigated.

378 Because magnetosomes tend to form linear chains when a magnetic field is applied, their  
379 collective behavior is similar to that of a collection of elongated particles for which shape  
380 anisotropy dominates. In the FC configuration, all chains are oriented in the direction of the  
381 field applied during the FORC measurement. The field is therefore applied along the easy

382 magnetization axis. As a consequence, during FORC measurement, individual magnetic  
 383 moments tend to switch direction for a field range that is narrower than in the ZFC  
 384 configuration (Fig. 8a, b) resulting in hysteresis and partial hysteresis curves to have a more  
 385 squared shape and causing FORC diagram contours to be less extended both vertically and  
 386 horizontally. By contrast, the FC90 configuration is equivalent to a measurement along the  
 387 hard magnetization axis leading to magnetic moments switching over a large range of fields  
 388 (Fig. 8c). Thus, hysteresis and partial hysteresis loops are more irreversible, and the FORC  
 389 diagrams cover a larger surface. The ZFC FORC diagrams have shapes intermediate between  
 390 the FC and the FC90 shapes. These results are fully consistent with the FORC diagrams  
 391 measured in ZFC, FC and FC90 configurations by [29] on oriented chains of hexagonal cobalt  
 392 nanoplates for which the easy axis was also aligned with the chain direction.



393  
 394 **Figure 8:** FORCs measured on Sample 5. a: ZFC; b: FC; c: FC90 configurations. d: Marginal  
 395 coercivity distributions. e: vertical profiles through the maximum of the FORC distribution  
 396 for the corresponding FORC diagrams.

397  
 398 These results can be further quantified by comparing the Full Width at Half Maximum  
 399 (FWHM) of the marginal distribution (Fig. 8d, and Table 1). The marginal distribution

400 FWHM of the five samples is lowest in the FC configuration and highest in the FC90  
 401 configuration. The same trend is found for the FWHM of the vertical distribution (Fig. 8e, and  
 402 Table 1), except for Sample 4, for which the ZFC FWHM is slightly higher than the FC90  
 403 FWHM. The difference in FWHM values is more striking for samples forming chains, i.e.,  
 404 Samples 1, 2 and 5. This could be used as a mean of quantifying the amount of chains  
 405 compared to the amount of individual particles, though further work would be needed to  
 406 investigate this effect.

407

408 **Table 1:** Values of  $H_c$  (horizontal) and  $H_u$  (vertical) fields for which the FORC distribution is  
 409 maximum; Full Width at Half Maximum (FWHM) of the marginal coercivity distributions  
 410 and the vertical field distributions, for all the measured FORC diagrams.

Sample	FC/ZFC/FC90	$H_c$ max (T)	$H_u$ max (T)	FWHM marginal (T)	FWHM vertical (T)
1	ZFC	0.067	0.000	0.045	0.011
	FC	0.060	0.000	0.040	0.008
	FC90	0.064	-0.001	0.056	0.016
2	ZFC	0.025	-0.001	0.036	0.026
	FC	0.020	-0.001	0.030	0.023
	FC90	0.024	-0.001	0.039	0.031
3	ZFC	0.035	0.000	0.045	0.039
	FC	0.032	-0.002	0.038	0.024
	FC90	0.032	-0.001	0.052	0.052
4	ZFC	0.038	0.000	0.047	0.029
	FC	0.036	-0.001	0.037	0.017
	FC90	0.040	-0.002	0.047	0.024
5	ZFC	0.035	-0.004	0.036	0.017
	FC	0.034	-0.002	0.023	0.009
	FC90	0.042	-0.002	0.052	0.024

411

#### 412 *4.3.Application to cancer treatment*

413 According to hyperthermia measurements, Sample 5 (coated with CA) presents better  
 414 hyperthermia properties than Sample 4 (coated with DOPC). The SAR and the heating  
 415 temperature are higher for Sample 5 than for Sample 4. As shown by TEM observations,

416 magnetosomes are arranged in chains forming rings in Sample 5, while there is no such  
417 organization in Sample 4. FORC diagrams, and especially the vertical FORC profiles indicate  
418 that magnetostatic interactions are weaker in Sample 5 than in Sample 4, which is expected  
419 based on the chain (Sample 5) versus cluster (Sample 4) configuration. This is consistent with  
420 the results of [30] and [31], in which the chain structure is shown to increase the SAR. This is  
421 also consistent with the fact that CA is a triacid, which allows the complexation of Fe with  
422 several magnetosomes, and therefore the re-formation of a chain-like structure [13].  
423 Our SAR values can also be compared with the SAR value of 217 W/g measured in a  
424 previous study on extracted magnetosomes produced in similar conditions as Sample 2 and  
425 measured with parameters identical to those used in this study (Le Fèvre, unpublished data).  
426 This value is intermediate between the SAR of the mostly unchained DOPC-coated  
427 magnetosomes and that of the partially chained CA-coated magnetosomes. As magnetosomes  
428 in Sample 2 are partially organized in chains, this is also consistent with the increase of SAR  
429 with chain structure.

430

431

## 5- Conclusion

432 The evolution of structural properties of magnetosomes across the production steps are  
433 consistent with the evolution of their magnetic properties and can be summarized as below:

- 434 1 Magnetosomes in MSR-1 bacteria are arranged in chains. The magnetic properties are  
435 typical of stable single domain particles forming chains;
- 436 2 After extraction, magnetosomes tend to form clusters, but some chains remain.  
437 Extracted and heated magnetosomes are composed of maghemite. The coercivity  
438 decreases, and magnetostatic interactions increase.

439 3 After purification, magnetosomes form clusters of particles, and it is more difficult to  
440 identify chains. Magnetostatic interactions increase with respect to extracted  
441 magnetosomes.

442 4 Magnetosomes coated with DOPC remain organized in clusters. This coating is not  
443 stable with respect to time and temperature. Magnetostatic interactions are comparable  
444 to those of extracted magnetosomes.

445 5 Magnetosomes coated with CA tend to form chains arranged in rings. The ability of  
446 particles to organize in larger structures has been recovered in this sample and  
447 magnetic properties show similarities with those observed in bacteria. The CA-coated  
448 sample displays enhanced hyperthermia properties compared to the DOPC-coated  
449 magnetosomes and therefore is more interesting for biomedical applications.

450 Lastly, differences in FORC diagrams acquired after zero-field cooling, field cooling co-  
451 axially to the measuring field and perpendicularly to the measuring field can be interpreted as  
452 partly resulting from the configuration of magnetosomes, whether chains are formed or not.  
453 Therefore, such measurements have the potential to help quantify the ability of magnetic  
454 particles to form chains, a critical characteristic in biological applications.

455



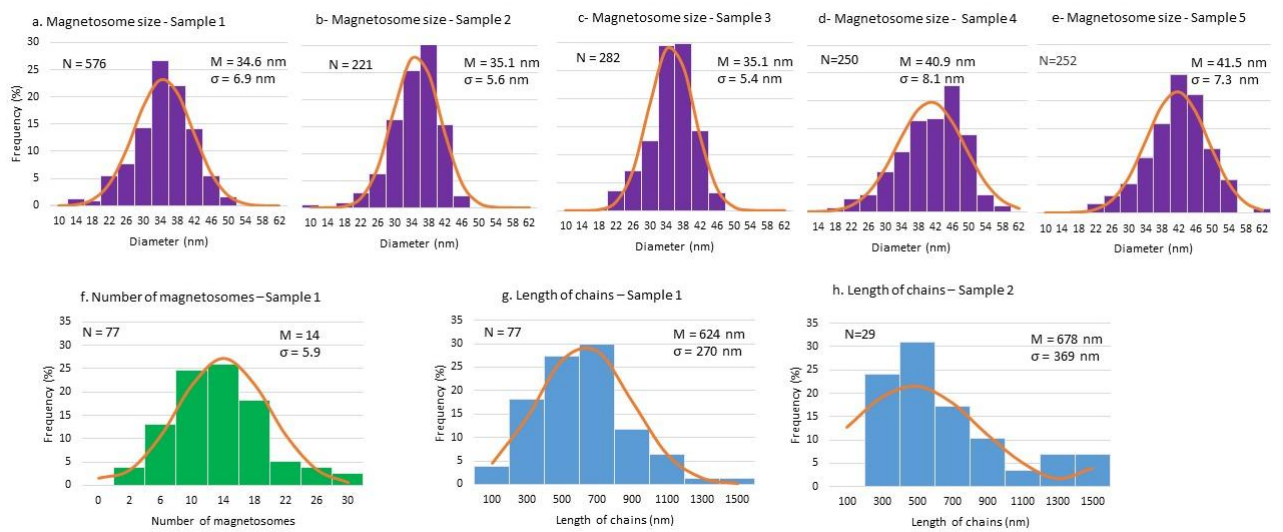
456 *Acknowledgements*

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459

460 **Appendix**

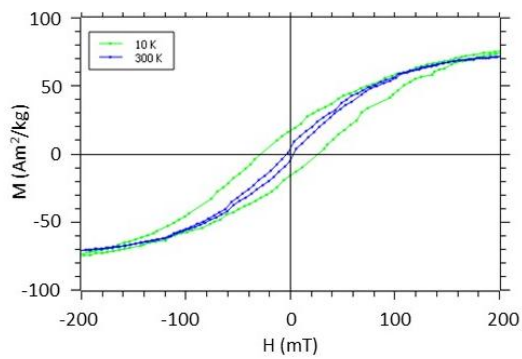


461

462 **Figure A-1:** Magnetosome size distributions for: a: Sample 1; b: Sample 2; c: Sample 3; d:

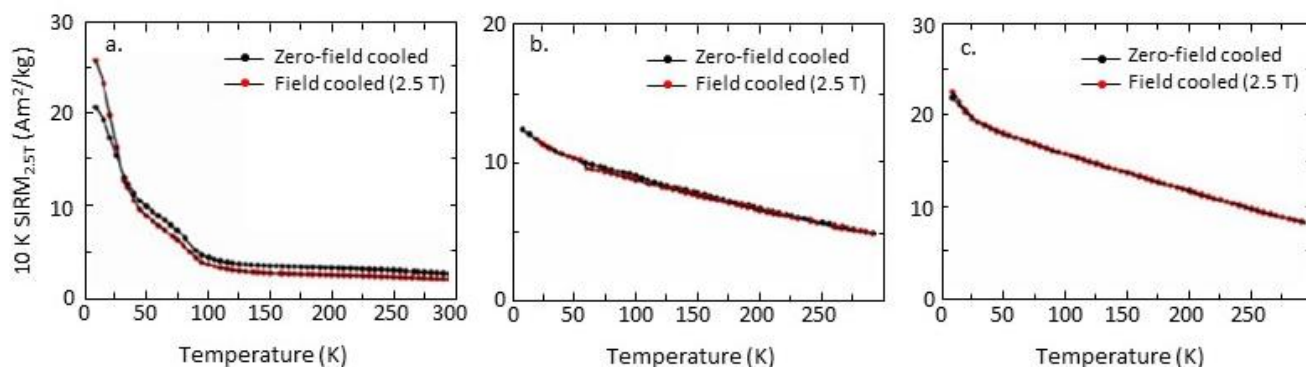
463 Sample 4; e: Sample 5; f: Distribution of the number of magnetosomes per chain for Sample

464 1; Length of chains distribution for: g: Sample 1; h: Sample 2.



465

466 **Figure A-2:** Magnetization curves measured for Samples 2 as a powder at 10 K (green curve)  
467 and at 300 K (blue curve).



468  
469 **Figure A-3:** Heating curve of SIRM acquired at 10 K in a 2.5 T field (Le Fèvre, unpublished  
470 data). a: Extracted magnetosomes; b: Extracted magnetosomes heated for 2 hours at 200°C; c:  
471 Purified magnetosomes (without membrane).

472

473

474           **References**

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