Intracellular Magneto-Spatial Organization of Magnetic Organelles Inside Intact Bacterial Cells

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Magnetotactic bacteria naturally produce magnetosomes, i.e., biological membrane bound nanomagnets, at ambient conditions. It is important to understand simultaneously the possible size variations and the magnetic behavior of nano-magnets inside intact bacterial cells for both applicational purposes as well as to enhance the basic understanding of biomineralization leading to intracellular nano-magnet synthesis. In this work, we utilize High-resolution Transmission Electron Microscopy and Near-field Scanning Optical Microscopy based measurements on intact non-fixed single cells to rigorously and quantitatively understand the intra-cellular magneto-spatial distribution of nano-magnets synthesized by Magnetospirillum gryphiswaldense. We demonstrate that it is possible to measure the relative magnetic moments along the intracellular magnetosomal chains for intact and non-fixed bacterial cells. Using our in vivo measurements on several single cells, we report that magnetic behavior of intracellular nano-magnets synthesized by magnetotactic bacteria depend on their relative location in the magnetosomal chains. Our work opens promising avenues in the direction of measuring the magnetic behavior of nano-magnets inside living systems by utilizing an operationally straight-forward approach.

Keywords: Magnetosome, Magnetotactic Bacteria, NSOM, Nanomagnets.

1. INTRODUCTION

The remarkable ability of magnetotactic bacteria for synthesizing uniform nano-magnets is well documented.1–5 However, complete mechanisms leading to this biomineralization are yet to be elucidated in spite of several studies that explore functional manifestations of intracellular magnetosomal assembly.6–10 While some excellent experimental observations on understanding magnetic properties of biologically synthesized nano-magnets have been primarily in aqueous suspensions of purified nano-magnets, they have required the use of capital intensive and sophisticated techniques.11,12 Studies on magnetotactic bacteria have provided us with substantial evidence of carrying out biological nano-magnet synthesis with somewhat uniform size distributions with low standard deviations. However precise control over biological synthesis of magnetic nano-crystals has not been possible till date. This is surprising considering the fact that, normally, biological synthesis is very robust and precise (and redundant to maintain these features). Magnetosomes are considered the “signature” organelles of magnetotactic bacteria, and sizes of functional cellular organelles are normally substantially uniform regardless of growth cycles. From an applicational point of view, an understanding into mechanisms leading to control of the size of the intracellularly synthesized nano-magnets is an important area. Till date, success in this direction has been limited in our scientific investigations by varying culture conditions to either scale-up the biological production of nano-magnets13–15 or, assess the impact on morphological features of bacterial cells and magnetosomal chains,16 or at best doping them with different minerals in efforts to change their compositions.12 While there are several fascinating insights into the genes and proteins that may be involved in controlling intracellular magnetosomal sizes,9,17–20 yet we have not advanced to controlling the size and magnetic characteristics during biological synthesis of nano-magnets even after nearly four decades of first discovery of the magnetotactic bacteria that naturally synthesize nano-magnets.21

There is an emerging consensus in the field in support that the newly-formed crystals are (i) at the end of the intracellular magnetosomal chains, and, (ii) are smaller compared to the existing crystals.22 However,
are the newly forming/formed crystals at the end of the intracellular magnetosomal chains solely responsible for heterogeneity in the sizes of the nano-magnets? To investigate this, we decided to measure exact intracellular size distributions of the nano-magnets by analyzing several single cells of the magnetotactic bacterium *Magnetospirillum gryphiswaldense*. More importantly, we were interested in understanding the contribution of the magnetization of individual nano-magnets to the overall magnetization of an intact magnetotactic bacterial cell. Some elegant studies have utilized Transmission Electron Microscopy (TEM)—holography to map the magnetic flux density along magnetosomal chains in bacterial cells.\textsuperscript{23, 24} Several other studies have also been carried out to elucidate magnetic characteristics of magnetotactic bacteria on a single cell level.\textsuperscript{25–27} However, limitations of low sample sizes, sophisticated sample preparation (including chemical fixation), and, lack of a straightforward interpretation of results have not allowed emergence of a robust correlation between magnetization of individual nano-magnets and their physical properties (size, crystal location, crystal density). Thus, we measured intracellular magnetic characteristics by using an experimental system recently developed by us\textsuperscript{28} for measurements on intact and non-fixed bacterial cells. Based on our analysis of several single cells of *Magnetospirillum gryphiswaldense*, we report the intracellular magnetospatial organization of magnetic organelles inside intact bacterial cells.

### 2. MATERIALS AND METHODS

*Magnetospirillum gryphiswaldense* was cultured exactly as described previously.\textsuperscript{3, 8, 28} High resolution transmission electron microscopy also done exactly as described previously.\textsuperscript{28} Elemental analysis by Energy Dispersive X-Ray (EDX) spectroscopy confirmed the presence of only Fe₃O₄ crystals inside the bacterial cells. *In vivo* observations of magnetic properties of magnetosomal chains were done using a Near-field Scanning Optical Microscopy/Atomic Force Microscopy (NSOM/AFM) system.\textsuperscript{28} Images were collected simultaneously by operating in the AFM mode for surface profiling, and, in the magneto-optic Kerr effect (MOKE) mode for obtaining the magnetization contrast of the sample. Here it is important to mention that resolution for any light-based technique is limited to approximately the wavelength of light, however this is applicable to mainly far-field diffraction-limited techniques. Near-field techniques can resolve much less than the wavelengths. For NSOM, the best possible resolution is ∼50 nm, limited largely by the curvature of the NSOM tip (the tapered optical fibre that launches the light close to the substrate). Specifically in our case, the field from the nano-magnets extends to a region larger than the magnetosomal size thereby limiting our ability to resolve down to individual nano-magnets separately. Therefore, our quantifications are done in terms of relative intracellular positions rather than specific magnetosomal positions.

### 3. RESULTS

#### 3.1. Magnetosomal Size Depends on Intracellular Position

Figures 1(A)–(F) show some representative images obtained from transmission electron microscopy (TEM), of intracellular nano-magnets at different magnification scales. From our TEM data, we carefully selected the 20 cells, each having its complete magnetosomal chain, with the images acquired at high enough resolutions for rigorous quantitative analysis of every single intracellular nano-magnet. From these selected images, we obtained the size distribution for 430 intracellular magnets, by analyzing each nano-magnet individually, as shown in Figure 1(G). Interestingly, while the size of the intracellular nano-magnets was 36.8 ± 9.1 nm, the number of magnetosomes per cell varied from 13 to 28 in our selected population. Further, while several cells apparently had larger nano-magnets in the center of the magnetosomal chains (as shown in Figs. 1(B, D)), a few cells appeared to have a more heterogeneous distribution of nano-magnet sizes in their magnetosomal chains (as shown in Fig. 1(F)). Thus, we hypothesized that the ∼25% standard deviation in the size distribution observed by us could result from either a consistent position-dependence of sizes of the intracellular nano-magnets (as in the former above), or, heterogeneity within individual magnetosomal chains (as in the latter above). To investigate this, we first focused on cells that had exactly the same number of intracellular nano-magnets. Figure 1(H) shows the sizes of nano-magnets as a function of position inside three bacterial cells having 23 magnetosomes each. The first observation is clear that size of all nano-magnets comprising the magnetosomal chains is not the same inside the cells, consistent with the apparent qualitative observations above. This strongly indicates that the variations in sizes of the biological nano-magnets arise out of intracellular variations in each cell, rather than existence of distinct populations of bacterial cells with each population having a constant size of intracellular nano-magnets.

The second observation in Figure 1(H) is clear positional dependence of the size of nano-magnets. Nano-magnets at the edges of the chains are smaller than those at the center of the chain for all the three cells. Was this observation generally applicable regardless of the number intracellular nano-magnets? To answer this question, we had to first devise an approach for comparing positions of the intracellular nano-magnets relative to each other, but independent of their actual number inside the cell. Thus, we randomly assigned the nano-magnet on one end of the magnetosomal chain in each bacterial cell as the first. That automatically assigned the nano-magnet at the other end...
of the magnetosomal chain as last. This way, we were able to scale the position of intracellular nano-magnets relative to each other from 0–100%, 1 being the nano-magnet at the first end and 100 being the nano-magnet at the other end of the magnetosomal chain. This scaling of position allowed a comparison of distinct magnetosomal chains of varying lengths (i.e., varying number of intracellular nano-magnets) inside different bacterial cells, independent of the actual number of nano-magnets in any given magnetosomal chain. Figure 1(I) shows that the dependence of size on the position of the nano-magnet in the intracellular magnetosomal chains for twenty individual bacterial cells, each with different numbers of intracellular nano-magnets, was similar to that observed previously for three cells with the same number of nano-magnets. The nano-magnets at the end of the magnetosomal chains were smaller than those at the center of the chains. Here, it is conceivable that significant differences in sizes of the nano-magnets are observed mainly at the ends of the magnetosomal chains due to averaging of positions from bacterial cells that could have been present in different stages of biomineralization at the time of sampling. For example, if biomineralization in a cell started later compared to another, then largest crystals in the middle of the former will be equivalent to or possibly smaller than the crystals of the latter.

Thus, to confirm whether our size dependence conclusions were not resulting from averaging cells at different stages of biomineralization, we re-plotted the data for the 20 cells in terms of percent size of the nano-magnet as a function of percent position. Percent size was defined for each individual with the largest crystal in a given cell being scaled as 100% for the given cell and rest scaled in relation to that crystal. Figure 1(J) confirms that the conclusions drawn previously from Figure 1(H) and (I).

To further strengthen our conclusions, results of statistical analysis for positional dependence of magnetosomal sizes are shown in Table I. Two-tailed homoscedastic t-tests were performed on data from Figure 1(J) to compare the sizes observed at each Percent Position with the size observed at 50% position (i.e., at the center of the chain). Clearly the nano-magnets at the ends of the chains (∼20% on both sides) are significantly different in sizes (see Table I) and smaller (see Fig. 1(J)) compared to those at the center of the chains.

3.2. In Vivo Measurements of Magnetosomal Magnetization

How is the positional dependence of intracellular magnetosomes relevant in the context of magnetization of the intracellular magnetosomal chains? To answer this we had to first develop a methodology that would allow observation of the magnetic properties inside intact, and preferably living, cells. Thus we developed a nanonics
Table 1. Magnetosomal size depends on intracellular position: Statistical analysis for positional dependence of magnetosomal sizes was carried out. p-values obtained from two-tailed homoscedastic r-tests performed on data from Figure 1(J), to compare the sizes observed at each Percent Position with the size observed at 50% position (i.e., at the center of the chain), are shown. *show statistically significant differences (p < 0.05).

<table>
<thead>
<tr>
<th>Percent position (%)</th>
<th>p-value</th>
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<tr>
<td>0−5°</td>
<td>8.85 × 10^{-12}</td>
</tr>
<tr>
<td>&gt;5 to 10°</td>
<td>2.77 × 10^{-16}</td>
</tr>
<tr>
<td>&gt;10 to 15°</td>
<td>1.20 × 10^{-16}</td>
</tr>
<tr>
<td>&gt;15 to 20°</td>
<td>1.51 × 10^{-16}</td>
</tr>
<tr>
<td>&gt;20 to 25</td>
<td>5.83 × 10^{-16}</td>
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<td>&gt;25 to 30</td>
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<td>7.30 × 10^{-16}</td>
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<td>9.66 × 10^{-16}</td>
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<td>&gt;55 to 60</td>
<td>7.69 × 10^{-16}</td>
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<td>8.28 × 10^{-16}</td>
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<tr>
<td>&gt;95 to 100°</td>
<td>1.28 × 10^{-16}</td>
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NSOM/AFM system to simultaneously image surface profiles and magnetization contrasts of single intact magneto-tactic bacterial cells using the magneto-optic Kerr effect, MOKE. The aim was to rigorously quantify the magnetization contrasts arising out of intracellular magnetosomal chains. Figures 2(A), (B) show the images of two bacterial cells acquired using the AFM mode of measurements. Figures 2(C), (D) show the magnetization contrasts observed by utilizing the MOKE effect of the same two cells. The clear black-and-white contrasts inside each of the two cells are due to magnetization of the nanomagnets aligned in the intracellular magnetosomal chains. Since this measurement does not allow visualization of the individual nano-magnets, rather it spans the whole magnetosomal chain, we carried out a line scan of the magnetization contrast across the whole bacterial cell. The blue and green lines in Figure 2(C) show one such line scan for each of the two cells. The pixel intensity profile along the two line scans are shown in Figure 2(E), (F) respectively. Clearly, the “sinusoidal” nature of the intensities allowed for a quantitative understanding of the magnetization along the line scan, providing measurable parameters of the magnetic width ($d_{MW}$) and the amplitude (M) in + and − directions. From these measured parameters we were able to calculate the magnetic moment $\mu$, in relative units (see figure legend for details). The quantitative assessment of the magnetic moment allowed us to investigate the positional distribution of the magnetization inside the intact bacterial cells. Figure 2(G) shows $\mu$, calculated from plots of all the line scans done across the two bacterial cells, as a function of the actual length ($L$) of the bacterial cell. Figure 2(H) shows the same $\mu$ as a function of the “magnetic length” ($L_M$) of the bacterial cell, that is simply the distance between the terminal ends of the black-and-white magnetization contrast. Clearly, and...
remarkably, the magnetic moment was found to depend on the intracellular location with the highest values in the middle and lowest values towards the edges of the magnetosomal chains. The next step was to extend this analysis to more number of cells for confirming whether the magnetic moment dependence on intracellular location was a general feature.

3.3. Magnetization of Magnetosomes Depend on Their Intracellular Positions and Magnetosomal Size

We calculated the magnetic moments from line scanning (as described in Fig. 2) for 9 individual bacterial cells. The data was analyzed as described for Figures 2(G) and (H). Figures 3(A) and (B) show that the magnetic moments along the magnetosomal chains are indeed dependent on their intracellular locations. It is somewhat important to note, as in Figure 2, that the dependence of magnetic characteristics of the magnetosomal chains on intracellular location is seen for both the whole cell length as well as just the magnetic length of the cells. Having confirmed the magnetic moment dependence on intracellular location as a general feature the magnetosomal chains, the next obvious question was to see whether it correlated with our previous observations (Fig. 1) on the size dependence of individual nano-magnets comprising the magnetosomal chains on intracellular location. Since we had already defined “Percent Position” in Figure 1, that is also indicative of the independent definition of the “Magnetic Length” defined in Figure 2, it was relatively straightforward to compare the size distribution of the nano-magnets comprising magnetosomal chains and distribution of the magnetic moments along the magnetosomal chains. For example, the magnetic moment of the magnetosomal chain at 50% of the magnetic length from the NSOM data was expected to represent the magnetic moment of the particular size of the nano-magnets at 50% position of the magnetosomal chain from the TEM data. Thus, we plotted the average magnetic moments of the magnetosomal chains for all the 9 single cells analyzed using NSOM, both in terms of the percent L and the percent \( L_M \) of the bacterial cells, against the average size of the nano-magnets in the magnetosomal chains corresponding to the same percentage position for all the 20 cells analyzed using TEM. By doing so in Figure 3(C), it was apparent that the magnetic moment of the intracellular magnetosomal chains was correlated to the size of the nano-magnets occurring at a given position, regardless of the intracellular location. Figure 3(D), showing the high correlation coefficients, confirmed that the magnetic moment of individual nano-magnets comprising a magnetosomal chain was directly correlated to size of the nano-magnet. Therefore, magnetization of the magnetosomes was dependent on the size of each magnetosome.

4. DISCUSSION AND CONCLUSIONS

Using rigorous analysis of high resolution TEM images of several single cells and individual nano-magnets forming intracellular magnetosomal chains in magnetotactic bacteria, along with utilization of a newly developed NSOM technique we have shown the following:

1. Size of individual magnetosomes is dependent on their relative location in the magnetosomal chains. Magnetosomes at the terminal ends of magnetosomal chains are smaller compared to magnetosomes in the middle.
2. It is possible to measure the relative magnetic moments along the intracellular magnetosomal chains for intact and living bacterial cells.
3. The magnetic behavior of a magnetosomal chain is not the same along the whole chain. It is the strongest in the middle and weakest at the edges.

Summarizing the above, we demonstrate that magnetic behavior of intracellular nano-magnets arranged in magnetosomal chains is correlated with the size of the individual nano-magnet. While at first glance this result may appear trivial, but it is certainly not. Visual inspection of
densities of nano-magnets shown in Figures 1(B)–(F) provides no apparent correlation with position. We confirmed this lack of dependence of magnetosomal density on intracellular position by rigorously quantifying the densities from our TEM data (not shown). On the one hand, one has to be careful in interpreting this result since all the magnetosomes are most probably not in the same plane while acquiring the data. On the other hand, the results do reflect the possibility that densities of nano-magnets inside individual magnetosomes can vary along the chain randomly. This would imply that some nano-magnets have more iron-oxide per unit area/volume compared to others. Thus, one would expect denser nano-magnets to have higher magnetization. Our findings do not indicate any such behavior.

Rather, the magnetic behavior of the magnetosomal chain appears to depend only on size (that depends on intracellular position). More importantly, a single bar magnet shows strongest magnetic behavior at its poles, where as magnetosomal chains show exactly the opposite behavior. This indicates that intracellular magnetosomal chains do not behave as a single bar magnet. We hope that this finding will be useful in better understanding intracellular behavior of magnetosomes, especially during magneto-aerotaxis, and possibly for exploring applicational possibilities.

References and Notes

5. A. Mittal, Women are from Venus, are magnetic bacteria from Mars? *Nature India* (2008), doi:10.1038/nihindia2008216.

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