Biomimetic production of precise nanomagnetic particles using magnetic bacteria and their biomineralisation proteins

Johanna Galloway, Masayoshi Tanaka, Jonathan Bramble, Andrea Rawlings, Stephen Baldwin, Stephen Evans and Sarah Staniland

Introduction

Scientific and economic interest in nanotechnology has grown in recent years. Within this the quest to produce tiny and highly tailored magnetic particles or nanomagnets is crucial. Nanomagnets have a range of practical uses such as: the development of 3D information storage systems providing high density data storage; medical applications such as site specific targeted therapies and image enhancers for diagnostic medicine.

However, as nanotechnology grows, so too does the need to develop precisely engineered nanomagnets. Different applications demand different shapes and sizes of particles and different magnetic properties. Producing nanomagnets with highly controlled; composition, size and shapes, in large enough amounts to be of use to these industries, have therefore become a key goal of researchers.

Magnetotactic bacteria are the simplest organisms that perform biomineralisation (Figure 1A). They take up iron ions from solution and produce nanoparticles of magnetite (Fe_3O_4) within lipid vesicles (called magnetosomes) with precise control, resulting in a strain specific uniform size and morphology. Interesting our recent TEM study has revealed how magnetic bacteria divide and what happens to the magnetosome chain within them during this process.

The aim of this group's research is to investigate, understand and then manipulate the biomineralisation process within these bacteria to enable the production of high-yields of customised nanomagnets for nanotechnological application using the genetic precision of nature.

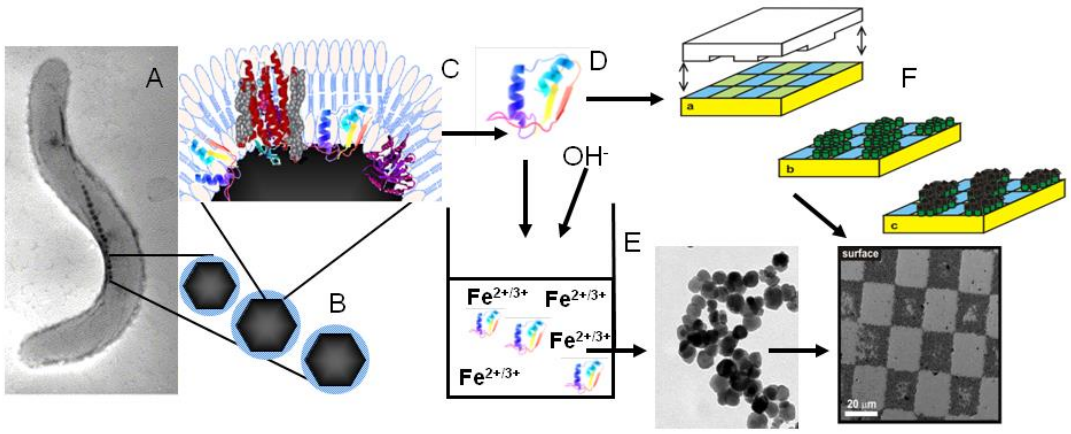

Figure 1: schematic of research activity. (a) a magnetotactic bacterium; **(b)** enlarged magnetosomes; **(c)** a close-up of the magnetosome membrane representing magnetosome membrane specific (Mms) proteins. **(d)** represents Mms6 with **(e)** depicting the *in vitro* precipitation. **(f)** Mms6 can be used to both control magnetic nanoparticle morphology and locate them in a pattern on a surface.

Results

The magnetic composition of magnetosomes as been successfully altered in vivo by doping the magnetosomes with cobalt resulting in magnetosomes with an increased magnetic coecivity compared to control magnetosomes. This was achieved with the addition of cobalt ions into the bacterial growth media which were taken up and incorporated into the magnetite mineral in approximately 1% quantities. More recently we have increased the

doping levels of cobalt to 3% and also achieved in vivo magnetosome doping with Mn (2.7 %) and Cu (15.6%). This has been achieved by a systematic study of growth and magnetosome over a range of concentrations of various transition metals to find the minimum inhibitory concentration for the microbes and the optimum doping levels. Additionally, we induced the production of independent nanoparticles of Te and Se within the cell. It must be noted that doping concentrations are restricted when magnetosomes are modified *in vivo* due to poisoning of the organism. We thus sought an *in vitro* route to offer more flexibility and higher-yields. Here, in collaboration with the lab of Prof. Matsunaga we build on their original method to develop a biomimetic route to more precise nanomagnets synthesized at room temperature using protein mediated precipitation of magnetic nanoparticles. The protein used was Mms6 (magnetosome membrane specific, 6 KDa) which was found to be unique to the magnetosome membrane and tightly bound to the crystal (Figure 1C & D). When this protein was expressed and purified and used *in vitro* it was found to control particle size and shape (Figure 1E).

Research is now being conducted in two parallel and complementary directions:
Firstly, the physical investigation of how Mms proteins interact with the forming mineral and control the magnetite's formation and morphology is being investigated (BBSRC funded). Several new proteins are being identified and expressed while their interaction with magnetite is being assessed using a range of spectroscopy, electron/force microscopy and neutron scattering techniques. Once key motifs, peptide and binding sites can be identified we could begin to design tailored additives for high-yield industrial nanomagnet production.

Secondly, we are developing a range of methods using the expressed Mms proteins *in vitro* for more advanced synthesis. We are enhancing this with the addition of membranes and vesicles to the systems (EPSRC funded). This is being furthered by experimenting with different proteins that affect the functionality of membranes. For example we are investigating novel metal ion transport proteins and vesicle deformation proteins which can be incorporation into vesicles along with Mms proteins to develop a range of novel, flexible biomimetic systems.

Finally, we have patterned a SAM surface with Mms6 and successfully mineralised morphologically controlled magnetic nanoparticles located in patterned on the surface (Figure 1F). Thus the protein has a dual purpose of controlling particle formation and locating the particles to the pattern. This is currently been advanced further to form customised nanomagnetic arrays. This is just one of many biomimetic systems we are developing to create several novel mineral/membrane assemblies, some tethered/free and attached to surfaces.

Publications
Galloway, J., Bramble, J., Rawlings, A., Burnell, G., Evans, S. & Staniland, S. (2012) Biotemplated magnetic nanoparticle arrays. *Small* **8:**204-208.

Galloway, J., Bramble, J., Rawlings, A., Burnell, G., Evans, S. & Staniland, S. (2012) Nanomagnetic arrays formed with the biomineralization protein mms6. *J. Nano Res.* **17:** 127-146.

Galloway, J. & Staniland, S. (2012) Protein and peptide biotemplated metal and metal oxide nanoparticles and their patterning onto surfaces. *J. Mater. Chem.* **22:** 12423-12434.

Rawlings, A., Bramble, J. & Staniland, S. (2012) Innovation through imitation: biomimetic, bioinspired and biokleptic research. *Soft Matter* **8:** 6675-6679.

Tanaka, M., Brown, R., Hondow, N., Arakaki, A., Matsunaga, T. & Staniland, S. (2012) Highest levels of Cu, Mn and Co doped into nanomagnetic magnetosomes through optimized biomineralisation. *J. Mater. Chem.* **22:** 11919-11921.

Tanaka, M., Critchley, K., Matsunaga, T., Evans, S. Staniland, S. (2012) Fabrication of lipid tubules with embedded quantum dots by membrane tubulation protein. *Small* **8:** 1590-1595.

Funding
This work has been funded by the EPSRC (Overseas Postdoctoral Life-science Interface Fellowship Scheme 2005-2008 EP/C53204X/1) while Masayoshi Tanaka is funded as a Royal Society Newton Fellow under the Newton international fellowship scheme. Research is being funded by the BBSRC (2010-1014 BB/H005412/1) and the EPSRC (2011-2015 EP/I032355/1).

Collaborators
External: B. Ward (University of Edinburgh, UK), A. Harrison (ILL, Grenoble, France), T. Matsunaga (Tokyo University of Agriculture and Technology, Tokyo, Japan), D. Cowan (University of the Western Cape, Cape Town, South Africa), A. Roychoudhury (University of Stellenbosch, Stellenbosch, South Africa), M. Edirisinghe and E. Stride (University College London, UK).
Leeds: C. Moisescu and L. Benning (School of Earth and Environment).