



## Using Magnetosome Genes to Enable Molecular Magnetic Resonance Imaging

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With its superb spatial resolution, magnetic resonance imaging (MRI) has great potential for monitoring the cellular activities that define early stages of disease progression. To this end, we are developing MRI reporter gene expression based on the magnetosome [1]. In magnetotactic bacteria (MTB), magnetosome formation is a stepwise protein-directed process that compartmentalizes iron biominerals in membrane-enclosed vesicles [2]. In mammalian cells, biosynthesis of magnetosome-like nanoparticles would provide an endogenous magnetic resonance (MR) contrast agent under genetic control [3] and enable MRI reporter gene.

Hypothesis: In mammalian cells, essential magnetosome proteins MamI and MamL co-localize on an intracellular membrane and interact to initiate formation of a rudimentary magnetosome-like nanoparticle.

MTB genes *mamI* and *mamL* were cloned from *M. magneticum* sp. AMB-1 and inserted into fluorescent vectors to create Mam fusion proteins. Green fluorescent protein (GFP)-MamI and Tomato-MamL were expressed in human melanoma cells, alone and in combination. Protein expression was verified by immunoblot. Subcellular location of fusion proteins was examined by confocal microscopy. Diffusion coefficient and apparent radius of fusion proteins were measured by fluorescence correlation spectroscopy (FCS) in cell-free samples. Analysis of variance (ANOVA) and post hoc tests were performed in GraphPad Prism 8.

Confocal microscopy of GFP-MamI and Tomato-MamL demonstrated fluorescence co-localization and protein-protein interactions. FCS data (Table 1) indicated a significant decrease in diffusion coefficient when GFP-MamI is co-expressed with Tomato-MamL compared to expression of GFP or GFP-MamI alone. This was consistent with a significant increase in apparent radius of co-expressed GFP-MamI/Tomato-MamL particles compared to GFP or GFP-MamI alone. The same trend was obtained with Tomato fusion protein/complexes.

**Table 1. FCS parameters in MDA-MB-435 cell lysates.**

	GFP	GFP-MamI	GFP-MamI/ Tomato-MamL	Tomato	Tomato- MamL	Tomato-MamL/ GFP-MamI
Diffusion coefficient ( $\mu\text{m}^2/\text{s}$ )*	108 ± 4% †	90 ± 3% ‡	43.2 ± 50% †,‡	51.4 ± 60%	49.2 ± 3%	40.1 ± 9%
Apparent radius (nm)*	1.7 ± 4% §	2.1 ± 3% ¶	4.4 ± 50% §,¶	3.7 ± 60%	3.8 ± 3%	4.7 ± 9%

\* Data are the mean ± standard deviation, expressed as a percentage (n = 5). †,‡  $p < 0.001$  §,¶  $p < 0.05$

Fluorescent fusion proteins of MamI and MamL co-localize and interact in a mammalian cell expression system, confirming the specificity of magnetosome protein interactions in a foreign environment.

### References:

- [1] Goldhawk *et al* (2017) Design and Applications of Nanoparticles in Biomedical Imaging, p. 187
- [2] Uebe & Schuler (2016) Nat Rev Microbiol 14:621
- [3] Sun *et al* (2019) Bioimaging: Imaging by Light and Electromagnetics in Medicine and Biology, accepted