Evaluation of Binding Properties of Functionalized Bacterial Magnetic Nanoparticles to Human Cancer Cells

Rebecca Mills and Denis Trubitsyn

Department of Biological and Environmental Sciences, Longwood University, Farmville, VA Email: trubitsynd@longwood.edu

Introduction

Bacterial nanoparticles, magnetosomes, are biomineralized by prokaryotic microorganisms known as magnetotactic bacteria **(Figure 1).** Magnetosomes are surrounded by a phospholipid bilayer membrane and contain an iron core. These characteristics make them unique and allow for application in biological and medical fields such as drug targeting, cells separation, and MRI contrast enhancement[1]. This can also be relevant in cancer therapy, specifically using magneto-hyperthermia technique[3]. They can be functionalized using foreign proteins that are genetically fused to magnetosome membrane anchor proteins. MamC is one of these proteins that is tightly associated with magnetosomes[2]. The overall aim of this study is to functionalize magnetosomes by creating a construct that will express the receptor protein, NKG2D, on the surface of magnetosomes **(Figure 2)**. This will allow them to specifically bind to cancer cells that over produce NKG2D ligand. NKG2D ligand is a protein overly abundant on the surface of cancer cells. In order to evaluate the binding properties of functionalized NKG2D magnetosomes to cancer cells, it first needs to be established that functionalized NKG2D is being expressed on magnetosomes.

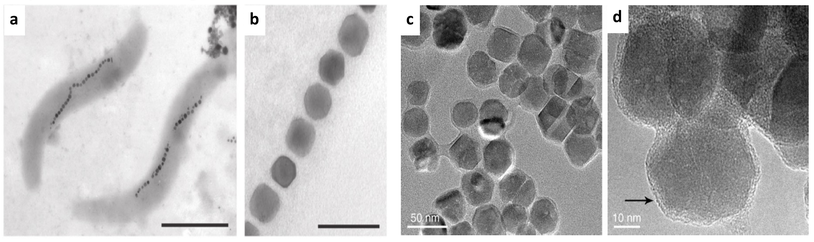


Figure 1. *Magnetospirillium gryphiswaldense* MSR-1 (a) and its magnetosomes (b, c, d)[1]

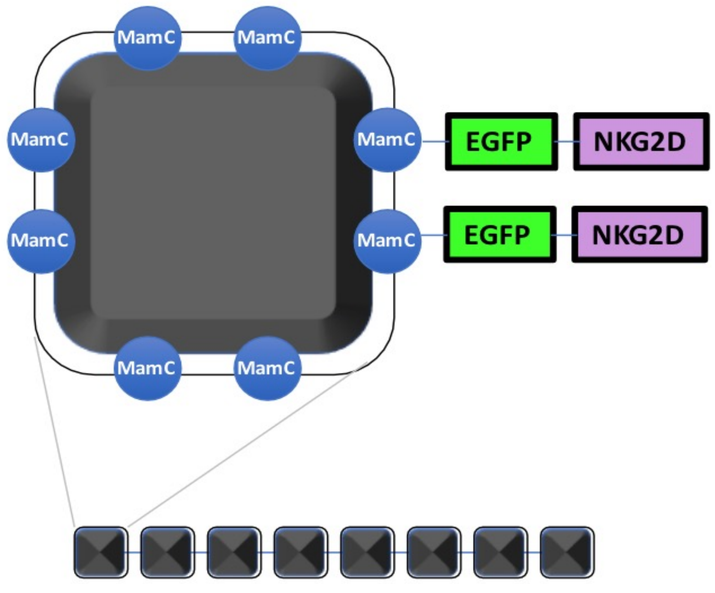
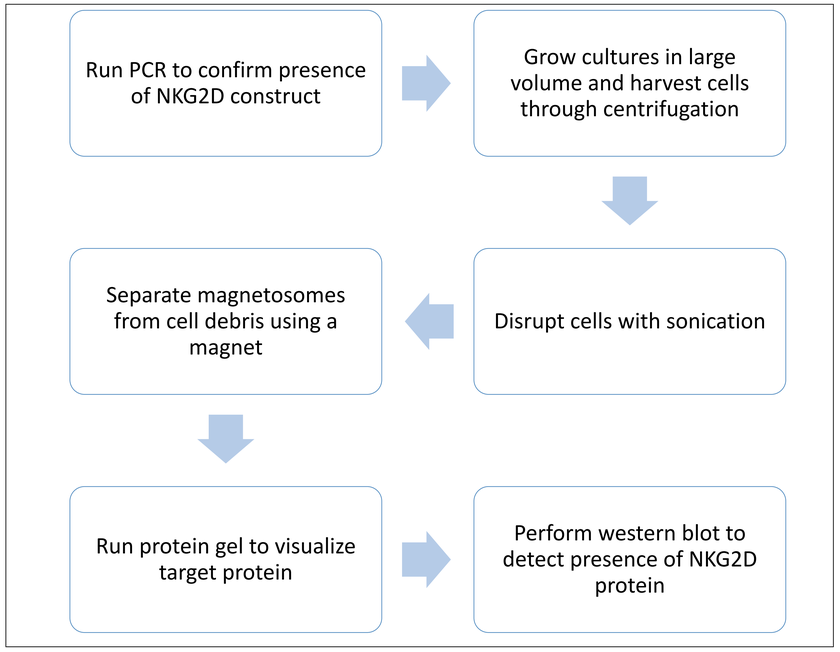


Figure 2. Schematic diagram showing the functionalization of magnetosomes with NKG2D receptor fused to MamC. MamC is a protein that fuses to the membrane of magnetosomes to allow functionalization. NKG2D is a receptor protein expressed on the surface of magnetosomes.

Materials and Methods



Results

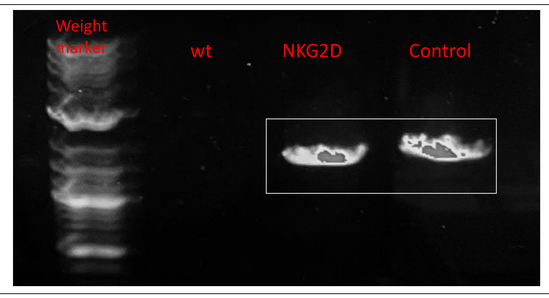


Figure 3. PCR to confirm target construct NKG2D. PCR products of expected size are highlighted above. Confirmation of the target construct includes the presence of a band in the NKG2D lane which is not present in wt.

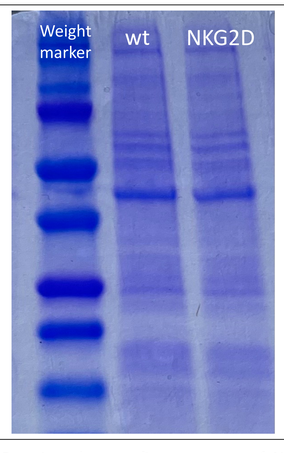


Figure 4. Protein gel to confirm presence of NKG2D protein. Difference in the two lanes would confirm expression of MamC-NKG2D.

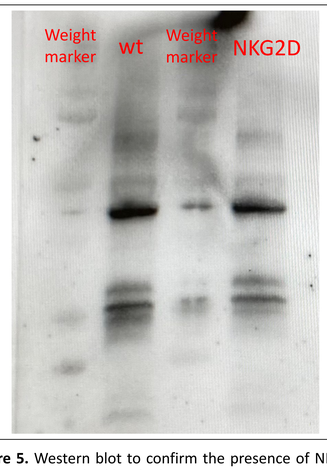


Figure 5. Western blot to confirm the presence of NKG2D protein. This method has higher sensitivity than protein gel and is aimed to see a difference in the two lanes

Results and Discussion

Presence of the genetic construct was confirmed through PCR, while wildtype (wt) strain in *Magnetospirillum gryphiswaldense MSR-1* did not contain the construct. A product with expected band size in NKG2D strain can be seen in **Figure 3**. The strains were cultured and harvested in order to isolate magnetosomes. Magnetosome proteins were stripped off magnetosome crystals and a protein gel was then performed to visualize MamC-NKG2D protein expression in the NKG2D strain. However, there was no difference in proteins observed in NKG2D and wildtype (**Figure 4).** In order to increase sensitivity, a western blot method was then used with primary anti-NKG2D and secondary anti-mouse antibodies to detect MamC-NKG2D fusion. The results in **Figure 5** show no difference in protein profile in NKG2D and wildtype. The next aim of the project will be to ensure expression of MamC-NKG2D fusion followed by experiments to evaluate specificity of binding properties of functionalized magnetosomes to human cancer cells.

Future Work

We welcome your ideas on how this project can be advanced. Please write in your comments below.

-Resequence the NKG2D construct to confirm that there are no mutations, specifically in the promoter region.

Acknowledgements

We are grateful to Amorette Barber at Longwood University for her expert advice on the use of NKG2D receptor proteins.

References

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2. Nudelman, H., Gonzalez, P., Kolushiva, S., Widdrat, M., Reichel, V., Peigneux, A., Davidov, G., Bitton, R., Faivre, D., Jimenez-Lopez, C., Zarivach, R. (2018). The importance of the helical structure of a MamC-derived magnetite-interacting peptide for its function in magnetite formation. *Acta Crystallografia: Structure Biology,* 74(1), 10-20.
3. Martinkova, P., Brtnicky, M., Kynicky, J., & Pohanka, M. (2018). Iron Oxide Nanoparticles: Innovative Tool in Cancer Diagnosis and Therapy. *Advanced healthcare materials*, 7(5), 1700932.