Investigating Ion-Protein Binding for Magnetite Formation Using NPS

Insplorion's Nanoplasmonic Sensing (NPS) technology enabled the monitoring of iron ion binding to a magnetosome protein and its mutants, allowing for a comparison of the two, ultimately providing insight into the specific iron-binding sites.

Introduction

Magnetotactic bacteria are capable of synthesizing single crystal nanoparticles of iron oxide, in the form of magnetite, inside organelles called magnetosomes. This process, biomineralization, gives rise to particles that are more uniform and have a narrower size distribution than those made using simple chemical synthesis methods. Uniformity is an important trait for nanoparticles used within the medical and semiconductor industries, making it essential to learn ways to better control nanoparticle formation in vivo and understand the biomineralization process. In this study, the activity of magnetosome membrane protein Mms6, involved in the loading, nucleation and growth of soluble iron ions into magnetite nanoparticles was investigated. Wild type and mutant Mms6 were used in order to decipher the specific residues responsible for the binding of iron ions.

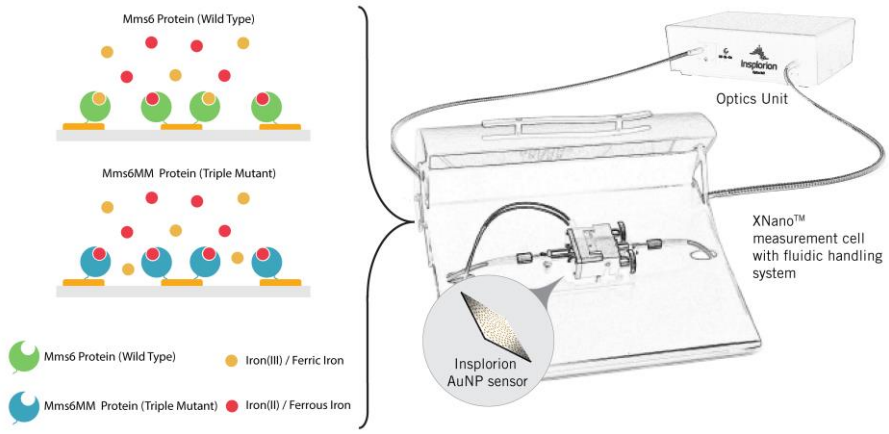

Figure 1: Insplorion system setup. The inset shows a schematic illustration of the sensor surface with the Mms6 wild type and mutant proteins, and their binding to Ferric and Ferrous Iron. Figure is not to scale.

Experimental Procedure

Mms6 mutants were generated by directed mutagenesis substituting negatively-charged residues suspected of influencing iron-binding with neutral ones. Wild type and mutant Mms6 were expressed in bacteria and purified. A triple mutant variant was then created comprising substitutions of three key residues identified after iron binding screening using a radiolabel-free iron binding assay. The triple mutant, called Mms6MM, was compared against the wild type protein (Mms6) using Insplorion's XNano. After obtaining a stable baseline in water with cleaned, bare gold sensors, the system was switched to Tris buffer (25mM, 7.4 pH, 500mM NaCl). The protein (0.3 mg/ml) was then applied; upon stabilization, buffer then water was flown through the system. Next, a solution of 2:1 Fe(III) to Fe(II), the stoichiometric ratio of magnetite, was introduced to the system. The system was subsequently returned to water. A 400 second window surrounding each transition point (buffer to protein, water to iron, and iron to water) was used in helping overlay the results from the wild type, mutated, and protein-free runs.

Results

NPS has several advantages in these types of studies. Firstly, the absence of the fluorescent tag means that any potential steric artefact interactions are avoided. Secondly, the self-assembly of the protein as immobilized on a gold surface is a better mimic of the magnetosome membrane's native environment. And finally, NPS makes it possible to study unstable proteins like Mms6MM in water.

The NPS signal was tracked as the buffer was changed to the protein solutions (Figure 2A), and again as water was changed to the Fe(III)/Fe(II)

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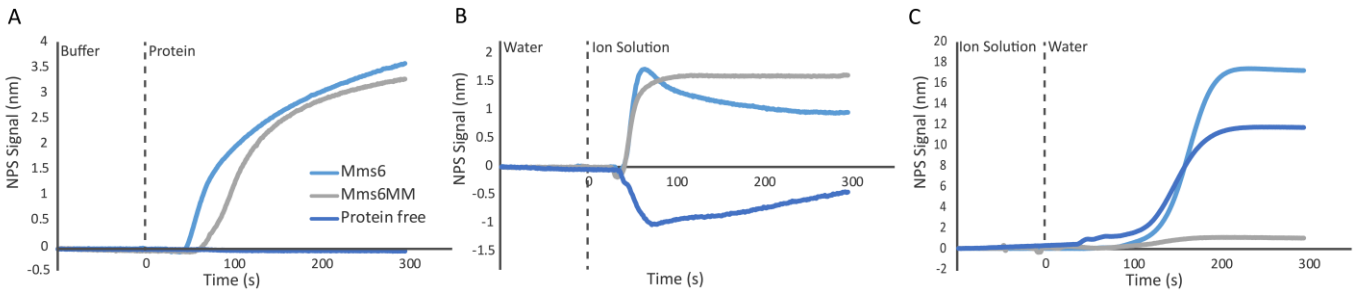

Figure 2: (A) Mms6, Mms6MM and negative control interaction with the NPS sensor's surface. (B) Interaction of iron(II)/iron(III) solution with the proteins on the surface after (A). (C) Transition from the iron solution to water and consequent iron precipitation.

solution (Figure 2B) and back (Figure 2C). Both Mms6 and Mms6MM showed a similar peak shift (Δλ) in 2A, indicating that both proteins attach to the gold surface in a similar manner, while saturation at around five minutes shows similar surface coverage. As expected, the negative control (protein-free buffer) did not change the NPS signal. When the mixed valence iron solution is applied (2B), both proteins react with a smaller magnitude. This small peak shift can be interpreted as interaction between iron ions and the protein, a conformation change in the protein, or a combination of both. Moreover, Mms6MM shows a decaying signal, suggesting weaker binding of the iron ions and clearly showing a difference between the triple mutant and wild type protein under the same condition. Figure 2C shows the peak shifts upon returning the system to water. Mms6 and the protein-free run have a sizable peak shift, signifying a large change in the immediate environment of the sensor surface. The authors suggest this is potentially due to the precipitation of iron oxide as the acidic pH of the mixed valence iron solution is replaced with that of neutral water. Note that iron(III) oxide can precipitate at pH ≤6 whereas iron(II) oxide is more soluble in water, precipitating only at >6. The triple mutant Mms6MM, however, does not show such a large shift during this change, indicating that the iron(III) precipitation on the surface of the protein at this pH was prevented. This could be due to iron-binding inhibition resulting from the mutations, or a change in the protein structure.

Monte Carlo simulations strengthened the hypothesis that the mutations did not cause major conformation changes, thus implying that the lack of change in the signal is likely due to iron(III) binding inhibition.

Conclusions

Insplorion's Nanoplasmonic sensing was used to investigate the effect of individual and multiple mutations in the Mms6 protein on its ability to bind iron for magnetite biomineralization. NPS offered several advantages for elucidating these results, namely: 1) a tag-free system offers no steric artefacts, allowing native self-assembly; 2) a more similar environment to the magnetosome membrane on a surface; and 3) a more stable environment for unstable biomolecules at biological pH solutions. This work shows how NPS can be used in the study of the mechanisms behind biomineralization which will ultimately allow better control of the biosynthesis of magnetic nanoparticles. This work shows one of many possible ways to use Insplorion's technology and research instruments to study bioinorganic interactions.

This study was performed by Prof. Sarah Staniland and coworkers at The University of Sheffield, United Kingdom.

References

[1] Rawlings et al. (2020) *Investigating the ferric ion binding site of magnetite biomineralisation protein Mms6.* DOI: https://doi.org/10.1371/journal.pone.0228708

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