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Metals IN BIOLOGY

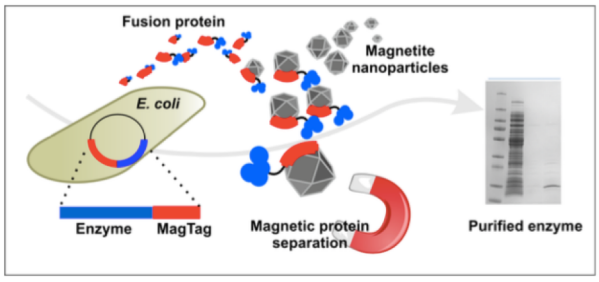
Mag-Tag: magnetite nanoparticle affinity tags for industrial biotechnology protein purification

"This PoC project has made a very fruitful industrial collaboration possible by means of a very simple and timely funding system. It will launch a whole new industrial research area for us." Sarah Staniland, University of Sheffield."

The University Of Sheffield. BIOCATALYSTS exceeding enzyme expectations

Sarah Staniland & Andrea Rawlings, University of Sheffield; Mark Blight, Biocatalysts Ltd

OUTCOMES: In this study, we used a protein that had high binding affinity and selectivity for certain magnetic materials that we previously identified. We used our affinity protein as a fusion tag (MagTag) to a test protein, GFP (green fluorescent protein), as this allowed us to track the binding and release of the target (the GFP fusion protein) via simple fluorescence measurements. During the course of the project we made the GFP–MagTag fusion construct and showed that the presence of the magnetic material binding tag had no detrimental impact on production of the GFP.



Schematic overview of the principle of the MagTag fusion protein purification system.

We optimised a simple synthetic route to the fabrication of cheap magnetic nanoparticles and demonstrated that the fusion protein could bind these under industrially relevant conditions, namely using crude cell lysate with a high optical density. Fluorescence measurements showed that we could successfully capture the GFP fusion protein from the lysate, out-competing other proteins within the sample. Importantly, we were able to show that it was possible to recover the GFP from the nanoparticles after binding and clean-up.

INITIAL AIMS: Enzyme catalysts are ideally suited to the industrial manufacture of foodstuffs, biofuels and pharmaceuticals, yet the current challenge to the widening the use of enzymes is the expense of producing them on a large scales due to the need for expensive, highly functionalised purification resins. We propose a revolutionary, cheap, universally applicable, enzyme purification method to widen the use of purified enzymes in industry. We will use protein fusion-tag technology to purify enzymes directly from crude preparations using cheap, unfunctionalised magnetic iron-oxide nanoparticles, meaning that the enzymes can then be bulk purified through magnetic separation. By substantially reducing the costs of purification we seek to make the use of enzymes an affordable, green and sustainable method of producing a wide range of products.

- **University funding awarded for further studies**
- **Awarded BBSRC follow-on funding for further development**
- **Seeking intellectual property protection**
- **Manuscript: Rawlings (2016) Biochem. Soc. Trans. 44: 790-795**

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