E3B The Elements of Bioremediation, Biomanufacturing & Bioenergy Metals in Biology

Durham University · University of Kent · Manchester 1824 The University of Manchester · UKRI Biotechnology and Biological Sciences Research Council

PROJECT PARTNERS: Mark Smales and David Beal, University of Kent; Paul Bennett, Sekisui Diagnostics; Charlotte Williams, CSIRO Manufacturing, Australia

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CSIRO · University of Kent · SEKISUI DIAGNOSTICS

Site-specific conjugation of alkaline phosphatase via metal-mediated His-tag complexation for biomanufacturing diagnostic and biotherapeutic reagents

"The funding allowed us to make new connections with the Kent group working on conjugation chemistry … and to discuss and explore different site-specific conjugation strategies that can potentially be utilised at CSIRO."

PROJECT AIMS: An increasing number of protein biotherapeutics and diagnostics require the linking of a protein to other molecules. But the use of specialised linker molecules often results in variable attachment in a non-site-specific manner.
To overcome this problem, we investigate using a histidine tag, attached to a model alkaline phosphatase enzyme, conjunction with metal ions to act as a bridge between the tag and another molecule.
We test the use of this approach to site-specifically link alkaline phosphatase to a model biotherapeutic antibody and determine the functionality of the product.

OUTCOMES & NEXT STEPS:

- Further experiments to increase the yield are being undertaken through MSc projects.
- The work will form the basis of grant applications to BBSRC and EPSRC. These will involve the collaborators, who are looking to exploit the technology.
- We are also exploring options to i) apply for funding to continue work with CSIRO ii) run a PhD studentship.
- The concepts of the work are included in our outreach work at local schools and University open days.
- Some results will form part of a wider paper on conjugation strategies for labelling.

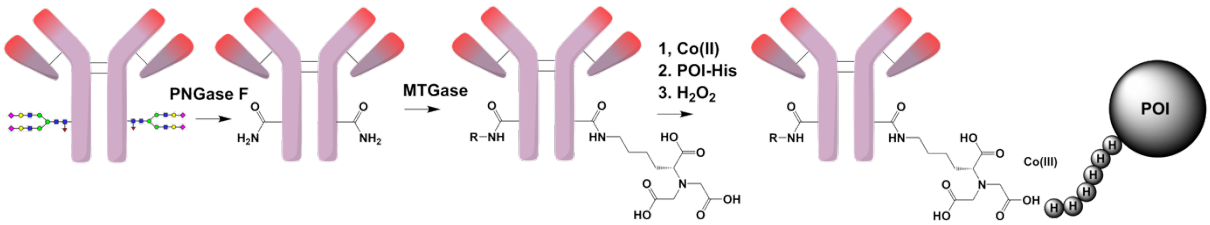
RESULTS:
Overall, we demonstrated that we can site-specifically conjugate His-tags on alkaline phosphatase to other molecules and moieties containing nitrilotriacetic acid functionality.

Using Sekisui's alkaline phosphatase containing a C-terminus His-tag as a model system, we showed that cobalt-mediated conjugation via the His-tag can be achieved:
(1) via nitrilotriacetic acid with a fluorescent marker (as initial proof of concept)
(2) via nitrilotriacetic acid with 5 KDa PEG (to determine the percentage conversion of the reaction)

We have shown that site-specific conjugation can be achieved directly to a His-tag, a tag ubiquitously used in recombinant protein applications, potentially allowing the site-specific conjugation of any His-tagged protein. By combining this approach with methodology to incorporate metal-binding elements to antibodies, we can produce antibody–recombinant protein reagents for diagnostics and as potential therapeutic molecules for treating disease.

Further work will focus on improving the yield, using a combination of surface immobilisation and conjugation.

Change in technology readiness level: 1 to 3/4



A schematic outlining the conjugation of a protein of interest (POI) containing a 6x His-tag to an IgG antibody using cobalt-mediated conjugation.