MACQUARIE University

Superior, site-specific and controlled IgM conjugation methods

EXECUTIVE SUMMARY

- Immunoglobulin M (IgMs) represent the first line of defense antibodies. Their therapautic and diagnostic use has been limited due to their characteristics - very large, complex and difficult to conjugate.
- We have developed a novel methodology that opens new avenues for therapeutic and diagnostic applications of conjugated IgMs. It enables strategic, planned and quantifiable site-specific conjugation of IgMs to small molecules, MRI/PET tracers or fluorescent probes, while maintaining recognition of antigen targets and not compromising structural antibody integrity.
- This methodology is superior over current methods used to functionalise IgG antibodies at specific sites, solving multiple challenges associated with the functionalisation of complex IgM antibodies. It is a biologically compatible process that offers uniformity and consistency without the use of harsh chemical reactions.
- An Australian provisional patent application has been filed. We are seeking an industry partner interested in applying this technology to functionalise IgM molecules through a collaboration or technology license.

BACKGROUND

IgMs are the first type of immunoglobulins to appear as part of the immune response to the initial exposure to an antigen, with strong, high avidity driven interactions. IgMs have demonstrated superior performance compared to IgGs in HIV (28-fold; *Wolbank et al 2003, J. Virology*) and in cancer (100-fold, *Piao et al 2016, OncoImmunology*). Antibody conjugation methods for therapeutic and diagnostic uses have mostly been applied to IgGs as the technological tools are available for their purification and conjugation. IgM molecules hold a great potential for further functionalisation, having ~400 lysines and 51 glycosylation sites available for coupling and engineering. However, known methods applied to IgGs are difficult or impossible to apply to IgM molecules. For example, site-specific cysteine labelling commonly applied to IgGs is not applicable due to disruption of the IgM polymeric structure, leading to loss of avidity and hence function.
The novel approach described here offers the opportunity of targeting antigens with a different antibody other than IgG that (i) recognises different types of antigens; (ii) is the first immune response of the body; (iii) has higher avidity than IgG due to its polyvalent structure and (iv) is able to be conjugated with many more (drug, imaging) molecules than IgGs.

SUPERIOR, NOVEL ENZYME-BASED CONJUGATION

- Allows precise attachment of small molecules, tracers and fluorescent probes;
- Retention of IgM function - attachment occurs outside antigen-binding sites;
- Therapeutically friendly process - IgM remains intact;
- Heavily glycosylated (51 glycosylation sites) - great candidates for site-specific antibody-drug (ADC) and imaging agent (MRI/PET) conjugations. Glycoconjugation methods have been applied to IgGs but are limited in their usefulness due to only two sites being available.
- High Drug-Antibody-Ratio (DAR) due to large number of glycosylation sites
- Tuneable attachment of different small molecules and ability to target different glycans (using specific glycosyltransferase enzymes and choosing either single or multiple transferases)
- Accurate determination of the amount and site of attachment of small molecules (quantifiable conjugation yield determined by mass spectrometry and/or UV/VIS spectrometry)

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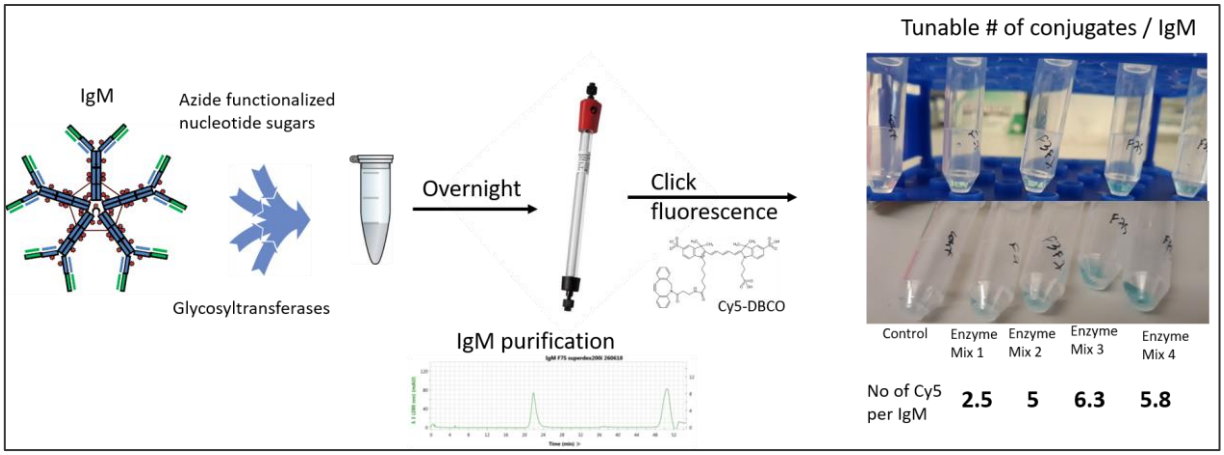

Figure 1. 2-step process workflow that yields site-specifically conjugated IgM molecules. The desired IgM is incubated with the chosen glycosyltransferases and their respective azide functionalized substrate in a single pot reaction to incorporate the azide groups onto the IgM. Following IgM purification, the desired small molecule functionalised with a cyclic alkyne can be readily incorporated onto the IgM. By choosing different glycosyltransferases, different amounts of incorporation can be achieved (differs for each IgM).

APPLICATIONS

- ✓ Therapeutics (small molecule IgM-ADCs)
- ✓ Diagnostics (tracer IgM-MRI, tracer IgM-PET)
- ✓ *In vitro* and *in vivo* assays (IgM-fluorophores)

CAPABILITIES AND EXPERTISE

The team from the Department of Molecular Sciences at Macquarie University has deep expertise in analytical technologies involving LC-MS/MS-based glycomics and glycoproteomics. Capabilities include mapping the micro- (glycoform distribution) and macro- (occupancy) heterogeneous glyco-signatures of IgMs in a detailed yet site-specific manner using top-end LC-MS/MS and software, which allows the analyses and interpretation of this type of data. The team has led the development and application of these technologies worldwide.

Key publications include: (i) Moh ES *et al*., (2016) Site-specific N-glycosylation of recombinant pentameric and hexameric human IgM. JASMS 27(7):1143-55; (ii) Arun V Everest-Dass *et al*., (2018) Human disease glycomics: technology advances enabling analysis of specific glycan structures on proteins – Part 1. Expert Reviews in Proteomics Feb;15(2):165-182.

INVENTORS

Dr Edward Moh, Dr Nima Sayyadi, Professor Nicolle Packer; Department of Molecular Sciences, Macquarie University

INTELLECTUAL PROPERTY POSITION

Australian provisional patent application filed

PARTNERING OPPORTUNITY

We are seeking an industry partner for further development and commercialisation of this technology through a research collaboration or technology licence. Opportunity to influence current provisional patent claims.

WOULD YOU LIKE TO KNOW MORE?

Gorjana Mitic, PhD; Commercialisation and Innovation Manager (Life Sciences) +61-2-9850-4542;
gorjana.mitic@mq.edu.au

mq.edu.au/commercialisation