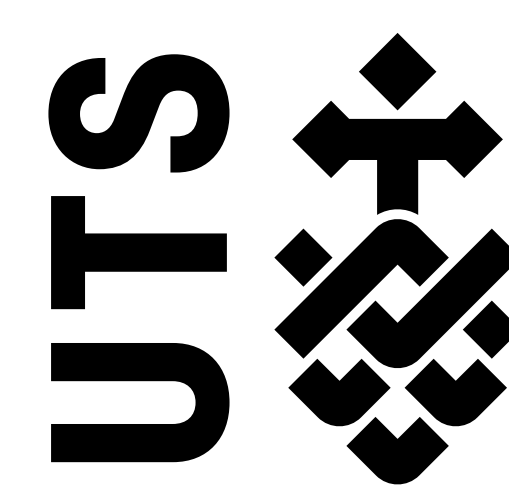


Metal Chelating Polymers as Homogenous Antibody Metal Conjugates for immuno-Mass Spectrometry Imaging



Anthony Thai ^a, Thomas Lockwood ^a, Karen Hakobyan ^b, Jake Violi ^c, Andrew McDonagh ^a & David Bishop ^a.

^a School of Mathematical and Physical Sciences, Faculty of Science, University of Technology Sydney, Sydney, NSW, Australia.

^b School of Chemical Engineering, Faculty of Engineering, University of New South Wales, Sydney, NSW, Australia.

^c School of Chemistry, Faculty of Science, University of New South Wales, Sydney, NSW, Australia.

INTRODUCTION AND BACKGROUND

Immuno-mass spectrometry imaging (iMSI) simultaneously images and quantifies biomolecules in tissue samples.¹

Tissue samples are stained with antibodies labelled with metals and then analysed with laser ablation-inductively coupled plasma-mass spectrometer (LA-ICP-MS).

For high sensitivity, antibodies are labelled with metal chelating polymers (MCPs) containing a metal chelator at each monomer unit.

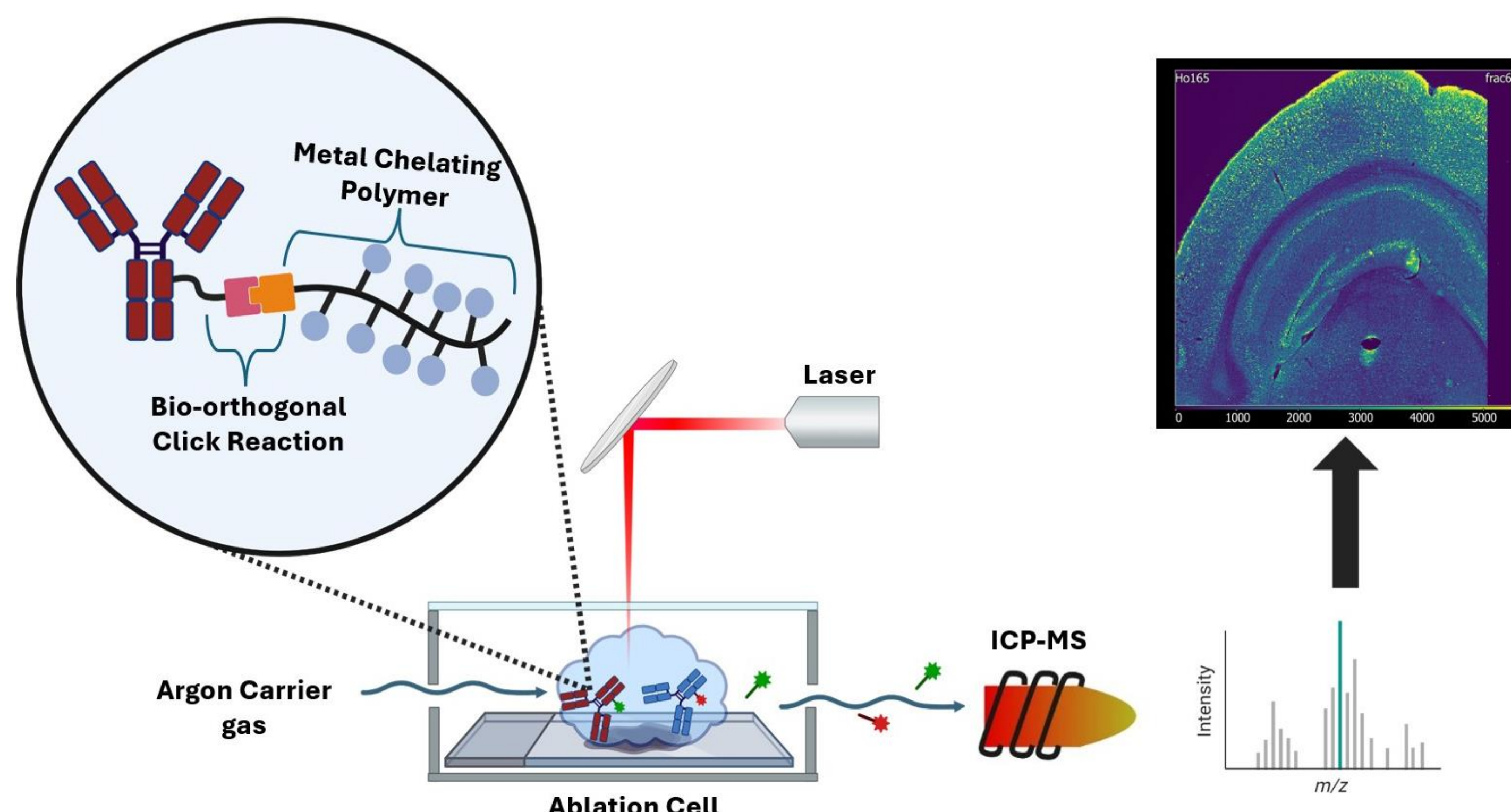
Reproducibility can be a barrier for quantitative analysis.

Current challenges:

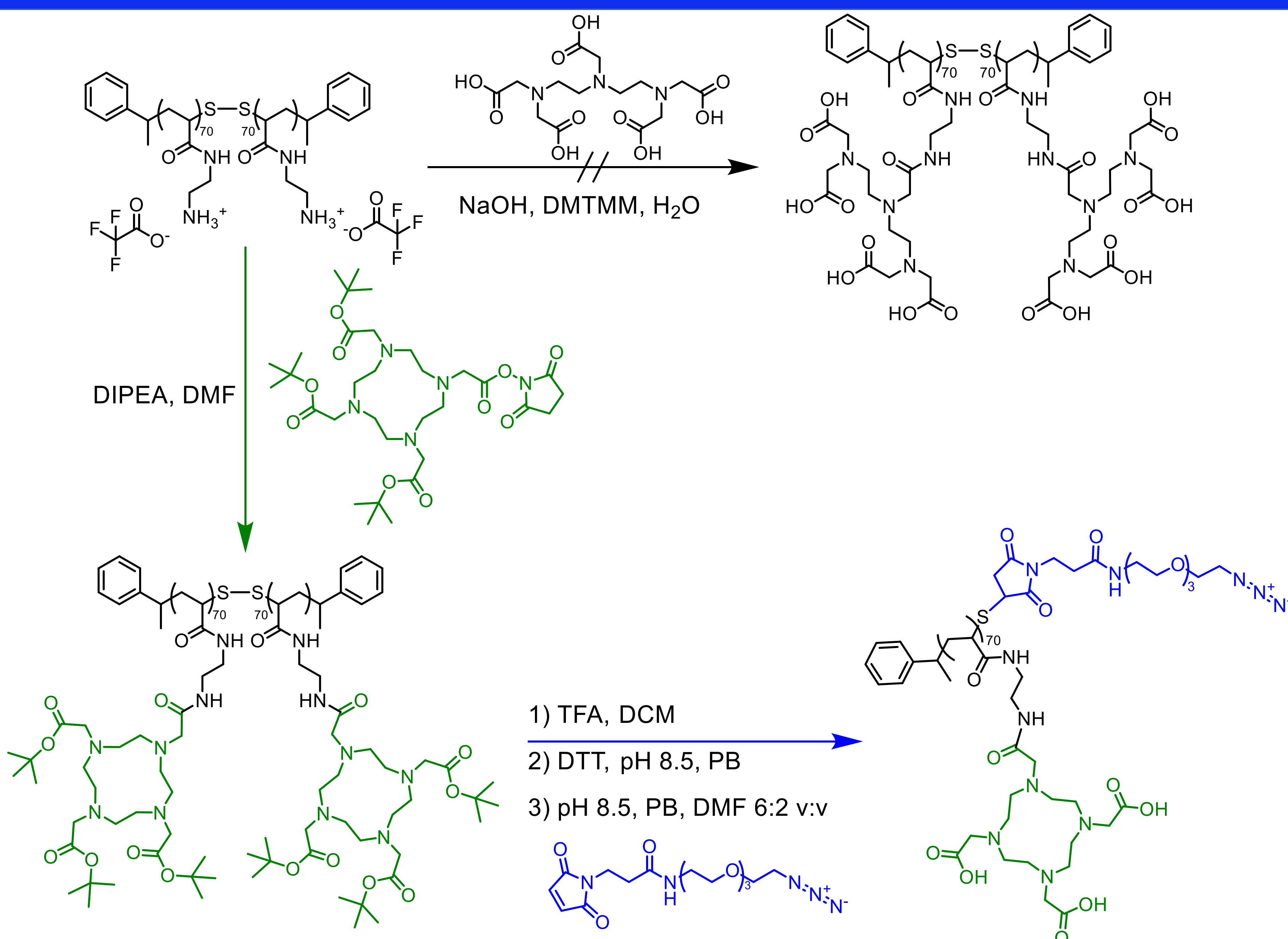
- Conjugating MCPs to antibodies using disulfide reduction of cystine residues for maleimide-thiol reactions produces irreproducible yields.²
- The number of metals per MCP can vary due to non-specific post-polymerisation reactions that incorporate the pendant chelator groups

Aims:

- Improve post-polymerisation modification reactions for introducing metal chelators.
- Introduce bio-orthogonal reactions to conjugate MCPs to antibodies



METHODS



Previous Method

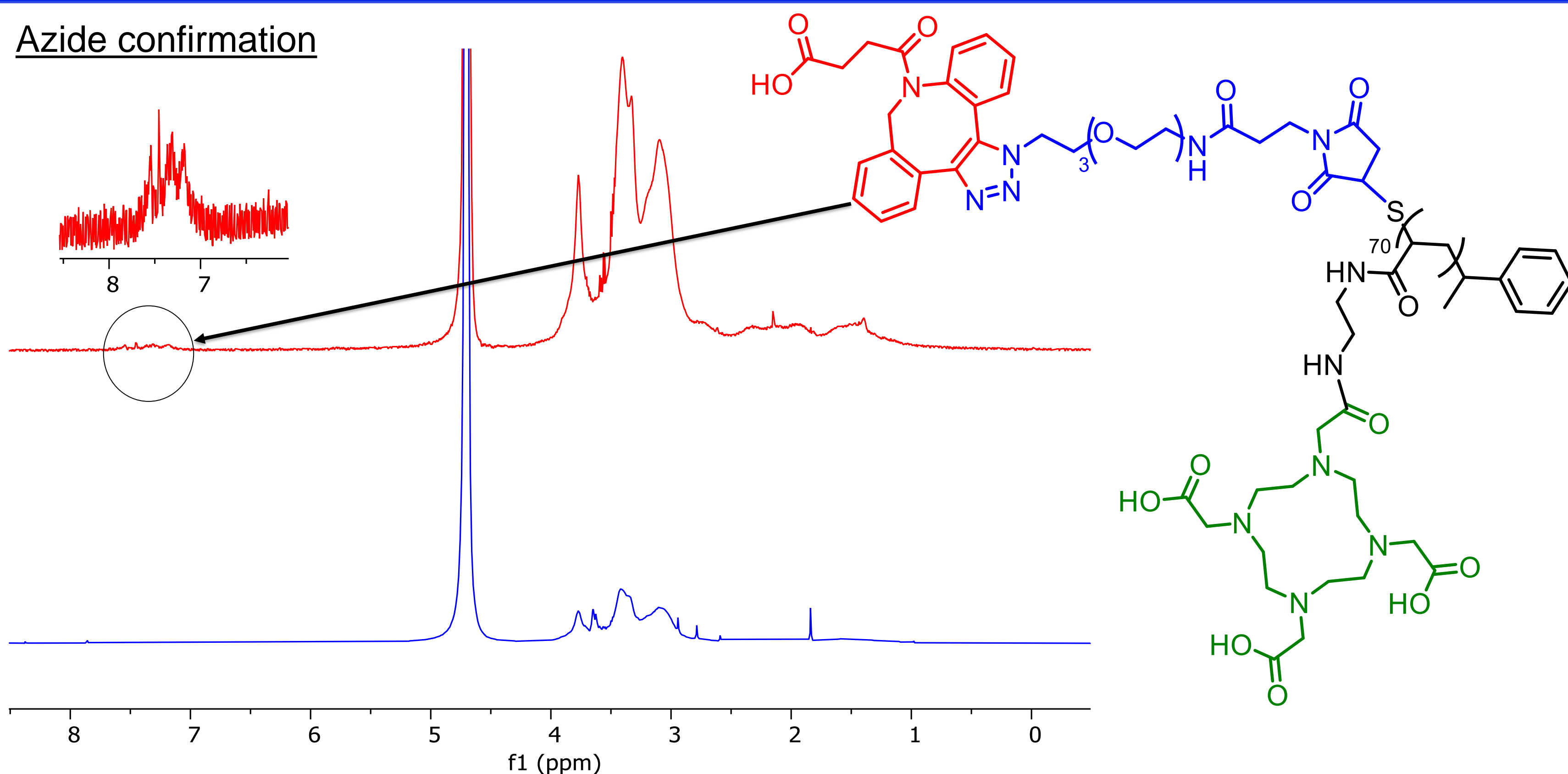
- 80 equivalents of metal chelator used per monomer unit
- Potential for unwanted cross reaction between acrylamide monomers and metal chelators
- Longer purification method required to remove the large excess of metal chelator
- Excess metal chelators affected the ¹H NMR integration

Current Method

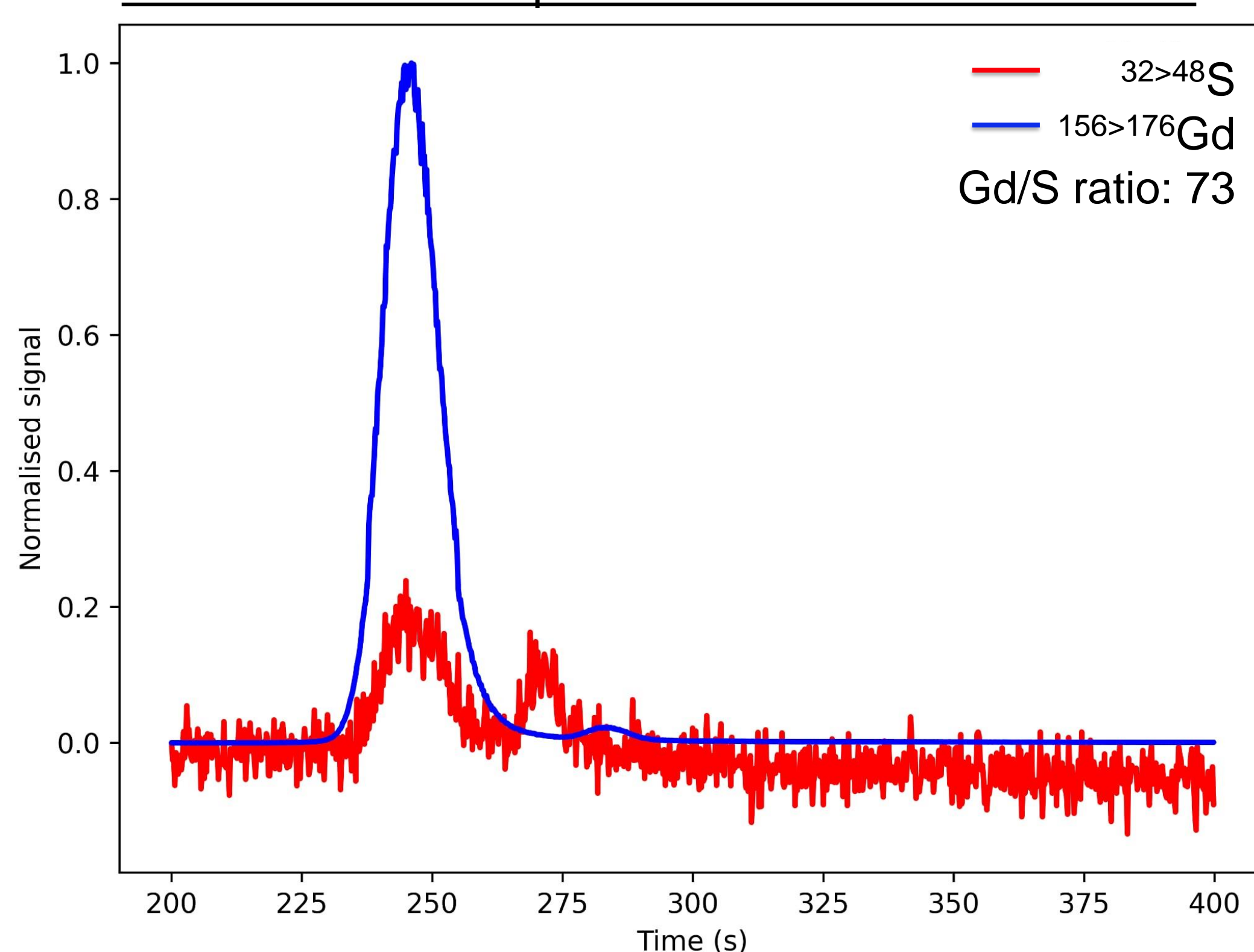
- Prevents inter- and intramolecular cross linking between monomers.
- Extra deprotection step with TFA required
- Polymer disulfide is cleaved with DTT followed by maleimide-thiol conjugation of the azide linker
- Presence of azide is confirmed by reaction with DBCO

RESULTS AND DISCUSSION

Azide confirmation



Number of metals quantification with SEC-ICP-MS



CONCLUSIONS AND FUTURE DIRECTIONS

- A new method was developed for introducing pendant DOTA chelators.
- Azide functionalisation of MCP was completed and confirmed by reacting with DBCO.
- Metals per MCP were quantified by comparing the ratio of sulfur to lanthanide.

Future directions

- Optimise the characterisation of lanthanides on each MCP.
- React MCP with alkyne modified antibody for iMSI analysis of tissue samples

References

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- 2) Liu, H.; Chumsae, C.; Gaza-Bulseco, G.; Hurkmans, K.; Radziejewski, C. H. Ranking the Susceptibility of Disulfide Bonds in Human IgG1 Antibodies by Reduction, Differential Alkylation, and LC-MS Analysis. *Anal. Chem.* **2010**, 82 (12), 5219–5226. <https://doi.org/10.1021/ac100575n>.

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