ADCBIO

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A Platform Approach for Enzymatic Remodelling of Antibodies and Conjugation to Branched Toxin Linkers

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Introduction

Innovative enzymatic approaches for modification of antibodies and conjugation to toxin linkers have gained attention for their ability to attach payloads site-specifically with no or only minor modifications to antibody primary structure.

Here, we evaluated a platform approach for one such technology, GlycoConnect™, that was applied to two human IgG_1 antibodies and a murine IgG_{2B} antibody with a variety of branched toxin linkers based on MMAE, Maytansine, Exatecan, and DXd (Figure 1). Remodelling incorporated an azide-carrying sugar into the glycosylation of the heavy chain, to which the toxin linkers conjugated via BCN-based strain-promoted click chemistry. Analysis of the resultant ADCs showed that the standard process achieved a high level of site-specific drug incorporation in all cases. RP-HPLC worked well to determine drug to antibody ratios (DARs) while analysis by HIC depended on hydrophobicity of the toxin linker.

A single conjugate was progressively scaled up from milligram to multigram scale. The ADC quality attributes were consistent across different scales and as purification methods changed from lab to manufacturing-compatible unit operations.

Standard Workflow

The antibodies were buffer exchanged into Tris-buffered saline and concentrated to 25 mg/ml. Remodelling was carried out with [P] = 15 mg/ml with 1.0% w/w EndoSH, 7.5% w/w Galactosyl transferase, 10 mM $MnCl_2$, and 25 molar equivalents UDP-*N*-acetylglucosamine-azide at 30°C for 16 to 18 hours. Enzymes and excess substrates/cofactors were removed by Protein A chromatography.

For the standard workflow, the pH of the antibody in formulation was adjusted to pH 7-8, and conjugation was carried out at [P] ≈ 9 mg/ml with 4 molar equivalents toxin linker and 50% Propylene glycol at 20°C for 16 to 18 hours. The conjugates were desalted into a standard formulation buffer by G25-based buffer exchange and analysed by SEC for monomer levels, and HIC and RP-HPLC for DAR.

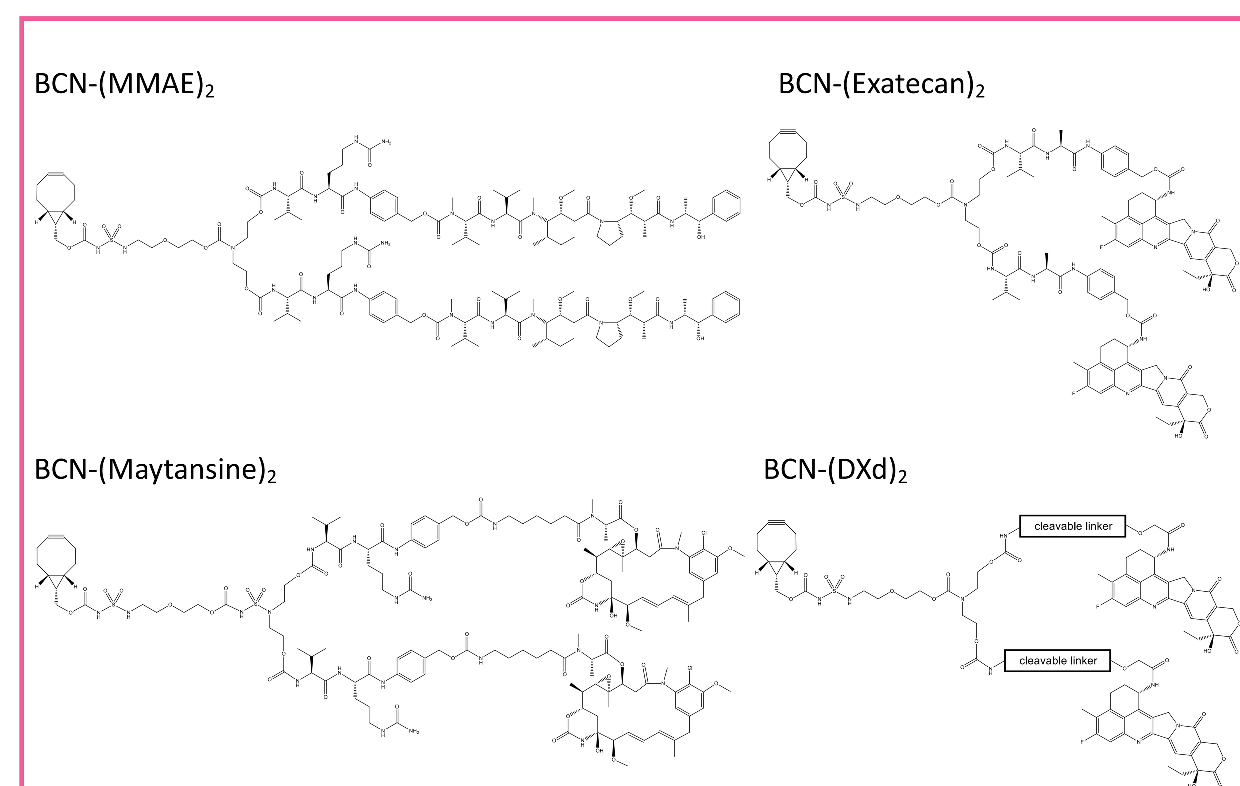

Figure 1: Structures of the branched toxin linkers.

IgG_1 mAb1 Conjugation Screen

IgG_1 mAb1 was remodelled using the standard protocol and stored in formulation buffer until use. This material was taken forward for conjugation with all branched toxin linkers following the standard protocol.

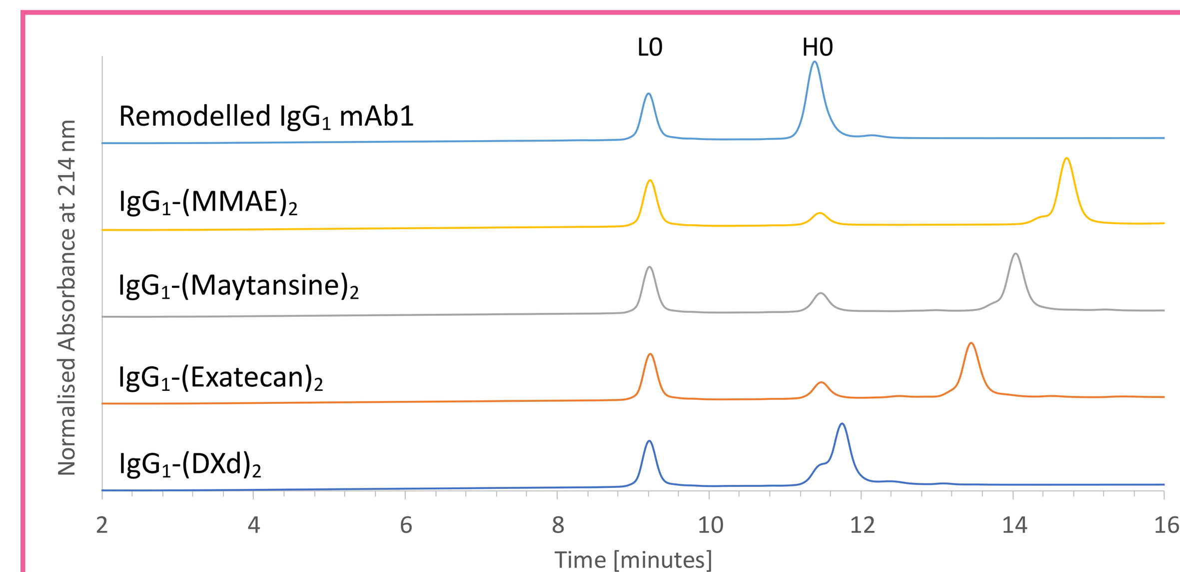

Figure 2: RP-HPLC traces of IgG_1 mAb1 and its conjugates with BCN-$(MMAE)_2$, BCN-$(Maytansine)_2$, BCN-$(Exatecan)_2$, and BCN-$(DXd)_2$. With exception of IgG1 X-$(DXd)_2$ all ADCs were well resolved, and conjugation only occurred on the heavy chain. The traces have been normalised to the L0 peak.

RP-HPLC showed that only the heavy chain was conjugated to. Good resolution was achieved for all IgG_1 mAb1 conjugates with exception of the $(DXd)_2$ where conjugated and unconjugated heavy chain species partially overlapped (Figure 2). Only IgG_1 mAb1-$(MMAE)_2$ was reasonably resolved well by HIC while all other conjugates eluted as single peaks (Figure 3).

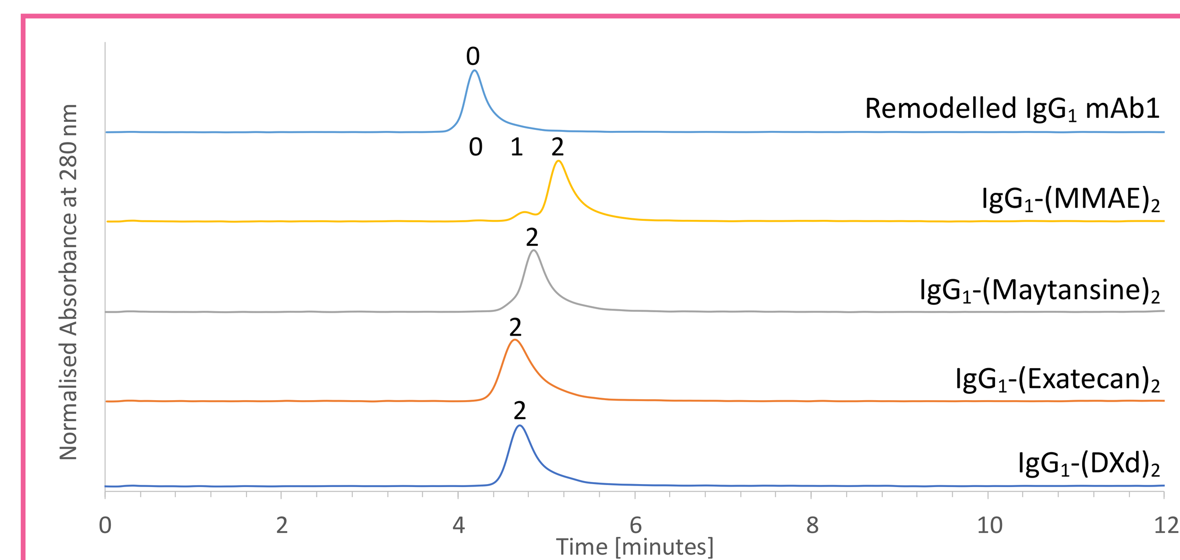

Figure 3: HIC traces of IgG1 mAb1 and its conjugates with BCN-$(MMAE)_2$, BCN-$(Maytansine)_2$, BCN-$(Exatecan)_2$, and BCN-$(DXd)_2$. Only the (MMAE)2 conjugate showed some resolution between DAR 0, 1, and 2, while all others eluted as a single peak. The traces have been normalised to the highest peak.

All toxin linkers conjugated via a BCN-azide click reaction to the expected extent (DAR 1.8) by RP-HPLC. SEC analysis revealed that they were tolerated well by the antibody with moderate reductions in monomer levels (Table 1).

Table 1: Quality attributes of the conjugates of IgG_1 mAb1.

Toxin Linker	DAR by RP-HPLC	DAR by HIC	Monomer by SEC
Modified IgG_1 mAb1	N/A	N/A	99.2%
$(MMAE)_2$	1.8	1.9	97.1%
$(Maytansine)_2$	1.8	N/D	97.3%
$(Exatecan)_2$	1.8	N/D	98.1%
$(DXd)_2$	1.8	D/D	96.9%

Mouse IgG_{2B} Conjugation Screen

The murine IgG_{2B} antibody was remodelled and conjugated with BCN-$(MMAE)_2$, BCN-$(Maytansine)_2$, and BCN-$(Exatecan)_2$ following the standard protocol.

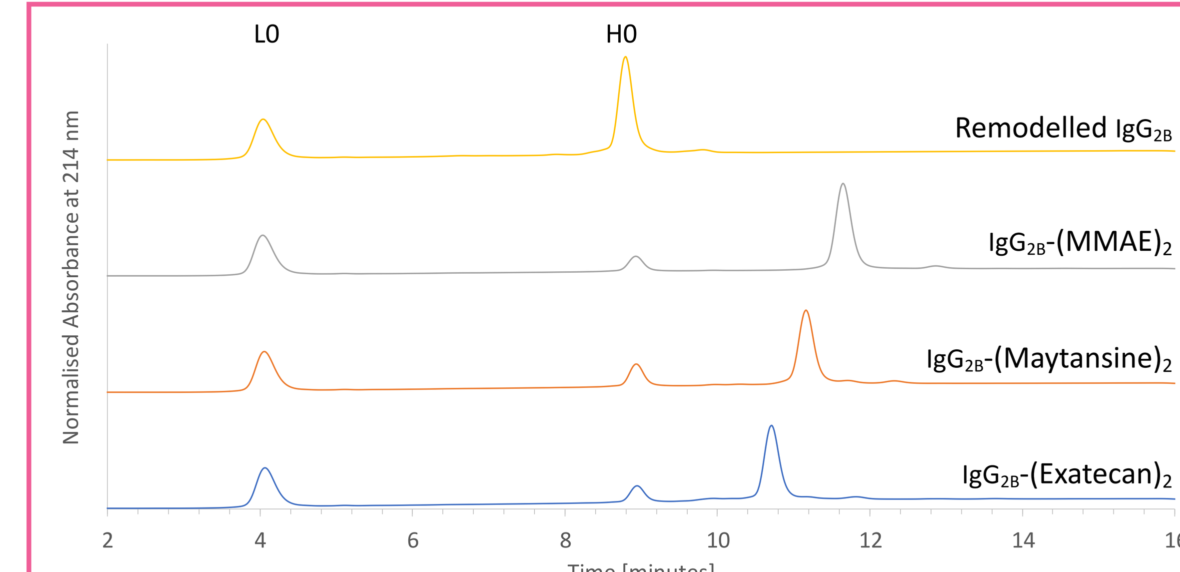

Figure 4: RP-HPLC traces of the remodelled murine IgG_{2B} and its conjugates with BCN-$(MMAE)_2$, BCN-$(Maytansine)_2$, and BCN-$(Exatecan)_2$. All ADCs were well resolved, and conjugation only occurred on the heavy chain. Traces have been normalised to L0.

An optimised RP-HPLC method was run to resolve the more hydrophobic species of the murine IgG_{2B} conjugates, which showed good separation between conjugated and unconjugated heavy chain species (Figure 4). This was mirrored in improved resolution of the different DAR species by HIC where for all but IgG_{2B}-$(Exatecan)_2$ the DAR 1 species could be detected (Figure 5).

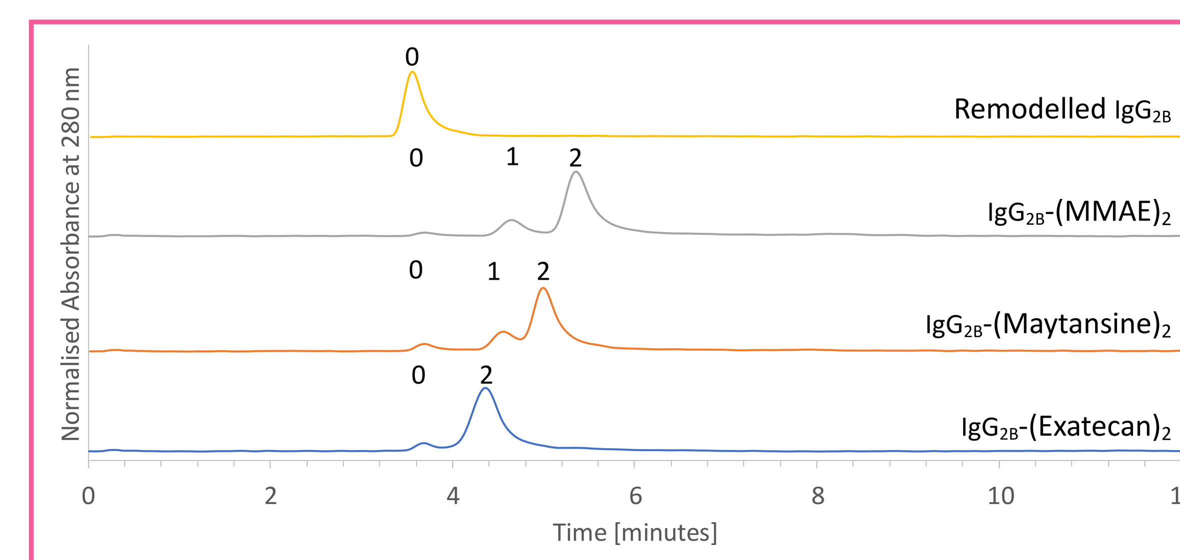

Figure 5: HIC traces of the murine IgG_{2B} and its conjugates with BCN-$(MMAE)_2$, BCN-$(Maytansine)_2$, and BCN-$(Exatecan)_2$. The IgG_{2B}-$(MMAE)_2$ and -$(Maytansine)_2$ conjugates showed some resolution between DAR 0, 1, and 2 species. The traces have been normalised to the highest peak.

Remodelling and conjugation of the murine IgG_{2B} yielded slightly lower DAR values by RP-HPLC and HIC than the IgG_1 mAb1 equivalents while maintaining higher monomer levels by SEC (Table 2).

Table 2: Quality attributes of the conjugates of the murine IgG_{2B}.

Toxin Linker	DAR by RP-HPLC	DAR by HIC	Monomer by SEC
Modified IgG_{2B}	N/A	N/A	98.5%
$(MMAE)_2$	1.7	1.7	98.9%
$(Maytansine)_2$	1.6	1.8	99.6%
$(Exatecan)_2$	1.8	N/D	99.3%

Scale-Up

Initial trials on a 10-mg scale were used to screen co-solvents and molar equivalents of the toxin linker for the conjugation reaction, settling on 7 molar equivalents BCN-$(MMAE)_2$ in 50% propylene glycol.

A proving run on a 0.5-g scale was performed to evaluate the efficiency and compatibility of TFF for purification and formulation.

The final manufacturing run at the 2.5-g scale was carried out using the learning from the initial trial and the proving run. Residual toxin was efficiently removed to a level of 0.1% free to total drug.

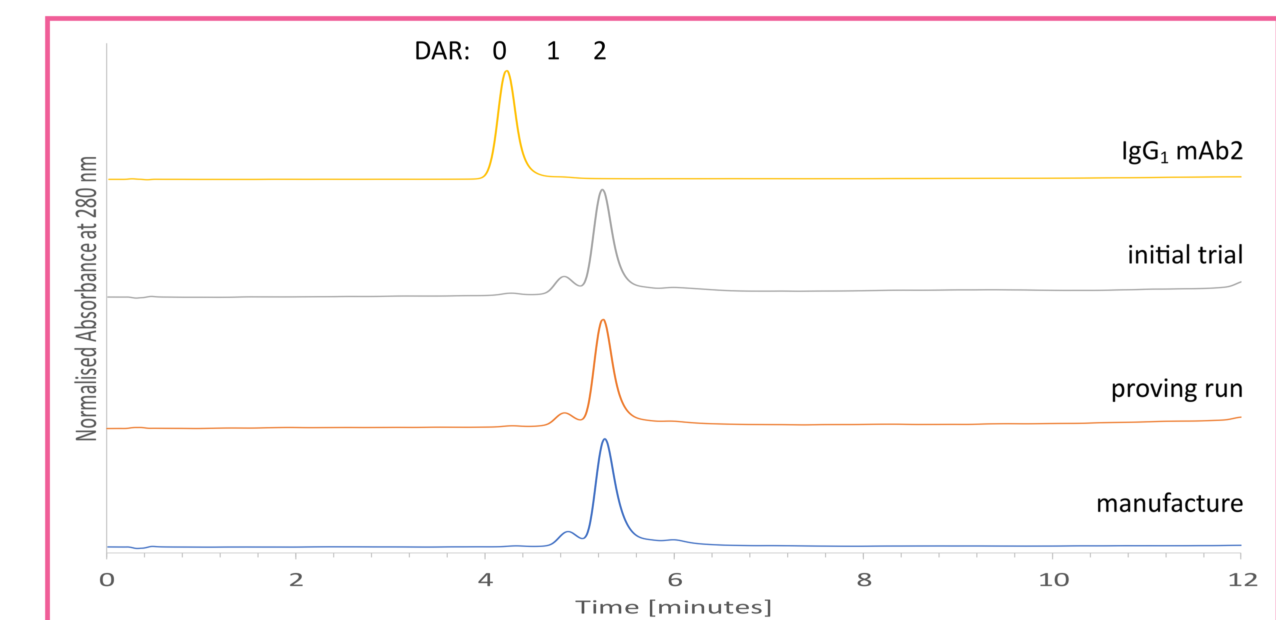

Figure 6: Quality attributes of the IgG1 and its BCN-(MMAE)2 conjugates of scaled-up IgG_1 Y. Traces have been normalised to highest peak.

Quality of the ADC was consistent between initial trial, proving run and manufacture across a 250-fold scale-up, with slightly increasing monomer levels from small to final scale (Figure 6 and Table 3).

Table 3: Quality attributes of the conjugates of scaled-up IgG_1 mAb2.

Stage	DAR by RP-HPLC	DAR by HIC	Monomer by SEC	Yield
Initial Trial	1.9	1.9	96.2%	N/A
Proving Run	1.9	1.9	97.3%	59%
Manufacture	1.9	1.9	98.9%	81%

Conclusions

We show that a platform approach for enzymatic remodelling and conjugation of antibodies can work as basis for screening campaigns with straightforward options to quickly scale from milligrams to multigrams of ADC. Optimisations might still have to be carried out to improve the quality of individual conjugates, but the process appears to be working with a broad range of antibodies and toxin linkers.

Acknowledgements

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