Preparation of bispecific antibody mimetics

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INTRODUCTION

Fab-PEG-Fab (FpF) molecules have been shown to act as IgG mimetics [1]. Each antibody Fab is conjugated at both termini of a *di-bis*-alkylation poly(ethylene glycol) (PEG) reagent site-specifically by disulfide bridging conjugation. We now wish to use the same alkylating approach to prepare bispecific FpFs mimetic. We aimed to compare the preparation of bispecific FpF using two *bis*-alkylation PEG reagents; *di-bis*-alkylation (Fig.1A) and *mono-bis*-alkylation functionalized with copper-free 1,3-dipolar cycloaddition (click reaction, Fig.1B) between an azide (N_3) and the strained alkyne in dibenzocyclooctyne (DBCO) [2]. For simplicity and proof of concept two identical Fabs were first used during this study. The Fab of choice was the anti-vascular endothelial growth factor (VEGF) therapeutic, Ranibizumab. The next step would be to compare the binding properties of anti-VEGF FpFs prepared from these two PEG reagents using Biacore X-100.

METHOD

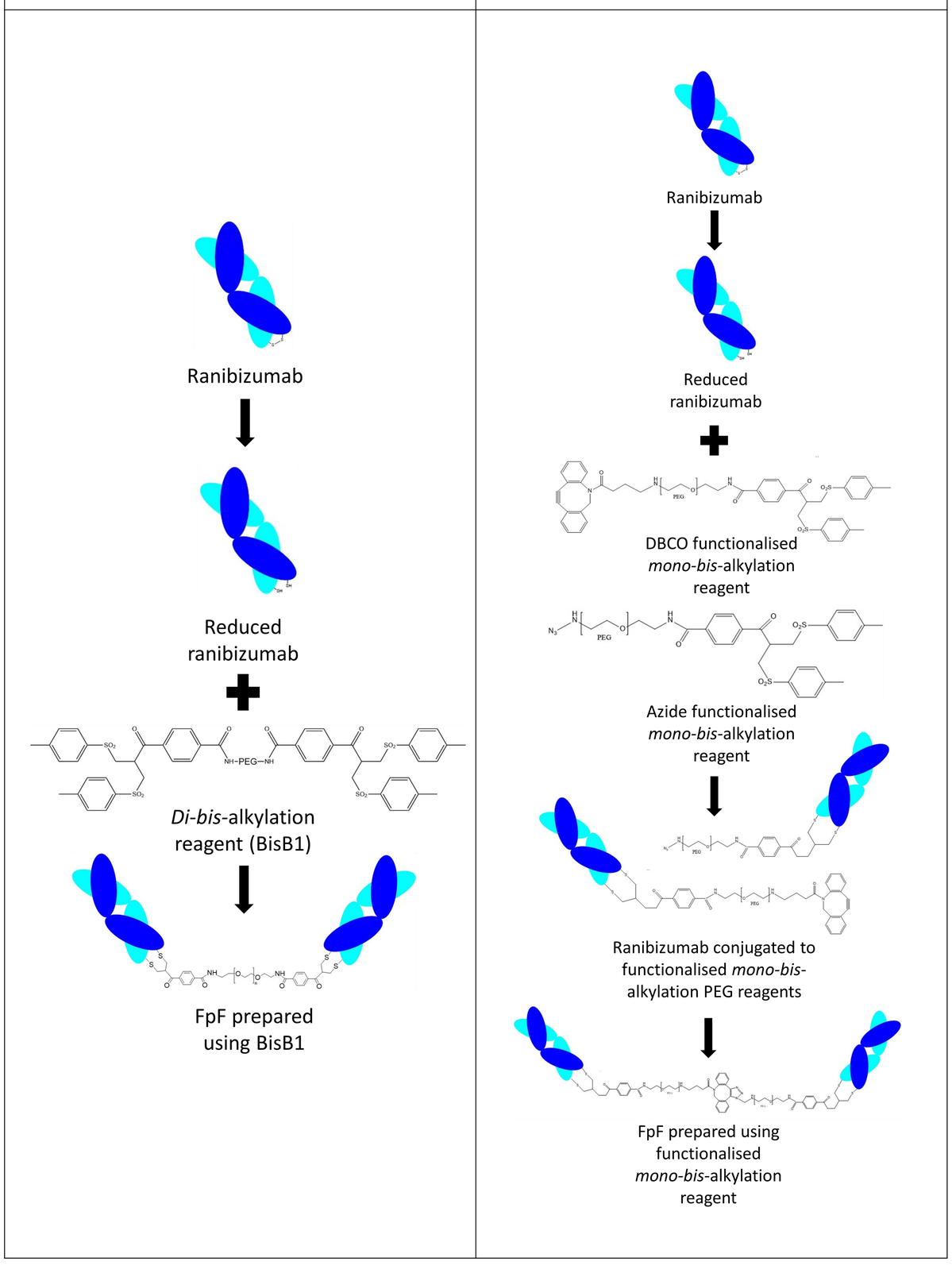
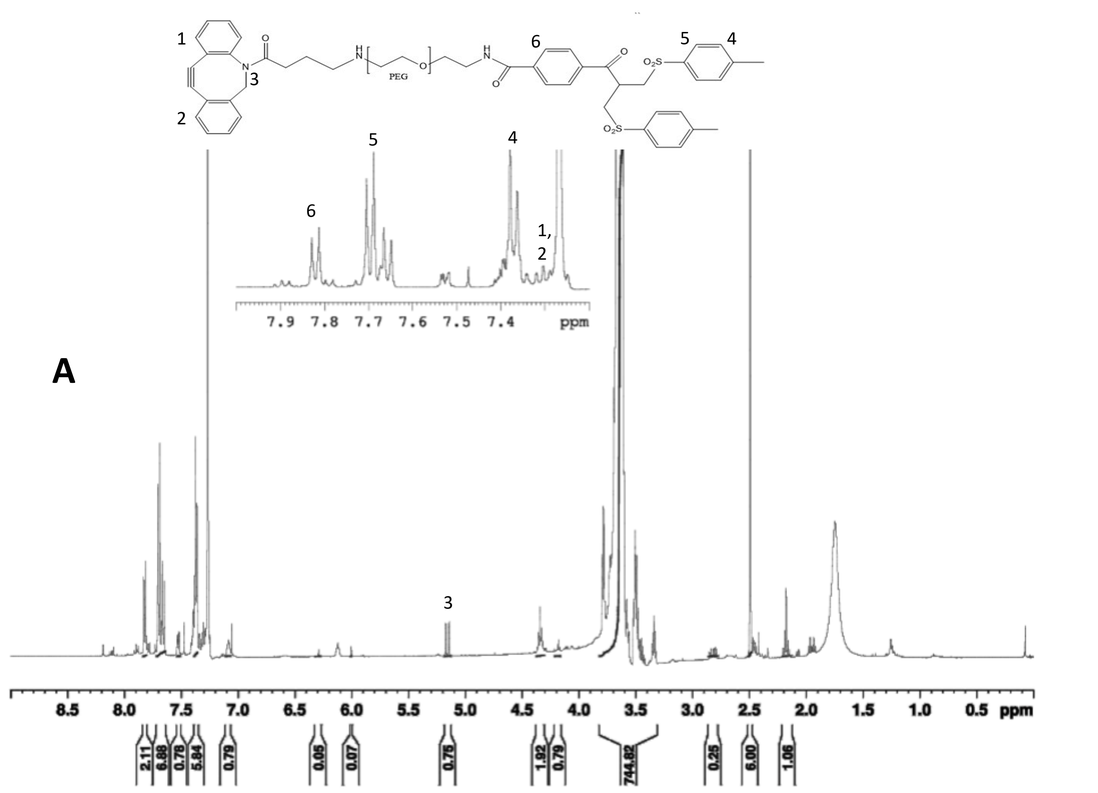


***Figure 1**: Flow diagrams summarising the methodologies used to prepare FpFs using a di-bis-alkylation reagent (A) and functionalised mono-bis-alkylation reagents (B)*

RESULTS

A *mono-bis*-alkylation reagent functionalized with a DBCO group was synthesised (Fig.2A). Fig.2B shows SDS-PAGE analysis for FpFs preparation using both alkylation reagents.



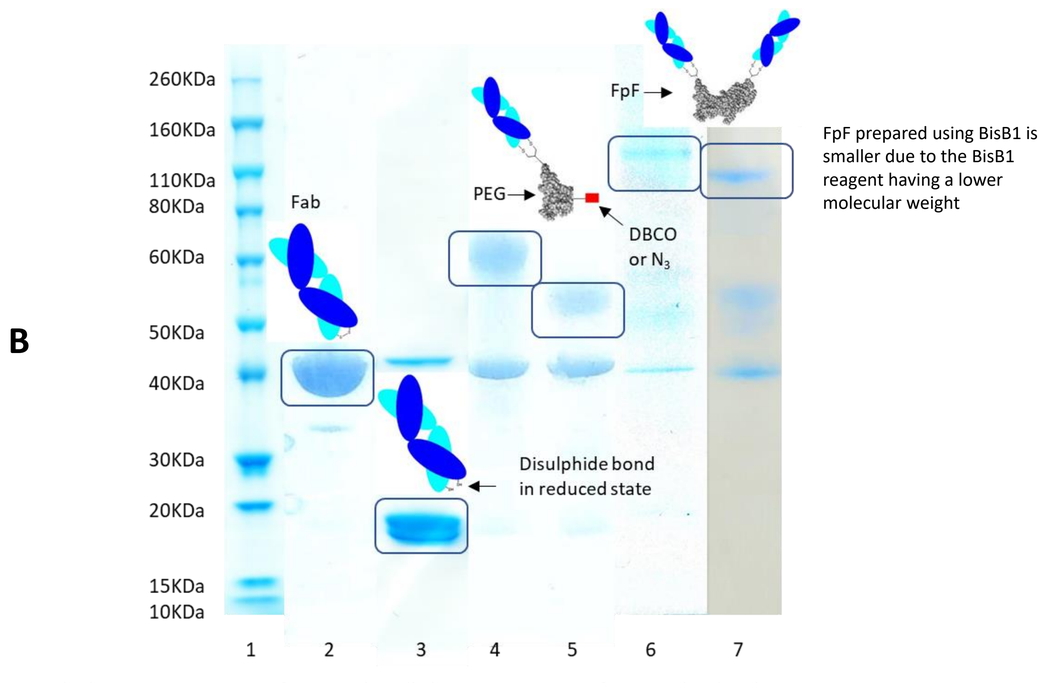


***Figure 2A**: A hydrogen NMR spectra of a mono-bis-alkylation PEG reagent functionalised with a DBCO group*

***Figure 2B**: A annotated SDS-PAGE gel detailing the results of the monospecific FpF preparations using two different alkylating reagents.* **Lane 1**: Molecular weight protein marker, **Lane 2:** 1mg/mL ranibizumab, **Lane 3:** Reduced ranibizumab **Lane 4:** Conjugation mixture – Reduced ranibizumab and functionalised *mono-bis*-alkylation reagent (10KDa molecular weight reagent), **Lane 5:** Conjugation mixture – Reduced ranibizumab and functionalised *mono-bis*-alkylation reagent (5KDa molecular weight reagent) **Lane 6:** Reaction mixture – Functionalised ranibizumab conjugates, 3 days incubation time **Lane 7:** Conjugation mixture – Reduced ranibizumab and d*i-bis*-alkylation PEG reagent (6KDa molecular weight reagent).

CONCLUSIONS AND FUTURE WORK

This proof of concept study found that FpFs can be prepared using two different methodologies based upon the use of bis-alkylation reagents with PEG backbones. The binding affinity of the molecules to their therapeutic target (VEGF), the yield of FpF obtained and the physicochemical stability of the FpFs will now be assessed to establish which reagent is preferred. We will then use the selected reagent make bispecific FpFs capable of targeting two different therapeutic targets simultaneously.

REFERENCES

[1] Khalili, H., Lee, R., Khaw, P.*et al.* An anti-TNF-α antibody mimetic to treat ocular inflammation. *Sci Rep* **6,** 36905 (2016). https://doi.org/10.1038/srep36905

[2] Agard, N., Prescher, J & Bertozzi, C. A Strain-Promoted [3+2] Azide/Alkyne Cycloaddition for Covalent Modification of Biomolecules in Living Systems. *J. Am. Chem. Soc*. **126**, 15046-150467 (2005) DOI: 10.1021/ja059912x