

Smaller but Smarter? A Nanobody Based Approach to Antibody Interference Mitigation

Background

Treatment with anti-CD38 antibodies represents an advanced therapy for patients with multiple myeloma and other diseases. However, therapeutic anti-CD38 antibodies in patient specimens often cause agglutination of red blood cells (RBCs) in indirect anti-human globulin tests (IATs), complicating the identification of irregular antibodies and the prompt transfusion of compatible blood.

Antigen masking by Fab or F(ab')₂ fragments has proven to be an effective way to mitigate anti-CD38 antibody interference and this method is now well established in immunohematological laboratories. With column agglutination technology (CAT) card systems such as Grifols DG Gel, however, **background issues can occur that might stem from light chain reactivity of the anti-human globulin (AHG) used**. We have developed a new approach to antigen masking, using nanobodies (Fig. 1) that do not react with anti-light chain antibodies.

Methods

Several nanobodies and fusion proteins were expressed in mammalian cells and used in antigen masking IAT (AMIAT) across several CAT systems.

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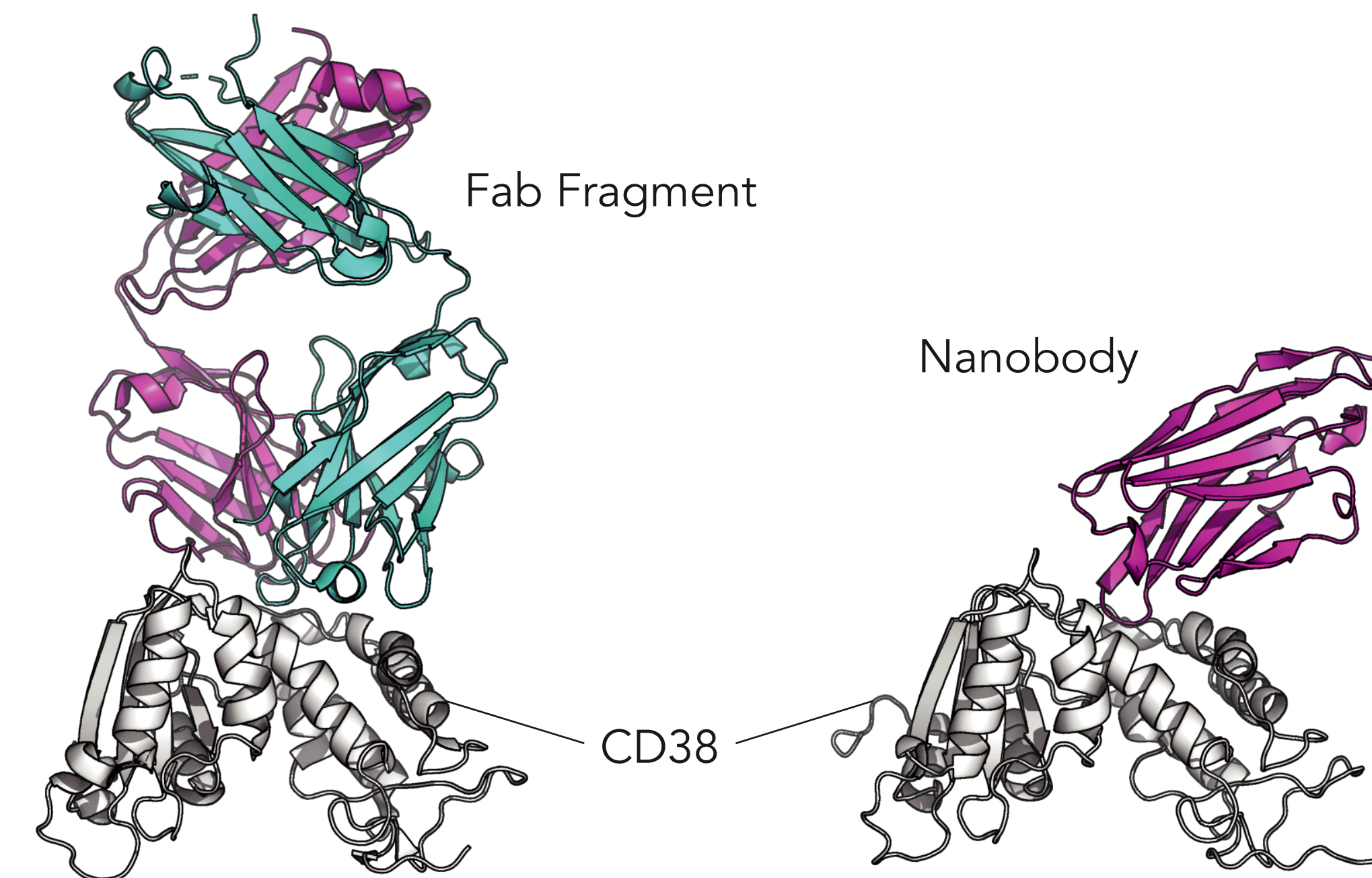


Figure 1. Structural comparison of a Fab fragment and a nanobody, both binding to CD38. The nanobody has no light chain (cyan) compared to the Fab fragment but covers a smaller area on CD38 (white). Magenta: heavy chain of Fab fragment and nanobody, respectively.

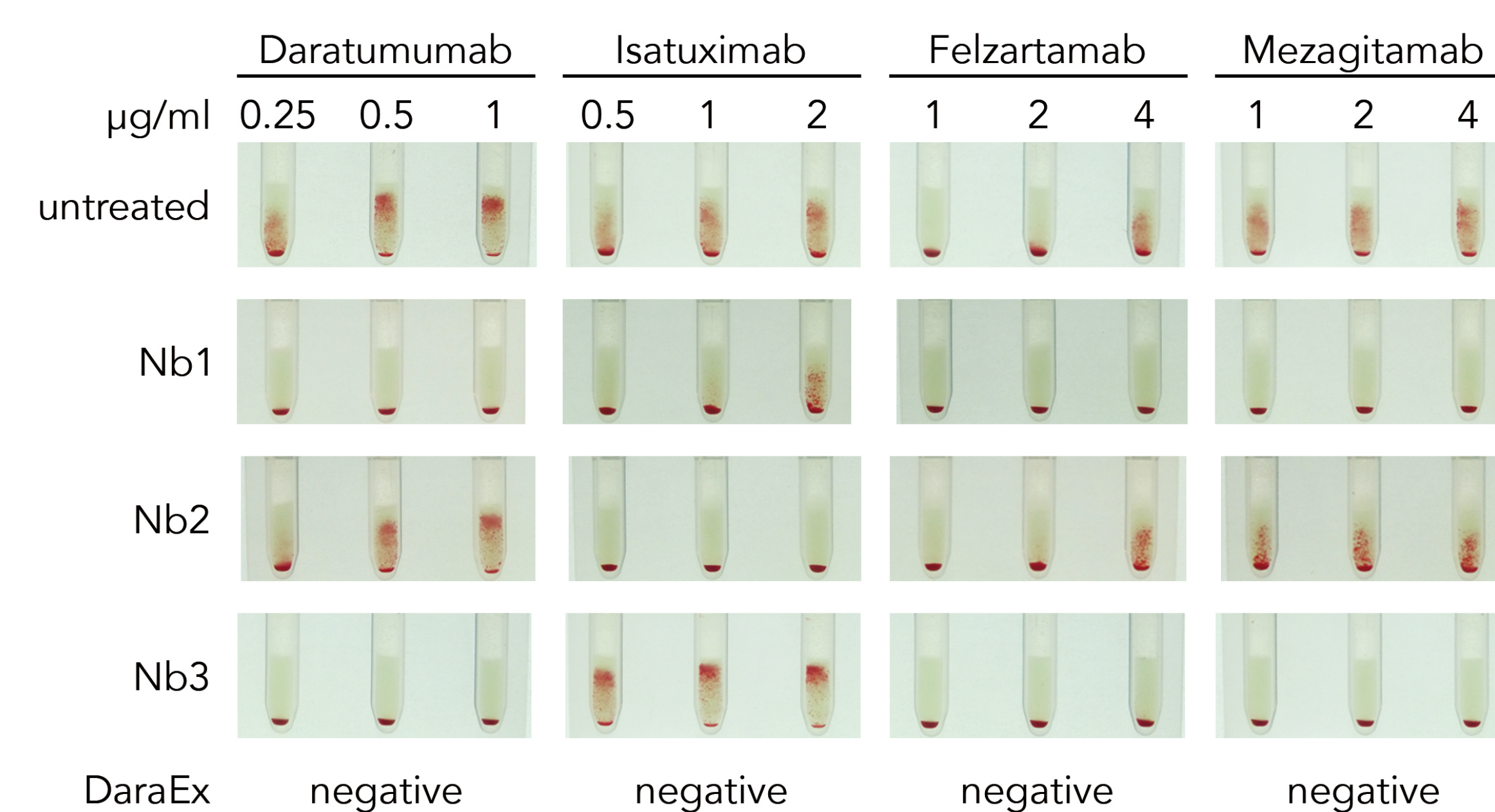


Figure 2. Nanobodies can inhibit binding of anti-CD38 antibodies to CD38 but cover less epitopes than DaraEx. RBCs were pre-treated with the nanobodies Nb1, Nb2, or Nb3, or were left untreated as indicated. Subsequently, anti-CD38 antibodies in the given concentrations were added and an IAT was performed. For comparison, results for DaraEx are indicated.

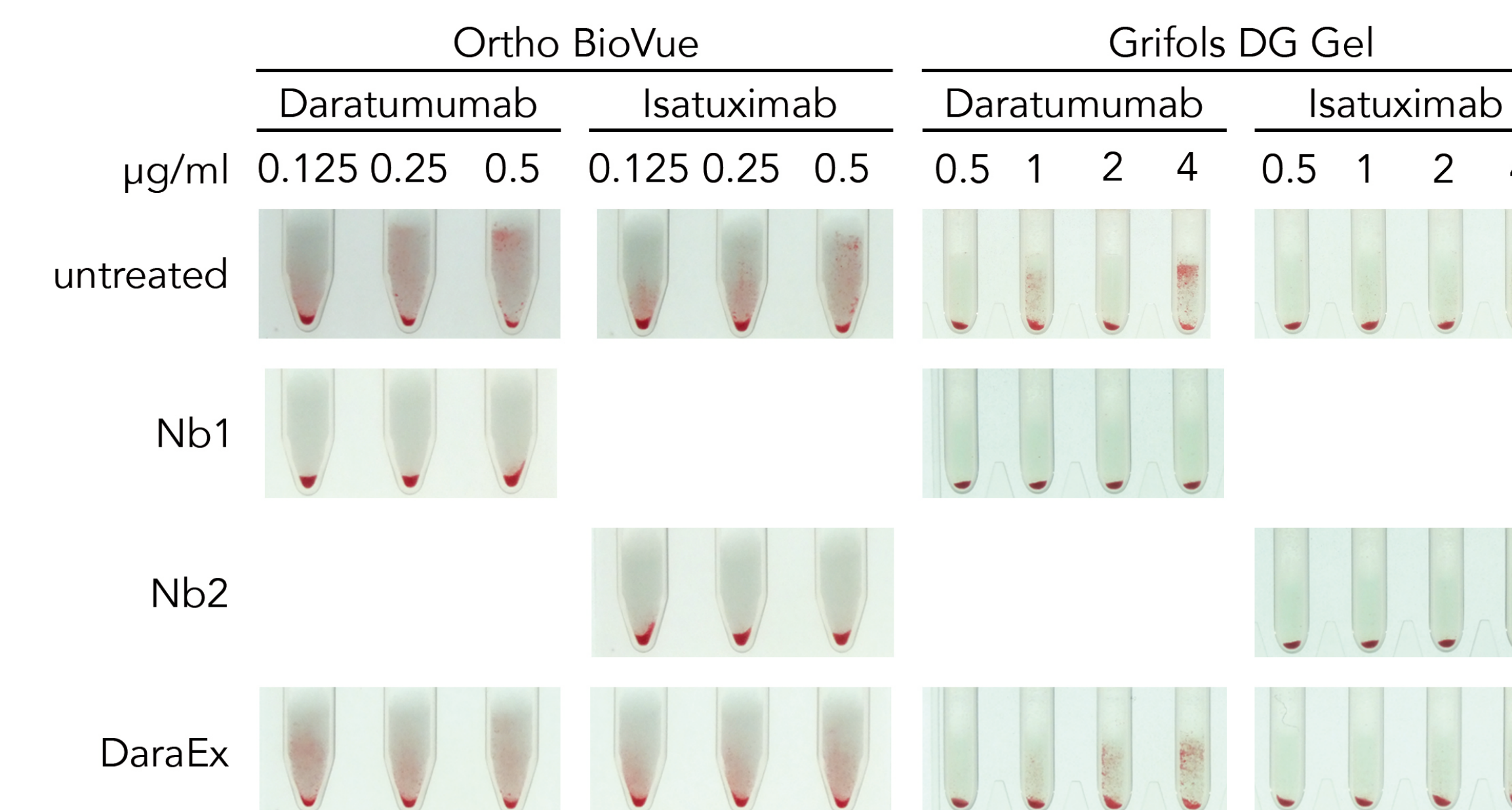


Figure 3. Nanobodies do not generate background in Ortho BioVue and Grifols DG Gel CAT. Daratumumab and isatuximab were incubated in increasing concentrations on either untreated RBCs or RBCs pre-treated with Nb1, Nb2, or DaraEx. Subsequently, an IAT was completed with the CAT systems indicated.

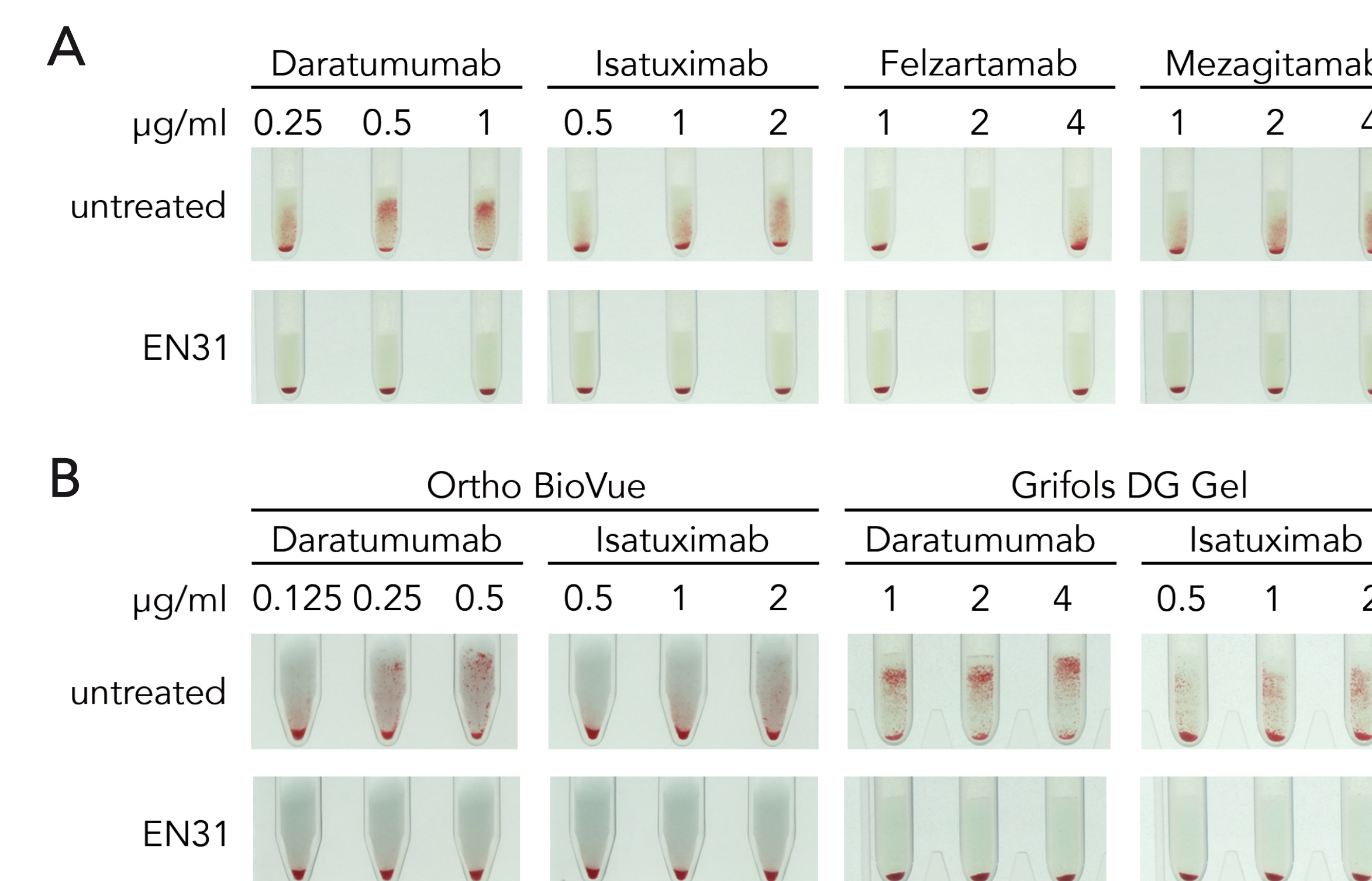


Figure 4. A newly designed engineered nanobody efficiently masks CD38 and shows no background in selected CAT systems. An AMIAT was performed with the engineered nanobody EN31 as masking reagent and the anti-CD38 antibodies at the concentrations as indicated. A: Bio-Rad ID System, B: Ortho BioVue and Grifols DG Gel.

Results

Single nanobodies were able to efficiently mask anti-CD38 antibody epitopes on RBCs (Fig. 2) but none did cover all epitopes. **Background in sensitive CAT systems was significantly reduced** compared to DaraEx (Fig. 3). All of the anti-CD38 antibodies tested (daratumumab, isatuximab, felzartamab, and mezagitamab) were inhibited from binding by a newly designed engineered nanobody (Fig. 4).

Conclusion

Nanobodies are a viable approach to mitigate therapeutic anti-CD38 antibody mediated interference in IAT and work across a broad spectrum of CAT systems. By protein engineering, we were able to generate **a modified nanobody that covers all epitopes and shows less background than Fab fragments**.

Conflict of Interest

SA, CH, PK, and CS are employees of imusyn GmbH & Co. KG. CH and CS are designated inventors of the technologies presented here.

Trademarks

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