

Characterization of a Novel T Cell Staining Reagent

Background

Accurate identification and characterization of antigen-specific T cells are essential for understanding immune responses in infection, autoimmunity, and cancer. T cell staining reagents serve as powerful and indispensable tools for precise identification of T cell specificities. In this study, a novel T cell staining reagent, Dyemer, was evaluated for its efficacy in T cell staining, peptide loading, and reversibility of the staining process.

Methods

T cell staining was performed on isolated peripheral blood mononuclear cells (PBMCs) to quantify CMV- and EBV-specific CD8⁺ T cell frequencies. Staining efficacy and quality were compared to conventional tetramers of the same specificity. Peptide loading onto peptide-receptive MHC molecules was assessed by analyzing the thermal stability of the MHC/peptide complex using differential scanning fluorimetry (DSF). By performing dual color multimer staining, reagent specificity was determined. Staining reversibility was evaluated by measuring complex dissociation after biotin incubation.

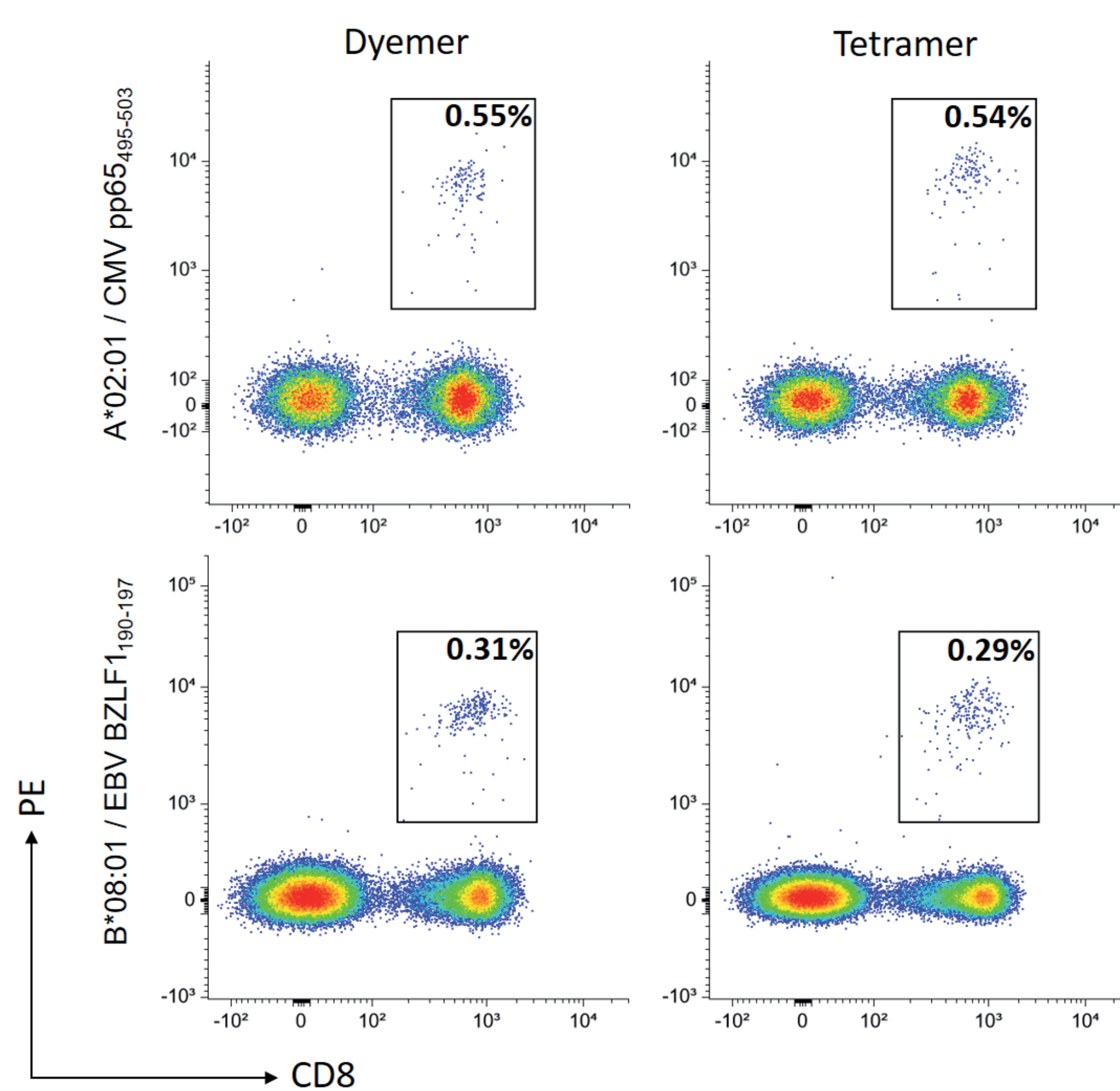


Figure 1. Comparison of staining efficiency between the Dyemer T cell staining reagent and conventional tetramers of the same specificity. PBMCs were gated on CD8⁺ T cells and incubated with PE-labeled Dyemer or tetramer. Frequencies of A*02:01/pp65₍₄₉₅₋₅₀₃₎ and B*08:01/BZLF1₍₁₉₀₋₁₉₇₎ specific CD8⁺ T cells are shown as percentage of total CD8⁺ T cells.

Results

Staining performance and reproducibility of Dyemer are comparable to or exceed that of conventional T cell staining reagents, such as tetramers (Fig. 1). Peptide loading is successfully achieved through single-step peptide addition to the peptide-receptive MHC molecule and subsequent overnight incubation (Fig. 2). Dual color multimer staining shows a high rate of double positive cells, indicating a high specificity of the T cell staining reagent (Fig. 3). The addition of biotin to the cells enables reversible staining by facilitating the dissociation of the staining complex from the cell surface, preserving cell integrity for further downstream analysis (Fig. 4).

Conflict of Interest

PT and CS are employees of imusyn GmbH & Co. KG. CS is a designated inventor of the technology presented here.

^a imusyn GmbH & Co. KG, Feodor-Lynen-Str. 5, 30625 Hannover, Germany
^b corresponding author: schneeweiss.clemens@imusyn.de

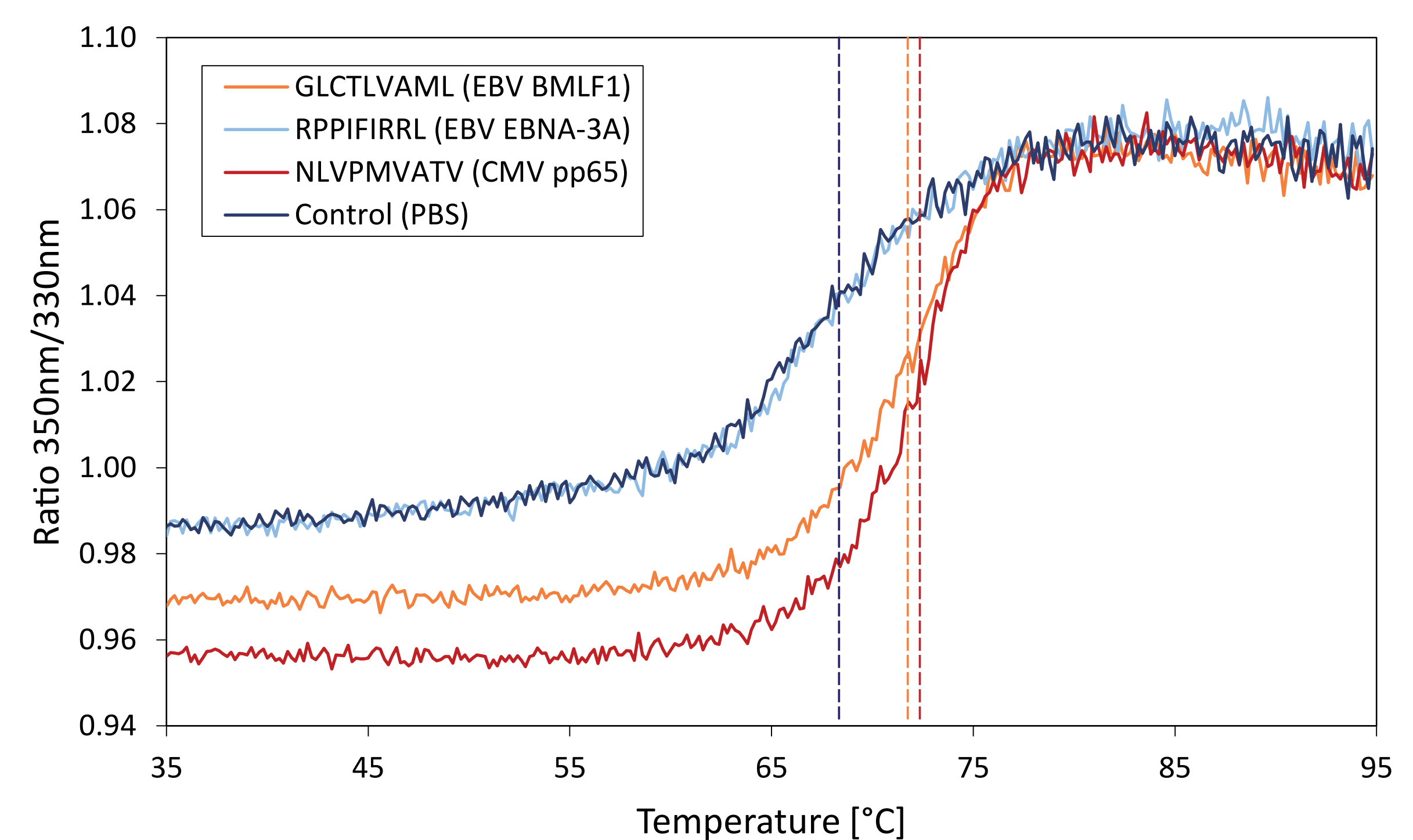


Figure 2. Melting curves of peptide-receptive HLA-A*02:01 incubated with selected peptides. HLA-A*02:01 was incubated with either A*02-specific peptide NLVPMVATV (red) or GLCTLVAML (orange), non-specific peptide RPIIFIRRL (light blue) or PBS (dark blue) at 4°C overnight. The thermal stability of the MHC/peptide complex was measured by differential scanning fluorimetry (DSF). An altered curve profile and changes in thermal stability indicate successful peptide loading. Dotted lines indicate the melting temperature T_m .

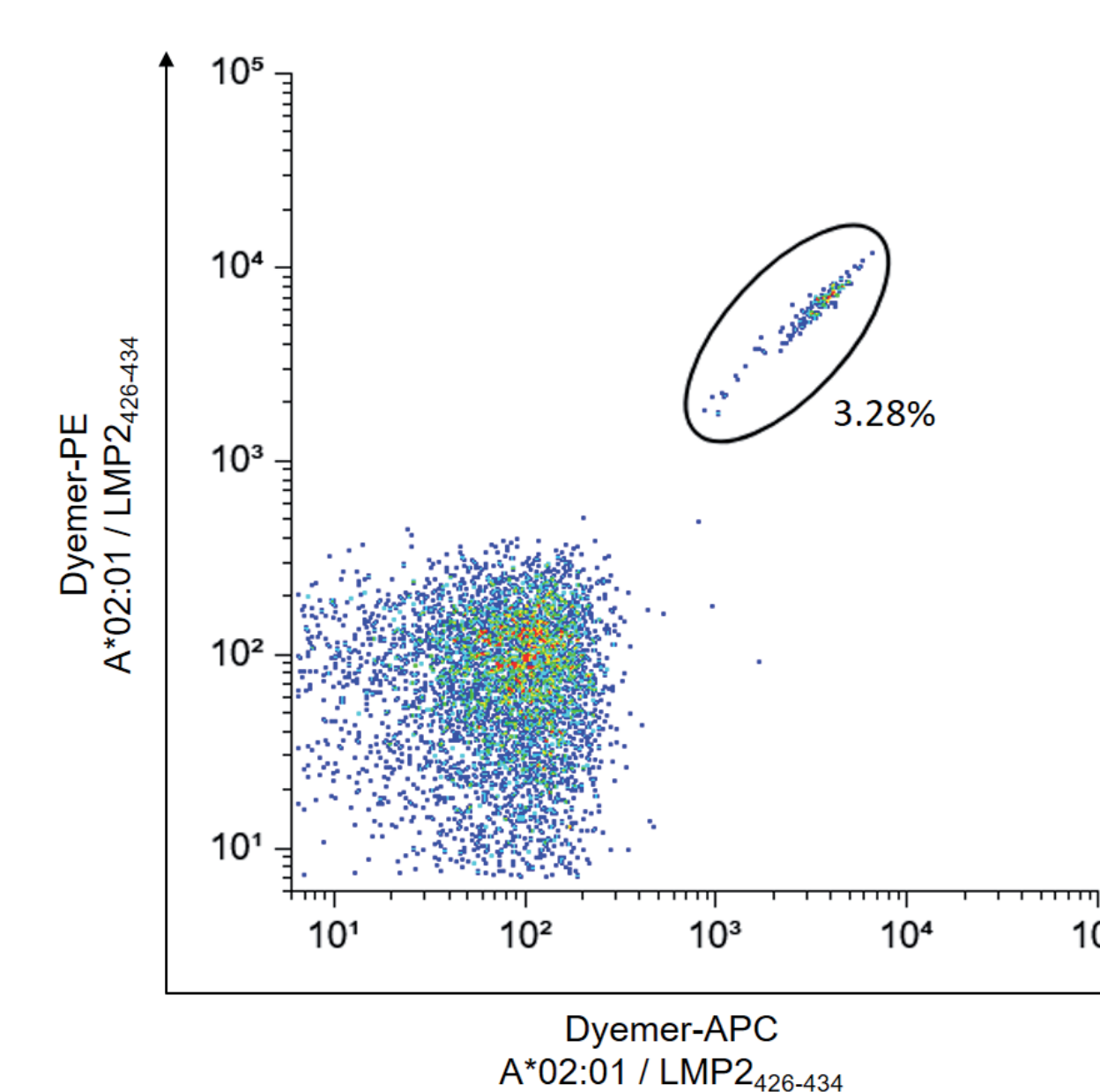


Figure 3. Dual color multimer staining using PE- and APC-labeled Dyemer of the same specificity. PBMCs were gated on CD8⁺ T cells and incubated with Dyemer A*02:01/LMP2₍₄₂₆₋₄₃₄₎. Frequencies of double positive CD8⁺ T cells are shown as percentage of total CD8⁺ T cells.

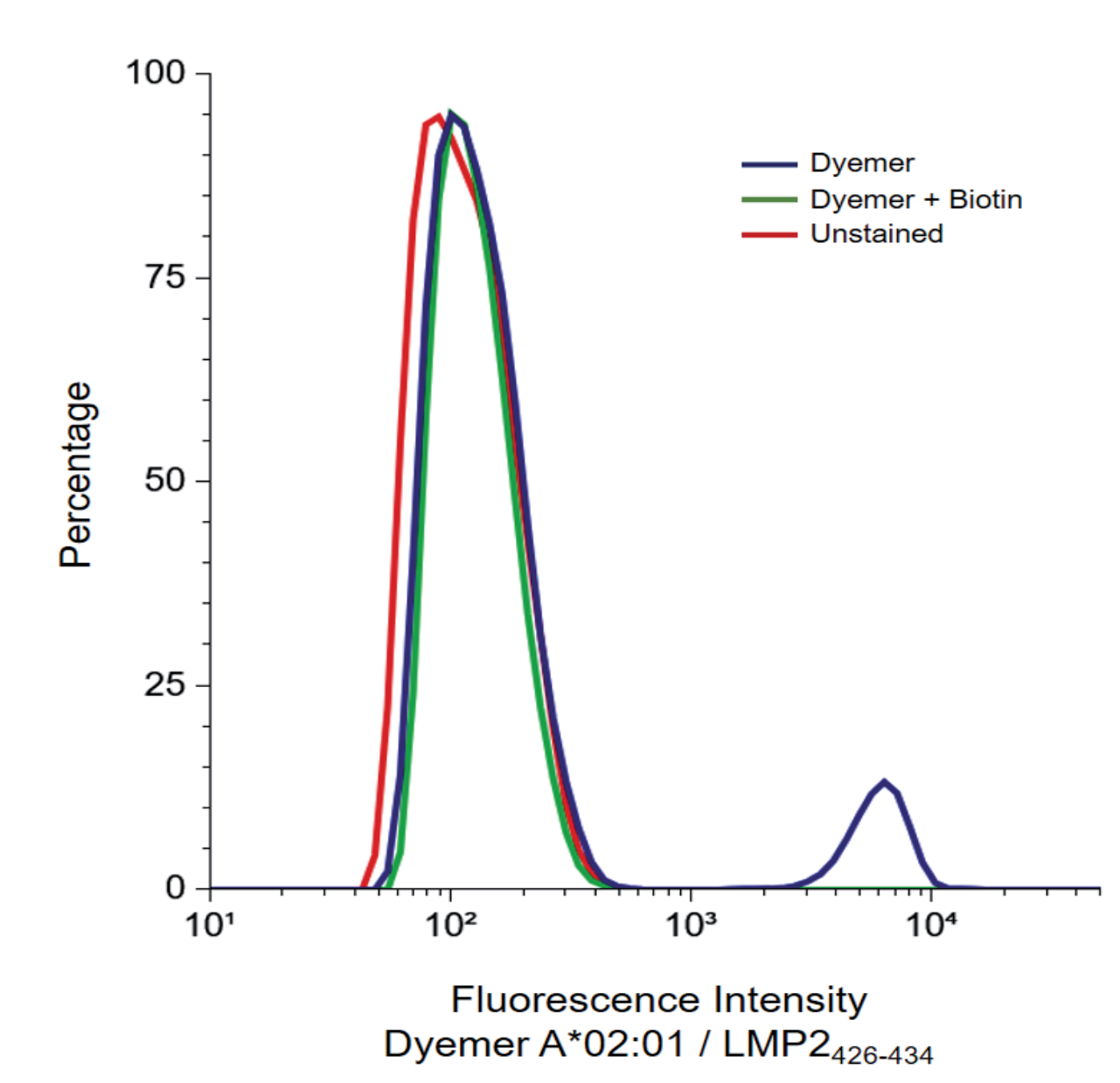


Figure 4. Reversibility of Dyemer T cell staining. PBMCs were gated on CD8⁺ T cells and incubated with Dyemer A*02:01/LMP2₍₄₂₆₋₄₃₄₎. After T cell detection, the staining complex was removed from the cell surface by biotin addition. Frequencies are shown as percentage of total CD8⁺ T cells.

Conclusion

The promising novel reagent Dyemer combines high specificity and competitive sensitivity with the option for flexible and fast in-lab peptide loading, enabling efficient detection of T cell specificities, immunogenicity screening, and identification of potential candidates for cancer therapy.



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