Developing a Platform for Screening of TCR Mimic Specificity

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Introduction

The field of antigen-specific immunotherapy is rapidly evolving and there is an increasing need for assessing specificity and potential cross-reactivity of TCR and TCR mimic (TCRm)-based therapeutics against related peptide epitopes. In this study we present the steps for developing a workflow allowing production and specificity screening of functionally validated TCRm monomers. Here we exploit the high sensitivity of Dextramer® reagents in combination with TCRm molecules to establish a flexible platform for screening of TCRm specificty in an artificial cell system using flow cytometry.

Conclusions

We demonstrate:

- The ability to produce TCRm monomers in an *E. coli*-based expression system.
- A method for making functional and sensitive TCRm Dextramer® reagents
- The development of a flow cytometry-based assay for validating specific binding of TCRm to their target pMHC complex.
- A flexible platform with the potential for high-throughput screening for fine-specificity and cross-reactivity.

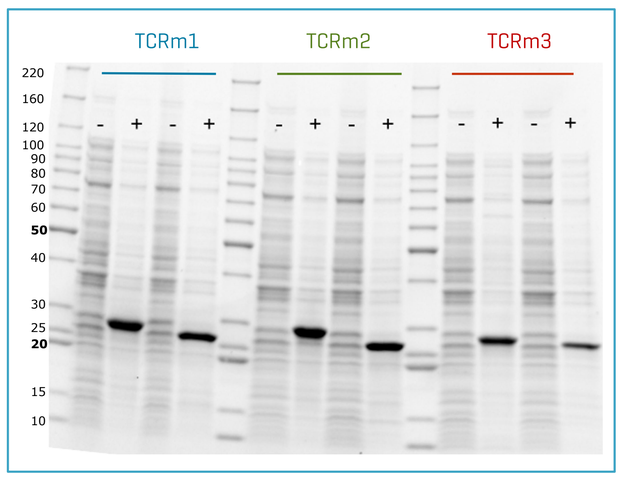
Experimental Procedure for Expression and Purification of TCRm

TCRm used in this study

The heavy and light chain sequences for three TCRm were extracted from the literature for cloning and expression.

TCRm	HLA Allele	Antigen	Target epitope	Protein size [light/heavy; kDa]	A280 @ 1mg/ml [light/heavy/dimer]
TCRm1	A*02:01	NY-ESO1	SLLMWITQV	24.3 / 25.1	1.69 / 1.60 / 1.66
TCRm2	A*02:01	WT-1	RMFPNAPYL	24.0 / 26.0	1.64 / 2.43 / 2.07
TCRm3	A*01:01	MAGE-1	EADPTGHSY	24.2 / 25.8	1.69 / 1.97 / 1.85

A



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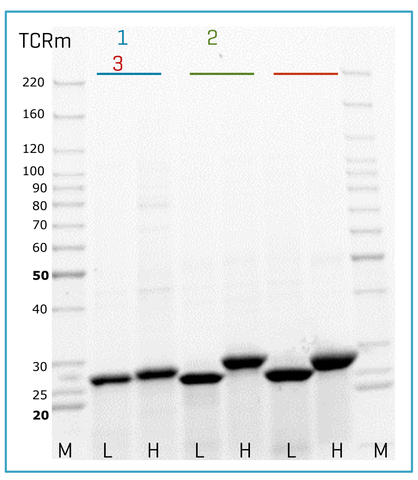

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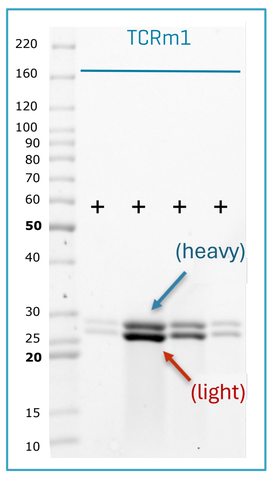
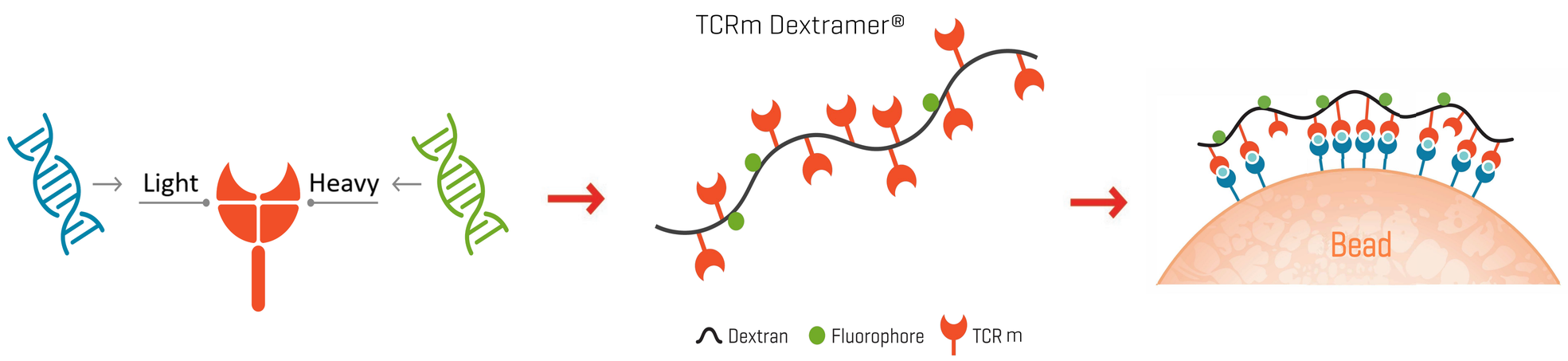


A) Heavy and light chains were successfully expressed in *E. coli* for all three TCRms. **B)** The expressed chains were purified and analysed by SDS PAGE demonstrating bands corresponding to the expected size of the light (L) and heavy (H) chains for all three TCRm constructs. **C)** The light and heavy chains for all three TCRm were refolded and purified by FPLC; Fractions from the purification of the refolded TCRms were analyzed by SDS PAGE. TCRm1 is shown as an example. Correct stoichiometry of heavy and light chain in the refolded TCRm1 is shown by the equal intensity of the two bands corresponding to the light and heavy chains of TCRm1 under reducing conditions.

TCRm Platform – Production and Validation



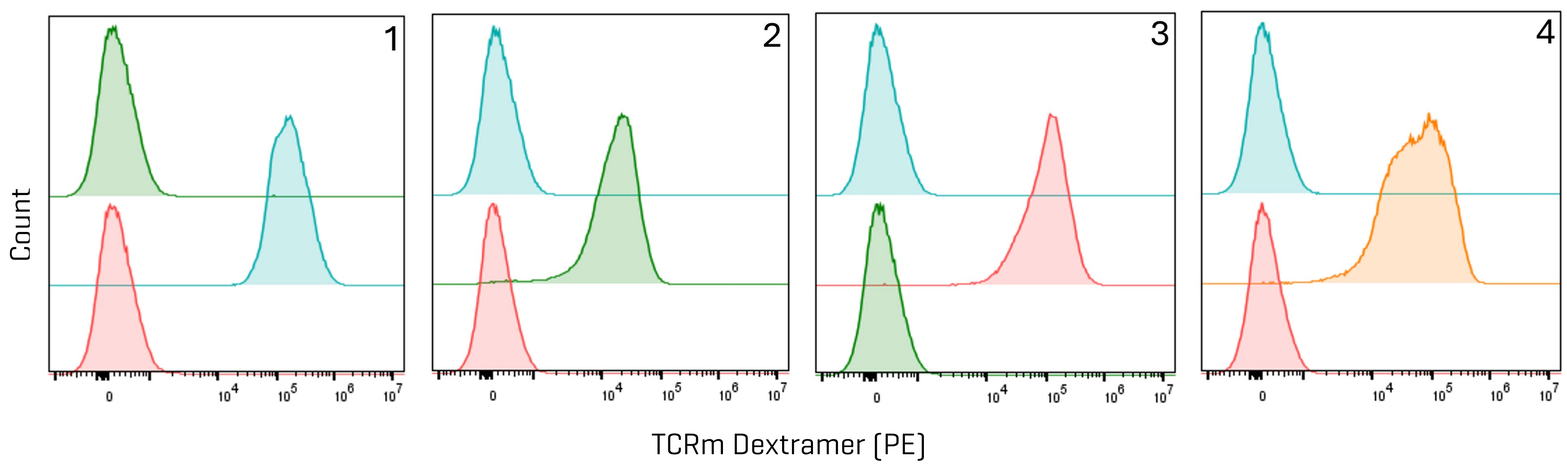
Separate chains of TCRm monomers were produced in *E. coli* and refolded *in vitro*. The resulting TCRm monomers were multimerized by attaching them to a fluorescent, PE-labelled Dextramer® backbone and finally tested for binding to pMHC-coated beads carrying either cognate or non-cognate pMHC complexes. The beads thus behave as an artificial cell display for validation of TCRm : pMHC binding. This platform is flexible and has the potential for high-throughput screening of TCRm : pMHC binding allowing analysis of fine-specificity and cross-reactivity.

Validation of TCRm Specificity

Screening Panel

The functionality and specificity of all three TCRm Dextramer® reagents were validated simultaneously by screening for recognition of cognate (X) and non-cognate pMHC (X) in a flow-based screening assay.

TCRm	NY-ESO1	WT-1	MAGE-1
TCRm1	X	X	X
TCRm2	X	X	X
TCRm3	X	X	X
TCR control	X	X	X



Successful recognition and binding of TCRm Dextramer® reagent to the target pMHC was measured as the increase in fluorescence intensity of PE. These data (1,2,3) show that all three TCRm were able to recognize and bind their target and not to an irrelevant pMHC complex. The control TCR was included as positive assay control (4).