Developing a Platform for Screening T cell receptor Specificity and Ranking of Affinity

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Introduction

Highly potent T cell therapy relies on the specificity as well as the affinity of the T cell receptor towards its MHC:peptide complex (MHCp).

Here we present a platform that enables both production and functional validation of soluble TCR and TCR mimic (TCRm) candidates. By exploiting the high sensitivity of Dextramer® reagents, this flexible platform also allows medium to high-throughput screening for fine specificity and cross-reactivity.

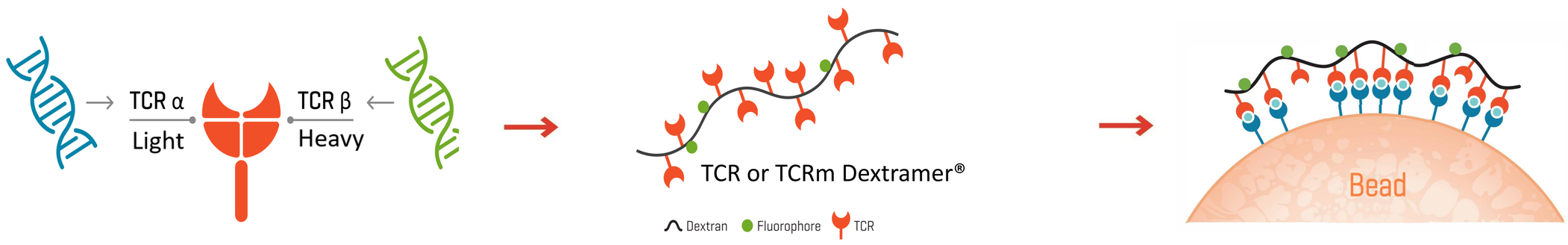
The advantages of this is furthermore that the binding characteristics for each TCR or TCRm Dextramer® reagent can be transformed into a binding score, which enables ranking of the candidates based on their affinity and overall avidity for MHCp complexes.

Conclusions

We have established a platform for production and functional validation of TCR and TCRm molecules of choice, which also enables:

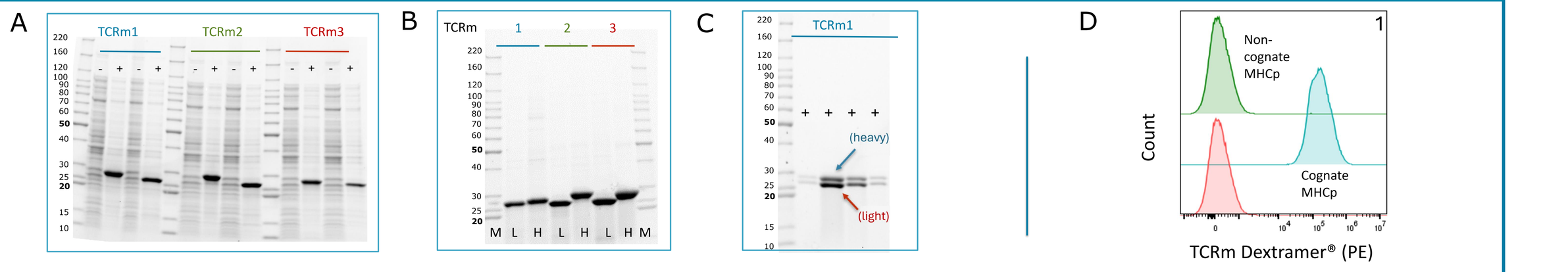
- Production of functional and sensitive TCR and TCRm Dextramer® reagents.
- Ranking of TCR molecules based on their affinity for any MHCp in an artificial cell system using flow-cytomety.
- A flexible platform for high-throughput screening for fine-specificity and cross-reactivity of TCR and TCRm candidates.

Platform for Production and Validation



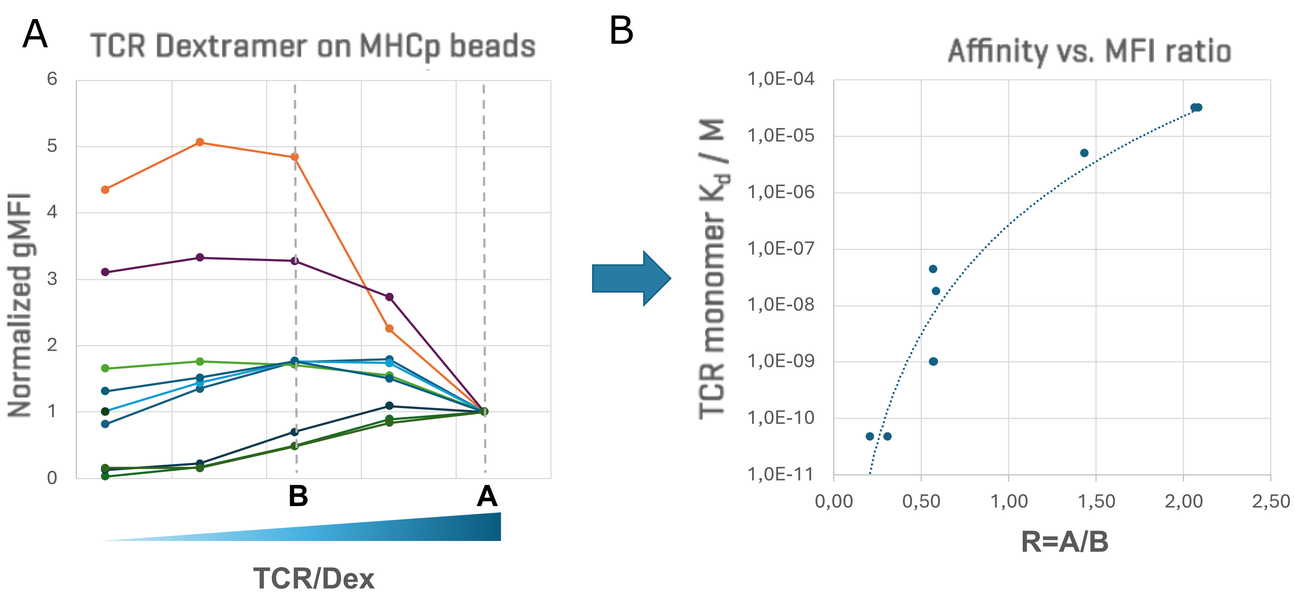
Separate chains of the monomer of choice, TCR or TCRm, were produced in *E. coli* and refolded *in vitro*. The resulting TCRm monomers were multimerized by attaching them to a fluorescent, PE-labelled Dextramer® backbone and finally tested for binding to MHCp-coated beads carrying either cognate or non-cognate MHCp complexes. The beads thus behave as an artificial cell display for validation of TCRm : MHCp binding.

Experimental procedure



A) Heavy and light chains were successfully expressed in *E. coli* for three TCRm's. **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 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. **D)** Successful recognition and binding of TCRm Dextramer® reagent to the target MHCp vs. binding to non-cognate MHCp complexes was measured as the increase in fluorescence intensity of PE.

TCR affinity ranking



By varying the number of TCR molecules on the TCR Dextramer®, the TCR Dextramer molecules binds to the MHCp-loaded beads bind in a manner, which depends on the TCR:MHCp monomer affinity.

A) Binding of fluorescent TCR Dextramer reagents (T cell surrogate) to MHCp-loaded beads (target cell surrogate) was assessed using flow cytometry. Binding characteristics of each TCR Dextramer reagent was transformed into a binding score (R) by taking the ratio of the bead (geometric mean fluorescence intensity (gMFI) values at points A and B. **B)** The binding score correlates with TCR:MHCp equilibrium dissociation constants, and the empirical fit can be used to assess affinity directly or to simply perform affinity ranking. By choosing the points A and B, the affinity ranking can be adjusted for maximal sensitivity at either low, medium, or high affinity.

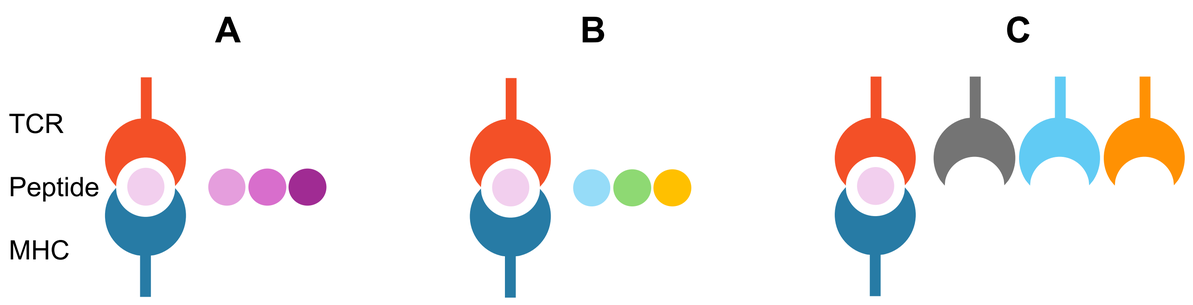
Perspectives

This platform enables affinity assessment of TCR and TCRm in a high throughput format

A. Assessment of fine specificity by screening against an array of MHCp complexes carrying variants of the target epitope

B. Cross reactivity assessment by screening specific TCR or TCR candidates against an array of different/unrelated pMHC complexes

C. Affinity ranking of chosen candidates by targeting the same or different MHCp complexes



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