Transforming Tumor Research: Engineering MHCs as Versatile Reagents

Shicheng Chen[1], Xiangzhong Zheng[1], Juan Liu[1], Jia Cui[1], Jiamin Wang[1], Yuting Shi[1], Huaiyu He[1], Mallory Griffin[1], Xiaoyuan Ran[1], Man-Hee Suh[1]

[1]KactusBio Inc.

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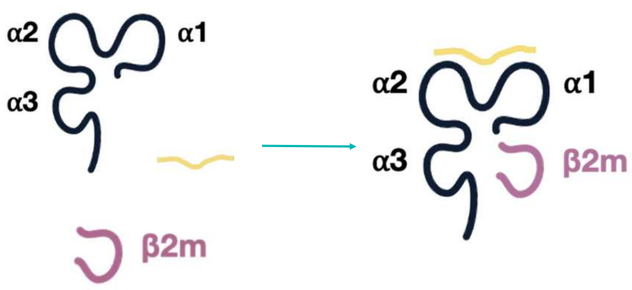
Introduction

The Major Histocompatibility Complex (MHC) is a group of cell surface molecules essential for adaptive immunity. MHC participates in antigen presentation to T cells and trigger downstream immune responses. The absence of dependable, multifaceted MHC reagents has been a hindrance in the therapeutic development against cancers. To address this unmet need, we developed MHC molecules in multiple formats with high versatility. These improved, highly active MHC molecules have the potential to facilitate T cell or TCR-based therapeutic research and development.

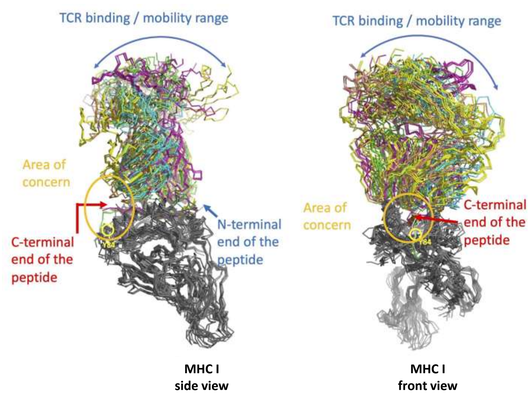
Form 1: Bacteria-Refolded MHC for Broad, High-Throughput Studies



Bacteria-refolded MHC molecules are produced by expressing the heavy chain and β2-microglobulin separately in *E. coli*, followed by an *in vitro* refolding process in the presence of synthetic peptides. The high flexibility and scalability enables large- scale, high-throughput production of customized pMHC complexes of multiple peptides, suitable for applications such as tetramer staining, T cell profiling, as well as structural studies.

Form 2: Mammalian-Expressed Single-Chain Trimer MHC with High Stability and Physiological Presentation

The single chain trimer (SCT) MHC molecule is an engineered construct in which the MHC heavy chain (HLA), β2-microglobulin, and a specific peptide are genetically linked into a single polypeptide chain. This design ensures stable, homogenous presentation of the peptide-MHC with normal post-translational modifications in mammalian cells that can overcome the caveats often seen in the *E.coli* refolded MHC-peptide complex. The SCTs are particularly advantageous for screening studies targeting specific peptides.

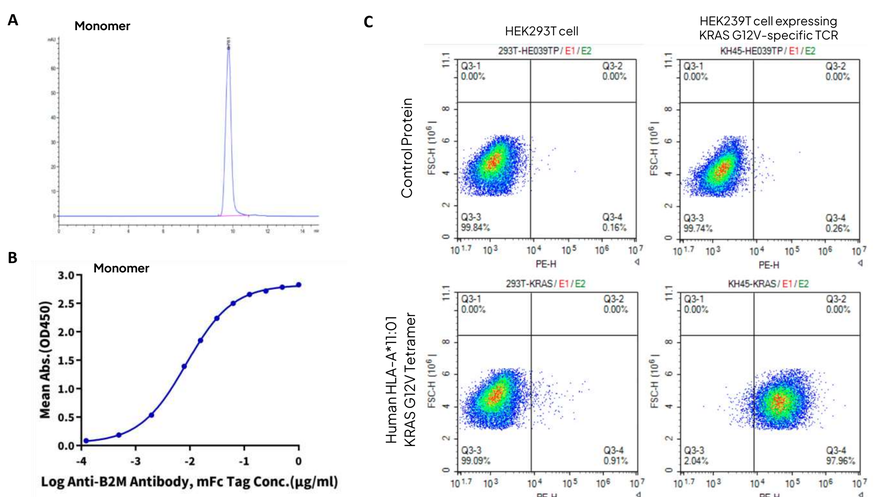


Both refolded and SCT monomers exhibit high purity and robust bioactivity in cell and non-cell based assays.

We evaluated the protein purity and the bioactivity of bacteria-refolded and mammalian-expressed SCT. As expected, both formats show high purity in HPLC and robust binding to their target TCRs in various assays, demonstrating their capability in diverse research applications.

Bacteria Refolded

Human HLA-A*11:01& B2M& KRAS G12V (VVVGAVGVGK)



Mammalian SCT

Human HLA-A*02:01& B2M& PRAME (SLLQHLIGL)

Figure 1. **Bacteria-refolded MHC:** **(A)** The purity of HLA-A*11:01 KRAS G12V Monomer is greater than 95%. **(B)** Immobilized HLA-A*11:01 KRAS G12V Monomer at 0.5 μg/ml (100 μl/well) on the plate. Dose response curve for Anti-B2M Antibody with the EC_{50} of 8.5 ng/ml. **(C)** KRAS G12V-specific TCR-HEK293T cells were stained with PE-Labeled HLA-A*11:01 KRAS G12V Tetramer. Non-transfected HEK293T cells and PE-Labeled proteins were used as a negative control. **Mammalian SCT MHC:** **(D)** The purity of Biotinylated HLA-A*02:01 PRAME Monomer is greater than 95% **(E)** Immobilized Biotinylated HLA-A*02:01 PRAME Monomer at 1 μg/ml (100 μl/well) on the streptavidin precoated plate. Dose response curve for HLA-A*02:01 PRAME-specific TCR with the EC_{50} of 4.8 ng/ml. **(F)** PRAME-specific TCR-HEK293T cells were stained with Biotinylated HLA-A*02:01 PRAME monomer, followed by SA-PE conjugation. Non-transfected HEK293T cells and PE-Labeled proteins were used as negative controls.

Human-Murine Chimeric MHC is potential for higher antibody screening efficiency.

Many TCR-mimic antibodies obtained in immunization are targeting MHC I α3 and β_2m domains, which will not bind to the critical peptide binding pocket. Here, we designed the chimeric human-murine SCT MHC by replacing the human α3 and β_2m domains with murine ones and performed further engineering to maintain the structural and functional integrity. This should increase the likelihood of generating peptide-specific antibodies, making screening less labor-intensive.

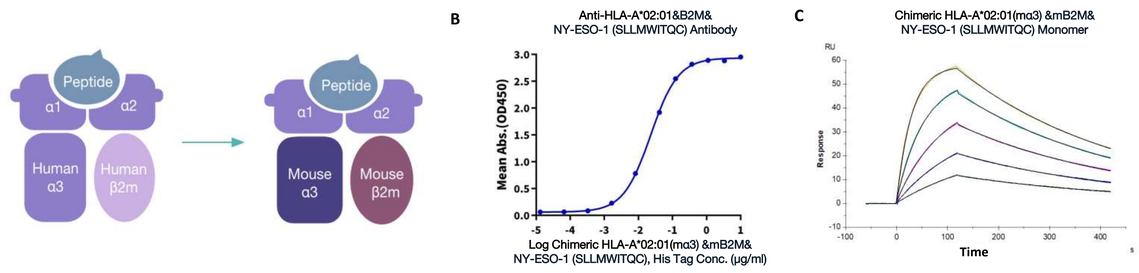
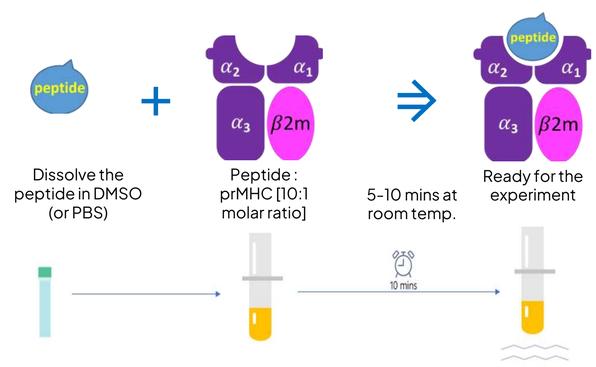


Figure 4. Function validation of human-murine chimeric MHC. **(A)** Design of α3 and β_2 M domain replacement from human to murine species. **(B)** Immobilized Anti-HLA-A*02:01&B2M&NY-ESO-1 (SLLMWITQC) Antibody at 5ug/ml (100ul/well) on the plate. Dose response curve for Chimeric HLA-A*02:01 (mα3) &mB2M&NY-ESO-1 (SLLMWITQC) Monomer with the EC_{50} of 23.0 ng/ml. **(C)** SPR analysis between Anti HLA-A*02:01&B2M& NY-ESO-1 (SLLMWITQC) Antibody captured on CM5 Chip and HLA-A*02:01(mα3)&mB2M&NY-ESO-1(SLLMWITQC) Monomer. Affinity constant:9.32 nM.

Form 3: Peptide-Ready MHC (prMHC) for Rapid Antigen Loading & Screening

We have developed a peptide-ready MHC in which MHC molecules are stabilized without the presence of peptides. These time-saving, ready-to-load MHCs allow convenient, rapid loading of in-house prepared peptide libraries just at room temperature with no UV-induced peptide exchange required. Our prMHC simplifies the process and enhances the scope of research, making it a versatile and invaluable tool in the field of immunology and beyond.



Peptide-loaded prMHC monomers perform equally to SCT pMHC monomers

We demonstrated, by ELISA and SPR, that the prMHC monomer, once loaded with the specific antigenic peptides, are able to bind to antigen-specific TCRs with equivalent performance to SCT monomers.

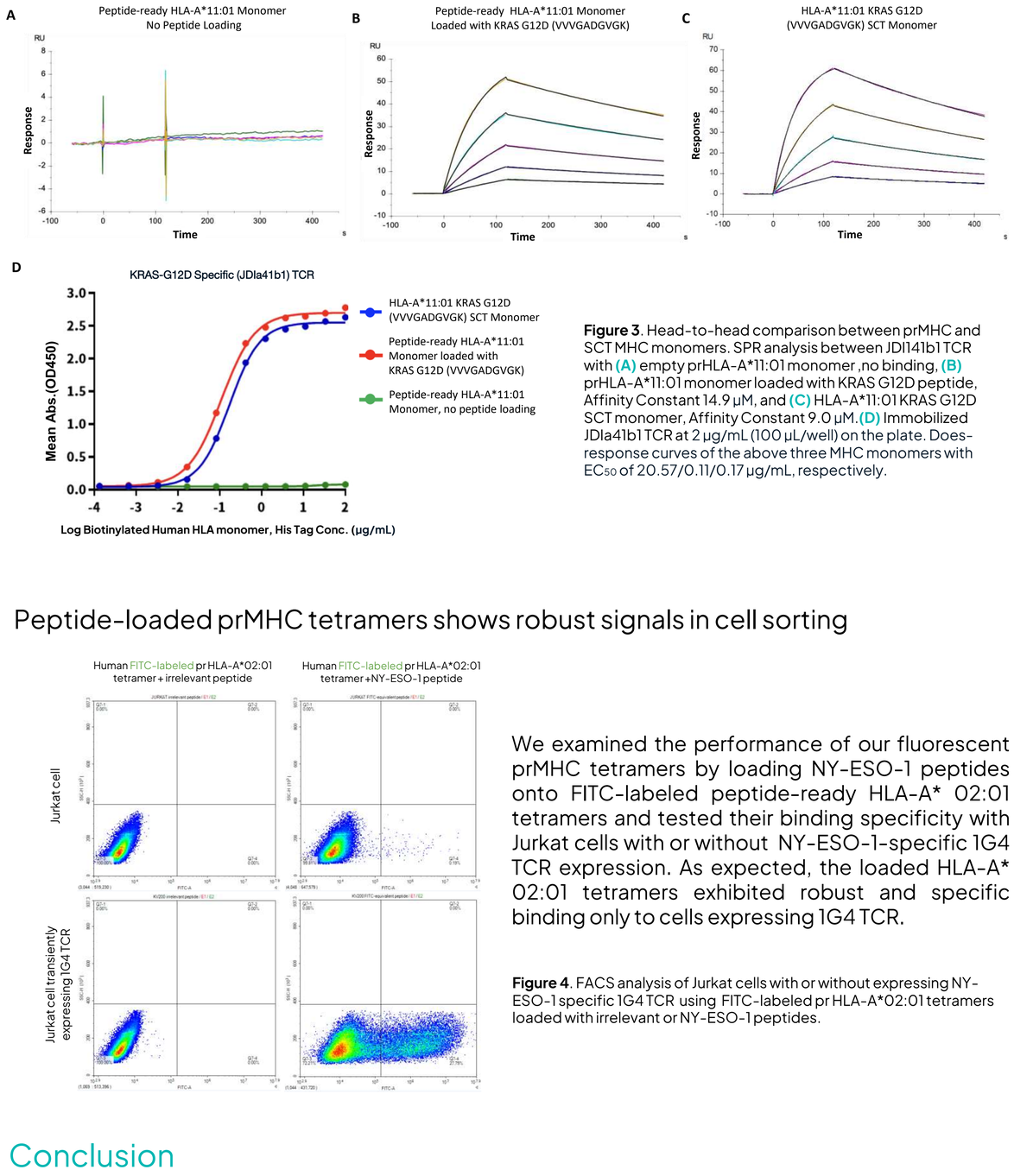


Figure 3. Head-to-head comparison between prMHC and SCT MHC monomers. SPR analysis between JDI141b1 TCR with **(A)** empty prHLA-A*11:01 monomer ,no binding, **(B)** prHLA-A*11:01 monomer loaded with KRAS G12D peptide, Affinity Constant 14.9 μM, and **(C)** HLA-A*11:01 KRAS G12D SCT monomer, Affinity Constant 9.0 μM. **(D)** Immobilized JDIa41b1 TCR at 2 μg/mL (100 μL/well) on the plate. Does-response curves of the above three MHC monomers with EC_{50} of 20.57/0.11/0.17 μg/mL, respectively.

Peptide-loaded prMHC tetramers shows robust signals in cell sorting

We examined the performance of our fluorescent prMHC tetramers by loading NY-ESO-1 peptides onto FITC-labeled peptide-ready HLA-A* 02:01 tetramers and tested their binding specificity with Jurkat cells with or without NY-ESO-1-specific 1G4 TCR expression. As expected, the loaded HLA-A* 02:01 tetramers exhibited robust and specific binding only to cells expressing 1G4 TCR.

Figure 4. FACS analysis of Jurkat cells with or without expressing NY-ESO-1 specific 1G4 TCR using FITC-labeled pr HLA-A*02:01 tetramers loaded with irrelevant or NY-ESO-1 peptides.

Conclusion

- KACTUS high-quality refolding and SCT MHCs have equal performances on bioactivity assays, making it a powerful tool for TCR-based therapeutic research applications.
- KACTUS proprietary prMHCs cover common HLA alleles such as HLA-A 11:01, HLA-A 02:01, HLA-A 02:03, HLA-A 03:01, etc., which can be utilized for highly efficient neoantigen loading, with robust binding performance in SPR, ELISA and FACS assays, equivalent to SCT MHCs