X-ray Crystallography of HLA Single Chain Trimers for Therapeutics Targeting HPV-induced Cancers

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Background and Motivation

- **Human major histocompatibility complex (MHC) class I proteins** or human leukocyte antigens (HLA-I) present peptide fragments from endogenous proteins on the cell surface for recognition by T cell receptors (TCRs) and NKG2x / CD49 or KIR natural killer cell receptors.[1]
- **Single chain trimers (SCTs)** couple all three components of peptide/HLA-I complex (pHLA) (the integral-membrane heavy- or α-chain, the invariant light β_2-microglobin chain, and the peptide fragment) into a single polypeptide, allowing recombinant expression via secretion from eukaryotic cells.[1]
- **Immunotherapies** can be designed to bind to pHLA complexes presented on the surface of HPV induced tumor cells based on X-ray crystallographic structures of SCTs.

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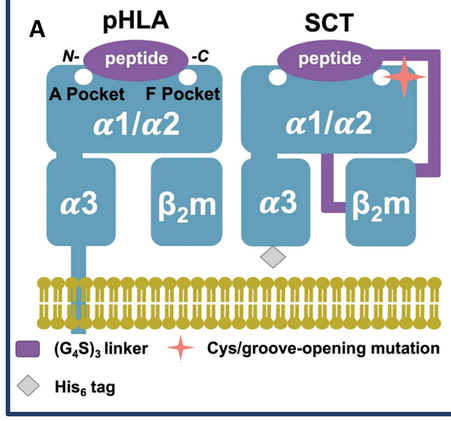

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HLA class 1 allele	HPV peptide sequence
HLA-A*11:01	SVYGTTLEQQYNK
HLA-A*03:01	SVYGTTLEQQYNK
HLA-A*29:02	SVYGTTLEQQY
HLA-A2	VLDFAPPGA

Figure 1. SCT design and structure. (A) Native pHLA Structure and SCT structure.[1] (B) Table containing the HLA alleles and peptides of the SCTs we hoped to produce and crystallize.

Crystal Screening

A

96 well screens	Precipitants	Buffers	Addatives
JCSG+	PEG 2k	MES (pH=6)	96 well additive screen
AmSO4	PEG 2k MME	HEPES (pH=7)	30% v/v Dimethyl sulfoxide
pHClear	PEG 3350	TRIS (pH=8)	0.1 M Urea
PEGs I	PEG 6k	TRIS (pH=8.5)	30% w/v D-Sorbitol
PEGs II			30% w/v D-Sorbitol
			30% v/v Ethylene glycol
			30% w/v 1,6-Hexanediol
			30% v/v Ethanol
			30% w/v Sucrose

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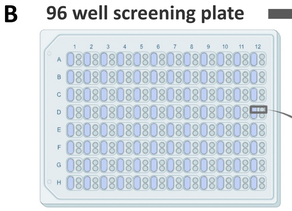
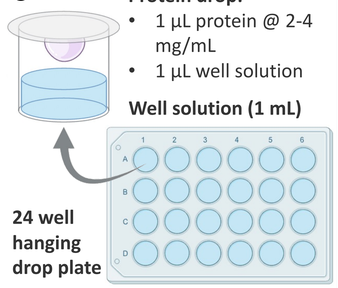


Protein drop:
- 100 nL protein @ 6-8 mg/mL
- 100 nL well solution

Well solution (80 µL):
- 12-28% w/v precipitant
- 0.1-0.2M buffer or salt
- Additives

C



Protein drop:
- 1 µL protein @ 2-4 mg/mL
- 1 µL well solution

Well solution (1 mL)

D.1 D.2 D.3

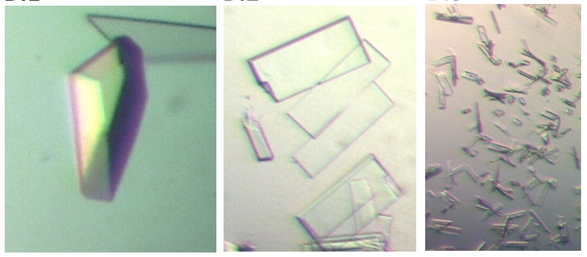


Figure 3. SCT crystallization. (A) Crystallization well condition screenings. (B) Diagram showing 96 well plate setup. At this stage the well volume is 80 µL and the drop is a 200 nL sitting drop. (C) 24 well plate set up. Here the well volume is 1 mL and the drop is a 2 µL hanging drop (D.1) Thick singular crystal. (D.2) Sheet crystals. (D.3) needle crystals.

Significance and Future Directions

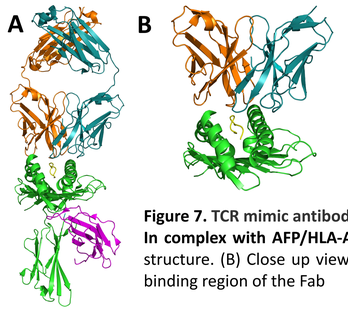
CAR/Adaptive T-cell Therapies

- Structures can inform the design of T-cell based therapeutics that target specific HLA class I complexes
- SCTs used to indicate which TCRs will bind to the target cell
- SCTs inform mutation of TCRs to create stronger binders

Figure 6. MHC class I TCR complex (4MS8).[2] Only the binding cleft (green) and the peptide (blue) of the MHC complex is shown. The T-cell receptor consists of two chains, the α-chain (purple) and the β-chain (yellow).

Antibody Therapies

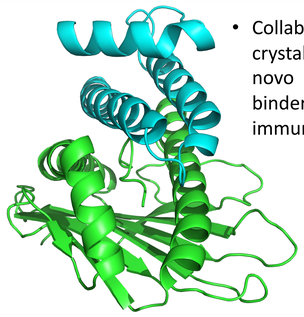
A B



- TCR mimic antibodies can be made as immunotherapies that target intracellular antigens presented by HLA class I proteins

Figure 7. TCR mimic antibody (Fab fragment) In complex with AFP/HLA-A*02 (7RE7).[3] (A) Overview of structure. (B) Close up view of the HLA peptide cleft and binding region of the Fab

De Novo Computational Modeling



- Collaborator Aaron Ring uses x-ray crystallographic structures for de-novo computational design of binders to be turned into immunotherapies.

Figure 8. Aaron Ring's model of a binder to HLA-A2 with an HPV peptide from E7 protein. Shown in green is the binding cleft and peptide of the HLA complex. Shown in blue is the designed binder.

SCT Production and Purification

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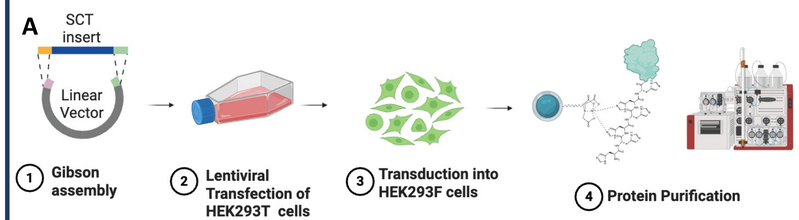

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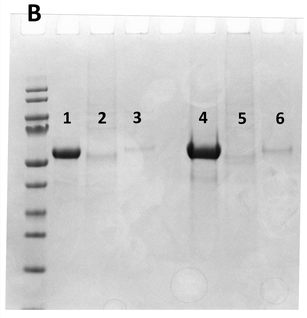

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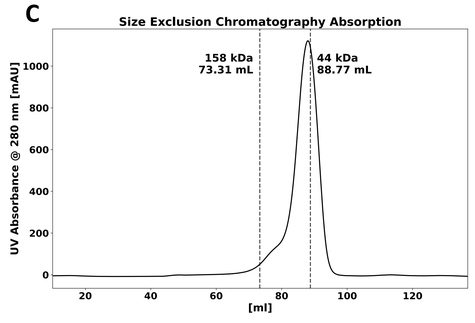
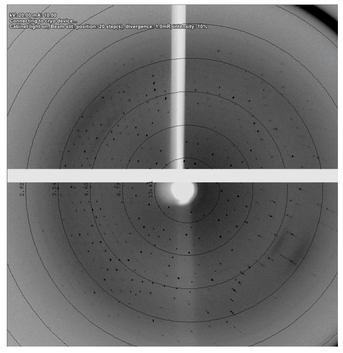


Figure 2. SCT expression and purification. (A) Process flow to produce SCTs for crystallization. (B) Protein PAGE gel indicating successful HisTag purification of two SCTs. Columns 1 and 4 are the protein elution. Columns 2 and 5 are the column flow through. Columns 3 and 6 are from the left-over nickel resin. (C) Size exclusion chromatography (SEC) UV absorbance [mAU] as a function of mL of PNE buffer passed through the column. Dotted lines are measured standards indicating that protein peak falls within the expected range for a ≈50 kDa protein.

Solving SCT Structures

Figure 4. SCT X-ray diffraction pattern. Reflections are visible out to past 2.95 Å



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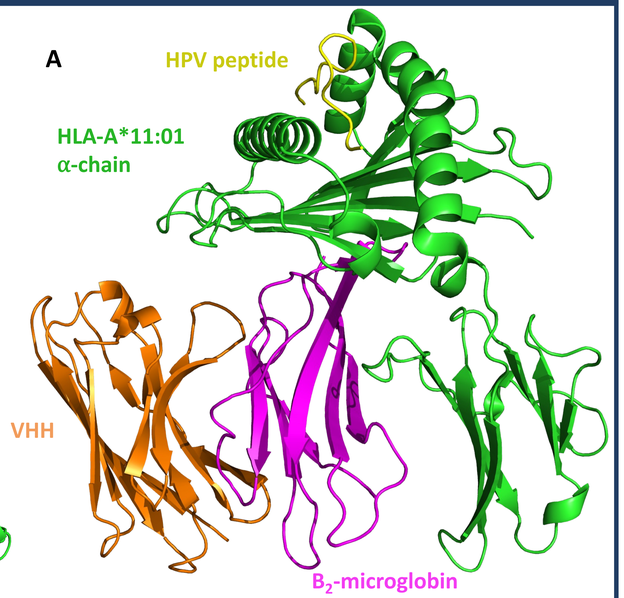

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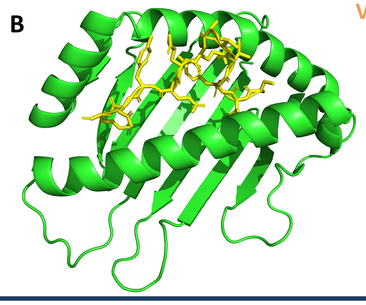

Figure 5. HLA HLA-A*11:01 with peptide sequence SVYGTTLEQQYNK. (A) overview of entire structure shown from three different angles. Crystal Structure was obtained with a resolution of 2.7 Å (B) Close up of binding cleft and specific HPV peptide. Structure shown is not fully refined and is a preliminary model.

References

1. Finton, Kathryn A. K., et al. "Effects of HLA Single Chain Trimer Design on Peptide Presentation and Stability." *Frontiers in Immunology*, vol. 14, May 2023. *Frontiers*, https://doi.org/10.3389/fimmu.2023.1170462.
2. Adams, Jarrett J et al. "Structural interplay between germline interactions and adaptive recognition determines the bandwidth of TCR-peptide-MHC cross-reactivity." *Nature immunology* vol. 17,1 (2016): 87-94. doi:10.1038/ni.3310
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Acknowledgments

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