From Antigen Recognition to Radionuclide Delivery: Structural Studies of HLA-A and Siderocalin-HOPO-Eu Complexes

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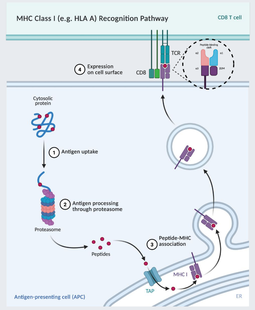
Background and Motivation: Structural Biology for Targeted Cancer Therapy

Targeted Cancer Therapy Requires Two Complementary Capabilities

1. We Must Define a Tumor Specific Target

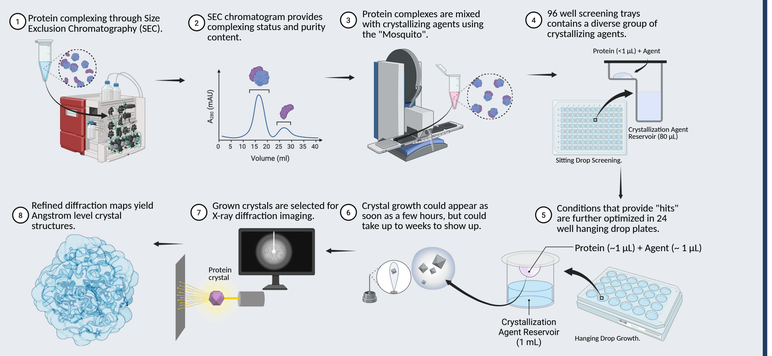
- Human papillomavirus **(HPV)** drives the production of oncogenic proteins, including the **E6** protein that functions as a **molecular glue** to the **ubiquitin ligase** for the tumor suppressor **p53**.
- Cell surface MHC Class I proteins **(HLA-A, -B, -C)** display peptides derived primarily from intracellular proteins on the cell surface for immune recognition. **(Figure 1: HLA-A Antigen Presentation)**



2. We Must Accurately Deliver the Therapeutic Payload

- Current radiotherapies run into **severe off target toxicity**, as the radioactive dose is **not** localized only to the tumor.
- **Siderocalin** (Scn) forms high-affinity noncovalent complexes with lanthanide loaded HOPO chelators.
- Structural based **engineering** could improve chelator retention and provide a potential modular platform for radioactive metal delivery when coupled to a **tumor targeting module**.

Crystallography Workflow



1. Engineered SCTs Stabilize Peptide-HLA Complexes

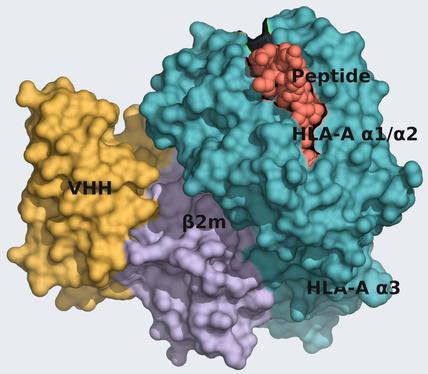


Figure 2: Single Chain Trimer Design. A Single Chain Trimer (SCT) is an **engineered mimic** of our pHLA A presentation system. The SCT links a peptide from HPV, β2-microglobulin (β2M), and the HLA-A heavy chain. An engineered disulfide between a linker and the heavy chain stabilizes peptide loading, while the AD01 VHH binds β2M as a peptide-independent **crystallization chaperone**.

2. HLA Allele Alters Presentation of the Same Peptide

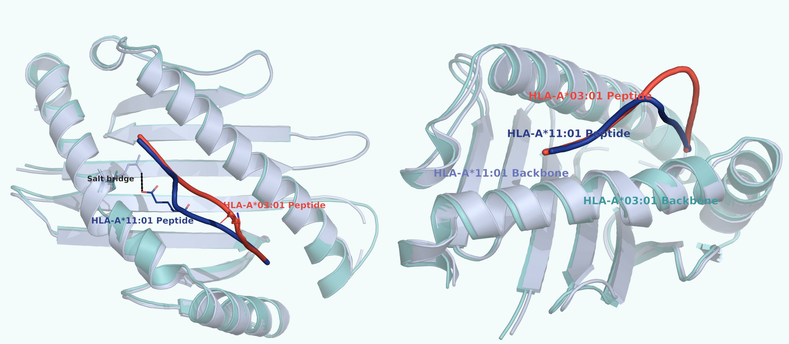


Figure 3: Allele dependent presentation of the HPV16 E6 13-mer. HLA-A*03:01 and HLA-A*11:01 are two **different** yet **structurally homologous** HLA alleles. However, when the same 13-mer peptide *SVYGTTLEQQYNK* is aligned to the two binding grooves, these peptides adopt different presentation conformations. Our structures **(Left: Side View, Right: Top View)** indicate that a distinct salt bridge in the HLA-A*11:01 α-helix may contribute to a tighter binding conformer.

Same peptide sequence ≠ Same antigenic surface.

3. Partial Peptide Disorder Observed in HLA-A*03:01-AD01 Crystal

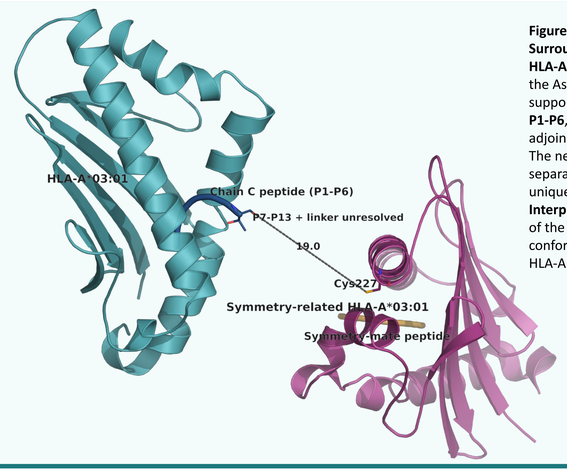


Figure 4: The Crystal Environment Surrounding a Partially Solved HLA-A*03:01 Peptide. In chain C of the Asymmetric Unit, our density supports the **positioning of residues P1-P6**, whereas P7-P13 and the adjoining linker remain unresolved. The nearby symmetry mate is separated by only ~19Å, suggesting a unique crystal packing contact. **Interpretation**: The C-terminal region of the 13-mer may retain substantial conformational flexibility within the HLA-A*03:01 construct.

4. Peptide Length Reshapes HLA-A*11:01 Antigenic Surface

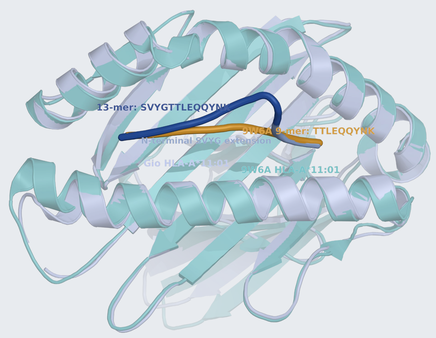
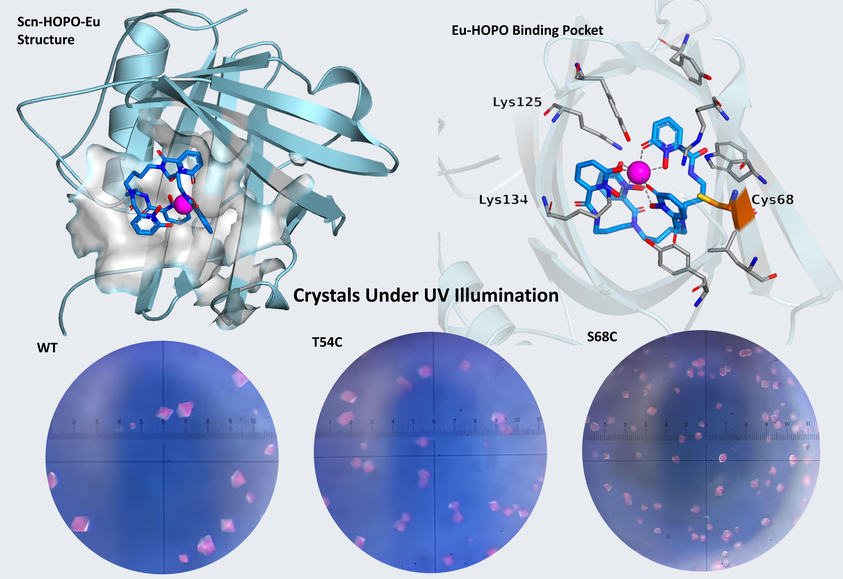


Figure 5: Structural comparison between nested HPV16 E6 peptides presented by HLA-A*11:01. HLA-A*11:01 recognizes and displays both the 13-mer *SVYGTTLEQQYNK* and the N-terminally reduced 9-mer *TTLEQQYNK* (PDB: 9W6A)[2]. The difference in **four** residues alter the peptide presentation conformation, where the 13-mer is more revealing and less enclosed than the respective 9-mer.

5. Siderocalin Retains Eu-HOPO Binding Across Cysteine Variants



6. Engineering a Covalent Lanthanide Chelator Platform

- **Why engineer siderocalin?** Siderocalin binds lanthanide 3,4,3 HOPO complexes with high affinity, providing a protein scaffold for transporting radioactive metals. However, despite being a nanomolar-affinity binder, the wild type interactions are **non-covalent**, which may result in the premature deposition of radioactive metals.
- **Why introduce cysteine?** Two coordinated cysteines form a **covalent** disulfide linkage. T54C and S68C are tested as engineered thiol handles. For disulfide bond formation with the HOPO chelator, a cysteine needs to be installed properly on 3,4,3 HOPO **as well**. Our first round test demonstrates that both mutations preserve the original **non-covalent** scaffold compatibility.
- **Why does it matter?** Subsequent testing with both mutant Scn and **engineered HOPO** could potentially result in a **covalent radiometal cargo delivery system** that might reduce unintentional payload dissociation.

References & Acknowledgements

References: [1] Finton KAK *et al. Front Immunol.* 2023;14:1170462. [2] Wang J *et al.* RCSB Protein Data Bank, **9W6A**.

We thank **Giovanni M. Loia and Kathryn A. Finton** for permission to use and compare unpublished HLA-A*11:01 SCT structural data, and **Roland Strong, Peter Rupert, Michael Stark, and Sabriyah Morshed** for experimental mentorship and collaboration. Molecular structures were visualized in **PyMOL (Schrödinger, LLC)**; schematics were created with **BioRender.com**. This work is supported by the NIAID. The Fred Hutch Summer Undergraduate Research Program is supported in part by the **University of Chicago, Fred Hutch Internship Program, and participating laboratories/research groups**.