# Comprehensive class II HLA cross-reactivity profiling of single peptides across DR, DQ, and DP molecules

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HLA Class II Binding Platform

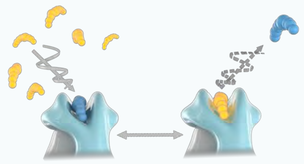
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## 1. BACKGROUND & AIM

- Prediction alone cannot establish whether a peptide forms a detectable, reproducible complex with a specific HLA Class II molecule.
- Most binding studies examine only a few alleles and therefore miss cross-reactivity across the broader DR, DQ, and DP repertoire.
- **Aim: establish one direct experimental platform that supports both targeted peptide–allele validation and broad allele-panel mapping.**

**From a single pair to the Class II landscape**

## 2. ONE WORKFLOW, TWO APPLICATION DESIGNS



Peptide exchange into load-competent soluble HLA-II

**1 Peptide$^{bio}$ design**
N-terminal biotin–Ahx peptide; sequence and solubility reviewed.

**2 HLA activation + loading**
Native soluble DR, DQ, or DP; proprietary in-house conditions.

**3 Conformation-sensitive capture**
Capture the correctly folded peptide–HLA complex.

**4 Streptavidin ELISA + modeling**
Quantify signal, curve quality, and apparent $EC_{50}$ when supported.

**Signal requires captured HLA and associated peptide$^{bio}$ → evidence of complex formation and persistence under the assay conditions**

## 3. MODEL PEPTIDES & PANEL

INFLUENZA Hemagglutinin [HA]

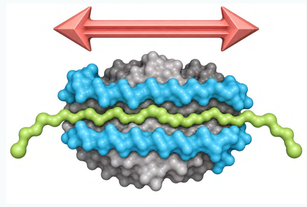
**Biotin–Ahx–PKYVKQNTLKLAT**

Well-characterized DR1 benchmark

CLIP CONTROL

**Biotin–Ahx–PVSKMRMATPLLMQA**

Physiologic groove placeholder; broad but allele-dependent behavior

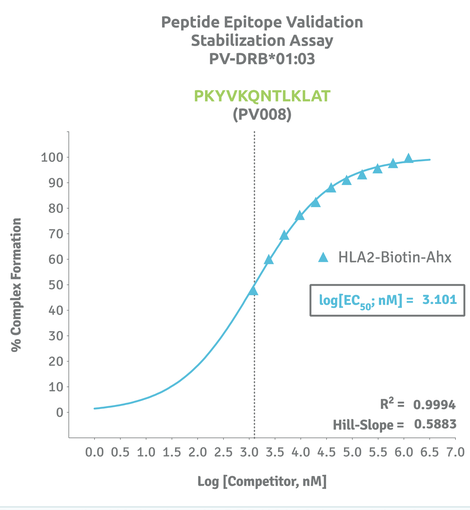


| CLASS II LIBRARY | PANEL BREADTH | REPORTING UNIT |
|---|---|---|
| >100 molecules | DR • DQ • DP | Exact α/β pair |

**Broad screening is followed by titration of prioritized positive, borderline, and representative negative interactions.**

## 4. APPLICATION 1 | DIRECT COMPLEX-FORMATION VALIDATION

**Does one peptide form a measurable, concentration-dependent complex with the target allele?**




*Representative HA titration with DRB1*01:03. Internal development data.*

**STUDY DESIGN**

- Target HLA plus an allele-matched positive control
- Concentration series with replicate wells
- Sigmoidal fit only when baseline and plateau are adequately bracketed

**PRIMARY OUTPUTS**

APPARENT POTENCY
**$EC_{50}$ / log$EC_{50}$**

ASSAY WINDOW
**Top response + slope**

DECISION QUALITY
**Fit + controls + precision**

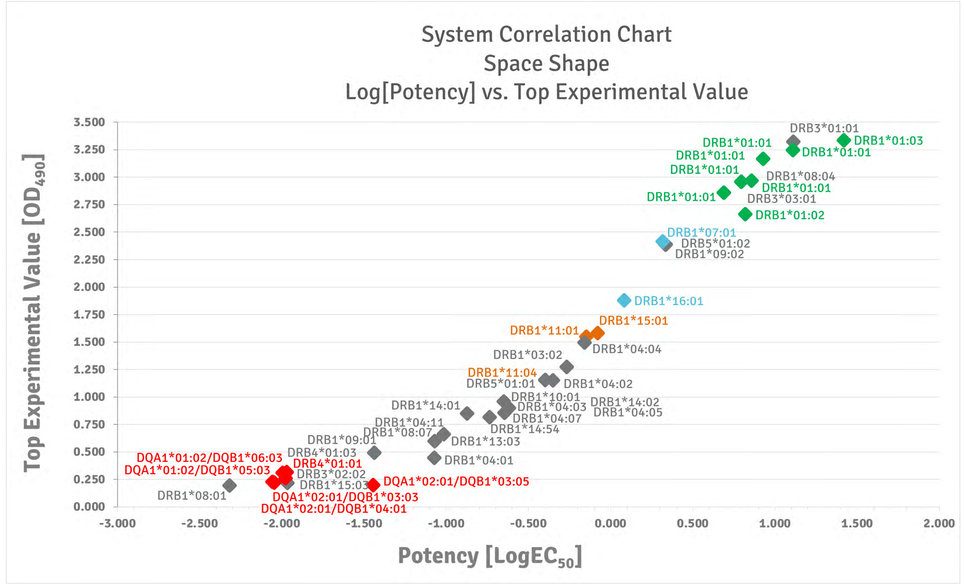
**REPRESENTATIVE RESULT**

**HA × DRB1*01:03 | $log_{10}EC_{50}$ [nM] = 3.101 | ≈1.26 µM | $R^2$ = 0.9994**

**The apparent $EC_{50}$ is an operational concentration–response value within this protocol—not a thermodynamic $K_d$ or a competitive $IC_{50}$.**

## 5. APPLICATION 2 | CLASS II CROSS-REACTIVITY PROFILING

**Which HLA-DR, -DQ, and -DP molecules show compatible peptide presentation potential?**




*HA Space–Shape profile: fitted potency (x) versus maximum experimental signal (y). Internal development data.*

**PANEL STRATEGY**

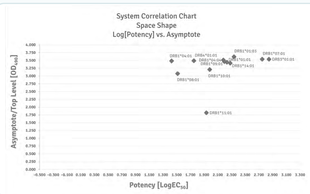
- Standardized screen of one peptide across >100 Class II molecules
- Confirm prioritized positive, borderline, and representative negative pairs by titration

**SPACE–SHAPE VIEW**

- X axis: apparent potency ($logEC_{50}$)
- Y axis: achievable complex-associated signal
- Upper-right region: strongest combined performance

**INFLUENZA HA FINDINGS**

- Expected DR1 behavior recovered
- Additional DR cross-reactivity resolved
- Most DQ and DP molecules remained in the low-response region




*CLIP showed consistently strong binding across the tested DR panel, whereas many DQ molecules showed reduced binding (not shown), indicating molecule-specific preferences for distinct CLIP sequences.*

**Interpret cross-allele differences using both potency and maximum signal.**

## 6. TAKE-HOME MESSAGE & APPLICATIONS

**VALIDATE**
Targeted peptide–allele evidence with concentration dependence

**MAP**
Empirical cross-reactivity across HLA-DR, -DQ, and -DP

**PRIORITIZE**
Peptide–HLA pairs for reagents, receptors, and functional studies

**One peptide. Map its Class II cross-reactivity landscape.**

**Immunotherapy • Vaccine design • Immune monitoring • Immunopeptidomics validation**