nature chemical biology

SUPPLEMENTARY INFORMATION

https://doi.org/10.1038/s41589-018-0130-4

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# T cell receptor cross-reactivity expanded by dramatic peptide–MHC adaptability

**Timothy P. Riley[1,2], Lance M. Hellman[1,2], Marvin H. Gee[3,4], Juan L. Mendoza[3,4], Jesus A. Alonso[3,4], Kendra C. Foley[5], Michael I. Nishimura[5], Craig W. Vander Kooi[6], K. Christopher Garcia[3,4] and Brian M. Baker[1,2]***

[1]Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN, USA. [2]Harper Cancer Research Institute, University of Notre Dame, Notre Dame, IN, USA. [3]Department of Molecular and Cellular Physiology, Stanford University School of Medicine, Stanford, CA, USA. [4]Department of Structural Biology, Stanford University School of Medicine, Stanford, CA, USA. [5]Department of Surgery, Cardinal Bernardin Cancer Center, Loyola University Chicago, Maywood, IL, USA. [6]Department of Molecular and Cellular Biochemistry, University of Kentucky, Lexington, KY, USA.
*e-mail: brian-baker@nd.edu

| Peptide | NCBI Reference Sequence(s) | | | | |
|---|---|---|---|---|---|
| MLI**DRG**LGLL | WP_082441782.1 | | | | |
| ILI**DRG**LGIM | YP_009336482.1 | | | | |
| QLM**DRG**LGVL | APQ42521.1 | | | | |
| ILN**DRG**LGML | XP_012938420.1 | XP_022519643.1 | XP_019768580.1 | KZS15328.1 | EFX89764.1 |
| LLF**DRG**TGLM | WP_061021764.1 | | | | |
| LLW**DRG**IGLL | OYT65050.1 | | | | |
| MML**DRG**LVLM | WP_067737417.1 | WP_008829097.1 | WP_043869125.1 | | |
| MML**DRG**LVMM | WP_008332046.1 | WP_090062244.1 | WP_074641131.1 | | |
| MMW**DRG**TLIL | WP_014126739.1 | | | | |
| IMF**DRG**LGLL | WP_062805772.1 | | | | |
| TLF**DRG**LGL | BAC79679.1 | | | | |
| MIF**DRG**VWL | WP_011053120.1 | | | | |
| MVF**DRG**HPL | CAD41203.1 | | | | |
| RLW**DRG**EIM | OAP17390.1 | | | | |
| LLW**DRG**QNL | WP_000380056.1 | | | | |
| ILW**DRG**REL | WP_084163317.1 | | | | |
| DIW**DRG**IHL | WP_103068111.1 | | | | |
| MML**DRG**QRL | OUT77584.1 | | | | |
| MML**DRG**KEL | WP_011095018.1 | WP_103523545.1 | | | |
| MMV**DRG**TTL | XP_021274615.1 | | | | |

**Supplementary Table 1. Naturally occurring peptides resembling the DRG class of peptides found in various genomes.** A non-comprehensive sampling of naturally occurring peptides from various proteomes with a DRG motif predicted to be compatible with the DMF5 TCR. BLAST searches required ideal anchor residues in positions 2 and/or 9 for nonamers. Decamers required an ideal anchor in position 2 and a large hydrophobic anchor in position 10 to facilitate the register shift.

| Peptide | $K_D$ (μM)[a] | ΔG° (kcal/mol)[b] | ΔΔG° (kcal/mol)[c] | $T_m$ (°C)[d] |
|---|---|---|---|---|
| ELAGIGILTV (MART-1) | 5.5 ± 0.5 | -7.17 ± 0.05 | -- | 65.0 ± 0.1 |
| SMAGIGIVDV | 202 ± 24 | -5.04 ± 0.07 | 2.13 ± 0.09 | 65.8 ± 0.1 |
| SMLGIGIVPV | 43 ± 13 | -5.96 ± 0.18 | 1.21 ± 0.19 | 66.1 ± 0.2 |
| NMGGLGIMPV | 7.3 ± 0.9 | -7.00 ± 0.07 | 0.17 ± 0.09 | 58.6 ± 0.2 |
| NLSNLGILV | 11 ± 1.4 | -6.76 ± 0.08 | 0.41 ± 0.06 | 66.3 ± 0.1 |
| IMEDVGWLNV | 121 ± 12 | -5.34 ± 0.06 | 1.83 ± 0.08 | 55.1 ± 0.3 |
| ILEDRGFNQV | 99 ± 9 | -5.46 ± 0.05 | 1.71 ± 0.07 | 46.1 ± 0.2 |
| LMFDRGMSLL | 123 ± 16 | -5.33 ± 0.08 | 1.84 ± 0.09 | 50.0 ± 0.4 |
| MMWDRGMGLL | 144 ± 20 | -5.24 ± 0.08 | 1.93 ± 0.09 | 45.4 ± 0.1 |
| MMWDRGLGMM | 32 ± 2 | -6.13 ± 0.04 | 1.04 ± 0.06 | 45.2 ± 0.1 |

[a]Error in $K_D$ is standard error from non-linear curve fitting of replicated datasets to a 1:1 equilibrium binding model as described in Methods.

[b]Binding free energy changes ($\Delta G° = RT\ln[K_D]$); errors reflect propagated $K_D$ error. T = 298K (25 °C).

[c]Difference in binding free energy relative to MART-1 ($\Delta\Delta G° = \Delta G°_{MART\text{-}1} - \Delta G°_{pep}$); errors are propagated from errors in $\Delta G°$.

[d]Error in $T_m$ is standard error from non-linear curve fit of temperature derivative data to a bi-Gaussian function as described in Methods.

**Supplementary Table 2. DMF5 binding affinities and pMHC $T_m$ values for GIG and DRG-class peptides.** For the DMF5 TCR binding HLA-A2 presenting each peptide in the DRG and GIG class, the table shows binding affinity and binding free energy values as determined by SPR. The right-most column shows the thermal stability of each peptide/HLA-A2 complex as determined by DSF.

| Peptide | $K_D$ (μM)[a] | ΔG° (kcal/mol)[a] | ΔΔG° (kcal/mol)[a] | $T_m$ (°C)[a] |
|---|---|---|---|---|
| MMWDRGLG**A**M | > 500[a] | < 4.5 | > 1.6 | 43.5 ± 0.5 |
| MMWDRGLGM**V** | NBD[b] | < 5.0 | > 2.0 | 56.7 ± 0.3 |
| MMWDRGLGM | 30 ± 2 | -6.17 ± 0.04 | 0.04 ± 0.06[c] | 49.1 ± 0.3 |
| MMWDRG**A**GMM | > 500[d] | < 4.5 | > 1.6 | 42.1 ± 0.3 |
| MMWD**A**GLGMM | > 500[d] | < 4.5 | > 1.6 | 44.5 ± 0.2 |

[a] $K_D$, ΔG°, ΔΔG°, and $T_m$ errors defined as in Supplementary Table 2.

[b] No binding detected; ΔΔG° value is an estimated lower limit.

[b] ΔΔG° relative to MMWDRGLGMM (data in Supplementary Table 2).

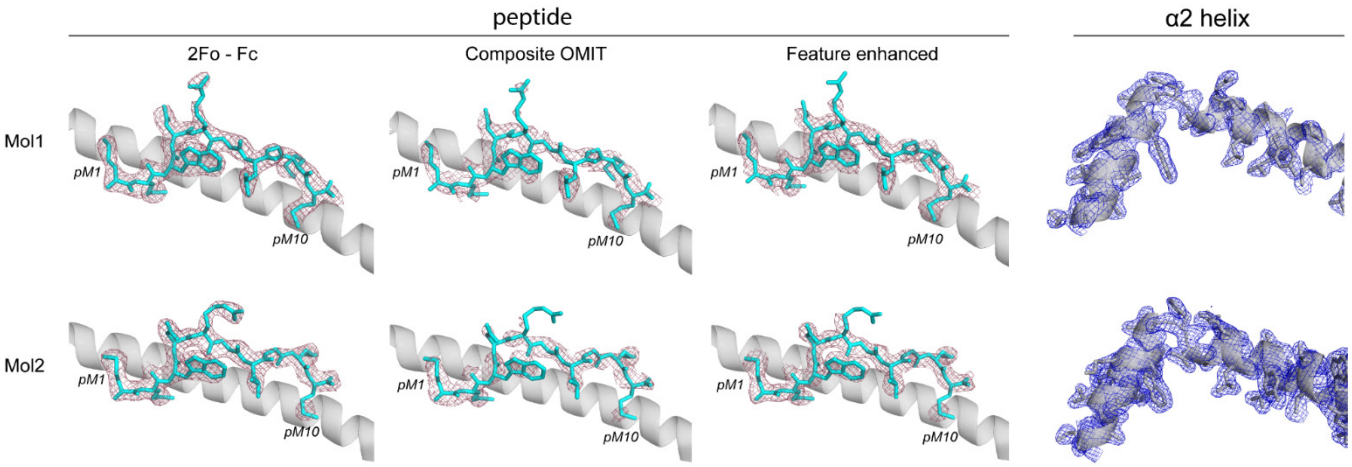
[c] Binding detected but too weak to quantify; $K_D$ and ΔΔG° values are estimated upper/lower limits.

**Supplementary Table 3. DMF5 binding affinities and pMHC $T_m$ values for MMWDRGLGMM variants.** For the DMF5 TCR binding HLA-A2 presenting each variant of the MMWDRGLGMM peptide, the table shows binding affinity and binding free energy values as determined by SPR. The right-most column shows the thermal stability of each peptide/HLA-A2 complex as determined by DSF.

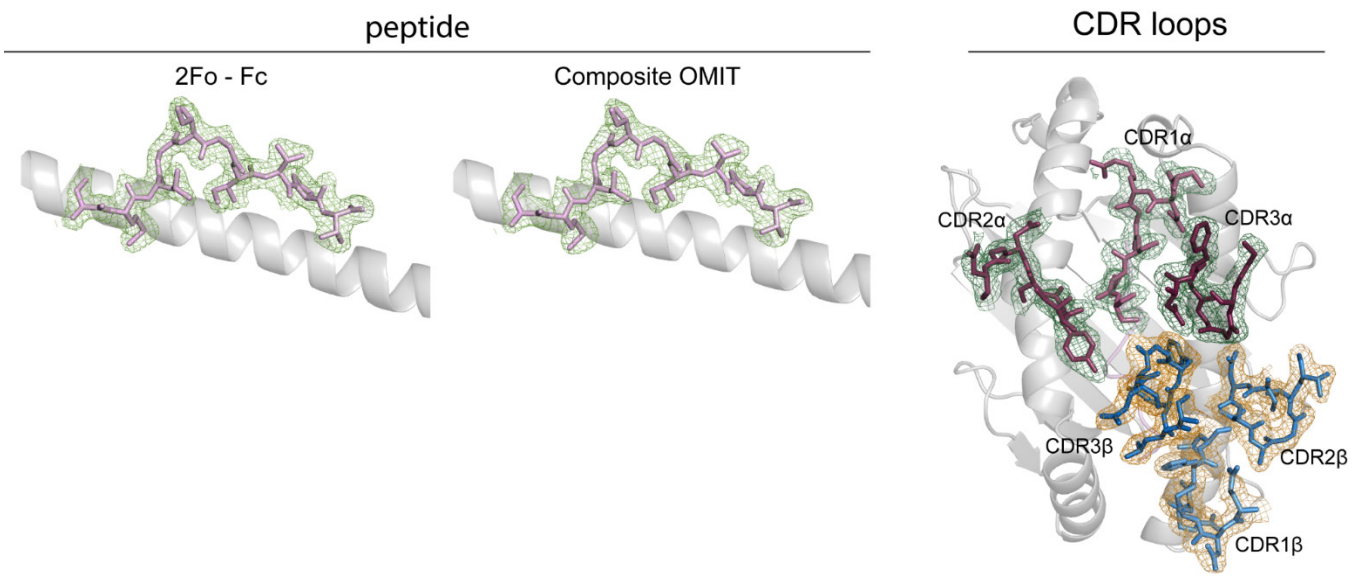
| | MMWDRGLGMM/HLA-A2 | DMF5-MMWDRGLGMM/ HLA-A2 | DMF5-SMLGIGIVPV/ HLA-A2 |
|---|---|---|---|
| **Data collection** | | | |
| Space group | $P2_1$ | C2 | C2 |
| Cell dimensions | | | |
| *a*, *b*, *c* (Å) | 58.30, 84.93, 84.32 | 232.08, 50.33, 84.36 | 228.11, 49.90, 92.45 |
| $\alpha, \beta, \gamma$ (°) | 90.00, 89.97, 90.00 | 90.00, 99.52, 90.00 | 90.00, 95.72, 90.00 |
| Resolution (Å) * | 19.95-2.50 (2.59-2.50) | 19.94-2.15 (2.23-2.15) | 38.75-2.39 (2.48-2.39) |
| $R_{sym}$ or $R_{merge}$ | 0.16 (0.69) | 0.12 (0.59) | 0.07 (0.62) |
| $I / \sigma I$ | 8.52 (2.64) | 10.98 (2.20) | 24.63 (2.12) |
| Completeness (%) | 98.5 (97.6) | 98.8 (96.4) | 98.0 (98.5) |
| Redundancy | 3.0 (3.0) | 2.7 (2.2) | 3.8 (3.7) |
| | | | |
| **Refinement** | | | |
| Resolution (Å) | 19.95-2.50 | 19.94-2.15 | 38.75-2.39 |
| No. reflections | 28114 | 49053 | 38730 |
| $R_{work}$ / $R_{free}$ | 0.211/0.249 | 0.180/0.226 | 0.201/0.244 |
| No. atoms | 6373 | 7007 | 6696 |
| Protein | 6288 | 6527 | 6484 |
| Ligand/ion | 0 | 0 | 0 |
| Water | 85 | 480 | 212 |
| *B*-factors | 54.0 | 39.2 | 52.31 |
| Protein | 54.6 | 39.4 | 52.95 |
| Ligand/ion | 0 | 0 | 0 |
| Water | 40.8 | 36.57 | 32.62 |
| R.m.s. deviations | | | |
| Bond lengths (Å) | 0.002 | 0.003 | 0.002 |
| Bond angles (°) | 0.45 | 0.60 | 0.58 |

*Highest-resolution shell is shown in parentheses.

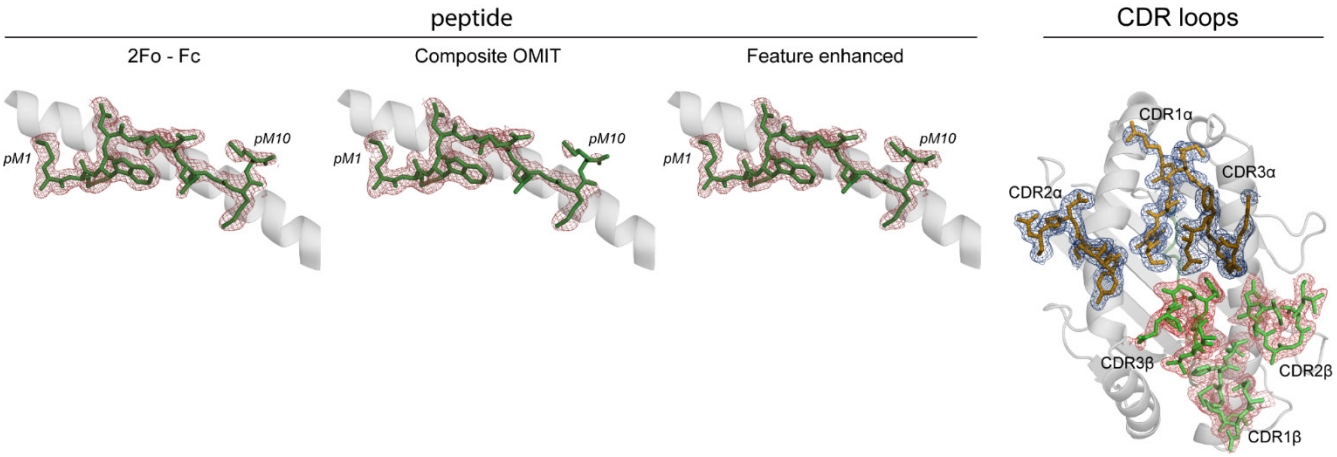
**Supplementary Table 4. X-ray data collection and refinement statistics for the indicated structures.**



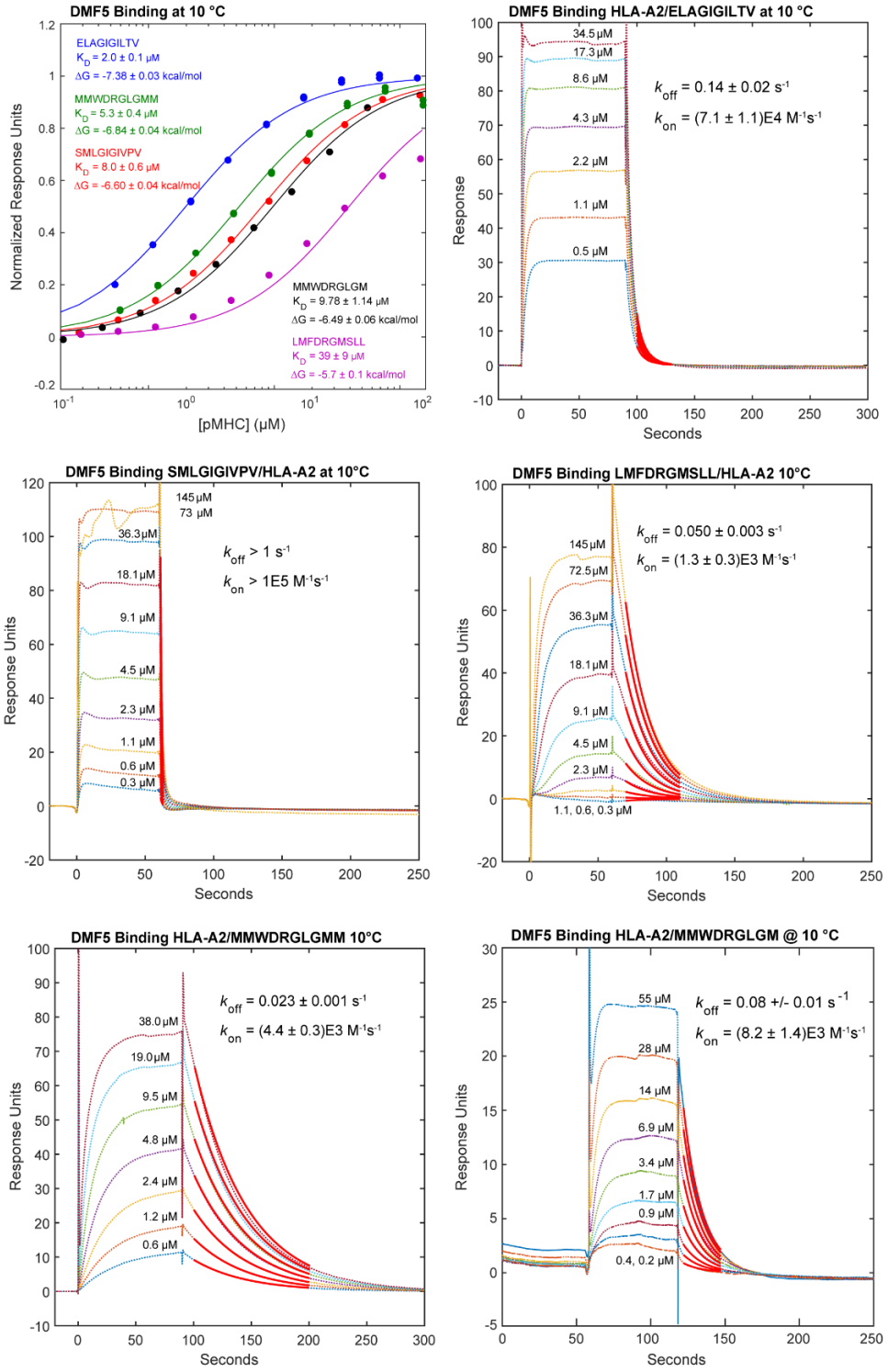

**Supplementary Figure 1A. Electron density images for the MMWDRGLGMM/HLA-A2 structure.** Density contoured at 1$\sigma$ is shown for $2F_o$-$F_c$, composite omit, and feature enhanced maps for molecule 1 (top) and molecule 2 (bottom). All three maps are shown for the peptide on the left. Only the $2F_o$-$F_c$ map is shown for the HLA-A2 $\alpha$2 helix on the right.



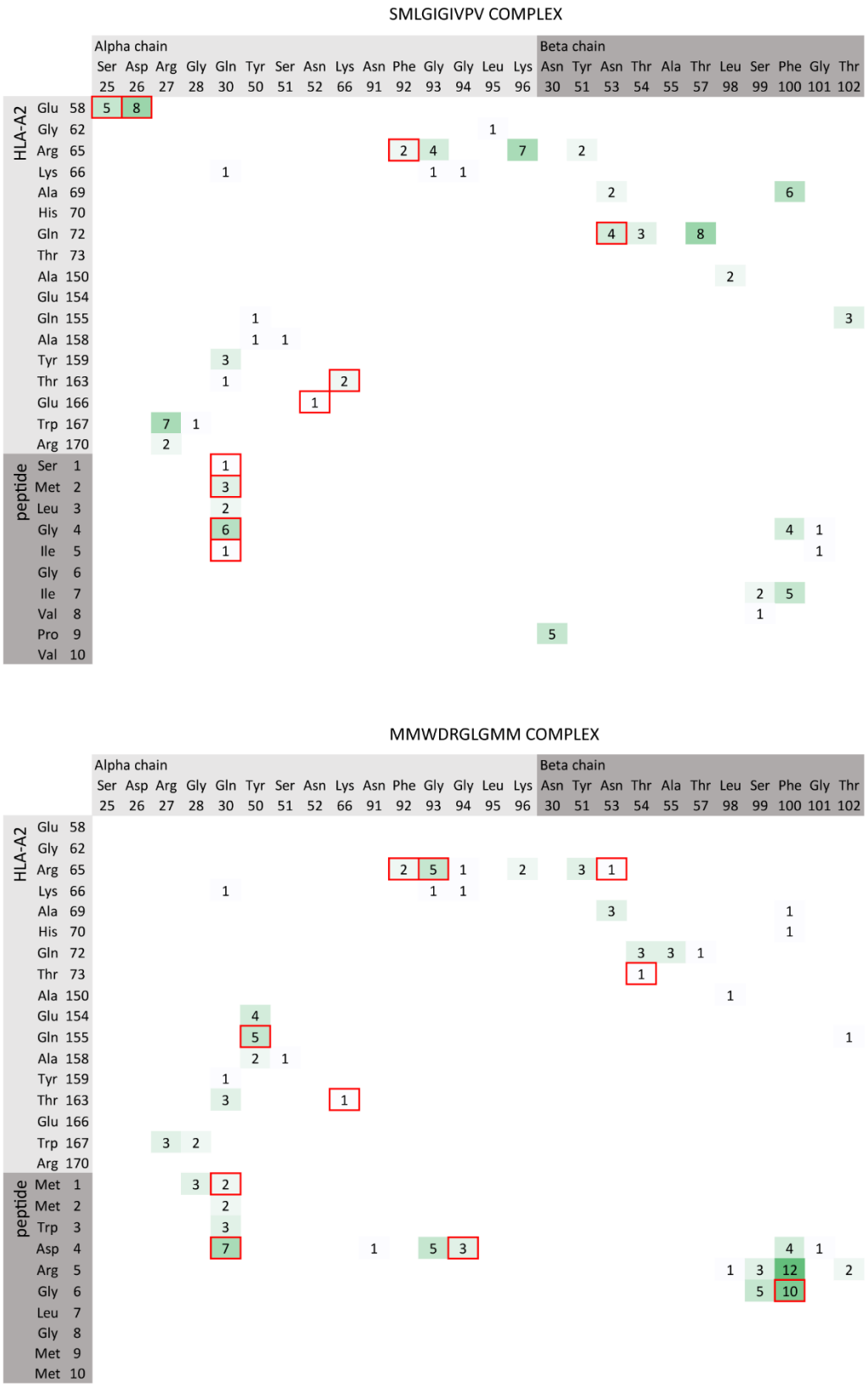

**Supplementary Figure 1B. Electron density images for the DMF5-SMLGIGIVPV/HLA-A2 structure.** Density contoured at 1σ is shown for $2F_o$-$F_c$ and composite omit maps. Both maps are shown for the peptide on the left. Only the $2F_o$-$F_c$ map is shown for the CDR loops on the right.



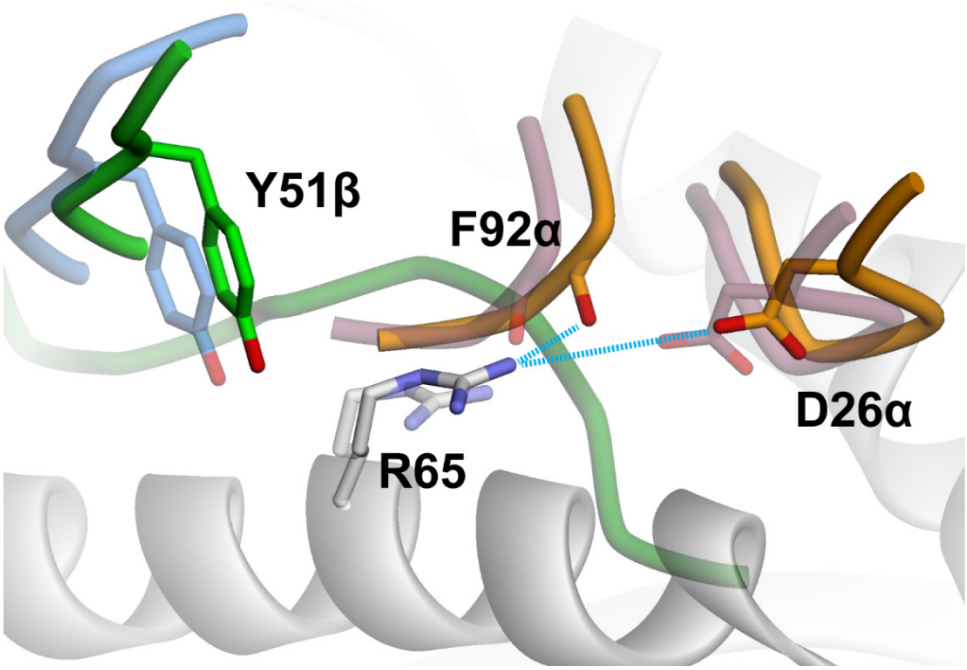

**Supplementary Figure 1C. Electron density images for the DMF5-MMWDRGLGMM/HLA-A2 structure**. Density contoured at 1$\sigma$ is shown for $2F_o$-$F_c$, composite omit, and feature enhanced maps. All maps are shown for the peptide on the left. Only the $2F_o$-$F_c$ map is shown for the CDR loops on the right.




**Supplementary Figure 2. SPR data for assessing the kinetics of DMF5 binding GIG, DRG, and the C-terminally truncated MMWDRGLGM peptides presented by HLA-A2.** The top panel shows steady state binding data used to determine $K_D$ values at 10 °C. The bottom four panels show kinetic data at 10 °C used to determine $k_{off}$ rates. Association rates were determined from $K_D$ and $k_{off}$ values ($k_{on} = K_D/k_{off}$). Errors are standard fitting errors for global analysis ($K_D$ and $k_{off}$) or propagated from errors in $K_D$ and $k_{off}$ ($k_{on}$). These experiments indicate that DMF5 binds the GIG peptides with faster kinetics than the DRG peptides, supporting the conclusion that TCR binding the DRG peptides occurs with a register shift. The truncated MMWDRGLGM nonamer is recognized with faster kinetics than the parental decamer, including a more rapid association rate.




**Supplementary Figure 3. Contact maps of the TCR-pMHC complexes with the SMLGIGIVPV (top) and MMWDRGLGMM (bottom) peptides**. Contacts are defined as interatomic distances ≤ 4 Å. Numbers indicate the number of contacts, with cells colored according to a white (min) or green (max) color scale. Elements with residues participating in interfacial hydrogen bonds are boxed in red. Amino acid numbering is adjusted from the PDB files to facilitate a direct comparison.




**Supplementary Figure 4. TCR electrostatic interactions with Arg65.** Interactions with Arg65 that help drive recognition of HLA-A2 and restrict its orientation over peptide/HLA-A2 complexes (ref. 34 in main text) are conserved in the MMWDRGLGMM and GIG interfaces. Coloring is as defined in Figure 6 in the main manuscript.

**Supplementary Video 1. Animation shown the transition of the MMWDRGLGMM/HLA-A2 complex from TCR-free to TCR-bound.** The video highlights the peptide's register shift and resulting C-terminal extension upon binding of the DMF5 TCR. The animation was generated via interpolation paired with a refinement step to minimize VDW clashes.