**Appendix e-1**

Molecular modelling of HLA class II-IgLON5 peptide interactions

This simulation protocol delivers a binding free energy score that can be used to approximately estimate the relative binding affinity of a peptide for a particular HLA allele, by building appropriate thermodynamic cycles.1

The structure of HLA-DRB1\*01:01 in complex with class II-associated invariant chain peptide (CLIP) fragment P103-P119 was obtained from the Protein Data Bank (PDB) entry 3PDO.2,3 Missing atoms in the crystallographic structure were modelled with the software Swiss-Pdb Viewer.4 The initial system configuration for the modelling of HLA-DRB1\*01:01 interactions with selected peptides included residues K2-I82 of DRA1\*01:01 and R4-Q92 of DRB1\*01:01, constituting the peptide-binding domain of the HLA molecule, and the nine CLIP residues (M107-M115) occupying positions P1 to P9 in the binding groove. The CLIP fragment was then converted to a nona-glycine peptide by removing the side chains of the nine residues. The chosen modelling approach generates the side-chain atoms corresponding to the query peptide (from the experimental set) via a molecular dynamics slow-growth technique under non-equilibrium conditions, thus transforming the nona-glycine peptide into the query peptide within the HLA-DRB1\*01:01 binding groove. Additionally, the method allows the assignment of a binding score. For each query peptide (LRLLAAAAL and IVHVPARIV) the side-chain atoms that were to be generated on the nona-glycine scaffold were defined as dummies in the biomolecular force field of the GROMOS96 simulation package.5 A dummy atom is one that has no interactions with the rest of the system. All other atoms in the system were assigned standard parameters from the 54B7 force field for vacuum boundary conditions, which includes the shielding of charge-charge interactions in a very crude way to mimic a basic solvent effect.6 The protonation state of ionisable residues was chosen to mimic a neutral pH. Following a steepest-descent energy minimization of the system in vacuum, a non-equilibrium slow-growth molecular-dynamics simulation (200 ps) at 10 K was performed. The low temperature was chosen to limit the extent of protein dynamics under the vacuum conditions. In the course of the simulation the dummy atoms were progressively transformed into regular atoms (i.e., atoms with standard force field parameters) using a soft-core potential to avoid singularities in the initial time steps, while the positions of HLA-DRB1\*01:01 backbone atoms were restrained with a harmonic potential. During the process, the growing atoms (i.e., atoms that were initially dummies) accommodate to the binding-groove environment under the sole influence of the force field. The simulation delivers the work involved in the transformation of the nona-glycine peptide into the query peptide under the specified conditions.7 To accumulate statistics on this non-equilibrium process, the simulation was repeated 1000 times with different initial velocity distributions. The free-energy change associated to the transformation was estimated from the work values by means of Jarzinsky’s equation:

Δ*G* = −*kBT* ln〈exp(−*W*/*kBT*)〉

where *kB* is Boltzmann’s constant, *T* is the absolute temperature, *W* is the work, and the angle brackets denote an average over the 1000 work values.8

To obtain a score that can be used to rank the binding of different peptides, the previous transformation (nona-glycine to query peptide) needs to be performed also for the isolated peptide in vacuum. The binding score can then be calculated as:

*Sbind* ≡ ΔΔ*Gbind* = Δ*Ggroove* – Δ*Gvacuum* ,

where Δ*Ggroove* refers to the transformation within the HLA-DRB1\*01:01 binding groove and Δ*Gvacuum* refers to the transformation of the isolated peptide. Note that, because of the conditions of the transformation, this score does not include the effects of solvation and entropy. It therefore responds only to the relative enthalpic affinity between the HLA molecule and the query peptide.

The same procedure was used to estimate the binding affinity of the two peptides for HLA-DRB1\*10:01. Since there is no crystallographic structure available for this protein, we generated a model for its binding domain using the software Modeller, with the structure of HLA-DRB1\*01:01 (PDB entry 3PDO) as template (available structure with most similar sequence).9 Peptide binding affinities were thereafter estimated with this model.

**References**

1. Muixí L, Carrascal M, Álvarez I, et al. Thyroglobulin peptides associate in vivo to HLA-DR in autoimmune thyroid glands. J Immunol 2008;181:795-807.
2. Berman HM, Westbrook J, Feng Z, et al. The Protein Data Bank. Nucleic Acids Res 2000; 28: 235-242.
3. Günther S, Schlundt A, Sticht J, et al. Bidirectional binding of invariant chain peptides to an MHC class II molecule. Proc Natl Acad Sci USA 2010;107:22219-22224.
4. Guex N, Peitsch MC. SWISS-MODEL and the Swiss-PdbViewer: An environment for comparative protein modeling. Electrophoresis 1997;18:2714-2723.
5. Scott WRP, Hünenberger PH, Tironi IG, et al. The GROMOS Biomolecular Simulation Package. J Phys Chem A 1999;103:3596-3607.
6. Schmid N, Eichenberger AP, Choutko A, et al. Definition and testing of the GROMOS force-field versions 54A7 and 54B7. Eur Biophys J 2011;40:843-856.
7. van Gunsteren WF, Daura X, Mark AE. Computation of free energy. Helv Chim Acta 2002;85:3113-3129.
8. Jarzynski C. Nonequilibrium Equality for Free Energy Differences. Phys Rev Lett 1997;78:2690-2693.
9. Šali A, Blundell TL. Comparative protein modelling by satisfaction of spatial restraints. J. Mol Biol 1993;234:779-815.