Supporting Information

Yin et al. 10.1073/pnas.1118801109

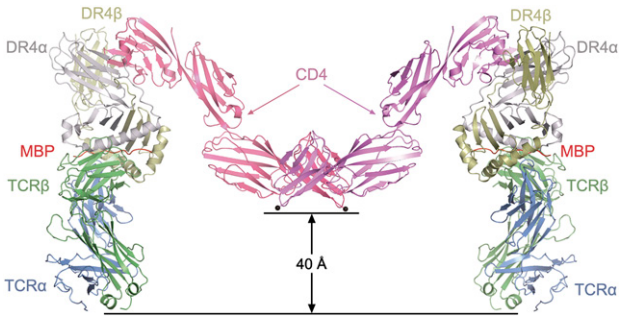

Fig. S1. Hypothetical model of a dimeric TCR–pMHC–CD4 complex based on CD4 dimerization. TCR α chain, blue; TCR β chain, green; HLA-DR4 α chain, gray; HLA-DR4 β chain, yellow; MBP peptide, red. The CD4 dimer is shown in light and dark pink, with the monomers associated through the D4 domain. The model was constructed by superposing the TCR–pMHC–CD4 structure onto the D4–D4-associated CD4 dimer observed in crystals of unbound human CD4 D1–D4 (PDB ID code 1WIO) (1). Cellular anchor points for the CD4 dimer are clearly nonplanar with anchor points for the TCRs, implying that this assembly is unlikely to be feasible on the T-cell surface.

1. Wu H, Kwong PD, Hendrickson WA (1997) Dimeric association and segmental variability in the structure of human CD4. *Nature* 387:527–530.

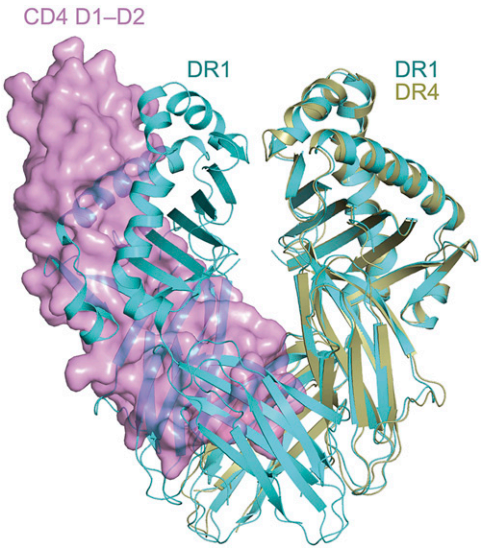

Fig. S2. CD4-binding site on HLA-DR coincides with putative HLA-DR dimerization site. CD4 D1–D2, pink; HLA-DR4, yellow; HLA-DR1 dimer, cyan. In the TCR–pMHC–CD4 structure, CD4 (surface representation) binds HLA-DR4 via its membrane-distal D1 domain at a concavity formed by the membrane-proximal α2 and β2 domains of the MHC class II molecule. In HLA-DR1 crystals, two HLA-DR1 molecules are seen to dimerize through their α2 and β2 domains (PDB ID code 1AQD) (1) at a site that overlaps the binding site for CD4.

1. Brown JH, et al. (1993) Three-dimensional structure of the human class II histocompatibility antigen HLA-DR1. *Nature* 364:33–39.

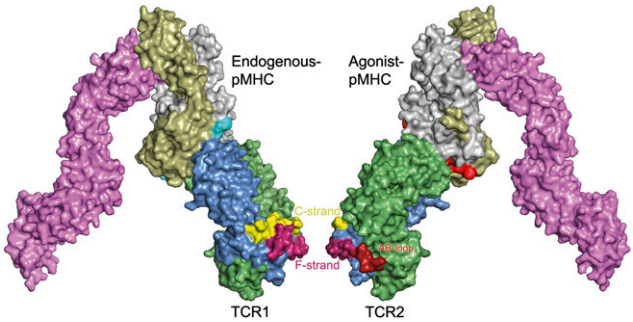

Fig. S3. Hypothetical model of a dimeric TCR–pMHC–CD4 complex based on TCR dimerization. TCR α chain, blue; TCR β chain, green; MHC α chain, gray; MHC β chain, yellow; agonist peptide, red; endogenous peptide, cyan; Cα C strand, yellow; Cα F strand, magenta; Cα AB loop, red. The TCR–pMHC–CD4 complex (TCR1) on the left was rotated ∼180° around a vertical axis to generate the complex on the right (TCR2). For clarity, the two complexes are shown pulled apart to expose the C and F strands and AB loop of Cα, which mediate TCR dimerization (1). In the original pseudodimer model of T-cell activation (2), the basic unit of T-cell activation is a heterodimer of agonist pMHC and endogenous pMHC complexes. In the modified pseudodimer model shown here, the basic unit of T-cell activation is a TCR dimer, in which one monomer engages an agonist pMHC complex and the other monomer engages an endogenous pMHC complex.

1. Kuhns MS, et al. (2010) Evidence for a functional sidedness to the alphabetaTCR. *Proc Natl Acad Sci USA* 107:5094–5099.
2. Krogsgaard M, et al. (2005) Agonist/endogenous peptide-MHC heterodimers drive T cell activation and sensitivity. *Nature* 434:238–243.

Table S1. Data collection and refinement statistics

Data collection	
Space group	$P4_32_12$
Cell dimensions	
a, *b*, *c* (Å)	146.2, 146.2, 231.4
α, β, γ (°)	90, 90, 90
Resolution limit (Å)	4.0
Unique reflections (*n*)	21,831
Completeness (%)[†]	99.9 (100)
$I/\sigma I$[†]	6.9 (2.0)
R_{merge} (%)[†,‡]	9.9 (47.6)
Refinement	
Resolution range (Å)	47.7–4.0
R_{work}/R_{free} (%/%)[¶]	23.8/30.5
Protein atoms (*n*)	9,231
R.m.s. deviations	
Bond lengths (Å)	0.005
Bond angles (°)	1.106

R.m.s., root mean square.
[†]Values in parentheses correspond to the highest resolution shell (4.18–4.0 Å).
[‡]$R_{merge} = \sum|I_j - \langle I\rangle|/\sum I_j$, where I_j is the intensity of an individual reflection, and $\langle I\rangle$ is the average intensity of that reflection.
[¶]R_{work} (R_{free}) = $\sum||F_o| - |F_c||/\sum|F_o|$; 5% of data were used for R_{free}.