Supplementary Information for:

Quantitative analysis of T cell receptor complex interaction sites using genetically encoded photo-crosslinkers

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Supplementary Figure 1. In-cell surface protein photo-crosslinking. Cells expressing TCRs with pAzpa incorporated were crosslinked under 365 nm UV light for 0 min, 15 min, 30 min, 45 min, 60 min or 90 min. Cells were lysed and TCR-CD3 complexes were immunoprecipitated with biotin-labeled anti-CD3ε and probed with anti-V5 antibody (to detect TCRβ) using Western blot. Lower bands between 37-50 KD represent non-crosslinked TCRβ chain and upper bands between 75-100 KD represent crosslinked TCRα and β chains.
| Distance Calculation | Mutation Sites on α chain | Control |
|----------------------|---------------------------|---------|
|                      | R39 | G40 | S41 | K65 |
| Clashes /Contacts    | 2.62 | 3.92 | 3.68 | -   |
| Center of Mass       | 7.63 | 5.56 | 4.78 | 22.26 |
| pAzpa                | 9.94 | 8.95 | 6.81 | 22.30 |
| pBpa                 | 8.17 | 8.46 | 8.86 | 27.98 |

**Supplementary Table 1. Distance of mutation sites on TCRα chain to β chain.** The types of distance measurements are given on the left: “Clashes/Contacts” is measured using the Find Clashes/Contacts feature of USCF Chimera; “Center of Mass” is measured using Molsoft ICM, and distance is measured from center of mass of the side chain to any backbone atom on the β chain; “pAzpa” and “pBpa” is calculated the same way as “Center of Mass”, except the atoms of the β chain are restricted to those of residues preferred by each crosslinking agent (see Supplemental Methods for details).
| Residue | Atom | Distance |
|---------|------|----------|
| Arg 9   | cb   | 9.0942   |
| Arg 9   | o    | 9.57274  |
| Tyr 10  | ce2  | 9.16581  |
| Tyr 10  | cd1  | 9.50254  |
| Leu 89  | cd1  | 9.33059  |
| Leu 89  | cg   | 9.91837  |
| Gly 106 | o    | 9.39234  |
| Gly 106 | c    | 9.59373  |
| Pro 107 | cd   | 9.41275  |
| Gly 108 | n    | 9.17931  |
| Gly 108 | o    | 9.54225  |
| Arg 110 | cg   | 9.0661   |
| Val 155 | c    | 9.83968  |
| Glu 156 | n    | 9.92877  |
| Glu 156 | c    | 9.93313  |
| Leu 157 | o    | 9.73332  |
| Leu 157 | n    | 9.85661  |
| His 167 | ce1  | 9.99008  |

Supplementary Table 2. Atoms on the TCRβ chain in a radius of 9-10 Å from selected residues on the TCR α chain. For Arg39, Gly40 and Ser41 on the α chain, distance was measured from the CB atom (CA in case of Gly40), and all atom types on the TCR β chain within this distance were included. Distances were calculated using Molsoft ICM (see Supplemental Methods).
Supplementary Methods
Distance Calculation
The 2B4 TCR structure (PDB 3QJF) was used for structural analysis and modeling. We used the Find Clashes/Contacts feature of USCF Chimera¹ to find the sites on the TCRα chain that are in close contact with the TCRβ chain, as given in Supplemental Table 1. Distances for “Center of mass”, “pAzpa”, and “pBpa” entries in Supplemental Table 1 were calculated using Molsoft ICM². In the center of mass calculations, side chains were defined by excluding the C, CA, N and O backbone atoms of each residue, except in the case of Gly40, where CA alone was taken as the side chain. Distances to all main chain atoms (C, CA, N, and O) on the TCRβ chain were measured and the minimum distance taken. For the UAA-preferred measurements, side chains on the TCRα chain were defined in the same way, but measurements were made only to main chain atoms of preferred amino acids of the TCRβ chain: for pAzpa Cys, His, Lys, Phe, Ser, Thr, Trp and Tyr; for pBpa Arg, Leu, Lys, Met, Val. For the calculations of the 9-10Å radius, the distances were measured in ICM, from the CB atom of each selected residue, except for Gly40, where the CA was used. All atoms in the TCRβ chain in this distance window were taken.

Reference
1. Pettersen, E. F., Goddard, T. D., Huang, C. C., Couch, G. S., Greenblatt, D. M., Meng, E. C., and Ferrin, T. E. (2004) UCSF chimera - A visualization system for exploratory research and analysis, J. Comput. Chem. 25, 1605-1612.
2. Abagyan, R., Totrov, M., and Kuznetsov, D. (1994) Icm - a New Method for Protein Modeling and Design - Applications to Docking and Structure Prediction from the Distorted Native Conformation, J. Comput. Chem. 15, 488-506.