

## **Altered peptide ligands induce delayed and reduced CD8:CD3 $\zeta$ interaction – a role for CD8 in distinguishing ligand quality**

**Pia Yachi**, Jeanette Ampudia, Tomasz Zal and Nicholas R.J. Gascoigne  
Department of Immunology, IMM1, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037, USA

Altered peptide ligands (APL) for the TCR have different effects on T cell activation and thymocyte development. We imaged fluorescence resonance energy transfer (FRET) between CD3 $\zeta$  and CD8 fused to cyan and yellow fluorescent protein (CFP, YFP), respectively, to investigate TCR-CD8 interactions during T cell activation in a system where APLs with different activation (agonist/antagonist) and thymocyte selection (negative/positive) phenotypes have been characterized, and their solution-binding kinetics determined. FRET between CFP and YFP can be used to investigate molecular proximity between two proteins when they are less than 10 nm apart. Recognition of agonist MHCp complexes triggers intermolecular interaction between CD8 and TCR, detectable across the T cell-APC contact area. We find that recruitment of TCR and CD8 to the synapse, and induction of FRET between these molecules is delayed with weak agonist, but that the interaction becomes strong with time. Antagonists have delayed TCR recruitment and very late, low intensity FRET. There is a close correlation of TCR recruitment and TCR-coreceptor interaction with the biological response, which accounts for anomalies in the correlation between TCR solution binding kinetics and the biological response.