

# Production of MHC Class I Tetramers

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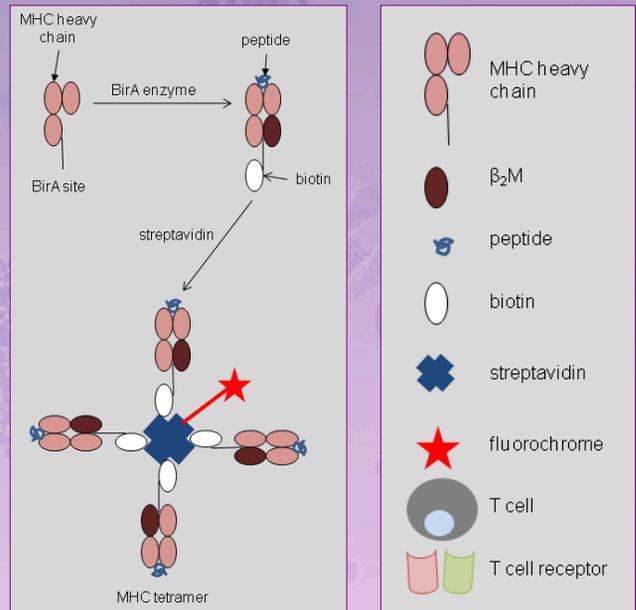
**Major histocompatibility complex (MHC) tetramers** were first described in the mid-1990s, and since then have been an invaluable tool to all T-cell immunologists. Identifying antigen-specific T cells is inherently difficult due to the low avidity of the interaction between T cell receptor (TCR) and MHC:peptide complex. The interaction has a half life of a mere 10 seconds!

The production of MHC class I tetramers is shown in **Figure 1**. First MHC heavy chains expressing a bacterial BirA-recognition site are synthesised. The MHC heavy chain is then folded with  $\beta_2$ -microglobulin ( $\beta_2$ M; the MHC light chain) and synthetic peptide. The enzyme **BirA** is then added to biotinylate the complex, adding a biotin molecule to each MHC monomer that is formed. In the presence of **streptavidin** which has four biotin binding sites per molecule, four MHC monomers are joined together to form a tetramer.

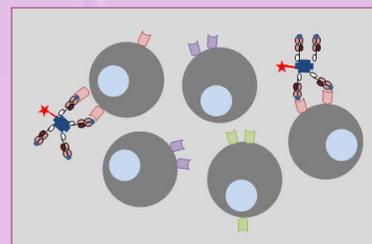
Whilst single MHC:peptide complexes and TCR bind weakly, MHC tetramers can bind up to four TCRs simultaneously, creating a much stronger interaction. MHC tetramers only bind to the relevant TCR (**Figure 2**), and tetramers specific for any MHC heavy chain (e.g. HLA A2, HLA B7) can be produced, coupled with any peptide epitope of choice (e.g. viral, bacterial, tumour and autoimmune antigens).

The streptavidin molecule is routinely linked to a **fluorochrome** such as **phycoerythrin**, to allow

for direct staining for use with flow cytometry. An example of tetramer staining is shown in **Figure 3**; here peripheral blood mononuclear cells (PBMCs) have been stained with an antibody specific for CD8 and also a tetramer of HLA-A2 molecules containing a cytomegalovirus (CMV) peptide. It can be seen that 1.3% of CD8+ cells are tetramer positive. Antigen-specific T cells as low as 0.05% of all T cells can be measured.

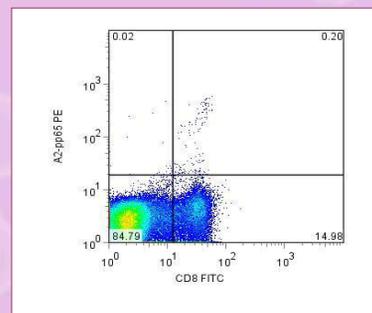


**Figure 1.** MHC tetramer production. Diagram showing how MHC tetramers are produced. The right panel shows a key for symbols used in all figures.



**Figure 2.**

MHC tetramers only bind TCRs specific for the HLA-peptide in the MHC-tetramer. Any irrelevant TCRs are left unbound.



**Figure 3.**

Flow cytometry analysis of a patient who has T cells specific for an A2 CMV epitope on the pp65 virus protein.

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