**Single-molecule and super-resolution investigation of chemokine receptor behaviour within the T cell immunological synapse**

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The immunological synapse (IS) serves as a highly dynamic yet tightly organised platform for the transfer of information between T cells and a number of different antigen-presenting cell (APC) types. It is required for the correct activation of T cells in response to infection, as well as the prevention of autoimmunity, and licensing of APCs for subsequent anti-microbial processes. Chemokine receptors are G-protein coupled receptors that recognise inflammatory and homeostatic chemokines, mediating leukocyte migration towards sites of infection or within specialised tissues of the immune system. In recent years it has become apparent that a number of chemokine receptors, particularly CXCR4, also contribute to the stability and longevity of the IS, and do so through signalling processes distinct from those involved in cell migration. Nonetheless, the spatiotemporal organisation of this behaviour within the complex architecture of the IS is unstudied, and hence its integration with the known signalling events of T cell activation is not understood. Here we use total-internal reflection fluorescence (TIRF) microscopy to examine CXCR4 and other chemokine receptors within primary human $CD4^+$ T cells during the process of IS formation on supported lipid bilayers. Single-molecule tracking demonstrated substantial changes in receptor dynamics over the lifetime of the synapse, both in terms of spatial location and nature of diffusion. Combining TIRF with super-resolution structured illumination microscopy (SIM) also revealed the segregation of some chemokine receptors from other signalling domains, most significantly those of the T cell receptor. These observations shed light on the mechanisms by which T cell activation is influenced by chemokine receptors, and hence how such processes are regulated during a coordinated immune response.