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Title: Optogenetic control of TCR activation

Abstract: T cell development, differentiation as well as activation are crucial events in adaptive immune responses and depend on interactions between T cell receptors (TCRs) and self or foreign peptides bound to major histocompatibility complexes (pMHCs) on antigen-presenting cells (APCs). The duration of the TCR-pMHC interaction is thought to play a pivotal role in determining the outcome of this stimulation event. IN fact, it has been proposed that T cells discriminate between self and non-self peptides via the ligand's different binding kinetics, called kinetic proof-reading. However, due to experimental limitations, nothing is known about the detailed temporal prerequisites deciding the T cell's fate.

Here, we constructed a synthetic TCR utilising optogenetic tools to precisely manipulate TCR-ligand interaction times by light illumination. We fused an *Arabidopsis* phytochrome B (PhyB)-interacting factor (PIF) to the extracellular part of the TCR. Light of red or far-red wavelengths is then used to precisely control the interaction time and intervals of the synthetic TCR with PhyB tetramers as the ligand.

We used a so far not employed property of Phytochrome B in optogenetics, namely that each individual molecule cycles between the binding and non-binding state at 660 nm light, the cycling rate being determined by the intensity of the light. Quantifying the calcium response as well as the amount of tetramer bound to the cells, we remarkably observed that ligand binding half-life plays the dominant role in T cell activation, largely independent of receptor occupancy. Thus, this is the first direct proof that T cells can measure the duration of the TCR-ligand interaction and respond accordingly.

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