Rewiring innate immune pathways by synthetic biology tools for the development of novel biosensors of microbial activity

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Inflammasomes are intracellular multiprotein complexes that form in response to various pathogenic and endogenous danger signals and execute pyroptosis, an inflammatory form of programmed cell death. CARD8 is a human sensor that upon activation with HIV protease or DPP8/9 inhibitors assembles an inflammasome through direct recruitment and activation of pro-caspase-1. Active caspase-1 activates proinflammatory cytokines and gasdermin D, which assembles pores in cellular membranes, leading to pyroptosis. CARD8 is composed of a disordered N-terminal tail, ZU5-UPA, and CARD domains. Upon cleavage of the N-terminus by HIV protease, the N-terminus becomes destabilized and undergoes proteasome degradation, while the UPA-CARD fragment remains intact and forms filaments that facilitate the recruitment and activation of pro-caspase-1. We aimed to develop a viral detection biosensor by reformatting CARD8 to act as a biosensor of microbial activity independently of its function as a component of the inflammasome. We directly fused the CARD8 molecule with an enzymatic component (i.e. split luciferase system) that becomes functional only upon UPA-CARD filament formation, which leads to a restoration of enzyme activity that can be used as a readout. We first demonstrated the functionality of the system using HIV protease and through the incorporation of other viral protease cleavage sites illustrated the high adaptability of CARD8-based biosensor for the detection of other viral proteases. Unlike methods that rely on detecting a specific nucleic acid sequence and can thus fail to detect mutated viruses, our biosensor detects the activity of pathogen proteases, which are crucial for virus propagation. Such biosensors have the potential to be used for diagnostics and also for the development of antiviral drugs targeting viral proteases.