

Determining the role of TIR domain of Interleukin-1 receptor 8 (SIGIRR) in regulating TLR4 signalling

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The TIR superfamily includes membrane receptors, Interleukin-1 receptors (IL-1Rs) and Toll-like receptors (TLRs) and also TIR-containing cytoplasmic adaptor proteins such as MAL and MyD88. These proteins play a major role in immune signalling and are vital to innate host defense, inflammation, injury and stress [1]. IL-1R8, also known as single immunoglobulin interleukin-1 receptor-related protein (SIGIRR) is an inhibitory receptor from IL-1R family which regulates signalling of both IL-1Rs and TLRs. The mechanism of inhibition is not yet known, but the only available genetic evidence suggests that the conserved intracellular TIR domain of IL-1R8 alone is necessary to inhibit LPS-induced TLR4 signalling [2]. The recent cryo-EM structure of the MAL protofilament has revealed the molecular mechanism of TIR-TIR interactions in the MAL and MyD88 dependent TLR4 signalling [3]. Based on this, we hypothesize that a similar TIR:TIR interaction between the TIR domain of IL-1R8 and the TIR domains of either TLR4/MAL/MyD88 would be involved in the inhibition mechanism.

The TIR domain of human IL-1R8 was cloned, expressed and purified using *E. coli* host system. Turbidity assays, negative-stain electron microscopy (EM) and single-molecule fluorescence spectroscopy (SMFS) analysis indicated a potential interaction between IL-1R8^{TIR} and MAL^{TIR}. MAL^{TIR} forms filamentous assemblies when incubated with IL-1R8^{TIR} (Fig. 1). We are currently focusing on solving the 3D structure of MAL^{TIR}/IL-1R8^{TIR} filaments using negative-stain EM and cryo-EM to obtain molecular insights into the interaction interfaces and binding sites of IL-1R8^{TIR} and MAL^{TIR}. This study will eventually lead to an understanding of how TLR4 signalling is regulated by IL-1R8 and can potentially pave way in development of new therapeutic agents in future.

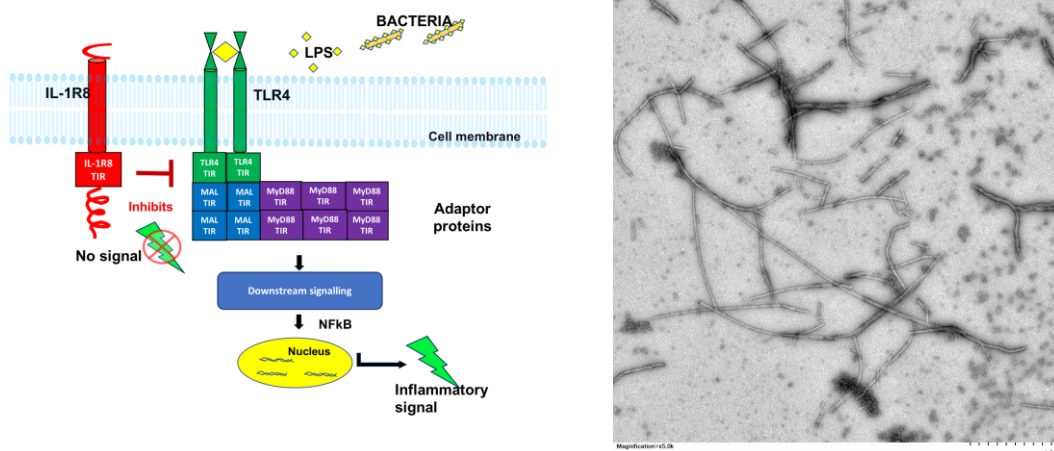


Figure 1. Left: Model representing the inhibition of TLR4 signalling by IL-1R8. Right: Negative-stain EM image of MAL^{TIR}/IL-1R8^{TIR} filaments taken using Hitachi HT 7700 TEM.

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Keywords: Interleukin-1 receptor 8; TLR4 signalling; MAL/TIRAP; TIR domain; cryo-EM