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TLR4 TIR domain higher-order assemblies reveal the structural basis of adaptor recruitment in Toll-like receptor signaling pathways

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Toll-like receptors (TLRs) are pattern recognition receptors that initiate immune responses and the production of pro-inflammatory cytokines in response to pathogen- or endogenous danger-associated molecules (PAMPs/DAMPs), such as microbial lipids and nucleic acids. TLRs serve as a first line of defence against various foreign agents; however, aberrant TLR signaling is linked to autoimmune and chronic inflammatory diseases. TLR signaling occurs through interactions between the TLR Toll/interleukin-1 receptor (TIR) domain and TIR domains of adaptor proteins, leading to activation of the transcription factors NF-κB and interferon regulatory factors [1]. Recognition of PAMPs/DAMPs by the TLR extracellular domain is thought to induce dimerisation of the cytosolic TIR domain, leading to recruitment of intracellular TIR domain-containing adaptor proteins such as MAL. The TIR domain of MAL (MAL^{TIR}) has previously been shown to self-assemble into filaments in vitro, with each proto-filament containing two parallel strands of TIR domains arranged head-to-tail [2].

The TLR4 TIR domain ($TLR4^{TIR}$) nucleates the formation of $TLR4^{TIR}$:MAL^{TIR} filaments. To elucidate the structural basis of TLR signaling and adaptor protein recruitment, we determined the structure of $TLR4^{TIR}$:MAL^{TIR} filaments and characterised the interactions between the receptor and adaptor TIR domains. Cryo-electron microscopy of $TLR4^{TIR}$:MAL^{TIR} filaments revealed two filament morphologies, with reconstructions resolved to resolutions of 2.9 Å and 3.4 Å. The $TLR4^{TIR}$:MAL^{TIR} filaments showed that MAL^{TIR} subunits form two-stranded “proto-filaments”, similar to those observed in MAL^{TIR} self-assemblies, with a single strand of $TLR4^{TIR}$ subunits found between the MAL^{TIR} proto-filaments. The structures suggest a lateral recruitment of adaptor TIR domains, which is contrary to previously proposed models. Subsequent mutagenesis of assembly interfaces and cell signaling assays demonstrated that the interactions within $TLR4^{TIR}$:MAL^{TIR} filaments reflect those of biological assemblies. As such, we present not only the first structure of the TLR4 TIR domain, but also the first structural evidence of TIR:TIR interactions between a TLR and its downstream signaling adaptor. In conclusion, our studies provide new insights on the structural basis of TLR and adaptor TIR domain interactions and the formation of TIR domain signalosomes in TLR signaling pathways. This will ultimately provide a greater understanding of the molecular mechanisms of immune system signaling and inflammatory disease.

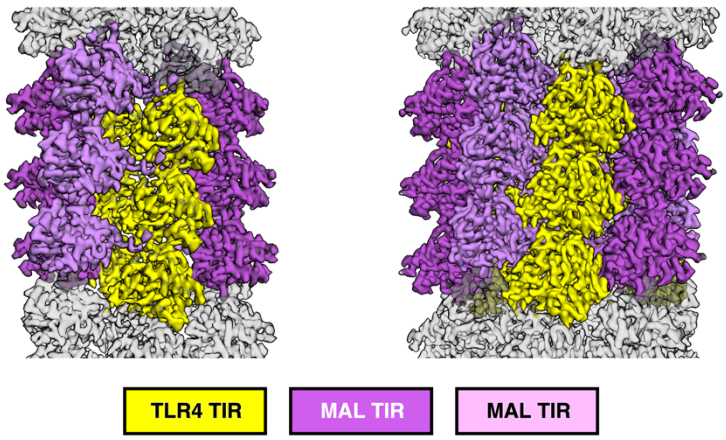

Figure 1. Cryo-EM reconstructions of $TLR4^{TIR}$:MAL^{TIR} filaments. Left: A six stranded morphology resolved to 3.3 Å resolution. Right: A nine stranded morphology resolved to 2.9 Å resolution.

[1] Nanson, J. D., Kobe, B., & Ve, T. (2019). Death, TIR, and RHIM: Self-assembling domains involved in innate immunity and cell-death signaling. J. Leukoc. Biol., 105(2), 363-375.

[2] Ve, T., et al. (2017). Structural basis of TIR-domain-assembly formation in MAL-and MyD88-dependent TLR4 signaling. Nat. Struct. Mol. Biol., 24(9), 743.