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# Structural evolution of TIR-domain signalosomes in mammalian, plant and bacterial immunity characterized by integrative structural biology

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TIR (Toll/interleukin-1 receptor) domains are widely distributed in animals, plants and bacteria, and function through self-association and homotypic interactions with other TIR domains [1]. Across phyla, these domains feature in proteins with immune functions - TLRs (Toll-like receptors), IL-1Rs (interleukin-1 receptors) and their adaptor proteins in animals; NLRs (nucleotide-binding, leucine-rich repeat receptors) in plants; and antiphage defence proteins in bacteria. Although long assumed to only have protein interaction functions, the TIR domains across kingdoms also feature self-association-dependent enzymatic activities, namely cleavage of nucleotides such as $NAD^+$ [2,3]. We used an integrated structural biology approach to characterize the signalosomes formed by different TIR domains. We reconstituted large assemblies of the TLR/adaptor TIR domains (not known to have enzymatic activities); the structures of the filamentous assemblies of the TIR domains of TLR adaptor MAL [4], TRAM and the TLR4:MAL complex (unpublished) were determined by cryo-electron microscopy (cryoEM) helical reconstruction, and the structures of crystalline arrays of MyD88 were determined by micro-electron diffraction and serial femtosecond crystallography [5]. We further stabilized the active assemblies of enzyme TIR domains from the mammalian protein SARM1 (involved in axon degeneration; octameric complexes) [6] and the bacterial protein AbTir from *Acinetobacter baumanii* [3] (filamentous assemblies) with $NAD^+$ mimics and determined their structures using single-particle cryoEM and helical reconstruction, respectively. We found that all these TIR domain assemblies feature a head-to-tail arrangement of TIR molecules, with the enzyme active site located in the interface between two molecules, explaining the requirement for self-association in enzyme activity. However, such head-to-tail row of molecules in stabilized by another row associating in an antiparallel fashion in enzyme assemblies such as those from SARM1 and plant NLRs, and in a parallel fashion in both scaffold assemblies (in TLR adaptors) and bacterial enzyme assemblies (Fig. 1). In all cases, we validated the observed interactions by structure-guided mutagenesis and functional assays (e.g. [7]). The products of enzymatic reactions have downstream signalling functions in immune pathways or their suppression. Our studies will form the foundation of applications ranging from the treatment of inflammatory disorders and bacterial infections in humans to the prevention of plant diseases.

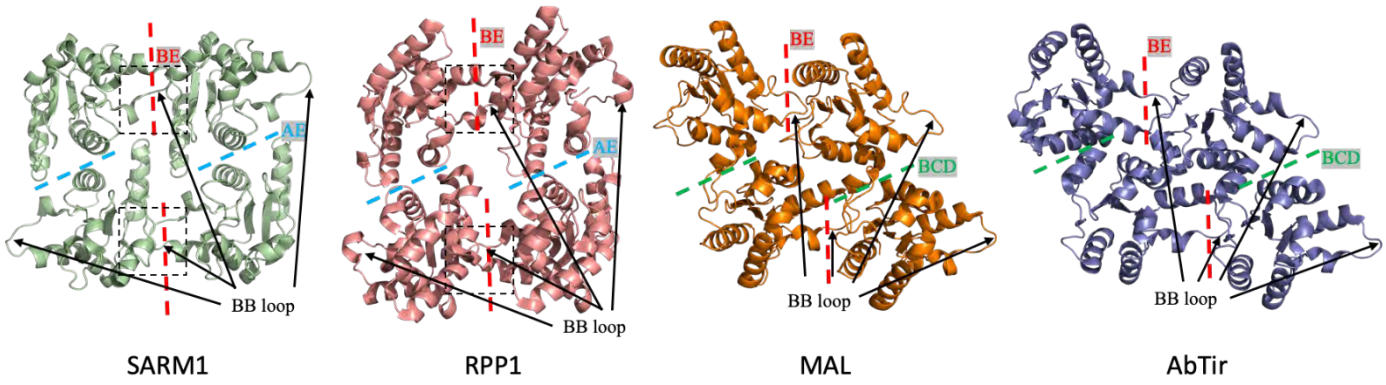


**Figure 1**. Structures of filamentous TIR-domain assemblies (four TIR domains are shown in each case, but the assemblies are open-ended from left to right of the page).

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