Towards the structure of the TIR-domain signalosome

Surekha Nimma[1‡], Thomas Ve[1,2‡], Simon J. Williams[1,3‡] and Bostjan Kobe[1*]

Addresses:

[1]School of Chemistry and Molecular Biosciences, Institute for Molecular Bioscience and Australian Infectious Diseases Research Centre, University of Queensland, Brisbane, QLD 4072, Australia.

[2]Institute for Glycomics, Griffith University, Southport, QLD 4222, Australia

[3]Research School of Biology, The Australian National University, Canberra, ACT 2601, Australia

*Corresponding author: Kobe, Bostjan (b.kobe@uq.edu.au)

‡These authors contributed equally to this work

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SUMMARY

TIR (Toll/interleukin-1 receptor/resistance protein) domains feature in animal, plant and bacterial proteins involved in innate immunity pathways and associated processes. They function through protein:protein interactions, in particular self-association and homotypic association with other TIR domains. Structures of TIR domains from all phyla have been determined, but common association modes have only emerged for plant and bacterial TIR domains, and not for mammalian TIR domains. Numerous attempts involving hybrid approaches, which have combined structural, computational, mutagenesis and biophysical data, have failed to converge onto common models of how these domains associate and function. We propose that the available data can be reconciled in the context of higher-order assembly formation, and that TIR domains function through signaling by cooperative assembly formation (SCAF).

INTRODUCTION

The TIR (Toll/interleukin-1 receptor (IL-1R)/resistance protein) domain was first defined after detecting sequence similarities between the intracellular regions of the mammalian IL-1R and the *Drosophila* protein Toll [1]. TIR domains typically function as protein interaction modules, and are mostly found in multi-domain proteins involved in innate immunity pathways in animals and plants, despite the proposed independent evolutionary origins for these pathways [2]. TIR domains also appear in many bacterial proteins, at least some of which are used by pathogenic bacteria to evade the host immune responses [3].

In mammals, TIR domains are found in Toll-like receptors (TLRs) and IL-1Rs as their cytosolic segments, as well as in the cytosolic adaptor proteins involved in signaling downstream from these receptors. TLRs (10 family members in humans: TLR1-10) are pattern-recognition receptors (PRRs) that defend against microbial infection and endogenous danger, by interacting with conserved pathogen- and danger-associated molecular patterns (PAMPs/DAMPs) [4]. These interactions lead to the TLR-selective recruitment of the TIR domain-containing adaptor proteins MyD88, MAL (TIRAP), TRIF (TICAM-1) and TRAM (TICAM-2) via TIR:TIR domain interactions [5]; these interactions trigger downstream activation of transcription factors such as NF-κB, AP-1 and IRFs to induce anti-pathogen signaling and inflammation [6]. An atypical TLR adaptor is SARM, which acts as a negative regulator of TRIF signaling [7], but also functions in neuronal axon degeneration [8,9] and cell-death pathways [10]. BCAP (B-cell adaptor for PI3K) has recently been proposed to be the sixth TIR domain-containing TLR adaptor [11,12]. IL-1Rs (10 family members found in humans: IL-1RI, IL-1RII, IL-1RaCP, ST-2, IL-1Rrp, IL-1Rrp2, IL-1RAcPL, IL-1RAPL, IL-1RAPL2 and SIGIRR) associate with proinflammatory cytokines, and like some of their TLR cousins, signal by recruiting the TIR domain-containing adaptor MyD88 [13].

In plants, TIR domains are found as the N terminal segments of a major subclass of cytoplasmic nucleotide-binding (NB)/leucine-rich repeat (LRR) resistance (R) proteins. NB-LRR proteins are typically referred to as plant NLRs due to their similarity to mammalian nucleotide-

55 binding oligomerization domain (NOD)-like receptors [14]. Plant NLRs directly or indirectly
56 recognize "effector" proteins introduced into the plant cell by plant pathogens during the invasion
57 of the plant. Effector detection by plant NLRs triggers defense responses, known as the
58 hypersensitive response, that often include localized cell death at the site of infection [15]. The TIR
59 domains are considered to be the signaling domains in plant NLRs, because they can cause cell
60 death autonomously when expressed ectopically *in planta* [16-18]. TIR-only (TIR-X) and TIR-NB
61 (TIR-N) proteins are also found in plants [19], and while their general functions are to date
62 unknown, a number of these proteins have been shown to induce cell death when transiently
63 expressed in tobacco and provide enhanced resistance when overexpressed in stable transgenics in
64 Arabidopsis [20].

65 TIR domains are also found in proteins from a wide range of bacterial species, where they
66 exist in combination with different types of domains [3]. Although the functions of most of these
67 proteins are unknown, some proteins such as TcpB from *Brucella melitensis* and TcpC from
68 uropathogenic *Escherichia coli* CFT073 suppress TLR signaling, possibly through interacting with
69 the host TIR domain-containing proteins [21].

70 In all these different organisms, TIR domains are thought to function through self-
71 association and homotypic association with other TIR domains. However, they can also engage in
72 heterotypic interactions with proteins not containing TIR domains (e.g. the vaccinia virus protein
73 A46 can bind MyD88, MAL, TRIF, TRAM and TLR4 [22]), and in intramolecular fashion with
74 other domains in TIR domain-containing proteins [14] (e.g. with both the NB and LRR domains in
75 the plant NLR RPP1 [23], and with an N-terminal helix in the bacterial protein TcpB [24]).

76 Currently, 32 structures corresponding to 16 different TIR domains from animals, plants and
77 bacteria have been deposited in the Protein Data Bank [25]. Structurally, TIR domains comprise
78 125-200 residues and contain a central parallel β-sheet surrounded by α-helices [25,26]. The
79 elements of secondary structure are usually referred to sequentially; for example the BB loop
80 connects strand βB with helix αB. Some of these structural elements correspond to conserved

sequence motifs called box 1–3 in mammalian TIR domains [25]. While the wealth of structural information has improved our understanding of TIR-domain function in individual systems, it is widely assumed that TIR-domain functions in different systems do not converge on a common mechanism of action. To date, no common self-association interfaces have been observed in the crystal structures of animal TIR domains, and numerous studies combining structural knowledge of TIR domains with computational docking, site-directed mutagenesis and other methods have proposed models that are different from each other [27-48]. By contrast, some common association modes are emerging for plant and bacterial TIR domains. Here, we review the key studies attempting to define the structural basis of TIR-domain function and suggest that both in plant and mammalian innate immunity pathways, it could be explained in the context of signaling by cooperative assembly formation (SCAF) (Box 1).

2. Self-association and homotypic association of TIR domains in mammalian proteins

TLR and IL-1R-dependent signal transduction is initiated by self-association of their intracellular TIR domains (hereafter denoted with superscript "TIR") upon binding of PAMPs (TLRs) or cytokines (IL-1Rs). The TLR^{TIR} dimer then acts as a scaffold to recruit downstream adaptor proteins through TIR:TIR domain interactions. The highly conserved BB-loop in TLR/IL-1R and adaptor TIR domains plays an important role in signaling. In TLR4, the BB loop is the site of a naturally occurring mutation P712H [49], which renders it non-responsive to the PAMP lipopolysaccharide (LPS). This mutation also abolishes signaling when introduced into other receptor or adaptor TIR domains.

MyD88 also contains a death domain (DD) that interacts with IRAKs (IL-1R-associated kinases) through DD:DD interactions, forming the oligomeric myddosome, consisting of six MyD88, four IRAK4 and four IRAK2 DDs [50] (Box 1c). Forced dimerization of $MyD88^{TIR}$ constitutively initiates signaling [51], suggesting that upon TLR activation, the TLR, MAL and MyD88 form an oligomeric platform through TIR:TIR domain interactions, which in turn promotes

the assembly of the myddosome via DD:DD interactions. In comparison to MyD88 signaling, less is known about TRIF signaling, but live-cell imaging and confocal immunofluorescence analyses have shown that TRIF alters its distribution profile from a diffuse cytoplasmic to a speckle-like structure in response to TLR3 interaction with dsRNA [52], suggesting the formation of TIR domain-dependent oligomeric TRIF complexes.

Crystal structures have been determined for the TIR domains of human TLR1, TLR2, TLR6, TLR10, IL-1RAPL, MAL, MyD88 [26,30,37,40-43,53] and Toll-related receptor TRR-2 from the lower metazoan *Hydra magnipapillata* (PDB ID 4W8G, 4W8H). NMR structures have also been determined for $MyD88^{TIR}$, $TRAM^{TIR}$ and $TRIF^{TIR}$ [32,36]. Attempts to form stable TIR-domain complexes have been unsuccessful, suggesting that weak interactions are a general feature of the mammalian TIR-domain complexes, and that membrane localization or the context of a large assembly stabilizes the interactions. Crystal contacts can reflect biological interactions [54]; analyses of crystal structures and combinations of computational modeling and docking studies, NMR and site-directed mutagenesis have led to several models of TIR domain assembly and although they are all different from each other [27-48], some common trends in the proposed TIR:TIR domain interaction modes are emerging (Figure 1, Table S1).

The BCD interface. Several of the crystal structures (TLR1, TLR2, TLR6, IL-RAPL, MAL and TRR-2) contain an interface involving the αC helices and either the αB/BB-loops or the αD regions, or both (the BCD interface) (Figure 1). In the $TLR1^{TIR}$, $TLR2^{TIR}$ and $TLR6^{TIR}$ structures, symmetric αC:αC helix interactions are found at the core of this interface, flanked on both sides by interactions between the BB-loop/αB region on one molecule and the DD-loop/αD region on the second molecule [26,43]. It has been questioned whether this interface is physiologically relevant, because in both TLR1 and TLR6, it is stabilized by a disulfide bond (between the C707 residues in TLR1 and the equivalent C712 residues in TLR6). However, a similar interface involving the same secondary structure elements is also observed in the IL-1RAPL crystal structure [30]. In the $TLR10^{TIR}$ dimer [40], one of the molecules has been rotated 90° compared to the $TLR1^{TIR}$, $TLR2^{TIR}$

and TLR6^{TIR} dimers, resulting in the two BB loops of TLR10^{TIR} interacting directly with each other. Many loss-of-function mutations in TLR4 localize to the surface regions involved in this interface and the TLR10^{TIR} homodimer has therefore been widely accepted as representative of TLR4^{TIR} dimerization following LPS recognition [27-29,31,36].

Crystal-contact analysis of the MAL structures revealed a symmetric interface comprising the αC and αD regions. Mutations of residues in this interface disrupt both MAL and MyD88 binding [37,41]. In one of the crystal forms of TRR-2^{TIR} (PDB ID 4W8G), one of the molecules has been rotated 180° compared to the MAL dimer, and the interface consists of the αC and αD helices of one molecule and the αC and αB helices of the second molecule. Although significant differences are observed between the interfaces described here, they are all centered around the αC helix and involve similar faces of the TIR domain. Furthermore, docking of TRAM^{TIR} NMR structures, using data based on mutagenesis coupled with yeast-two-hybrid (Y2H) assays as restraints, suggested that TRAM^{TIR} can self-associate using a similar configuration to the TLR10^{TIR} dimer [36], while MyD88^{TIR} can self-associate via a MAL^{TIR}-like dimer interface [35].

The BE interface. The MyD88^{TIR} crystal structure and the two different crystals forms of TRR-2^{TIR} contain an asymmetric head-to-tail TIR:TIR domain interaction involving the BB-loop of one molecule and the surface encompassing the βE/EE loop/αE region of the second molecule (the BE interface; Figure 1c). Extensive mutagenesis using the mammalian-two-hybrid (MAPPIT) methodology combined with docking also provides support for an asymmetric BE interface involved in MyD88 self-association [35]. Furthermore, site-directed mutagenesis data identify both the BB-loop (R196/D197) and helix αE (K282/R288) as MAL-binding sites, suggesting that MAL^{TIR} and MyD88^{TIR} may interact through a similar head-to-tail mode [32].

Some lines of evidence suggest that purified TLR adaptor TIR domains may form higher-order oligomers at high protein concentrations. For example, the ^{15}N-labeled signals from MyD88^{TIR} uniformly decreased upon titration with MAL^{TIR} [32]. Furthermore, TRAM and TRIF

158 oligomerized and precipitated out of solution at concentration above 200 μM [36]. Precipitation was
159 prevented by the introduction of a BB-loop mutation (C117H in TRAM and P434H in TRIF),
160 which has previously been shown to disrupt self-association in Y2H assays and to have a dominant
161 negative effect in IFN-β reporter assays; this enabled the NMR structures of the monomeric
162 proteins to be determined.

163 Many of the TIR-domain assembly models have assumed a 2:1 or 1:1 receptor/adaptor
164 TIR:TIR domain stoichiometry [27,28,31], but more recent models try to rationalize how a single
165 TLR^{TIR} dimer can recruit >6 MyD88 molecules required for myddosome assembly. In one study
166 [29], the PRISM algorithm combined with existing crystal structures and experimental data was
167 used to model MyD88 and TRIF signalosomes. Several different plausible models are presented,
168 but it is argued that the most likely is a model consisting of a symmetric BCD-interface TLR4
169 dimer (similar to the $TLR10^{TIR}$ dimer) that interacts with two symmetric BCD-interface MAL
170 dimers, which in turn recruit two symmetric MyD88 dimers; this would result in clustering of 8
171 MyD88 DDs, enabling myddosome formation. A completely different model, based on MAPPIT
172 mutagenesis data and docking, is presented in another study [35], where it is proposed that MyD88
173 oligomerization is a result of self-association through both a symmetric BCD interface (similar to
174 the MAL^{TIR} crystal dimer) and an asymmetric BE interface . By combining the two types of
175 interactions, it is proposed that $MyD88^{TIR}$ molecules can assemble into a left-handed helix, bringing
176 the DDs together for myddosome assembly. This model displays similarities to the open-ended
177 pyrin domain (PYD)/CARD assemblies recently described for other innate immunity pathways (e.g.
178 the inflammasomes [55] and MAVS-dependent RIG-I/MDA-5 signaling [56]), and extension of the
179 left-handed helix would presumably enable a single TLR dimer to assemble multiple myddosomes,
180 which is consistent with the ability of TLRs to activate a large transcriptional response based on
181 extremely low concentrations of PAMPs. Although this model is consistent with observed TIR:TIR
182 domain interaction modes, the observed variations could give rise to different oligomeric TIR-
183 domain architectures. For example, in one of the TRR-2 crystal forms (PDB ID 4W8G), we also

observe a combination of BCD and BE interfaces, which results in a formation of a linear parallel two-stranded head-to-tail array of TIR domains within the crystal (Figure 1d). This architecture would also enable MyD88 DD clustering and myddosome formation. The BCD interface in this linear assembly differs from the MAL-based BCD interface in [35] by a 180° rotation of one of the molecules. However, it involves the αC and αD helices and can thus explains the reported MyD88 mutagenesis data. Our analyses illustrate that care must be used in interpreting docking results with limited structural information, and to fully elucidate the molecular mechanisms of TIR-domain assembly formation and the exact nature of the interfaces, structural information on stable oligomeric assemblies will be required. Furthermore, TIR-domain proteins usually contain other domains and can be attached to membranes; however, the TIR-domain linker sequences are usually of sufficient length (>20 residues) to enable the proposed interactions on cell-membranes or in the presence of other domains.

3. Self-association and homotypic association of TIR domains in plant proteins

The Arabidopsis TIR-X protein AtTIR (AT1G72930) provided the first plant TIR-domain structure [57]. It revealed a similar fold to those observed for mammalian TIR domains; however, an extended αD region is found. This feature appears to be unique to the plant TIR domains and present in most, but not all. AtTIR was report to be monomeric in solution [57]; however, this data was inferred from size-exclusion chromatography (SEC) alone, which, as subsequent studies have revealed, is unlikely to detect transient self-association. The first TIR-domain structure from a plant NLR came from the flax protein L6. $L6^{TIR}$ can self-associate according to Y2H and in-solution assays (SEC/multi-angle laser light scattering (MALS) and analytical ultracentrifugation (AUC)) [17]. Crystal-contact analysis, combined with mutagenesis, in-solution self-association assays and Y2H assays, revealed that the $\alpha D_{1/3}$, βE and αE regions mediate $L6^{TIR}$ self-association (the DE interface; Figure 2a, Table S1). Self-association is linked to the cell death-inducing activity association of $L6^{TIR}$ [17].

The RPS4^{TIR}:RRS1^{TIR} complex is the only crystal structure available for a complex of two different TIR domains. RPS4 and RRS1 are jointly responsible for NLR-mediated resistance to three different pathogens in Arabidopsis. The regions that mediate the heterodimer interaction (αA, αE and the AA and EE loops - the AE interface) are also observed in the structures of RRS1^{TIR}, RPS4^{TIR} and AtTIR as individual proteins [18]. RPS4^{TIR}, but not RRS1^{TIR} can induce cell-death signaling responses. The AE interface has been recently also observed in the crystal structures of the TIR domains from the wild grape NLR RPV1 [58] and the Arabidopsis NLRs SNC1 [59,60] and RPP1 [60].

The self-association of plant TIR domains observed to date is weak and transient; the dissociation constants measured for L6^{TIR} and RPS4^{TIR} by AUC experiments are in the high μM range. RPV1^{TIR} did not appear to self-associate *in vitro* under the conditions tested. It is speculated that TIR:TIR domain interactions would be stabilized, in the activated NLRs, by self-association of other domains such as the NB domains, based on comparisons with the related mammalian NLRs [14]. By contrast, the heterodimer formed between RPS4^{TIR}:RRS1^{TIR} is ~100x stronger (455 nM) than any self-associations of plant TIR domains. RRS1^{TIR} suppresses RPS4^{TIR} cell-death signaling in plants and suggests that the RPS4^{TIR}:RRS1^{TIR} interaction represents a repressed state of the pair [18].

The interfaces that mediate self-association in L6^{TIR} and RPS4^{TIR} are distinct, but they could co-exist (Figure 2). Mutations in Arabidopsis RPP1^{TIR} that map to both the DE and AE interfaces affect RPP1^{TIR} self-association, and a correlation between the degree of self-association *in vitro* and cell-death signaling has been observed [23]. These data suggest that both interfaces may facilitate self-association and signaling in RPP1 and potentially other plant TIR domains. Recent structures of SNC1^{TIR} and RPP1^{TIR} [59,60] revealed both AE and DE self-association interfaces within the crystal structures. Both interfaces also appear to control self-association, and we speculate that these interfaces may facilitate SCAF in the plant TIR domains (Figure 2).

4. Self-association and homotypic association of TIR domains in bacterial proteins

A common self-association interface has been observed in the available crystal structures of bacterial TIR domains, $PdTLP^{TIR}$ from the non-pathogenic *Paracoccus denitrificans* [61] and TcpB from the pathogenic *Brucella melitensis* [24,53,62] (Figure 3, Table S1). The dimer interfaces in both involve the DD and EE loops (different interface than the DE interface in plant TIR-domains) and leave the BB loops exposed on the surface of the molecules. While $TcpB^{TIR}$ associates transiently, full-length TcpB forms a stable dimer [62] and in one of the crystal structures, a helix corresponding to the sequence N-terminal to the TIR domain has been found to stabilize the interaction [24]. PdTLP and TcpB, as well as a number of other bacterial TIR-domain proteins, interact with MyD88, and some have been shown to interact with other mammalian TIR domains, including MAL^{TIR} and $TLR4^{TIR}$, and interfere with NF-κB signaling [21,42,62].

5. Reconciliation of structural data in the context of higher-order assembly formation

While common trends in association modes are emerging in plant and bacterial TIR domains, this is still not the case in animal TIR domains, despite the more extensive research. What could be the possible reasons for this? For the domains functional in innate immunity signaling, the associations need to be weak by design, so that responses are not too easily triggered in the absence of a pathogen or danger inducer. The specific conditions required for crystallization may therefore easily destabilize these interactions. Furthermore, the domains may have a tendency to assemble into higher-order oligomers not compatible with crystal formation. Indeed, higher-order assembly is an emerging feature of signaling in diverse innate immunity pathways. Protein domains from the DD family, in particular, appear to be able to form large, often open-ended helical structures [63,64]. Signaling through cooperative assembly formation (SCAF) explains the ultrasensitive, all-or-none response that is required in immune responses.

We propose that the available data on TIR-domain interactions can be reconciled by the hypothesis that TIR domains that function in immunity pathways signal by cooperative assembly formation (SCAF). The structures available to date likely provide snapshots into this assembly, but the structures may, for reasons outlined above, vary in their biological relevance. Reconstitution of stable complexes and their structural analysis, in combination with complementary cell biology approaches, should reveal the interactions relevant to the signalosomes that occur *in vivo*.

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484

FIGURE LEGENDS

Box 1. (a) In the classical concept of receptor-mediated signaling, the activated receptor (R; for example, activated by binding to the ligand L, blue) initiates signal transduction inside the cell through successive steps of activation of signaling proteins (E; for example, enzymes that perform post-translational modifications, such as protein kinases, or enzymes that produce second messengers, such as adenylyl cyclases). This leads to signal amplification in a cascade-like fashion. Red and green represent inactive and activated proteins, respectively. **(b)** In the case of signaling by cooperative assembly formation (SCAF), the activated receptor initiates signal transduction through higher-order assembly formation, which involves cooperative interactions with adaptor proteins (A) and eventually enzymes (E) to form a signalosome. The large assembly can lead to rapid activation of enzymes such as protein kinases or proteases through proximity-induced activation. The cooperativity is the result of conformational changes and new binding sites generated by the assembly architecture. SCAF appears to operate in most innate immunity pathways, including the ones involving TIR domains. Most higher-order assemblies characterized to date are mediated by members of the death-domain (DD) fold (DD, CARD, PYD, death-effector domain). The DD-mediated helical assembly containing 6 MyD88 (shades of red), 4 IRAK4 (shades of green) and 4 IRAK2 (shades of blue) DDs [50] is shown as an example in **(c)** in cartoon representation.

Figure 1. Representative TIR:TIR domain interactions based on structures of mammalian TIR domains.

(a) Crystal contact-based TIR-domain dimers [26,30,37,40,43] (PDB ID 4W8H). The protomers depicted on the right are all shown in analogous orientations.

(b) Superposition of one of the protomers from all the dimers shown in (a). The superimposed protomer of the $TLR2^{TIR}$ is shown in surface representation), with the other protomer from all the

TIR domains shown in different colors in ribbon representation.

(c) Head-to-tail arrangement of TIR domains in the crystals of $MyD88^{TIR}$ [42] and $TRR2^{TIR}$ (PDB ID 4W8G and 4W8H).

(d) Two stranded parallel head-to-tail arrangement of TIR domains in the crystals of $TRR2^{TIR}$ (PDB ID 4W8G).

Figure 2. TIR:TIR domain interactions mediated by the DE and AE interfaces in plant TIR-domain proteins.

(**a**) Crystal contact-based TIR-domain dimers observed for the $L6^{TIR}$ (blue) [17] and the heterodimer of $RPS4^{TIR}$ (dark green) and $RRS1^{TIR}$ (green) [18], revealing the DE and AE interfaces, respectively.

(**b**) Superimposed $RPS4^{TIR}$ and $L6^{TIR}$ dimers, revealing that the DE and AE interface can coexist.

(c) A hypothetical AE and DE interface-mediated assembly of plant TIR domains (individual domains are shown in different colours). Note that this model does not account for other domains in NLR proteins, such as the NB and LRR domains, which could influence the arrangement and stoicometry of predicted assembles of plant NLRs, based on comparisons with the related mammalian NLRs [14].

Figure 3. TIR:TIR domain interactions in bacterial TIR-domain proteins. The crystal structures of $PdTLP^{TIR}$ (red) [61] and $TcpB^{TIR}$ (blue) [24] reveal an analogous dimer interface. In one of the structures of $TcpB^{TIR}$ (PDB ID 4LZP) [24], the dimer is stabilized by a helix corresponding to the sequence N-terminal to the TIR domain (light blue).

Table S1

Reports on the characterization of homotypic TIR-domain interactions. Only binary interactions are listed in the table in cases where higher-order complexes have been analyzed in the original publications.

Interacting TIR domains	Organism	Interface and interaction mode	Methods	Reference
Animal TIR domains				
$TLR2^{TIR\text{-}C713S}$:$TLR2^{TIR\text{-}C713S}$	*Homo sapiens*	Asymmetric dimer; involves αB, αC, αD, CD and DD (molecule A) and αB and BB (molecule B)	X-ray crystallography, mutagenesis	[33]
IL-$1RAPL^{TIR}$:IL-$1RAPL^{TIR}$	*Homo sapiens*	Symmetric dimer; involves αB, αC and αD	X-ray crystallography, mutagenesis	[30]
$TLR2^{TIR}$: $MyD88^{TIR}$	*Homo sapiens*	Involves BB and αA of both molecules	Computational docking, mutagenesis	[39]
$TLR2^{TIR}$:$TLR2^{TIR}$, $MyD88^{TIR}$:$MyD88^{TIR}$		Symmetric dimer; involves αE		
$TLR1^{TIR}$:$TLR2^{TIR}$	*Homo sapiens*	Two interacting regions; region I: involves TLR1 BB, TLR2 DD; region II: involves TLR1 αA (His646) and αC, TLR2 CD (Asn700)	Mutagenesis, computational docking	[44]
$TLR4^{TIR}$:$TLR4^{TIR}$	*Homo sapiens*	Symmetric dimer; involves BB	Modeling, docking, mutagenesis	[31]
$TLR10^{TIR}$:$TLR10^{TIR}$	*Homo sapiens*	Symmetric dimer; involves BB, DD, αB and αC	X-ray crystallography, mutagenesis	[40]
$MyD88^{TIR}$:MAL^{TIR}	*Homo sapiens*	Two interacting sites on MyD88 (site 1 corresponds to BB (R196) and site 2 to αE	NMR spectroscopy, mutagenesis, docking	[32]

		(R288)		
$TLR4^{TIR}$:$TLR4^{TIR}$	*Homo sapiens*	Symmetric dimer: involves BB, DD, αC	Computational docking and modeling	[45]
$TLR7^{TIR}$:$TLR7^{TIR}$		Asymmetric dimer: involves BB (molecule A), αE (molecule B)		
$MyD88^{TIR}$:$MyD88^{TIR}$		Symmetric dimer: involves BB, αC		
$TLR4^{TIR}$:$SIGIRR^{TIR}$		3 patches; patch 1: involves TLR4 CD, and BB, SIGIRR αB; patch 2: involves TLR4 αB and αC, SIGIRR αC; patch 3: involves TLR4 BB, SIGIRR αD		
$TLR7^{TIR}$:$SIGIRR^{TIR}$		Involves SIGIRR BB and αB, TLR7 αE, CD, βD, βE and DE		
$MyD88^{TIR}$:$SIGIRR^{TIR}$		Involves MyD88 BB, and αC, SIGIRR BB, AA and αC		
MAL^{TIR}:MAL^{TIR}	*Homo sapiens*	Symmetric dimer: involves αC, αD	X-ray crystallography, docking, mutagenesis	[37]
MAL^{TIR}:$MyD88^{TIR}$		Involves MAL D96 (AA) and S180 (DD), MyD88 R196 (BB)		
$TLR4^{TIR}$:$TLR4^{TIR}$	*Homo sapiens*	Symmetric dimer: involves BB, αC	Molecular dynamics (MD) simulations, molecular docking	[46]
$TLR2^{TIR}$:$TLR1^{TIR}$		Asymmetric dimer: involves TLR2 DD,		

		TLR1 BB		
$TLR2^{TIR}$:$TLR6^{TIR}$		Involves TLR2 DD, TLR6 BB		
$ST2L^{TIR}$:MAL^{TIR}		Involves ST2L AB and BB, MAL BB, βA and βB		
$ST2L^{TIR}$:$MyD88^{TIR}$		Involves ST2L BB, AA and αA, MyD88 BB, αA		
MAL^{TIR}:MAL^{TIR}	*Homo sapiens*	Symmetric dimer: involves αC, αD	X-ray crystallography, mutagenesis	[41]
$TLR4^{TIR}$:$TLR4^{TIR}$	*Homo sapiens*	Symmetric dimer: involves BB, DD, αC	Mammalian protein-protein interaction trap (MAPPIT), homology modeling, mutagenesis	[28]
$TLR4^{TIR}$:MAL^{TIR}, $TLR4^{TIR}$:$TRAM^{TIR}$		Involves TLR4 αA, αB, BB, BC		
MAL^{TIR}:MAL^{TIR}	*Homo sapiens*	Asymmetric dimer: involves DD, DE, αD (molecule A), N-terminal region (molecule B)	X-ray crystallography	[47]
MAL^{TIR}:MAL^{TIR}	*Homo sapiens*	Symmetric dimer: involves αC, αD	Random mutagenesis, MAPPIT	[27]
MAL^{TIR}:$MyD88^{TIR}$		Involves MAL AB loop and two surface areas (area 1: Q135, W156; area 2: Y195, R215)		
MAL^{TIR}:$TLR4^{TIR}$		Involves MAL AB loop and three surface areas (area 1: Q135, W156; area 2: Y195, R215; area 3: Q153, R184, R192)		
$TRAM^{TIR}$:$TRAM^{TIR}$	*Homo sapiens*	Symmetric dimer: involves BB, αC	NMR spectroscopy, mutagenesis, docking	[36]

MyD88^{TIR}:MyD88^{TIR}	*Homo sapiens*	Symmetric dimer: involves βA, AA, αA, AB, CD, BB, αC	Site-directed mutagenesis, computational modeling	[48]
MyD88^{TIR}:MyD88^{TIR}	*Homo sapiens*	Two asymmetric dimers: dimer 1 involves αC, αD (molecule A), αA, EE and αE (molecule B); dimer 2 involves αB, BB (molecule A), DD, αD, EE and αE (molecule B)	X-ray crystallography	[42]
MAL^{TIR}:MAL^{TIR}	*Homo sapiens*	Symmetric dimer: involves αC, αD	X-ray crystallography, mutagenesis	[53]
TLR6^{TIR}:TLR6^{TIR}	*Homo sapiens*	Symmetric dimer: involves CD, DD, αB αC	X-ray crystallography, MALS	[43]
TLR4^{TIR}:TLR4^{TIR}	*Homo sapiens*	Two symmetric dimers; both involve BB	Modeling, *in silico* mutagenesis	[29]
MAL^{TIR}:MAL^{TIR}		Symmetric dimer: involves AB		
TRAM^{TIR}:TRAM^{TIR}		Symmetric dimer: involves BB		
ST2^{TIR}:TLR4^{TIR}, ST2^{TIR}:TRIF^{TIR}	*Homo sapiens*	Involves BB	Modeling, *in silico* mutagenesis	[38]
MyD88^{TIR}:MyD88^{TIR}	*Homo sapiens*	Asymmetric dimer: involves BB (molecule A), αE (molecule B); symmetric dimer: involves αD, αC	MAPPIT, mutagenesis, docking	[35]
TLR4^{TIR}:TLR4^{TIR}	*Mus musculus*	Asymmetric dimer: involves BB (molecule A), αE (molecule B)	Decoy peptides, modeling	[34]

TRR-2^{TIR}:TRR-2^{TIR}	*Hydra magnipapillata*	Asymmetric dimer: involves BB (molecule A), βD, βE, DE, αE (molecule B); symmetric dimer: involves αB, αC, αD	X-ray crystallography	Weisse & Scheidig, unpublished; PDB ID 4W8G
TRR-2^{TIR}:TRR-2^{TIR}	*Hydra magnipapillata*	Asymmetric dimer 1: involves BB (molecule A), βD, βE, DE, αE (molecule B); asymmetric dimer 2: involves αA, αE (molecule A), αB, αC, αD (molecule B)	X-ray crystallography	Weisse & Scheidig, unpublished; PDB ID 4W8H
Plant TIR domains				
L6^{TIR}:L6^{TIR}	*Linum usitatissimum (flax)*	Symmetric dimer: involves αD_1, αD_3, αE, βE , DE, EE (DE interface)	X-ray crystallography, MALS, analytical ultracentrifugation (AUC), yeast two-hybrid (Y2H) analysis	[17]
RRS1^{TIR}:RPS4^{TIR}	*Arabidopsis thaliana*	Pseudo-symmetric dimer: involves αA, αE, EE (of both RRS1^{TIR} and RPS4^{TIR}) and DD (RRS1^{TIR}) (AE interface)	X-ray crystallography, MALS , SAXS, Y2H analysis	[18]
RPS4^{TIR}:RPS4^{TIR}	*Arabidopsis thaliana*	Symmetric dimer: involves αA, αE, EE (AE interface)	X-ray crystallography, MALS, SAXS, AUC, Y2H analysis	[18]
RRS1^{TIR}:RRS1^{TIR}	*Arabidopsis thaliana*	Symmetric dimer: involves αA, αE, EE (AE interface)	X-ray crystallography	[18]
RPV1^{TIR}:RPV1^{TIR}	*Muscadinia rotundafolia* (wild grapevine)	Symmetric dimer: involves αA, αE, EE (AE interface)	X-ray crystallography	[58]

$SNC1^{TIR}$:$SNC1^{TIR}$	*Arabidopsis thaliana*	Two dimer interfaces; interface 1 (AE interface), involves αA, αE, EE; interface 2 (DE interface): involves αD_1, αE, βE, DE, EE	X-ray crystallography	[59]
$SNC1^{TIR}$:$SNC1^{TIR}$	*Arabidopsis thaliana*	Two dimer interfaces; interface 1 (AE interface), involves αA, αE, EE; interface 2 (DE interface): involves αD_1, αE , βE, DE, EE	X-ray crystallography, MALS, SAXS	[60]
$RPP1^{TIR}$:$RPP1^{TIR}$	*Arabidopsis thaliana*	Two dimer interfaces; interface 1 (AE interface), involves αA, αE, EE; interface 2 (DE interface): involves αD^1, αE , βE, DE, EE	X-ray crystallography, MALS	[60]
Bacterial TIR domains				
$PdTLP^{TIR}$:$PdTLP^{TIR}$	*Paracoccus denitrificans*	Symmetric dimer: involves DD, EE	X-ray crystallography, hydrogen/deuterium exchange mass spectrometry (DXMS)	[61]
$TcpB^{TIR}$:$TcpB^{TIR}$	*Brucella melitensis*	Involves DD, EE, αC, αD	X-ray crystallography, MALS	[24]
$TcpB^{TIR}$:$TcpB^{TIR}$	*Brucella melitensis*	Symmetric dimer: involves DD, EE	X-ray crystallography, SAXS, MALS	[62]

$TcpB^{TIR}$:$TcpB^{TIR}$	*Brucella melitensis*	Symmetric dimer: involves DD, EE	X-ray crystallography, DXMS	[53]

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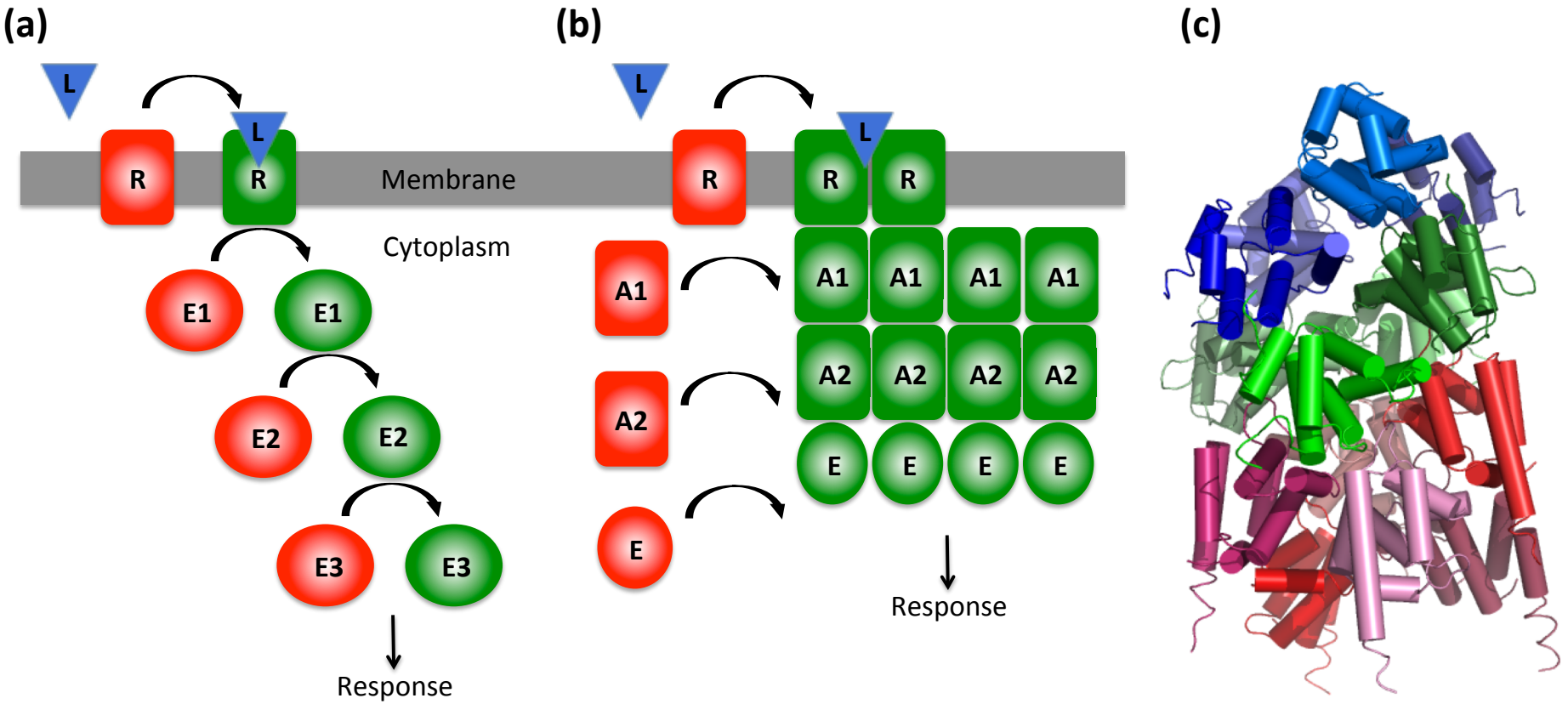
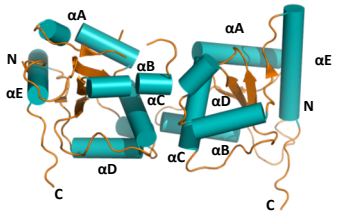
Box 1

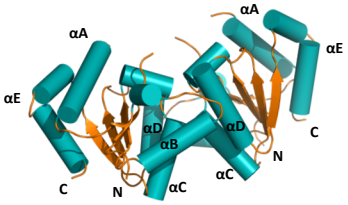

(a)
L
R
L
R
Membrane
Cytoplasm
E1
E1
E2
E2
E3
E3
Response
(b)
L
R
L
R
R
A1
A1
A1
A1
A1
A2
A2
A2
A2
A2
E
E
E
E
E
Response
(c)

(a)

TLR1



IL-1RAPL



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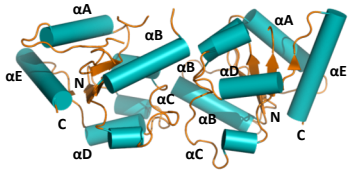
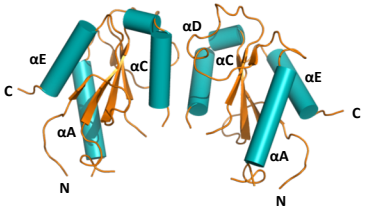


MAL



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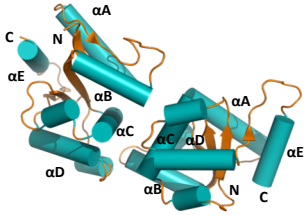
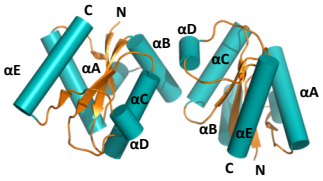


TRR2



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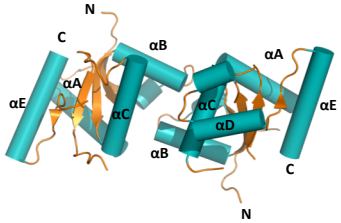
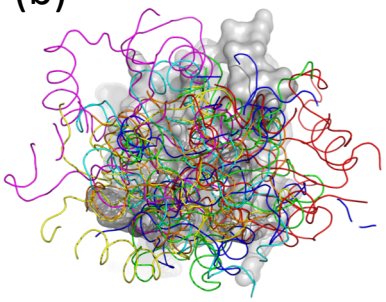
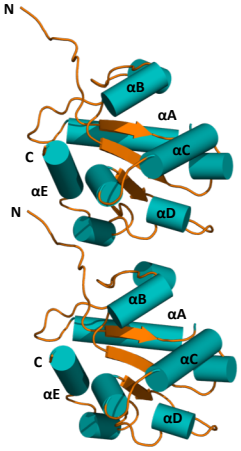


(b)



(c)

MyD88



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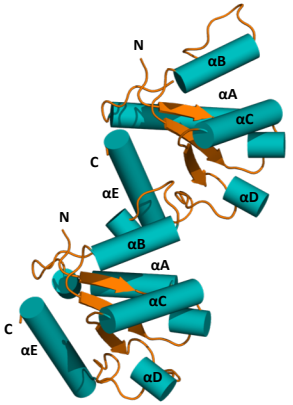


(d)

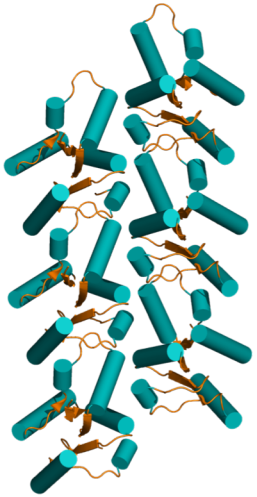
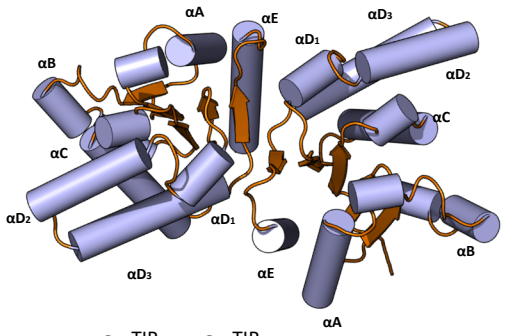


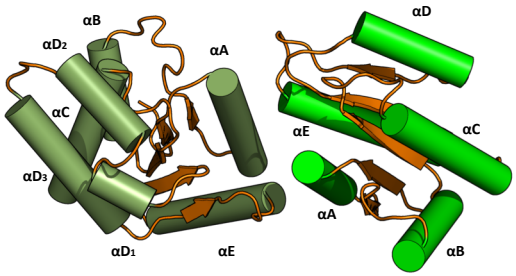
Fig. 2

(a)

L6^{TIR}

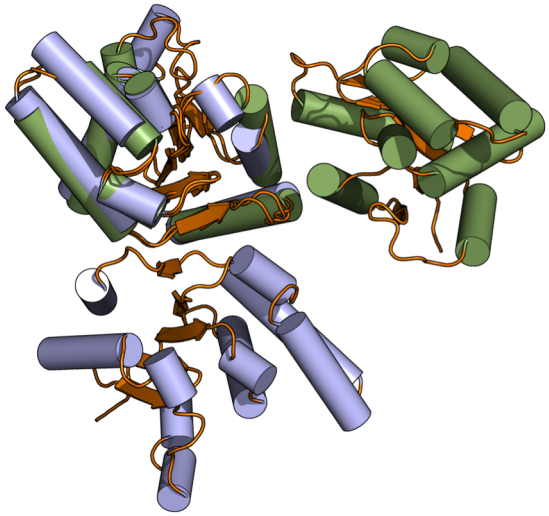


RPS4^{TIR}:RRS1^{TIR}



(b)

DE (L6^{TIR}) and AE (RPS4^{TIR}) interfaces



(c)

A hypothetical AE/DE interface-mediated oligomer

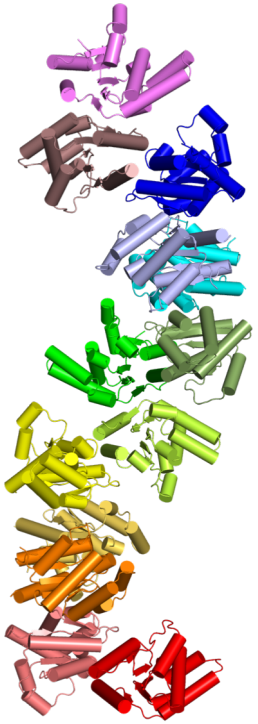
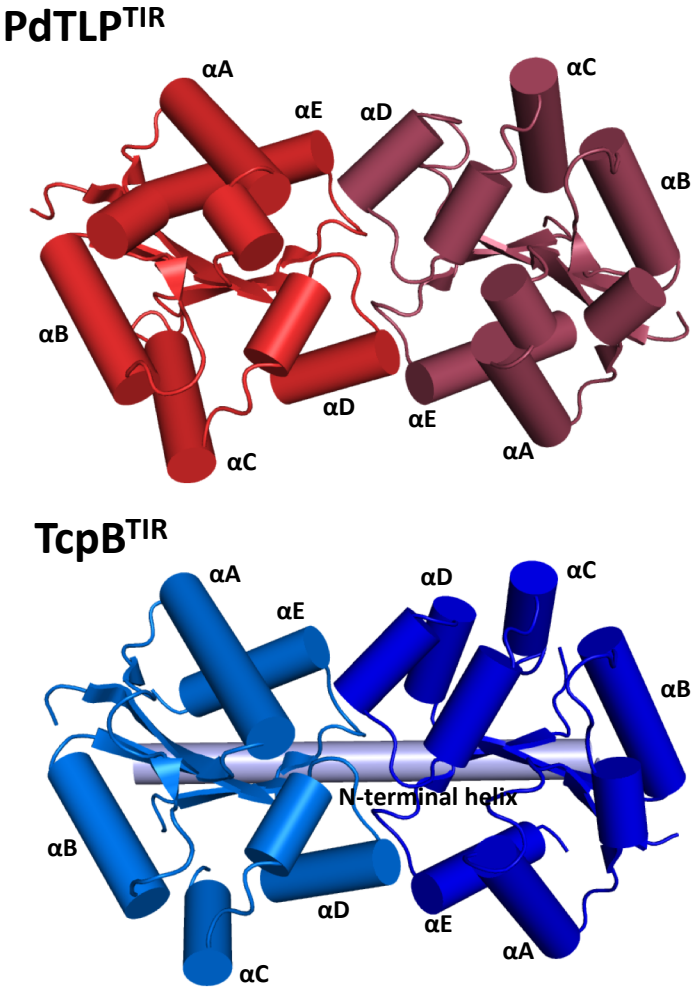


Fig. 3


PdTLP^TIR
αA
αE
αD
αC
αB
αB
αD
αE
αC
αA
TcpB^TIR
αA
αE
αD
αC
αB
N-terminal helix
αB
αD
αE
αC
αA