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Subversion of Innate Immune Signaling Through Molecular Mimicry

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Abstract

Innate immune signaling is mediated by a number of membrane-anchored or cytosolic receptor or sensor molecules. Several receptor families utilize conserved signaling domains such as the Toll/interleukin-1 receptor (TIR) domain and Pyrin domain (PYD) to link microbe recognition to induction of proinflammatory cytokines and interferons. Recent studies have identified a number of bacterial and viral TIR domains and PYD domains that directly target the signaling function of their host homologues. Emerging biochemical and structural studies of these microbial TIR and PYD domains suggest that they are mimics of their host counterparts at the sequence and structure levels. Unraveling the mechanisms of such molecular mimicry is crucial to our understanding and clinical intervention of infectious diseases and inflammatory disorders.

Keywords

Microbial subversion of innate signaling; TIR domain; PYD domain; molecular mimicry

The innate immune system is the first line of defense against infection. Several protein families have evolved to function as receptors or sensors of pathogen invasion, such as the Toll-like receptors (TLRs), nucleotide binding and oligomerization domain (NOD)-like receptors (NLRs), and retinoic acid-inducible gene 1 (RIG-I)-like receptors or helicases (RLRs or RLHs) [1]. Such molecular recognition events activate innate signaling pathways and secretion of proinflammatory cytokines or interferons to both limit initial infection and orchestrate adaptive immunity for long-term protection. The innate immune signaling pathways evolved through a combinatorial employment of several protein domains for pathogen sensing and subsequent signal transduction [2]. The signaling domains couple upstream pathogen sensing by the receptors to downstream effector expression and secretion through homotypic protein–protein interaction. There are two types of signaling domains for the above receptors: the TIR domain for the TLRs [3], and the Pyrin domain (PYD) or caspase recruitment domain (CARD) for the NLRs and RLRs. The TIR domains contain five α helices and five parallel β strands and belong to the Rossmann-fold family as characterized by the CATH database [4] (Fig. 1a, b). PYD and CARD belong to the same death domain superfamily of six-helical bundle fold [5] (Fig. 1c, d). Both TIR domains and the death domain superfamily are known to form specific domain–domain complexes to mediate protein–protein interactions. Recent studies have revealed a number of microbial homologues of these host signaling modules and suggested potential interference mechanisms through mimicry of host proteins. In particular, this review will focus

on several bacterial and viral TIR domains that sabotage TLR signaling, as well as poxvirus PYD-only proteins that target cytosolic receptors for inflammatory responses.

TIR Domains as Central Signaling Modules for TLRs

Five TIR adaptors have been discovered in human, including myeloid differentiation factor-88 (MyD88) as a central adapter for most TLRs, TIR domain-containing adapter protein inducing interferon- β (TRIF) as a second adapter for TLR3 and TLR4, TIR domain-containing adapter protein (TIRAP) or MyD88 adapter-like protein (MAL) as a bridging adapter for MyD88, TRIF-related adapter molecule (TRAM) as a bridging adapter for TRIF, and sterile α - and armadillo-motif-containing protein (SARM) [3]. SARM is the only negative regulator that inhibits signaling from TRIF. Through mostly sequence homology search, TIR domain-containing proteins have been uncovered in a number of bacteria such as *Salmonella enterica* [6], uropathogenic *Escherichia coli* strain CFT073 and *Brucella melitensis* [7], and *Brucella abortus* [8]. This new class of virulence factors has been shown to interfere with host TLR signaling, as discussed below.

Several TIR domain structures have been experimentally determined by X-ray crystallography or nuclear magnetic resonance spectroscopy, such as those for TLR1 and TLR2 [9], TLR10 [10], IL-1RAPL [11], or MyD88 [12], as well as TIR domains from bacteria *Paracoccus denitrificans* [13] and plant *Arabidopsis thaliana* [14]. A prominent feature of the TIR domain sequences is the three conserved box 1, box 2, and box 3 regions [9]. These three regions map to the hydrophobic core of the domain structure, as well as a long “BB” loop that was proposed to be the primary protein–protein interaction site.

TcpC Modulates MyD88 Signaling

TcpC from uropathogenic *E. coli* strain CFT073 was identified in a bacterial genomic sequence search [7]. Even with minimal sequence similarity to the human TIR domain proteins, the typical box 1, 2, and 3 sequences are present within the C-terminal ~200 residues of TcpC. This allowed for prediction of its tertiary structure that is very similar to its mammalian homologues. Pull-down assays demonstrated direct interaction between TcpC TIR domain and full-length MyD88, presumably through their respective TIR domains [7]. TcpC was shown to directly impair signaling through TLR2 and TLR4, reduce proinflammatory cytokine secretion, and increase bacterial burden in murine urinary tract and renal tissue infection models. The significance of TcpC as a virulence factor was highlighted by the correlation of TcpC-expressing bacteria strains from clinical samples with severity of urinary tract infection [7]. Surprisingly, even though TcpC does not contain a typical secretion signal, it was secreted by bacteria and taken up by macrophages to dampen cytokine production [7]. As the host TIR domain proteins are only known to be present in the cytosol, the above observation represents a novel shuttling ability of TIR domain proteins through lipid membrane, perhaps aided by cryptic localization signals and/or other transport proteins.

TcpB Facilitates Degradation of TIRAP/MAL

TcpB and Btp1 are identical proteins from *Brucella* bacteria except for a single mutation A138V within the predicted α A helix of their TIR domains [7,8]. Both proteins were shown to downregulate TNF production by macrophages [7] or dendritic cells [8]. Unlike TcpC, however, infection with Btp1-deficient bacteria did not alleviate symptoms of murine brucellosis [8], suggesting other redundant mechanisms of immune evasion. Because of the retarded maturation of dendritic cells upon *Brucella* infection, it was proposed that *Brucella* might play a role in facilitating the induction of immune tolerance and chronic infection [8], although the exact role of Btp1 or other *Brucella* proteins in this process is not well understood.

Recent studies suggest that TcpB targets signaling by TIRAP/MAL through at least two mechanisms. The first line of evidence by Radhakrishnan and colleagues demonstrated the localization of TcpB at the plasma membrane through its association with phosphoinositides (PtdIns) [15]. This is reminiscent of the binding of phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P₂) by the N-terminal region of TIRAP/MAL and its recruitment to the plasma membrane. The membrane localization is essential for its role as a bridging adapter between TLR2 or TLR4 and MyD88 [16]. Nonetheless, the implicit mimicry of TIRAP/MAL by TcpB has not been substantiated by direct binding of TcpB with TIRAP/MAL partner proteins such as MyD88 or TLR4, either through their TIR domains or otherwise.

Earlier studies demonstrated that phosphorylation of TIRAP/MAL by Bruton's kinase at Tyr86, 106, and 187 was important for its signaling and NF- κ B activation [17–19]. Furthermore, phosphorylation of TIRAP/MAL resulted in its association with suppressor of cytokine signaling (SOCS)-1, a negative regulator of cytokine signaling [20]. SOCS-1 is an E3 ligase that contains an SH2 domain, which binds phosphorylated tyrosines. Interaction of phosphorylated TIRAP/MAL with SOCS-1 causes polyubiquitination at residues Lys15 and Lys16, followed by its degradation and downregulation of TLR2 and TLR4 signaling [20]. In a striking resemblance, a new study by Sengupta and colleagues showed that TcpB promoted polyubiquitination and subsequent degradation of TIRAP/MAL, and this degradation process seemed more efficient for phosphorylated TIRAP/MAL [21]. It is currently unclear whether TcpB is an adapter for other proteases, is a protease itself, or contains E3 ligase activity like SOCS-1 to mediate proteasome-mediated degradation. The above two mechanisms of TIRAP/MAL inhibition by TcpB are not necessarily independent of each other: localization of TcpB to the plasma membrane can facilitate the binding, ubiquitination, or degradation of TIRAP/MAL by TcpB or other associated ubiquitination enzymes or proteases.

PdTLP Contains a TIR Domain that Binds MyD88

Through similar genomic sequence mining, a TIR domain-containing protein PdTLP was identified in *P. denitrificans* [22]. GST pull-down and co-immunoprecipitation studies demonstrated direct binding of the PdTLP TIR domain (PdTIR) with human and mouse MyD88 TIR domain [13,22]. The binding affinity between PdTIR and MyD88 appeared to be relatively low based on the GST pull-down assay [22]. The crystal structure of PdTIR is the first among microbial TIR domains to be determined [13] (Fig. 1b). It adopts a typical TIR domain fold, with a unique homodimerization interface mediated by the DD and EE loops. The exposed BB loop was proposed to be the site of heterotypic interaction with partner TIR domains, such as that from MyD88 [13]. The function of PdTLP as a virulence factor has not been characterized, as it is unclear whether its ability to interact with MyD88 dampens signaling from TLRs, or offers any advantage for bacterial infection, replication, or dissemination.

TlpA is an Essential Virulence Factor During *Salmonella* Infection

The *Salmonella* protein TlpA was shown to be requisite for suppression of NF- κ B activation as well as IL-1 β secretion during *Salmonella* infection. The fatality of mice infected with wild-type bacteria strain was significantly higher than those infected with TlpA-deficient strain [6]. Even though there are detectable sequence similarity between TlpA and TIR domains from human and other bacterial proteins, particularly at the box 1 and box 2 regions, direct interaction of TlpA with any of the TIR domain signaling proteins has not been demonstrated and preliminary report suggested that it does not bind MyD88 directly [6]. On a cautionary note, a caveat of identifying TIR domains with marginal sequence similarity is that some of the proteins with certain TIR domain signature sequences may turn out to adopt completely different folds in their tertiary structures.

Vaccinia Virus A46R Targets TIR Domain Adapters

A46R was first cloned as a TIR domain-containing protein from vaccinia virus [23]. It was later shown to suppress signaling from multiple TLRs [24]. In particular, A46R downregulated NF- κ B and mitogen-activated protein kinase activation induced by IL-1 α but not TNF α . Perhaps more importantly, A46R blocked IRF3 activation by TRIF-mediated signaling and thus prevented the secretion of IFN- β , which functions to suppress viral replication [24]. Intranasal infection model showed A46R-deficient virus had diminished virulence, consistent with the above observations. GST pull-down and co-immunoprecipitations indicated that transfected A46R was present in protein complexes containing host TIR domain proteins MyD88, TIRAP/MAL, TRIF, or TRAM but not SARM. This suggests that A46R specifically targets signaling but not negative regulator TIR domains. Recombinant GST-tagged A46R was shown to interact with TIRAP/MAL directly, but its binding to other TIR domain proteins has not been demonstrated.

The mechanisms of A46R's broad-spectrum antagonism to TLRs are unclear. It was suggested that the uniquely long BB loop at the box 2 region of its TIR domain might contribute to its ability to bind multiple TIR domains of diverse sequences [24]. Currently, mutagenesis studies of this loop or structural characterization of A46R have not been reported.

Pyrin-Only Proteins from Poxviruses Suppress Inflammasome Activation

Poxviruses encode a plethora of immune evasins that modulate both innate and adaptive immune responses [25]. Several poxviruses encode PYD-only proteins such as M013L from Myxoma virus [26,27] (Fig. 1d) and gp013L [28] from Shope fibroma virus. Both proteins were shown to downregulate secretion of inflammatory cytokines IL-1 β and IL-18, which bears intriguing similarity to the function of PYD-only proteins from hosts as negative regulators [29]. Compared with wild-type virus, infection by M13L-deficient virus was characterized by diminished viral load and rapid resolution of infection without mortality [26]. Transfected M013L and gp013L had distinctive cytosolic punctate distributions and were co-localized with ASC specks [26,28]. ASC (apoptosis-associated speck-like protein containing a CARD) is a crucial signaling adapter for NLRs, of which several members are known to form inflammasomes to induce IL-1 β and IL-18 secretion [30]. The co-localization and association between M013L and gp013L with ASC appeared to be mediated by their respective PYD domains. The mechanism of inflammasome inhibition may be attributed to viral protein association with ASC resulting in a disconnection between receptor/sensor activation and downstream effector caspase-1 activation.

The cytosolic receptor for poxvirus DNA has recently been identified as absent in melanoma 2 (AIM2) [31], a protein of the PYHIN/HIN200 family that contains both a PYD domain and a HIN200 domain known to bind DNA [32]. Because a number of cytosolic receptors other than AIM2 also utilize ASC as a central signaling adapter, the anti-inflammatory effect of poxvirus PYD proteins may not be restricted to the AIM2 pathway. The pleiotropic anti-inflammatory effect of these viral proteins makes them potential immuno-modulatory therapeutics for inflammatory disorders such as rheumatoid arthritis and multiple sclerosis.

Conclusion

A recent phylogenetic analysis of TIR domains identified nearly 1,200 eukaryotic TIR domain-containing proteins, as well as almost 500 bacterial homologues (Zhang and Godzik, personal communication). Surprisingly, the majority of the bacterial genomes contain a single TIR domain-encoding gene. This suggests that some of these bacterial TIR domains may have binding partners from other organisms, perhaps from hosts in which the bacteria reside as either

commensal or pathogenic species. This presents vast opportunities for microbe–host interaction and coevolution mediated by TIR domains.

In summary, a new class of virulence factors from bacteria and viruses has been identified that manipulate host innate immune signaling pathways through molecular mimicry. These microbial proteins contain signaling domains that bear sequence and structural similarity to their host targets, thereby potentially sabotage host immunity by hijacking crucial signaling pathways and uncouple receptor activation from effector induction. Unraveling the mechanisms of this molecular mimicry and host–pathogen interactions are essential to our understanding of microbial pathogenesis and medical intervention of relevant infectious diseases. Furthermore, immuno-modulatory molecules from microbes could serve as templates for development of therapeutics against inflammatory disorders.

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References

1. Kawai T, Akira S. The roles of TLRs, RLRs and NLRs in pathogen recognition. *Int Immunol* 2009;21:317–337. [PubMed: 19246554]
2. Palsson-McDermott EM, O'Neill LA. Building an immune system from nine domains. *Biochem Soc Trans* 2007;35:1437–1444. [PubMed: 18031241]
3. O'Neill LA, Bowie AG. The family of five: TIR-domain-containing adaptors in Toll-like receptor signalling. *Nat Rev Immunol* 2007;7:353–364. [PubMed: 17457343]
4. Orengo CA, Michie AD, Jones S, Jones DT, Swindells MB, Thornton JM. CATH—a hierarchic classification of protein domain structures. *Structure* 1997;5:1093–1108. [PubMed: 9309224]
5. Park HH, Lo YC, Lin SC, Wang L, Yang JK, Wu H. The death domain superfamily in intracellular signaling of apoptosis and inflammation. *Annu Rev Immunol* 2007;25:561–586. [PubMed: 17201679]
6. Newman RM, Salunkhe P, Godzik A, Reed JC. Identification and characterization of a novel bacterial virulence factor that shares homology with mammalian Toll/interleukin-1 receptor family proteins. *Infect Immun* 2006;74:594–601. [PubMed: 16369016]
7. Cirl C, Wieser A, Yadav M, Duerr S, Schubert S, Fischer H, et al. Subversion of Toll-like receptor signaling by a unique family of bacterial Toll/interleukin-1 receptor domain-containing proteins. *Nat Med* 2008;14:399–406. [PubMed: 18327267]
8. Salcedo SP, Marchesini MI, Lelouard H, Fugier E, Jolly G, Balor S, et al. Brucella control of dendritic cell maturation is dependent on the TIR-containing protein Btp1. *PLoS Pathog* 2008;4:e21. [PubMed: 18266466]
9. Xu Y, Tao X, Shen B, Horng T, Medzhitov R, Manley JL, et al. Structural basis for signal transduction by the Toll/interleukin-1 receptor domains. *Nature* 2000;408:111–115. [PubMed: 11081518]
10. Nyman T, Stenmark P, Flodin S, Johansson I, Hammarstrom M, Nordlund P. The crystal structure of the human toll-like receptor-10 cytoplasmic domain reveals a putative signaling dimer. *J Biol Chem* 2008;283:11861–11865. [PubMed: 18332149]
11. Khan JA, Brint EK, O'Neill LA, Tong L. Crystal structure of the Toll/interleukin-1 receptor domain of human IL-1RAPL. *J Biol Chem* 2004;279:31664–31670. [PubMed: 15123616]
12. Ohnishi H, Tochio H, Kato Z, Orii KE, Li A, Kimura T, et al. Structural basis for the multiple interactions of the MyD88 TIR domain in TLR4 signaling. *Proc Natl Acad Sci USA* 2009;106:10260–10265. [PubMed: 19506249]
13. Chan SL, Low LY, Hsu S, Li S, Liu T, Santelli E, et al. Molecular mimicry in innate immunity: crystal structure of a bacterial TIR domain. *J Biol Chem* 2009;284:21386–21392. [PubMed: 19535337]
14. Chan SL, Mukasa T, Santelli E, Low LY, Pascual J. The crystal structure of a TIR domain from *Arabidopsis thaliana* reveals a conserved helical region unique to plants. *Protein Sci* 2010;19:155–161. [PubMed: 19845004]

15. Radhakrishnan GK, Yu Q, Harms JS, Splitter GA. Brucella TIR domain-containing protein mimics properties of the Toll-like receptor adaptor protein TIRAP. *J Biol Chem* 2009;284:9892–9898. [PubMed: 19196716]
16. Kagan JC, Medzhitov R. Phosphoinositide-mediated adaptor recruitment controls toll-like receptor signaling. *Cell* 2006;125:943–955. [PubMed: 16751103]
17. Jefferies CA, Doyle S, Brunner C, Dunne A, Brint E, Wietek C, et al. Bruton's tyrosine kinase is a Toll/interleukin-1 receptor domain-binding protein that participates in nuclear factor kappaB activation by Toll-like receptor 4. *J Biol Chem* 2003;278:26258–26264. [PubMed: 12724322]
18. Liljeroos M, Vuolteenaho R, Morath S, Hartung T, Hallman M, Ojaniemi M. Bruton's tyrosine kinase together with PI 3-kinase are part of Toll-like receptor 2 multiprotein complex and mediate LTA induced Toll-like receptor 2 responses in macrophages. *Cell Signal* 2007;19:625–633. [PubMed: 17020802]
19. Gray P, Dunne A, Brikos C, Jefferies CA, Doyle SL. A MyD88 adapter-like (Mal) is phosphorylated by Bruton's tyrosine kinase during TLR2 and TLR4 signal transduction. *J Biol Chem* 2006;281:10489–10495. [PubMed: 16439361]
20. Mansell A, Smith R, Doyle SL, Gray P, Fenner JE, Crack PJ, et al. Suppressor of cytokine signaling 1 negatively regulates Toll-like receptor signaling by mediating Mal degradation. *Nat Immunol* 2006;7:148–155. [PubMed: 16415872]
21. Sengupta D, Koblansky A, Gaines J, Brown T, West AP, Zhang D, et al. Subversion of innate immune responses by Brucella through the targeted degradation of the TLR signaling adapter, MAL. *J Immunol* 2010;184:956–964. [PubMed: 20018612]
22. Low LY, Mukasa T, Reed JC, Pascual J. Characterization of a TIR-like protein from *Paracoccus denitrificans*. *Biochem Biophys Res Commun* 2007;356:481–486. [PubMed: 17362878]
23. Bowie A, Kiss-Toth E, Symons JA, Smith GL, Dower SK, O'Neill LA. A46R and A52R from vaccinia virus are antagonists of host IL-1 and toll-like receptor signaling. *Proc Natl Acad Sci USA* 2000;97:10162–10167. [PubMed: 10920188]
24. Stack J, Haga IR, Schroder M, Bartlett NW, Maloney G, Reading PC, et al. Vaccinia virus protein A46R targets multiple Toll-like-interleukin-1 receptor adaptors and contributes to virulence. *J Exp Med* 2005;201:1007–1018. [PubMed: 15767367]
25. Seet BT, Johnston JB, Brunetti CR, Barrett JW, Everett H, Cameron C, et al. Poxviruses and immune evasion. *Annu Rev Immunol* 2003;21:377–423. [PubMed: 12543935]
26. Johnston JB, Barrett JW, Nazarian SH, Goodwin M, Ricciuto D, Wang G, et al. A poxvirus-encoded pyrin domain protein interacts with ASC-1 to inhibit host inflammatory and apoptotic responses to infection. *Immunity* 2005;23:587–598. [PubMed: 16356857]
27. Rahman MM, Mohamed MR, Kim M, Smallwood S, McFadden G. Co-regulation of NF-kappaB and inflammasome-mediated inflammatory responses by myxoma virus pyrin domain-containing protein M013. *PLoS Pathog* 2009;5:e1000635. [PubMed: 19851467]
28. Dorfleutner A, Talbott SJ, Bryan NB, Funya KN, Rellick SL, Reed JC, et al. A Shope Fibroma virus PYRIN-only protein modulates the host immune response. *Virus Genes* 2007;35:685–694. [PubMed: 17676277]
29. Stehlik C, Dorfleutner A. COPs and POPs: modulators of inflammasome activity. *J Immunol* 2007;179:7993–7998. [PubMed: 18056338]
30. Schroder K, Tschopp J. The inflammasomes. *Cell* 2010;140:821–832. [PubMed: 20303873]
31. Hornung V, Ablasser A, Charrel-Dennis M, Bauernfeind F, Horvath G, Caffrey DR, et al. AIM2 recognizes cytosolic dsDNA and forms a caspase-1-activating inflammasome with ASC. *Nature* 2009;458:514–518. [PubMed: 19158675]
32. Ludlow LE, Johnstone RW, Clarke CJ. The HIN-200 family: more than interferon-inducible genes? *Exp Cell Res* 2005;308:1–17. [PubMed: 15896773]

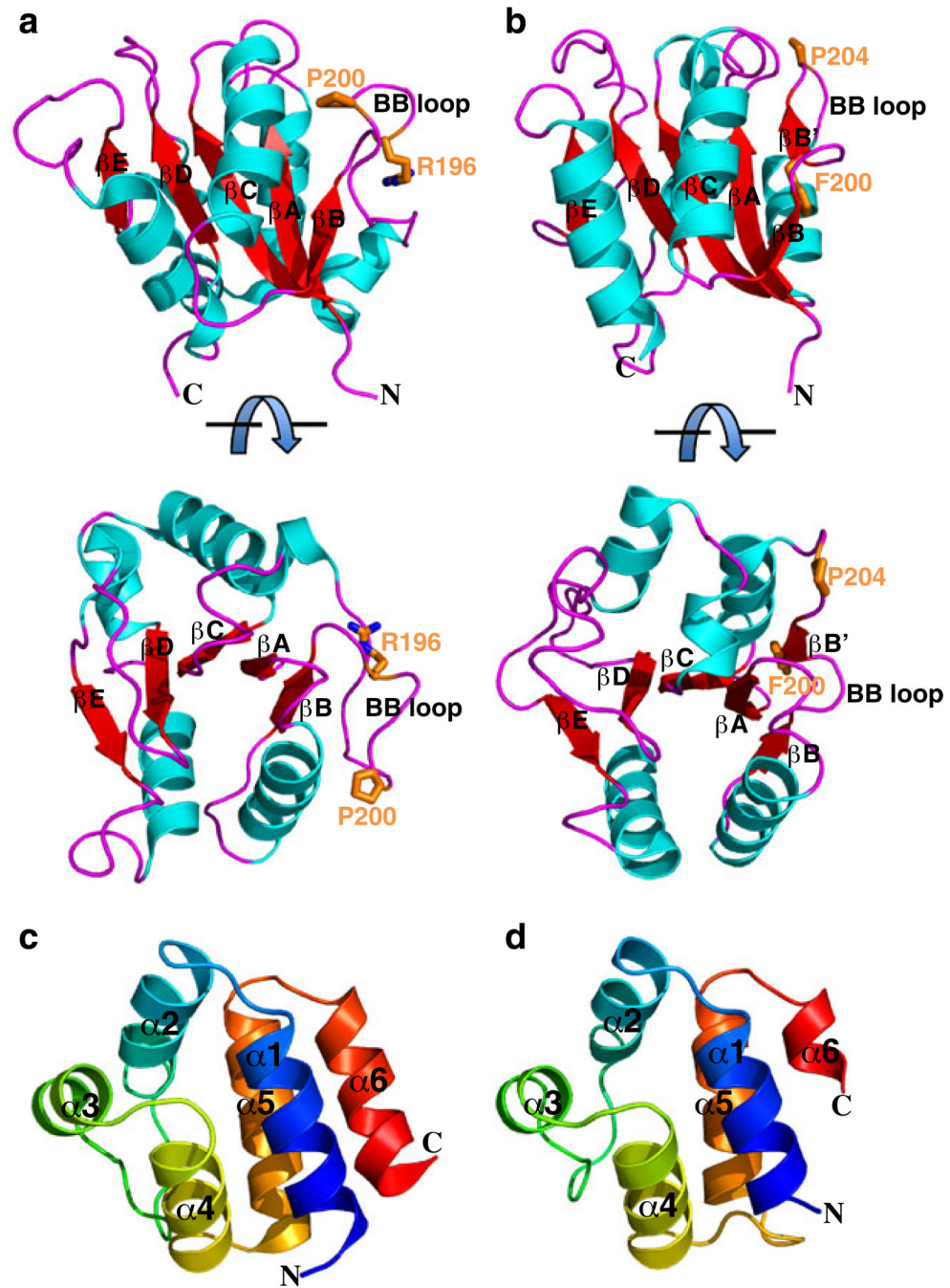


Fig. 1. Structures of the host and microbial TIR domains and PYD domains. The TIR domains of MyD88 (PDB code 2JS7) and PdTLP (PDB code 3H16) are shown as *ribbons* in **a** and **b**. The α helices are colored in *cyan* and β strands in *red* and labeled. The two different views of the structures are related by an approximately 90° rotation along the horizontal axis. The BB loop and two residues at the BB loop known to be indispensable for TIR domain function (R196 and P200 in MyD88 and the equivalent F200 and P204 in PdTLP) are labeled. The PYD domains of ASC (PDB code 1UCP) and Myxoma virus M013L (a structural model) are shown in **c** and **d**. The structures are in *rainbow colors* from their N-termini to C-termini. The six α helices are labeled