

Characterization of *Borrelia burgdorferi* tetratricopeptide repeat (TPR) containing proteins

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Abstract

Borrelia burgdorferi is the causative agent of Lyme disease, the most common vector-borne illness in the United States, affecting around 476,000 people annually with no human vaccine currently available. Unlike other pathogens, *B. burgdorferi* lacks classical virulence factors; instead, its pathogenesis depends on the ability to invade, disseminate, and persist in host tissues. Proteins containing tetratricopeptide repeats (TPRs) in *B. burgdorferi* have been shown to contribute to pathogenesis through motility, immune evasion, persistence, and membrane integrity. TPR motifs are 34 amino acid-long, alpha-helical structural motifs found in a variety of proteins that mediate protein-protein interaction. It is defined by a consensus sequence of small and large hydrophobic residues that forms a scaffold for protein binding. In bacteria, TPR-containing proteins have been shown to play roles in numerous cellular processes, including virulence. *B. burgdorferi* has 23 proteins annotated as putatively containing TPRs, out of which six have been previously characterized as important in pathogenesis. I hypothesize that the 17-remaining TPR-containing proteins of *B. burgdorferi* also have roles in pathogenesis. This research focuses on characterizing these putative TPR-containing proteins in *B. burgdorferi*. I began by confirming that all 17 of these proteins contain TPR motifs through the identification of consensus sequences and examination of predicted protein structures using web-based toolkits. My analysis suggests that each *B. burgdorferi* protein possesses between one and nine TPR motifs which form characteristic antiparallel α -helical structure that are potential sites for protein binding. The identified TPR motifs include variably conserved consensus residues which may prove useful in future studies to determine the contribution of such residues in protein binding. Moreover, I was successful in purifying one of these *B. burgdorferi* TPR-containing proteins, BB0133. BB0133 is a hypothetical protein containing 5 TPRs that is predicted to reside in the cytoplasm. Purified BB0133 will be used in future studies for the identification of its binding partners. Finally, I investigated the transcriptional changes of the 17 TPR-containing *B. burgdorferi* proteins in response to environmental conditions. I found that two of the TPR-containing genes, *bbj23* and *bbj24*, were upregulated under *in vitro* mammalian-mimicking conditions, suggesting they are potential candidates for further studies into their roles in mammalian infection. Overall, this research has helped to build a foundation in the characterization of *B. burgdorferi* TPR-containing proteins and in the future determining their roles in pathogenesis.

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Chapter 1 - Introduction

Lyme disease

Epidemiology of Lyme disease

Lyme disease is the most common vector-borne disease in the United States and Europe, with annual cases of approximately 476,000 in the United States and 650,000-850,000 cases in Europe (Alojz Peterle et al., 2018; Kugeler et al., 2021). The disease is mostly prevalent in the Northeastern, Mid-Atlantic, and upper Midwest regions of the United States as shown in Figure 1-1 (Mead, 2015). Lyme disease is caused by the bacterial pathogen *Borrelia burgdorferi* which is transmitted through the bite of an infected *Ixodes scapularis* tick. Lyme disease is spreading due to climate change, which has influenced the number of tick populations, their survival, and the availability of hosts (Dumic & Severnini, 2018). In the coming decades, it is predicted that cases of Lyme disease will increase by 20 percent in the United States (Dumic & Severnini, 2018).

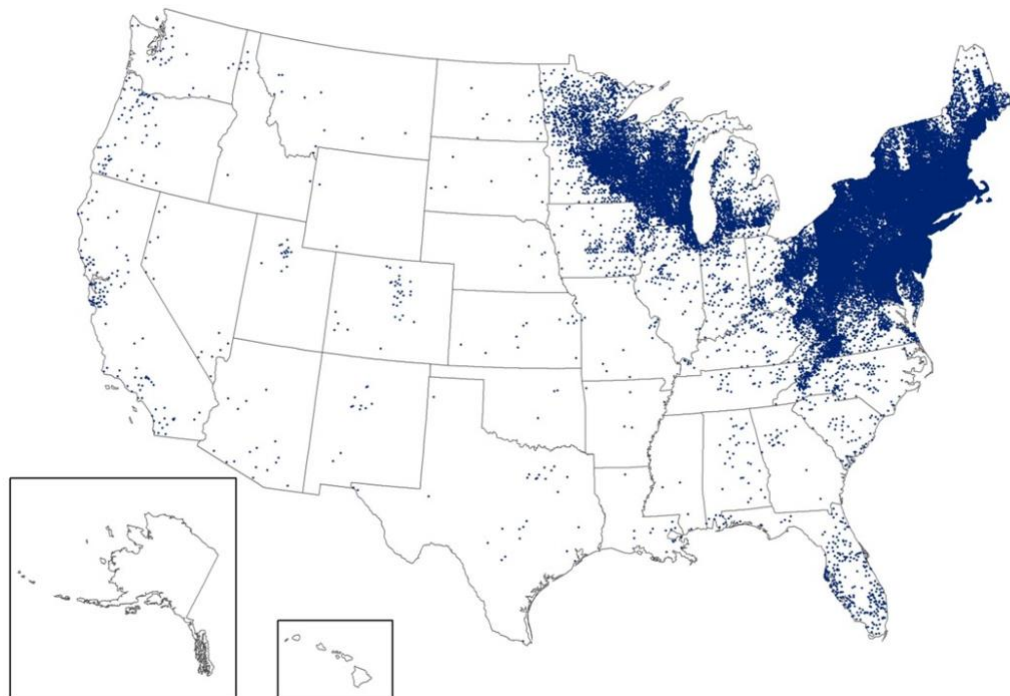


Figure 1-1. Reported cases of Lyme disease in the United States in 2023 according to the Center of Disease Control and Prevention.

Each dot represents the reported cases from the infected person's county of residence.

Transmission of Lyme disease

Multiple species of *Ixodes* ticks can transmit *B. burgdorferi* among animals; however, only four species commonly bite humans. In the United States the blacklegged or deer tick, *Ixodes scapularis*, is known as the principal vector for transmitting *B. burgdorferi*. In Europe, *Ixodes ricinus* is responsible for transmitting *B. burgdorferi* whereas in Asia, it is *Ixodes persulcatus* (Kurtenbach et al., 2006).

The nymphal *Ixodes* tick is responsible for the majority of human infections because of their small size which makes them harder to detect and remove before transmission occurs (Helble et al., 2021a; Radolf et al., 2012). The tick needs to remain attached and feed for an extended period, typically around 36 to 48 hours, before the *B. burgdorferi* spirochete migrates from the tick's midgut to its salivary glands and into the host (Helble et al., 2021b).

Symptoms of Lyme disease

After the spirochete is transmitted into the human body, Lyme disease progression occurs in three different stages: early localized Lyme disease, early disseminated Lyme disease, and late disseminated Lyme disease, with diverse symptoms and different clinical manifestations at each stage (Steere et al., 2004; Strnad et al., 2023). Early localized Lyme disease appears within 3 to 30 days after the bacterial transmission through the bite of an infected tick and begins with a skin lesion, called erythema migrans, that forms at the site of the tick bite in around 70% of patients (Schwartz et al., 2017). This initial phase of Lyme disease is accompanied by flu-like symptoms such as fever, fatigue, headache, and musculoskeletal pain (Steere et al., 1983).

However, if the disease remains left untreated, 3 to 12 weeks after initial infection, *B. burgdorferi* will disseminate into secondary tissues and organs via the bloodstream, leading to early disseminated Lyme disease (Biesiada et al., 2012). During this stage, bacteria may enter the joints, tendons, and cardiac tissues, leading to musculoskeletal pain, swelling of large joints, and carditis - also known as Lyme carditis (Bitar & Lally, 2008; Schwartz et al., 2017). The clinical symptoms of early disseminated Lyme disease may include cardiac symptoms (chest pain, heart palpitation, and shortness of breath) and neurological symptoms (headache, dizziness, meningitis, and facial palsy) (Skar et al., 2025).

Months after the initial infection, the late stage of Lyme disease may occur. The typical features at this stage include neurological and musculoskeletal issues like Lyme arthritis. Patients may also experience cognitive defects like loss of memory and difficulty in concentration (Bitar & Lally, 2008; Gray et al., 2002; Steere et al., 1983).

Diagnosis of Lyme disease

Lyme disease is diagnosed by exposure history and laboratory testing. In early stages of infection, a patient with an erythema migrans and plausible tick exposé receives an immediate clinical diagnosis, since antibody tests are often negative during this stage (R. P. Smith et al., 2002; Wright et al., 2012). For patients without the rash, diagnosis relies on a two-tiered serologic test. The first tier is a sensitive enzyme immunoassay that screens for antibodies against *B. burgdorferi*. Samples that test positive or unclear move to a second tier, a western immunoblot (Branda et al., 2017). This two-step process keeps specificity above 99% and screens out false positives caused by antibodies related to other bacteria and viruses. The test works well once the immune response builds up in later stages of the disease, correctly identifying >90% of the cases. However, in the

early stage it catches only 30-50% of early infections (Waddell et al., 2016). This gap in early-stage detection highlights the need of better diagnostic.

Treatment and prevention of Lyme disease

Antibiotic treatment is used to treat early infection and prevent progression to later stages. In the early stages of Lyme disease, a course of oral antibiotics, most commonly doxycycline or a beta-lactam such as cefuroxime axetil or amoxicillin, is used for 14 to 28 days to resolve the symptoms (R. P. Smith et al., 2002; Wright et al., 2012). However, months after the antibiotic treatment, approximately 10–20% of patients continue to experience fatigue, musculoskeletal pain, and cognitive difficulties which is known as post-treatment Lyme disease syndrome (PTLDS) (Aucott et al., 2013; Marques, 2008). The etiology of PTLDS remains unresolved. The proposed mechanisms include persistence of residual bacterial debris that sustain immune stimulation, autoimmune responses, and tissue damage that incurred during active infection (Bobe et al., 2021; Rebman & Aucott, 2020).

Since there is no human vaccine currently available, the disease could be prevented by avoiding tick-infested habitats, particularly wooded areas during spring and summer, using repellants such as DEET, and wearing protective clothing such as long sleeves and light-colored fabric to limit skin exposure and ease tick detection (Biesiada et al., 2012; Gray et al., 2002). Likewise, regular tick checks and removal of ticks before 24 hours of attachment is helpful given that transmission of *B. burgdorferi* typically requires around 36-48 hours of attachment (Gray et al., 2002).

Enzootic cycle

Ixodes scapularis undergoes four developmental stages during its life cycle: egg, larva, nymph, and adult as shown in Figure 1-2 (Helble et al., 2021a; Radolf et al., 2012). The life cycle

spans approximately two years, during which the tick takes a single blood meal at each stage. Ticks hatch from uninfected eggs, as *B. burgdorferi* is not transmitted transovarially from adult ticks to their offspring. Instead, ticks acquire *B. burgdorferi* while feeding on infected reservoir hosts, primarily mice, other small mammals, and birds (Steere et al., 2004).

Once infected, ticks can transmit the pathogen to subsequent hosts, including humans (Radolf et al., 2012). Humans are considered incidental or dead-end hosts, as they do not contribute to further transmission of the pathogen (Helble et al., 2021a; Kurtenbach et al., 2006). Adult ticks feed preferentially on deer, which are regarded as incompetent hosts because they do not sustain infection and can clear the pathogen (Radolf et al., 2012). Nevertheless, deer play a critical role in the ecology of Lyme disease by supporting tick reproduction and facilitating the geographic dispersal of ticks (Radolf et al., 2012).

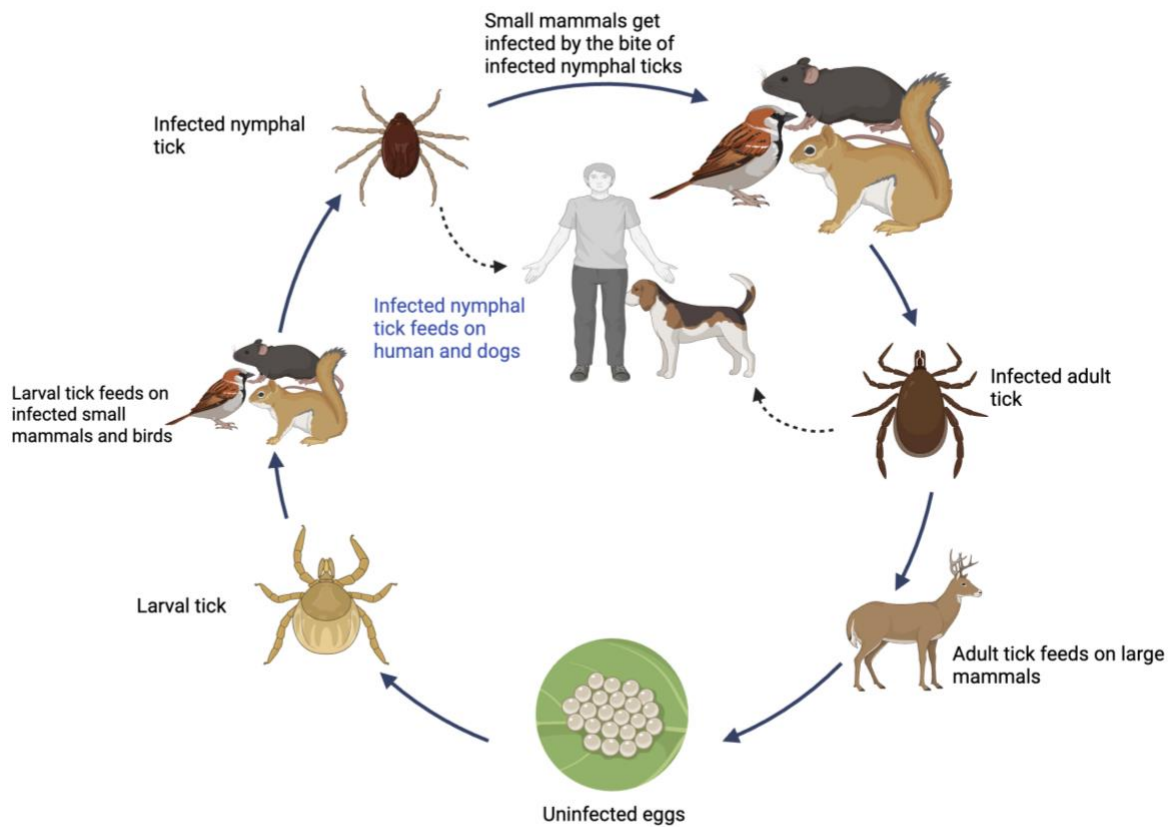


Figure 1-2. Enzootic cycle of *B. burgdorferi*.

Newly hatched uninfected larval ticks seek a host to feed on such as small mammals or birds. Since *B. burgdorferi* is not transmitted transovarially, this is the first opportunity for *B. burgdorferi* to enter the tick as it feeds upon infected small mammals. Once fed, the six-legged larva molts into an eight-legged nymph which may be infected with *B. burgdorferi* acquired during their first blood meal. Infected nymphal ticks can transmit *B. burgdorferi* to subsequent hosts that include humans and domestic animals. After the fed nymph molts into an adult stage, they search for large animals like white tailed deer for a final blood meal and mating. Infected adult ticks can also feed upon humans and domestic animals to transmit *B. burgdorferi*. This figure is adapted from Radolf et al., 2012 and was made using BioRender.

Borrelia burgdorferi

***B. burgdorferi* genomics**

The unusual genome architecture of *B. burgdorferi* underlies much of its ability to persist and adapt within tick vectors and mammalian hosts. Unlike most other bacteria, which carry a single circular chromosome, *B. burgdorferi* possess a linear chromosome of approximately 910 kilobases accompanied by 21 additional plasmids comprising 12 linear and 9 circular plasmids (Casjens et al., 2000). Together, these plasmids constitute more than 40% of the organism's total genetic material, the largest plasmid complement documented in any characterized bacterium (Casjens et al., 2000).

Research indicates that plasmid-derived functions are essential to spirochete infectivity and transmission, however many of the genes encoded by these plasmids are still poorly understood and their roles are unknown (Schwan et al., 1988). Although the plasmid content varies somewhat among natural isolates, circular plasmid cp26 is essential under all growth conditions tested (Byram et al., 2004). Likewise linear plasmid lp25 is required for the survival of spirochete in mice (Purser et al., 2003).

***B. burgdorferi* pathogenesis**

Unlike other bacterial pathogens, *B. burgdorferi* lacks classical virulence factors, such as toxins and secretion systems, to cause disease (Coburn et al., 2021). Notably, *B. burgdorferi* does not produce or secrete any known toxins, and its outer membrane lacks the lipopolysaccharide found in most Gram-negative bacteria. Instead, *B. burgdorferi* pathogenesis depends on its ability to invade, disseminate, and persist in host tissues (Brissette & Gaultney, 2014; Coburn et al., 2021; Strnad et al., 2023).

B. burgdorferi exhibits a dual nature of being able to survive in both mammalian and tick hosts by carefully modulating its gene expression (Radolf et al., 2012). This allows the spirochete to adapt to changes in the environment and nutritional availability during transmission between two different hosts. These transitions are largely governed by two regulatory circuits: the Hk1/Rrp1 pathway that includes c-di-GMP and controls gene expression within the tick, and the RpoS pathway that activates the genes required for mammalian infection as shown in Figure 1-3 (Rogers et al., 2009; Ouyang et al., 2012).

Once deposited into the skin, *B. burgdorferi* adheres to host tissues, disseminates through the bloodstream, and evades the immune response to establish infection (Radolf et al., 2012). The spirochete produces numerous surface adhesins, that mediate binding to host cells and extracellular matrix components and enable dissemination into host tissue (Fischer et al., 2003; Probert & Johnson, 1998; Radolf et al., 2012). Once there, *B. burgdorferi* can survive in protected tissues for months or years (Auwaerter, 2011; Radolf et al., 2012). The lasting infection slowly damages nearby tissue which drives the late-stage symptoms of Lyme disease such as Lyme arthritis.

RpoS pathway

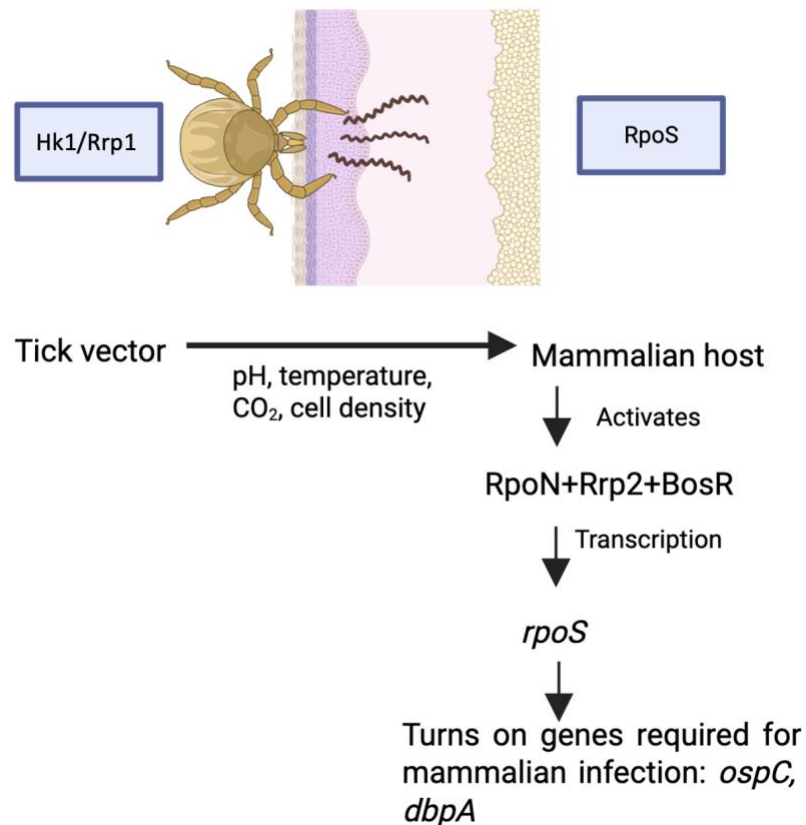


Figure 1-3. The RpoS pathway activates *B. burgdorferi* mammalian infection genes.

The ability of *B. burgdorferi* to adapt to the mammalian environment is governed by the RpoS pathway. Sigma factor RpoN, along with Rrp2 and BosR, control the transcription of *rpoS*. RpoS then goes on to activate numerous important mammalian infection genes. This figure was made using BioRender.

One factor that influences *B. burgdorferi* pathogenesis is the regulatory pathway RpoS that is involved in mammalian infection. The RpoS pathway is switched on *in vitro* by environmental cues that mark the transition from tick to mammal, including increased temperature, reduced pH, higher cell density, and host-derived signals encountered during tick feeding, Figure 1-3 (Caimano et al., 2007; Ouyang et al., 2008). This pathway is a regulatory cascade built around two alternative sigma factors, RpoN (σ_{54}) and RpoS (σ_S) (Hübner et al., 2001; Yang et al., 2003). RpoN, together with the response regulator Rrp2 and BosR transcribes the *rpoS* gene as shown in Figure 1-3

(Hyde, 2017; Ouyang et al., 2009, 2011). The resulting RpoS sigma factor directs RNA polymerase to a specific set of promoters, turning on the genes required for mammalian infection (Caimano et al., 2007; R. P. Smith et al., 2002). RpoS functions as the gatekeeper of host adaptation. It activates mammalian-phase lipoproteins such as *ospC* and *dbpA* while simultaneously repressing tick-phase genes such as *ospA*, producing the reciprocal OspA-to-OspC switch that accompanies transmission to the host (Caimano et al., 2007).

Tetratricopeptide Repeats (TPRs)

TPR structure

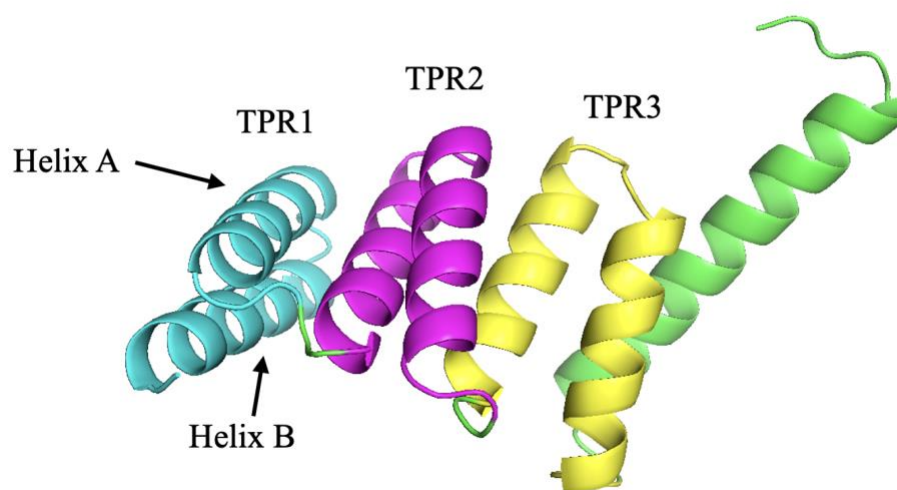


Figure 1-4. Ribbon diagram of the TPR-containing protein phosphatase 5 (PP5).

PP5 was the first TPR-containing protein whose structure was determined by X-ray crystallography at 2.3 Å resolution (PDB ID 3ICF). This protein illustrates the general structure of TPRs, with one shown in cyan, magenta, and yellow color. Each TPR motif is composed of two antiparallel α -helices, designated helix A and helix B, and all three motifs are structurally nearly indistinguishable from one another. They are arranged in parallel, so that neighboring α -helices in sequence run antiparallel to each other. Helix A and helix B are connected by a small linker which gives a helix-turn-helix model of TPR motifs. This figure was made using pyMOL Molecular Graphics System, version 3.1.

The tetratricopeptide repeat (TPR) is a 34-amino acid-long structural motif. TPR motifs were first recognized in the cell-division cycle proteins of *Saccharomyces cerevisiae* (D'Andrea & Regan, 2003; Sikorski et al., 1990). TPR motifs are arranged in tandem arrays of three to sixteen (or more) copies that fold together into a single elongated domain (Main et al., 2003). TPR motifs serve as a molecular scaffold that mediates protein-protein interactions and coordinates the assembly of larger multiprotein complexes (Blatch & Lassle, 1999; D'Andrea & Regan, 2003; Sikorski et al., 1990). TPR motifs have a characteristic pattern of small and large hydrophobic residues distributed across the 34 amino acids, with the TPR sequence centered around W₄-L₇-G₈-Y₁₁-A₂₀-F₂₄-A₂₇-P₃₂. Small hydrophobic residues such as alanine or glycine are found at positions 8, 20, and 27, while large hydrophobic molecules are found at the position 4, 7, 11, 24, and 32 (de Boer et al., 1994; Scheufler et al., 2000a; Sikorski et al., 1990). Each TPR motif folds into a helix-turn-helix unit composed of two antiparallel α -helical structures designated as helix A and helix B, connected by a small turn as shown in Figure 1-4.

The two antiparallel regions of a TPR fold and at a characteristic angle of approximately 24° (Das et al., 1998; Main et al., 2003; Scheufler et al., 2000a). The helix-turn-helix model of the TPR motif is packed together into a right-handed superhelix, generating a domain with both concave and convex surfaces (Cervený et al., 2013; Das et al., 1998). Ligand binding occurs mainly on the concave surface, which form a groove that accommodates the binding of the target protein or ligand, whereas the convex surface is largely solvent exposed and is generally not involved in canonical ligand recognition (Das et al., 1998; Scheufler et al., 2000b).

TPR function

TPR-containing proteins participate in a wide range of cellular processes like cell cycle regulation, transcriptional control, chaperon activity, protein translocation, and vesicular transport

(Blatch & Lässle, 1999; D'Andrea & Regan, 2003; Edqvist et al., 2006a; Sikorski et al., 1990). In bacteria, proteins containing TPRs possess similarly widespread functions and are frequently linked to pathogenesis (Cervený et al., 2013).

TPR-containing proteins in microbes

TPRs play numerous roles in microbes. One role is protein folding and delivery: TPR motifs serve as a chaperone of the type III secretion system in gram-negative bacteria like *Pseudomonas aeruginosa* and *Yersinia pestis*, to facilitate the establishment of infection by delivering effector proteins into host cells (Brooks et al., 2006; Edqvist et al., 2006b; Kondo et al., 2010). These chaperones use their concave groove to bind unfolded effector proteins and for secretion. Substrate binding by the TPR motif is the mechanistic step that helps make secretion possible, without this binding step, effectors degrade or aggregate before they ever reach the host cell (Dieppedale et al., 2011; Edqvist et al., 2006).

Beyond their role as secretion-system chaperones, TPR-containing proteins act directly as virulence factors at several distinct steps of pathogenesis, and in each case the TPR motif supplies the protein-protein interaction surface on which the function depends (Blatch & Lässle, 1999). For example, TPR-containing proteins contribute towards pathogenesis through factors like motility, immune invasion, and multiprotein complex assembly. For example, flagellar component A (FlcA) of *B. burgdorferi* helps in the assembly of flagellar components that modulate the motility of the pathogen (Xu et al., 2020). Another example found in multiple microbes is BamD, which uses TPR motifs to help assemble the β -barrel assembly machinery that inserts proteins into the outer membrane (Gatsos et al., 2008).

TPR-containing proteins are also involved in mediating invasion of pathogens into host cells. In *Legionella pneumophila* the Sell-repeat TPR-containing protein LpnE promotes entry

into macrophages and epithelial cells and directs the bacterium into a replication-permissive vacuole. Truncating its C-terminal repeat units abolishes invasion, showing that the TPR repeat array itself is involved in mediating entry (Newton et al., 2007). Once internalized, bacteria must avoid detection by the host innate immune system, which would otherwise trigger inflammatory clearance. The *L. pneumophila* periplasmic TPR-containing protein EnhC accomplishes this by binding the soluble lytic transglycosylase SltL and restraining peptidoglycan turnover, thereby lowering release of the muramyl-peptide fragments sensed by the cytosolic receptor Nod1. This dampens NF- κ B driven immune activation and is required for efficient replication within macrophages (Song et al., 2012). In some pathogen, persistence into host cell depends on TPR-containing proteins, as seen for the TPR-like proteins of *Francisella tularensis* that are required for replication and virulence within the macrophage cytosol (Cervený et al., 2013; Dieppedale et al., 2011).

TPR motifs also contribute to pathogenesis by organizing the regulation of virulence genes: in the periodontal intracellular pathogen *Porphyromonas gingivalis*. Three TPR motifs of TprA assemble a complex with the associated proteins TapA, TapB, and TapC that is required for full virulence, and loss of *tprA* both downregulates this gene cluster and impacts on bacteria's virulence ability in the murine model (Kondo et al., 2010). These examples of host-cell invasion, immune evasion, persistence, and virulence-gene regulation highlight the importance of TPR-mediated protein-protein interactions in bacterial pathogenesis and supports their potential as an attractive target for therapeutic intervention. (Blatch & Lässle, 1999; Cervený et al., 2013).

Chapter 2 - Characterization of *B. burgdorferi* tetratricopeptide repeat (TPR) containing proteins

Introduction

Borrelia burgdorferi, is the causative agent of Lyme disease. For *B. burgdorferi* to cause disease, it needs to be able to adhere to host tissues, disseminate through the bloodstream, evade the host immune response, and persist within host tissue across its dual-host enzootic life cycle (Brissette & Gaultney, 2014; Coburn et al., 2021). One factor that influence the pathogenic ability of *B. burgdorferi* is the presence of TPR motifs. There are six proteins in *B. burgdorferi* that have previously been identified as containing TPRs and were found to be involved in pathogenesis directly or indirectly through functions like motility, immune invasion, persistence, and membrane integrity (Chang et al., 2021; Groshong et al., 2014; Moon et al., 2018; Xu et al., 2020; Yang et al., 2016a; Zhuang et al., 2018a).

TPR-containing proteins FlcA (BB0326), FlcB (BB0058), and BB0236 contribute towards *B. burgdorferi* pathogenesis through motility (Chang et al., 2021; Moon et al., 2018; Xu et al., 2020). Motility in *B. burgdorferi* is driven by periplasmic flagellum which possess a unique component, the flagellar collar, that is essential for assembly of flagella and motility of the spirochete (Chang et al., 2021). The TPR motif present in FlcA (flagellar collar protein A) provides a surface for protein-protein interaction, resulting in two protein binding partners, MotB and FliL, which are also components of the flagellar collar (Xu et al., 2020). FlcB (flagellar collar protein B) binds with another flagellar component, FlcC, which together are important for generation of torque that is required for spirochete motility (Chang et al., 2021). Another flagellar TPR-containing protein, BB0236, is required for the proper assembly of the collar and overall flagellar complex. BB0236 binds with FlaB, which is the base component of the collar supporting the

spirochete in motility (Moon et al., 2018). Absence of flagellar TPR-containing proteins affects motility in *B. burgdorferi* which likely also has a downstream impact on its ability to colonize and disseminate in host tissues.

The *B. burgdorferi* TPR-containing protein BamD (BB0324) contributes to pathogenesis through impacting membrane permeability and integrity (Knowles et al., 2009). BamD is an accessory lipoprotein of the β -barrel assembly machine (BAM) which helps to catalyze the folding and insertion of β -barrel outer membrane proteins into the outer membrane (Dunn et al., 2015a). *B. burgdorferi* BamD consists of conserved TPR motifs comparable to the BamD homologs in *Neisseria meningitidis* and *Escherichia coli*. Previous studies have shown that *B. burgdorferi* BamD binds with other BAM components BamA (BB0795) and BB0028 (Brangulis, 2026a; Lenhart et al., 2012; Volokhina et al., 2009). This interaction was further supported by structural modelling of the *B. burgdorferi* BAM complex which predicted that BamD binds directly to the periplasmic domain of BamA (Brangulis, 2026a). In *B. burgdorferi*, loss of BamD increases the membrane permeability and sensitivity to antimicrobials, indicating its role in membrane integrity and outer membrane protein localization (Dunn et al., 2015b; Lenhart et al., 2012).

BB0238 is a TPR-containing protein that contributes to *B. burgdorferi* immune evasion. BB0238 is unique to *B. burgdorferi* with the N-terminal carrying the TPR motifs. Through this motif, BB0238 binds to the N-terminal region of BB0323, which is itself an essential virulence determinant required for persistence in mice (Kariu et al., 2015; Thakur et al., 2017). Protein-protein interaction of BB0323-BB0238 complex is necessary for long term survival of *B. burgdorferi* in mammals (Bista et al., 2026). The BB0238 TPR motif helps in stability of the overall protein and is involved in enhancing pathogen transmission from tick to mouse by promoting spirochete evasion of early host immune defense (Groshong et al., 2014; Kariu et al.,

2015). BB0238 also interacts with another *B. burgdorferi* protein, BB0108, which is a chaperone (Thakur et al., 2017).

Another TPR-containing protein is Lmp1 (surface-located membrane protein 1, BB0210) that contributes towards *B. burgdorferi* persistence in mice (Yang et al., 2016a; Zhuang et al., 2018a). Lmp1 also plays an essential role in spirochete transitions between mammalian host and tick vector (Koci et al., 2018). Lmp1 is divided into three regions: a N-terminal region (Lmp1N), a middle region (Lmp1M), and a C-terminal region (Lmp1C), among which Lmp1C consists of seven TPR motifs (Yang et al., 2016,). Lmp1 binds with a periplasmic serine protease BbHtrA (high-temperature requirement A) which is involved in stabilization of Lmp1 (Coleman et al., 2013; Kariu et al., 2015; Zhuang et al., 2018b). *B. burgdorferi* Lmp1 contributes to evasion of the host adaptive immune response and host-pathogen interaction (Koci et al., 2018; Yang et al., 2009, 2016b; Zhuang et al., 2018a).

Although six TPR-containing proteins have been characterized, *B. burgdorferi* encodes 17 more putative TPR-containing proteins (Table 2-1). The involvement of previously characterized TPR-containing proteins in *B. burgdorferi* pathogenesis through motility, persistence, immune evasion, and membrane integrity leads us to hypothesize that the remaining 17 putative TPR-containing proteins are also involved in pathogenesis. This chapter focuses on *in silico* characterization and transcriptional analysis of the 17 putative TPR-containing proteins, and recombinant expression of one of these putative TPR-containing proteins: BB0133.

Table 2-1. List of putative *B. burgdorferi* TPR-containing proteins.

TPR-containing protein
BB0106
BB0126
BB0133
BB0134
BB0171
BB0195

BB0209
BB0261
BB0298
BB0315
BB0398
BB0459
BB0537
BB0542
BB0714
BBJ23
BBJ24

Methods

In silico analysis

TPR motifs in each identified *B. burgdorferi* protein sequence were predicted using the web-based server TPRpred (Karpenahalli et al., 2007). The protein secondary structures were predicted using AlphaFold3 (Kurgan & Yang, 2025). PSORTb 3.0 was used to predict the potential location of a protein (Yu et al., 2010). SignalP6.0 was used to predict signal peptides present in proteins (Teufel et al., 2022). DeepTMHMM was used to predict transmembrane regions present in a protein structure (Hallgren et al., 2022). InterProScan was used to predict protein domains (Jones et al., 2014).

Bacterial strains and growth conditions

Strains used for this study are listed in Table 2-3. *B. burgdorferi* was grown in Barbour-Stoenner-Kelly II (BSKII) media supplemented with 6% normal rabbit serum. *E. coli* was grown in Luria-Bertani (LB) broth or Rich media (LB broth with 0.2% glucose) or supplemented with 100µg/mL ampicillin when needed.

Reverse transcriptase (RT) quantitative polymerase chain reaction (qPCR)

B. burgdorferi strain B31-A3 was grown under microaerophilic (32°C pH7.5 1%CO₂) or mammalian mimicking conditions (37°C pH6.8 5%CO₂) (Elias et al., 2002). The bacteria was grown to midlog phase ($5-8 \times 10^7$ *B. burgdorferi*/field) and then centrifuged at 4000 ×g for 20 minutes at 4°C. The supernatant was removed and the RNA pellet snap frozen before isolating RNA. *B. burgdorferi* RNA was isolated using a hot phenol chloroform isolation method as described in Lybecker et al., 2014. Total RNA was treated with DNase I (Takara) and 1µg of the DNase-treated RNA was converted to cDNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystem) according to the manufacturer's instructions. A no RT control was included for each sample when making cDNA. RT-qPCR was performed with 5ng cDNA using PerfeCTa SYBR Green FastMix Low ROX (Quantabio) according to manufacturer's instruction on a QuantStudio 5 Real-Time PCR system (Thermo Fisher). Primers used for RT-qPCR are shown in Table 2-2. *flaB* was used as an internal control and fold-change was calculated relative to 32°C pH7.5 1%CO₂ growth condition from three biological and two to three technical replicates using $2^{-\Delta\Delta CT}$ method (Livak & Schmittgen, 2001).

Table 2-2. List of primers used in this study.

Primer name	Sequence (5' to 3')
<i>flaB</i> -RT-qPCR-F	CAGCTAATGTTGCAAATCTTTTCTCT
<i>flaB</i> -RT-qPCR-R	TTCCTGTTGAACACCCTCTTGA
<i>bb0106</i> -RT-qPCR-F	TCTGAAAAAGACTATGAAGAAGCGG
<i>bb0106</i> -RT-qPCR-R	CTCACGCTCATAGGGGGTAG
<i>bb0126</i> -RT-qPCR-F	ACTTTGGAAATAAACAGCTCTGGC
<i>bb0126</i> -RT-qPCR-R	GGATTATGTAAAGAGTGGCGGAG
<i>bb0133</i> -RT-qPCR-F	AGCAAGCTCTGCAACAACATC
<i>bb0133</i> -RT-qPCR-R	GGTATGGGAAATTACGAGAGAGCA
<i>bb0134</i> -RT-qPCR-F	TCGTATCGGGAACCTTGGC
<i>bb0134</i> -RT-qPCR-R	AGCTTTTTCTTCTTTGCCCTGG
<i>bb0171</i> -RT-qPCR-F	ATTCAAACTATCGCGCACCG
<i>bb0171</i> -RT-qPCR-R	ATAAGGCCGAGGTGGCTTTC
<i>bb0195</i> -RT-qPCR-F	AGCTTCCTGTAAGATGCCGC
<i>bb0195</i> -RT-qPCR-R	TGTCTTGTAAGCATCCGAGC
<i>bb0209</i> -RT-qPCR-F	GGGGTGAAATCGACTACATA

<i>bb0209</i> -RT-qPCR-R	GAAGTTATTTTGCCCTGTTG
<i>bb0261</i> -RT-qPCR-F	AAAGATGTCCTAAAACACCTGGG
<i>bb0261</i> -RT-qPCR-R	CCAAAAGAGCTTCAATGTCGC
<i>bb0298</i> -RT-qPCR-F	CCAAGATCGCCCAGCGAATA
<i>bb0298</i> -RT-qPCR-R	ACTTGGTGATATGGGTGAGGAC
<i>bb0315</i> -RT-qPCR-F	AGCGGTAAAGAATTGTTTCAGAGA
<i>bb0315</i> -RT-qPCR-R	ACAATGTGTCGTCATTGAGTGC
<i>bb0398</i> -RT-qPCR-F	AATATGGTCCCAGCCCCTTGA
<i>bb0398</i> -RT-qPCR-R	CCACTATGATTAGGCTTGGGGG
<i>bb0459</i> -RT-qPCR-F	TGTTGGATAACAAAGACGAGGC
<i>bb0459</i> -RT-qPCR-R	TGCTGCTTGAAGAGATGCAC
<i>bb0537</i> -RT-qPCR-F	ATCGCCTCTTTTTCAGCTCTC
<i>bb0537</i> -RT-qPCR-R	TTTGGACAATCACCGCCTAAAG
<i>bb0542</i> -RT-qPCR-F	TTGCGCCTGCAAATAAAGAGG
<i>bb0542</i> -RT-qPCR-R	TTCCGCACCACTTGGTATAGG
<i>bb0714</i> -RT-qPCR-F	GCTTTGGAGTTTGGCTTATGTTAAT
<i>bb0714</i> -RT-qPCR-R	AGCACTGTCATAATCGCCTTT
<i>bbj23</i> -RT-qPCR-F	GGAGGTGCAGCTTTAAGCAAG
<i>bbj23</i> -RT-qPCR-R	GCAAGAGAAGTGTTGGCAAATG
<i>bbj24</i> -RT-qPCR-F	GGCATTGGGAGAGCTTGCTA
<i>bbj24</i> -RT-qPCR-R	GCTGTTTCTAAATGCCTCTTGGC
M13-F	GTAAAACGACGGCCAG
M13-R	CAGGAAACAGCTATGAC
pMAL-F	GGTCGTCAGACTGTTCGATGAAGCC
pMAL-R	TGTCCTACTCAGGAGAGCGTTCAC

Recombinant protein design

To produce recombinant BB0133 protein, the open reading frame was codon optimized without the start and stop codons for expression in *E. coli* through Integrated DNA technologies (IDT). The generated sequence was “gcgcgatatc TCA AGC GAG CGC ATC GTT GAA CTG ATC AAG GAA ATT TAT CTG GAT TTC CGC AAA GGC AAC TTC AAA GAA GCT CTG GTG AAA TCT GAA GAA GCG CAT TCA CTG GAC TTT GAT AAT ATC GAA ATT CTG ACC GCA CTG AAA TCG TCA GTT TAC TGG AAT GGC CAA GTA GAA AGC TTG GAC CGC ATT GGG CAG GAT TAT GAG AAG GCA GAA TTT CTG ATC CGT GAG TGG AAT AAT TTC GCA GGC CGC TAT CTG AAA AAA ATG GGC TGC AAC TTC CTT CAG GGT

CGT AAT AGT ATC AAA TAC TTC GTG TTT CAG ACG TGC CTG TAT ATT TAC AAA
AAC ATT TAC AAG ATG CAC CCG GAG AAT TTA GAC TTG CTG ATT AAA ATT GCA
AAA AGC TAT AAA GGC ATG GGT AAC TAC GAG CGT GCC ATC AAT GTT TTC CTG
CAA ATT CTG GGT GAC TTT AAA GAC AAC AGC GAC GTC GTA GCG GAA TTA GCA
GAT TCC TAC GCA CTT GTG GAC GAG ATC AAA GAA GCG AAA GTG CTT TTT CGC
GAA GCT TTT TTT ATT AAC CCG CAG AAA ATC GAT ATT TAT TCC CTG GAG AGT
GAC ATG ATC CTG CGT CTG ATT GAC CTT ATT AAG TCC GAT CGC AAC ATC TCC
GAC GAT CTG ATC AAA GAA TGG ATT CCA GTG TAT GGT AGT TTG AAT GGA GTT
TTC AAC ATT AAA CGC GAG TTA CGT CCT ATT GAA TTA GGT CAG CTT AAA CAG
AGC GTT TAT TCA CTT CGC AAT GAG CTG AAA GAA AAA AGT TAT CGC AGC ATT
AAT GAA TCT ATT CTG CTT CCT CGT CTG ATT AAC AAA TAT TTC TGG CTG ATC
GAT CAT TAT GTT CGT ATT AAA GAA GAC CGT GCC CGT ATC GAT GAA ATT TTA
CTG TAC ATT AAA GAA ATC GAT CTT GGA ATT TAC CAG CAA TAT GTT AAT
gaattcgcg”. The uppercase letters in the above sequence represent the codon optimized
nucleotides and the lower case represents the restriction enzyme EcoRI and EcoRV sites plus
buffer regions. Codon optimized BB0133 was inserted into pCR-Blunt to create pPB1 and elect-
transformed into Stellar *E. coli*. The resulting colonies were screened for BB0133 insertion using
M13-F and M13-R primers (Table 2-2). Once confirmed through colony PCR, pPB1 and pMAL-
c6T were digested using EcoRI and EcoRV. The resulting fragments were ligated together and
electro-transformed into NEBExpress *E. coli*. The resulting colonies were screened for insertion
using pMAL-F and pMAL-R primers (Table 2-2) and pPB30, pPB31, and pPB32 were selected to
move forward with for induction analysis. pPB30 was confirmed sequenced.

Table 2-3. List of strains and plasmids in this study.

Strain	Genotype	Reference
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<i>B. burgdorferi</i> strains		
B31-A3	wild type	Elias et al., 2002
<i>E. coli</i> strains		
Stellar	F-, endA1, supE44, thi-1, recA1, relA1, gyrA96, phoA, Φ 80d lacZ Δ M15, Δ (lacZYA-argF) U169, Δ (mrr-hsdRMS-mcrBC), Δ mcrA, λ -	Takara
NEBExpress	fhuA2 [lon] ompT gal sulA11 R(mcr-73::miniTn10--Tet ^S) [dcm] R(zgb-210::Tn10--TetS) endA1 Δ (mcrC-mrr)114::IS10	NEB
Plasmids		
pCR-Blunt	TOPO cloning vector	Invitrogen
pPB1	pCR-Blunt::BB0133	This study
pMAL-c6T	Protein purification vector	NEB
pPB30	pMAL-c6T::BB0133 Isolate #1	This study
pPB31	pMAL-c6T::BB0133 Isolate #2	This study
pPB32	pMAL-c6T::BB0133 Isolate #3	This study

IPTG induction and Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis (SDS-PAGE)

NEBExpress *E. coli* with pPB30, pPB31, pPB32, and pMAL-6cT were grown in Rich media containing 100 μ g/mL ampicillin. The overnight cultures were sub-cultured at 1:50 ratio and incubated for 2 hours until an OD₆₀₀ of ~0.5. Cultures were then induced using 0.5mM isopropyl β -D-1-thiogalactopyranoside (IPTG) and incubated at 37°C for 4 hours, or 18°C or room temperature overnight. The induced cells were harvested by centrifugation at 5000 xg for 10 minutes at 4°C and the pellets resuspended in 100 μ L of protein sample loading buffer. The prepared samples were run on a 12.5% SDS-PAGE. The gel was stained using Coomassie stain for 30 minutes on a shaker and destained overnight before being visualized.

Protein purification

NEBExpress *E. coli* with pPB30 or pMAL-c6T were grown overnight in Rich media containing 100 μ g/mL ampicillin. The overnight culture was sub-cultured at 1:50 ratio in LB broth

and was incubated for 2 hours until an OD600 ~0.5. The cultures were then induced with 0.5mM IPTG and incubated overnight with shaking at 18°C. Cells were harvested by centrifugation at 5000 xg for 20 minutes at 4°C. Collected cell pellets were resuspended in 100mL cold column binding buffer (20mM Tris-HCl pH7.4, 200mM NaCl, 1mM EDTA, 1mM DTT) per 1L of the induced culture. Cells were lysed using a microfluidizer at 15000 psi. The cell lysates were further diluted by adding around 200mL of column buffer into the microfluidizer. Cell lysates were clarified by centrifugation at 15000 xg for 30 minutes at 4°C and the supernatants collected. In a gravity column (Bio-Rad #7374011), 4mL of amylose resin (NEB #E8021) was added and allowed to settle before the cell lysate was run through at 4°C. The column was washed with 12 column volumes of cold column binding buffer. Bound recombinant protein was eluted with elution buffer (20mM Tris-HCl pH7.4, 200mM NaCl, 1mM EDTA, 1mM DTT, 10mM maltose). Eight sequential 1mL elution fractions were collected and analyzed on a 12.5% SDS-PAGE with subsequent Coomassie stain.

Bicinchoninic acid (BCA) assay

BCA assays were carried out for quantification of total protein concentration for all the collected protein elution fractions using a Pierce BCA Protein Assay Kit (Thermo Fisher) according to manufacturer's instructions. Absorbance was measured at 562 nm using an MR9600 microplate reader (Accuris SmartReader).

Statistical analysis

Statistical analysis was done using one-way ANOVA in GraphPad Prism (GraphPad Software, Inc., La Jolla, CA, United States). Significance was determined by p-value equal to or less than 0.05.

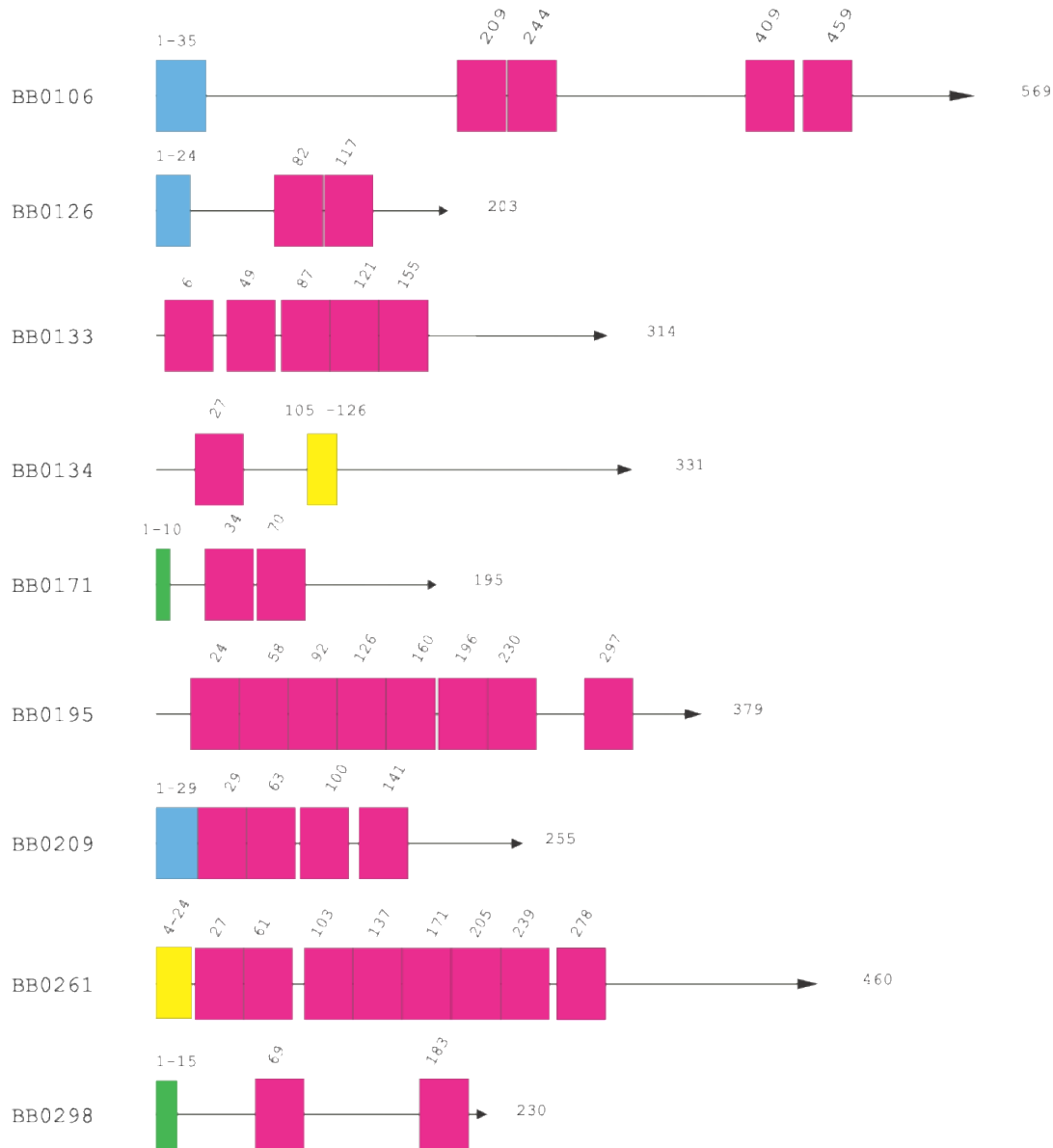
Result

Localization of TPR motifs across the predicted TPR-containing proteins

To begin the characterization of the 17 putative TPR-containing proteins Table 2-1, TPR sequences were identified using TPRpred and found to be a variable number per protein, ranging from 1 to 9 highlighted in magenta Figure 2-1. TPR motifs consist of 34 amino acids that center around W₄-L₇-G₈-Y₁₁-A₂₀-F₂₄-A₂₇-P₃₂ composed of small and large hydrophobic residues that form a scaffold for protein binding (Cervený et al., 2013; Scheufler et al., 2000b). We found that the TPR motifs of all 17 proteins had semi-conserved consensus residues as shown in Appendix Figure 4. The presence of semi-conserved consensus residues in all the 17 putative TPR-containing proteins suggest that these might form a groove for other proteins to interact. We also found that these identified sequences were present in tandem and aligned with the alpha helical antiparallel structure characteristic to TPRs as shown in Appendix Figure 5, which may be the potential site for protein-binding.

Further characterizing, we investigated additional domains found in the *B. burgdorferi* TPR-containing proteins. We found that eight of the TPR-containing proteins encode a N-terminal signal sequence, highlighted in blue in Figure 2-1 as predicted by SignalP, indicating these proteins are exported through the inner membrane to the periplasm or through the outer membrane. Two of the TPR-containing proteins contain a transmembrane region, highlighted in yellow in Figure 2.1 as predicted by DeepTMHMM, indicating they are membrane spanning proteins. Four of the TPR-containing proteins were identified as lipoproteins, highlighted in green in Figure 2.1 as predicted by PSORTb, indicating that they are embedded in the inner or outer membrane. It is predicted that the remaining three proteins, BB0133, BB0195, and BB0459, are cytoplasmic. These predicted protein localizations are supported by another web-based tool, InterProScan, indicating that a

subset of the TPR-containing proteins are cytoplasmic, some periplasmic, and some membrane-associated. Together, these predicted features suggest that the TPR-containing proteins in *B. burgdorferi* are distributed across multiple subcellular compartments and may participate in diverse cellular processes.



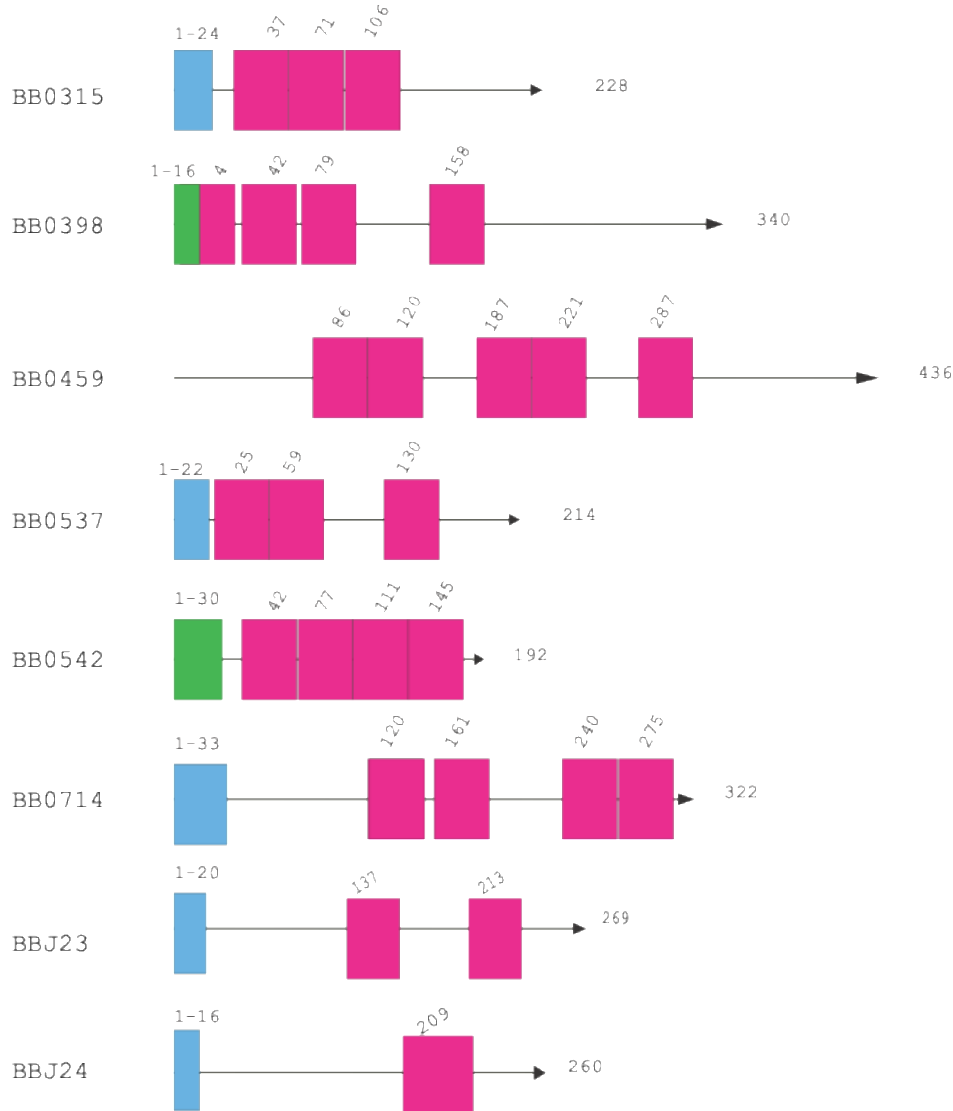


Figure 2-1. Localization of TPR motifs in *B. burgdorferi* proteins.

The name of each protein is given to the left and the total number of amino acids in the protein given to the right. The TPR motifs determined by TPRpred are depicted in magenta color. Numbers above the magenta-colored boxes refer to the first residue of the 34 amino acid long TPR sequence. Additionally, proteins have predicted transmembrane regions highlighted in yellow, signal sequences highlighted in blue, and lipoprotein regions highlighted in green.

Influence of environmental factors on transcription of *B. burgdorferi* TPR-containing proteins

To determine whether the TPR-encoding genes of *B. burgdorferi* are transcriptionally responsive to environmental cues, *B. burgdorferi* wild type strain was cultured under microaerophilic conditions (32°C pH7.5 1%CO₂) and mammalian-mimicking conditions (37°C pH6.8 5%CO₂). Transcript levels of each TPR-containing target were quantified by RT-qPCR. *flaB* was used as an internal control to normalize the expression and reported is the fold-change under mammalian-mimicking conditions relative to microaerophilic conditions, as shown in Figure 2-2. The chromosomally encoded TPR-containing targets showed a fold-change value at or below approximately one, indicating that their transcription was largely unchanged under mammalian host-like conditions. In contrast, two lp38 plasmid-encoded genes, *bbj23* and *bbj24*, were strongly upregulated under host-like conditions, with transcript levels rising approximately 70-fold and 12-fold, respectively. Together, these findings suggest that *bbj23* and *bbj24* are markedly induced under mammalian host-like conditions, identifying them as environmentally responsive genes that could be good candidates for future studies in mammalian virulence.

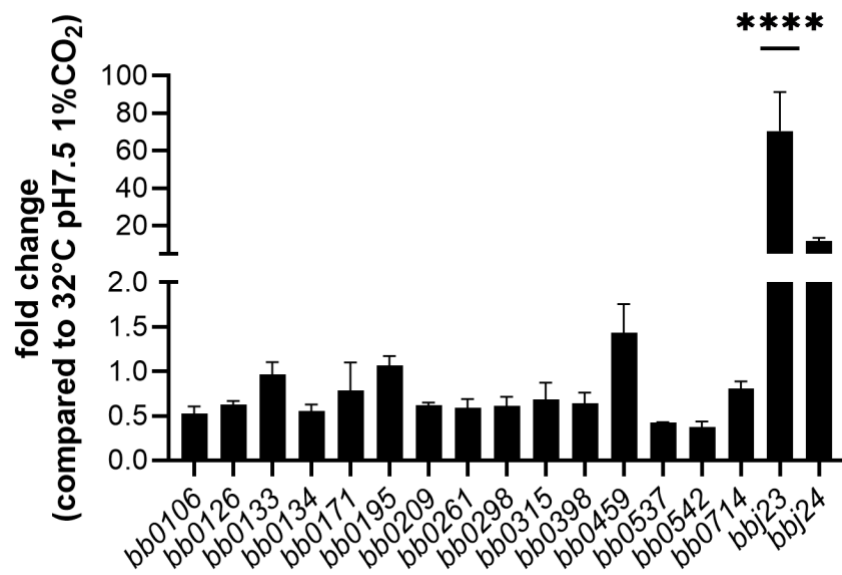


Figure 2-2. *B. burgdorferi* TPR-encoding genes *bbj23* and *bbj24* are regulated by environmental conditions.

B. burgdorferi wild type was grown in BSKII media to mid logarithmic phase under 32°C pH7.5 1%CO₂ (microaerophilic growth condition) and 37°C pH6.8 5%CO₂ (mammalian host-like condition). Transcript levels of the TPR-containing target genes were measured by RT-qPCR and are expressed as the fold change relative to 32°C pH7.5 1%CO₂. *flaB* served as the internal normalization control. Shown are the average and standard error of three biological replicates. ****=p-value <0.0001.

BB0133 protein purification construct

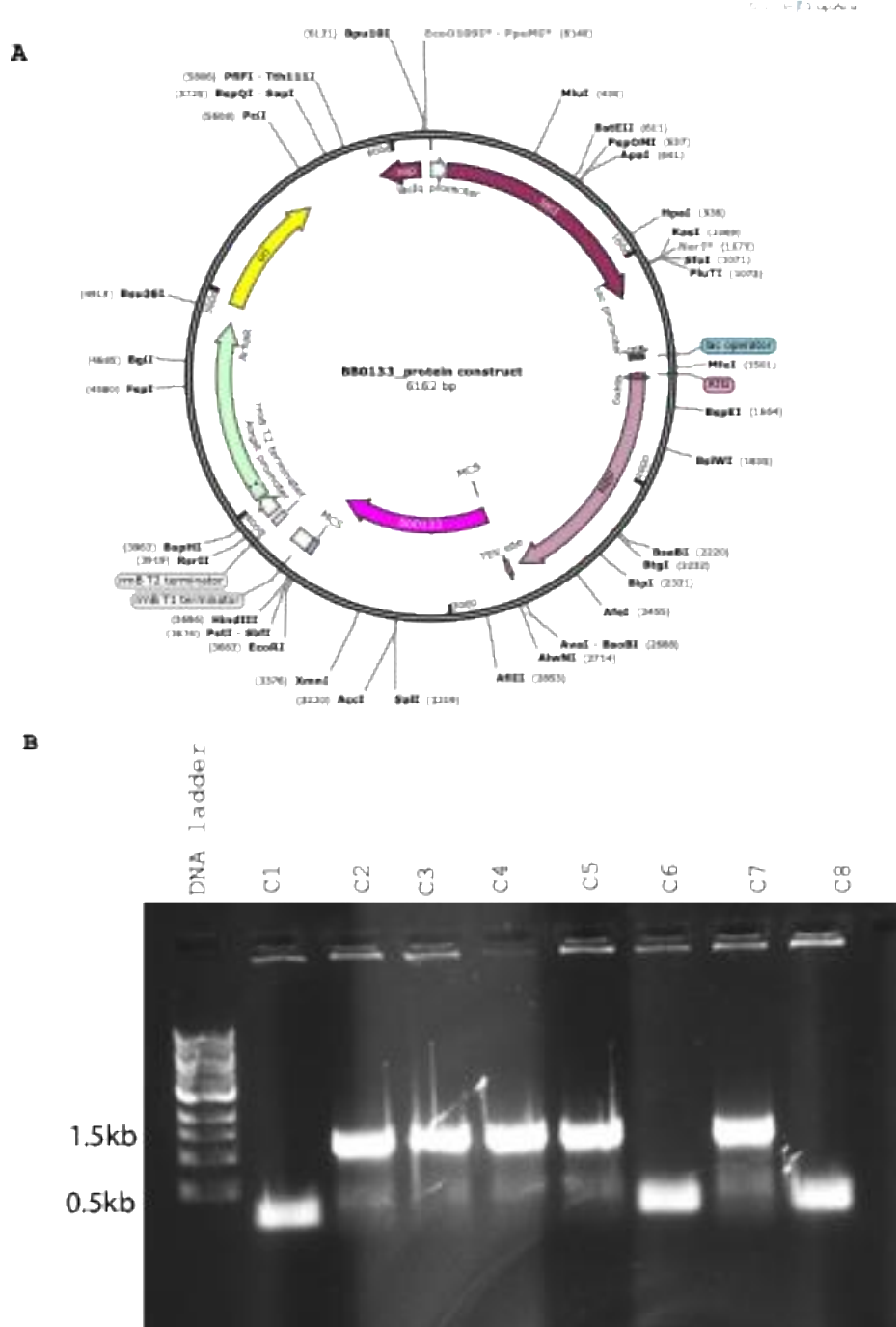


Figure 2-3. Recombinant protein construct for TPR-containing protein BB0133.

(A) Schematic of pPB30 plasmid with target protein BB0133 N-terminally tagged with MBP. (B) Colony PCR to screen for the BB0133 insert inside of the pMAL-c6T vector resolved on a 0.7% agarose gel. Colony (C) number is labeled on top of the gel image.

To N-terminally tag the codon optimized BB0133 protein fragment with MBP, protein purification construct pPB30 was designed as shown in Figure 2-3A. To generate this plasmid, we first cloned the codon optimized BB0133 gene fragment into a TOPO intermediate vector to create pPB1. The resulting pPB1 and pMAL-c6T were then restriction digested and the resulting fragments ligated together to create the BB0133-MBP fusion protein purification plasmid. This plasmid was introduced into an *E. coli* strain for protein purification and the colonies obtained were screened for BB0133 insertion into pMAL-c6T by PCR as shown in Figure 2-2B. The expected PCR product size of BB0133 insertion was 1184 bp. Colonies 2, 3, and 4 showed the expected size bands and were designated pPB30, pPB31, and pPB32 respectively.

BB0133 protein expression yield is highest at 18°C

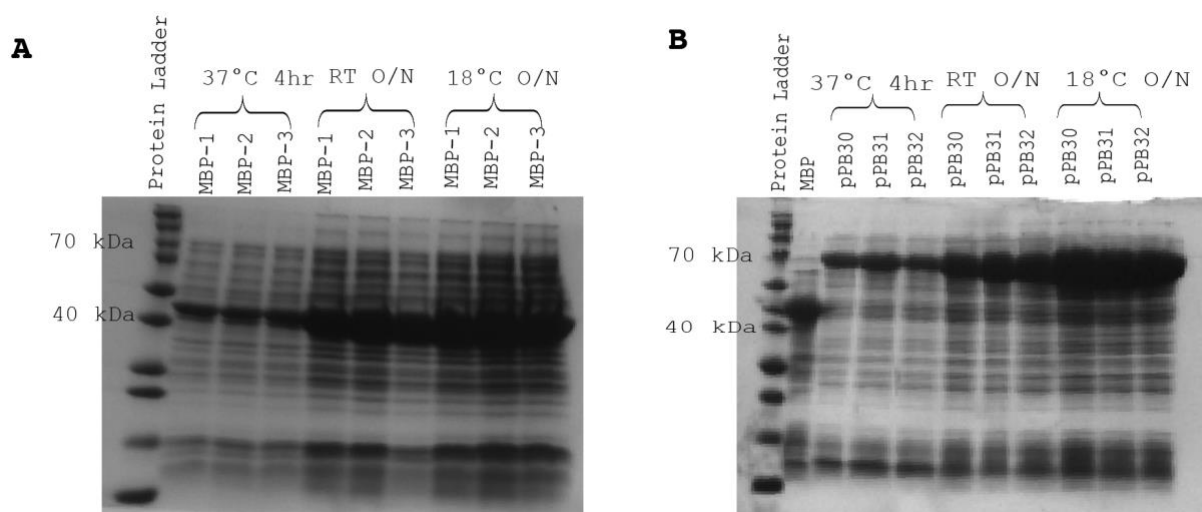


Figure 2-4. *B. burgdorferi* BB0133 production at various temperatures in *E. coli*.

(A) Three plasmid purification isolates of pMAL-c6T to produce MBP and (B) of pPB30, pPB31, and pPB32 to produce MBP fused BB0133 were induced with 0.5mM IPTG at different temperatures (37°C, room temperature (RT), and 18°C) to analyze protein production. Whole cell lysates were resolved by 12.5% SDS-PAGE. The temperature and time of induction are shown on top of the lanes and lane 1 is the molecular weight marker.

To evaluate recombinant protein expression, the maltose-binding protein (MBP) alone and the MBP-BB0133 fusion protein were induced with 0.5mM IPTG, and expression was screened under three conditions: 37°C for 4 hours, room temperature overnight, and 18°C overnight. Whole-

cell lysates were resolved by 12.5% SDS-PAGE and visualized with Coomassie stain Figure 2-4. As shown in Figure 2-4A, MBP alone produced a prominent band at approximately 42 kDa, across all three induction conditions. The intensity of the MBP band was found to increase under the overnight conditions, with the strongest expression observed at 18°C overnight, indicating that a lower-temperature induction extended overnight favored higher accumulation of the recombinant protein.

Similar induction for MBP-BB0133 fusion protein (Figure 2-4B) showed distinct band migrating at approximately 70 kDa in all induced samples, in all conditions consistent with the predicted mass of the fusion (BB0133 ~27 kDa, fused to MBP ~42 kDa), whereas the MBP-only control lane resolved at 42 kDa. Induction was highest at 18°C overnight, where the ~70 kDa band was most intense. Together, these results confirm that both MBP and the MBP-BB0133 fusion protein are successfully expressed in the NEBExpress *E. coli* host and that protein yield is highest under overnight induction at 18°C.

Purification and quantification of BB0133

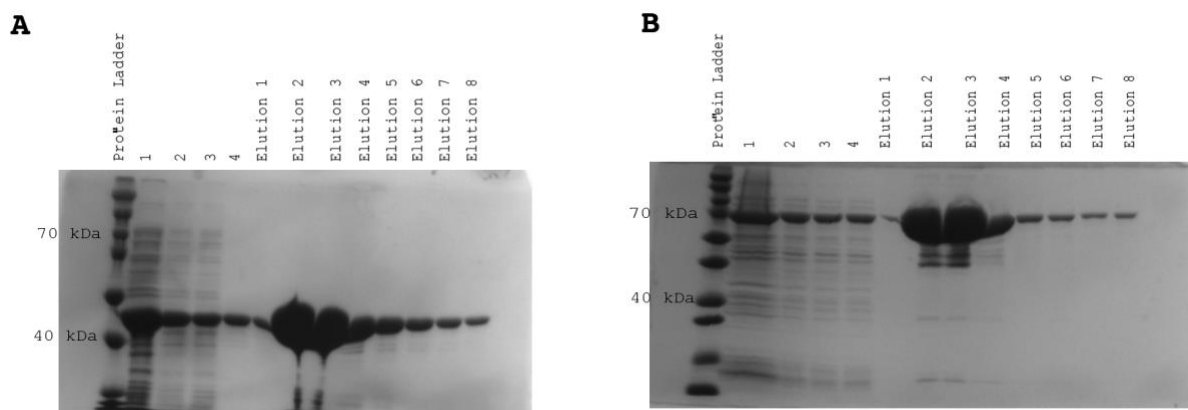


Figure 2-5. Purification of MBP and MBP-BB0133 fusion protein by amylose affinity chromatography.

(A) pMAL-c6T which produces MBP and (B) pPB30 which produces MBP-BB0133 were induced with 0.5mM IPTG at 18°C overnight and proteins purified from clarified whole-cell lysate on an amylose resin gravity column. Lanes 1-4 are quality control samples for induction, cell lysate,

column flow through, and column binding, respectively. Eight sequential fractions of protein were eluted (Elution 1-8) with maltose-containing elution buffer. Samples were resolved by 12.5% SDS-PAGE.

Following induction at 18°C overnight, MBP and the MBP-BB0133 fusion protein were purified from clarified cell lysate by amylose affinity chromatography. Eight sequential fractions were eluted with elution buffer containing maltose. SDS-PAGE analysis of the elution fractions (Figure 2-5) showed that both proteins were recovered at the expected molecular weights, approximately 42 kDa for MBP and approximately 70 kDa for the MBP-BB0133 fusion, with few contaminating bands, confirming that the amylose resin selectively captured the MBP-tagged proteins. Total protein concentration in each elution fraction was determined by BCA assay. The recombinant proteins eluted predominantly in the early fractions, with peak concentrations of approximately 20mg/mL for MBP and 10mg/mL for the MBP-BB0133 fusion. Together, these results demonstrate that both MBP and the MBP-BB0133 fusion protein can be purified by amylose affinity chromatography and recovered at quantifiable yields suitable for downstream applications.

Discussion

Previously characterized TPR-containing proteins in *B. burgdorferi* have contribute to pathogenesis through motility, membrane integrity, and immune evasion (Chang et al., 2021, 2021; Groshong et al., 2014; Lenhart et al., 2012; Moon et al., 2018; Zhuang et al., 2018a). The working hypothesis of this chapter is that the remaining putative TPR-containing proteins also play roles in *B. burgdorferi* pathogenesis. In this research we have used a combination of bioinformatic and experimental approaches to gain a basic understanding of the uncharacterized TPR-containing proteins encoded by *B. burgdorferi*. These approaches include identification of TPR sequences in

the putative TPR-containing proteins, genes that are transcriptionally responsive to *in vitro* environmental cues, and purifying protein BB0133.

The TPR sequences identified in this study were found to be variable in number ranging from 1 to 9, which falls within, but at the lower end of, the 3-16 tandem repeats usually reported for TPRs (Main et al., 2003). However, this range overlaps closely with what has already been described in *B. burgdorferi* which ranges from one TPR motif found in BB0238 (located in N-terminal region) to seven TPR motifs in Lmp1 (located in the C-terminal region) (Groshong et al., 2014; Yang et al., 2016a). Thus, putative TPR-containing proteins with only one or two motifs may still have biologically relevant protein-interaction roles.

The presence of semi-conserved consensus residues in all the 17 putative TPR-containing proteins suggest that these might form a concave groove for other proteins to interact with (Scheufler et al., 2000b). Moreover, the identified TPR sequences align well to the characteristic alpha-helical antiparallel structure of the TPR motif in the protein structure. The alpha-helical helix-turn-helix structure of the TPR sequence with semi-conserved residues, might be potential protein-binding locations (Cervený et al., 2013; Scheufler et al., 2000b).

The 17 putative TPR-containing proteins were predicted to localize to multiple subcellular compartments, suggesting that they may contribute to diverse cellular processes. This pattern is consistent with previously characterized *B. burgdorferi* TPR-containing proteins, which are found in different cellular locations as such FlcA, FlcB, and BB0236 which are periplasmic components of the flagellar collar, BamD is an outer membrane lipoprotein, and Lmp1 is a surface-exposed lipoprotein (Chang et al., 2021; Dunn et al., 2015b; Lenhart et al., 2012; Moon et al., 2018; Xu et al., 2020; Yang et al., 2016a; Zhuang et al., 2018a). Knowing the predicted location for these

previously uncharacterized TPR-containing proteins will be helpful in designing future protein purification experiments and in the identification of protein-binding partners.

TPR-containing genes *bbj23* and *bbj24* showed significant induction under *in vitro* mammalian-mimicking growth conditions. This is not necessarily surprising, given that previous studies using RNA sequencing of a *rpoS* mutant showed that *bbj23* and *bbj24* were positively regulated and that RpoS has been found to be activated in response to environmental cues such as temperature shifts, changes in pH, and cell density (Grassmann et al., 2026). It is interesting to speculate that *bbj23* and *bbj24* may be components of the RpoS regulatory pathway, that helps *B. burgdorferi* modulate its genome during and survive while transmission from the tick to the mammalian host (Caimano et al., 2007; Ouyang et al., 2011). Additionally, a recent study proposed that *bbj23* and *bbj24* may function as chaperones in the periplasm (Nixon et al., 2026). *bbj23* and *bbj24* are proposed to be in a seven gene operon consisting of *bbj23-bbj29* which encode for components of an ABC transporter. These findings suggest *bbj23* and *bbj24* are potential candidates for further studies in mammalian virulence.

One of the TPR-containing proteins we have been characterizing, BB0209, lies immediately upstream of the previously characterized TPR-containing protein Lmp1. BB0209 is co-transcribed with known virulence factor Lmp1, which suggest to us that BB0209 might also be involved in *B. burgdorferi* virulence (Lamb et al., 1995; Yang et al., 2009; Yang et al., 2016a).

In all six characterized *B. burgdorferi* TPR-containing proteins, pull-down assays were used to identify binding partners which ranged from 1 to 3 (Groshong et al., 2014; Kariu et al., 2015; Thakur et al., 2017; Yang et al., 2016a; Zhuang et al., 2018a). The TPR domain of FlcA located in the C-terminal region was directly shown to provide the binding surface for flagellar components MotB and FliL (Xu et al., 2020). Flagellar TPR-containing protein FlcB binding to

FlcC and BB0236 binding to FlbB were detected using pull-downs with full-length proteins (Chang et al., 2021). The structural modeling of the BAM complex indicates that the TPR motif of BamD docks with another component of the BAM complex- BamA (Brangulis, 2026b). Notably, for BB0238, initial studies attributed its interaction with BB0323 to its N-terminal TPR motif but subsequent structural and mutagenesis work suggest that this region is not involved in binding with BB0323 but rather plays a role in the proteins structural integrity (Foor et al., 2023; Kariu et al., 2015; Thakur et al., 2017). Together, this indicates that although the TPR motif is structurally conserved across these proteins, its role in mediating the identified protein-protein interactions is not uniform.

Successful purification of BB0133 as a MBP fusion protein builds a foundation for future downstream experiments like pull-down assays to identify the proteins binding partner(s). As the major role of the TPR motif is to mediate protein-protein interaction, identifying a characterized binding partner may give insight into the TPR-containing protein's function (Blatch & Lässle, 1999; Thakur et al., 2017). In overall, this study gives a foundational framework for future functional studies of the 17 identified TPR-containing proteins of *B. burgdorferi*.

Chapter 3 - Conclusion

B. burgdorferi persists in nature through the complex enzootic life cycle that requires adaptation in regulation of gene expression as the spirochete moves between tick and mammalian hosts (Radolf et al., 2012). Pathogenesis in *B. burgdorferi* depends upon its ability to invade, adapt, disseminate, and persist in host tissues (Brissette & Gaultney, 2014; Coburn et al., 2021; Strnad et al., 2023). There are multiple factors that influence the pathogenic ability of *B. burgdorferi*. One of the factors that influences *B. burgdorferi* pathogenesis is the presence of TPR-containing

proteins, important in protein-protein interaction. Six of the TPR-containing proteins previously characterized in *B. burgdorferi* were found to contribute towards pathogenesis through motility, immune evasion, persistence, and membrane integrity (Chang et al., 2021; Groshong et al., 2014; Moon et al., 2018; Xu et al., 2020; Yang et al., 2016a; Zhuang et al., 2018a). There are 17 more putative TPR-containing proteins in *B. burgdorferi* that have yet to be characterized.

Chapter 2 of this dissertation is directed towards characterizing these proteins to set up a foundation for future research in determining the role of these proteins. In this research 17 TPR-containing proteins in *B. burgdorferi* were characterized through a combination of *in silico* prediction analysis, transcriptional profiling, and recombinant protein expression. The presence of predicted signal peptides or transmembrane regions indicated that these proteins are distributed across multiple subcellular compartments. Mapping of the identified TPR sequences into the AlphaFold3 generated protein structure found that these TPR sequences aligned with the characteristic alpha-helical antiparallel structure. Additionally, MBP-BB0133 protein was successfully purified using amylose affinity chromatography and will be later used downstream in pull-down assays to identify protein binding partner(s). This information could be useful in future experimental designs for protein purification and identification of TPR-containing protein binding partners.

Transcriptional profiling by RT-qPCR further showed that the chromosomally encoded TPR-containing genes were unchanged under *in vitro* mammalian host-like conditions. Whereas the plasmid-encoded genes *bbj23* and *bbj24* were found to be markedly upregulated. These findings suggest that TPR-containing genes *bbj23* and *bbj24* are environmentally responsive and could be important during mammalian infection. These findings establish an initial framework for

characterizing the TPR-containing proteins in *B. burgdorferi* and highlight BBJ23, BBJ24 as priority candidates for further investigation into their roles in the enzootic cycle and pathogenesis.

Appendix A is focused on investigating the impact of *B. burgdorferi* *cyaB* at the host-pathogen interface. This study provides an initial screening of the co-cultivation *B. burgdorferi* wild type, *cyaB* mutant, and *cyaB* complement strains samples for strain confirmation and plasmid content. These confirmed samples will be used for RNA sequencing downstream to define the role of *cyaB* at the host-pathogen interface.

In Appendix B we sought to determine the antimicrobial activity of polymer and microgel compounds. Through inhibition of growth for both Gram positive and Gram negative bacteria, these compounds showed antimicrobial properties at concentration ranges of 1-0.5mg/ml for polymers and at 60mg/ml for microgel compounds. Although the effective concentrations of the polymer compounds were higher than those typically required for conventional antibiotics, future studies could evaluate whether combining these polymers with antibiotics enhances their antimicrobial activity.

References 2023 AST: CLSI M100 ED33 Updates | News | CLSI. (n.d.). Retrieved July 7,

2026, from <https://clsi.org/about/press-releases/ast-news-update-june-2023-new-clsi-m100-ed33-updated-aminoglycoside-breakpoints-for-enterobacterales-and-pseudomonas-aeruginosa/>

Alojz Peterle, Daciana Octavia Sârbu, Jadwiga Wiśniewska, Frédérique Ries, Merja Kyllönen,

Michèle Rivasi, Mireille D'Ornano, & Sylvie Goddyn. (2018). *MOTION FOR A*

RESOLUTION on Lyme disease (Borreliosis) | B8-0514/2018 | European Parliament.

https://www.europarl.europa.eu/doceo/document/B-8-2018-0514_EN.html

- Ante, V. M., Farris, L. C., Saputra, E. P., Hall, A. J., O'Bier, N. S., Oliva Chávez, A. S., Marconi, R. T., Lybecker, M. C., & Hyde, J. A. (2021). The *Borrelia burgdorferi* Adenylate Cyclase, CyaB, Is Important for Virulence Factor Production and Mammalian Infection. *Frontiers in Microbiology*, 12, 676192.
<https://doi.org/10.3389/fmicb.2021.676192>
- Aucott, J. N., Rebman, A. W., Crowder, L. A., & Kortte, K. B. (2013). Post-treatment Lyme disease syndrome symptomatology and the impact on life functioning: Is there something here? *Quality of Life Research*, 22(1), 75–84. <https://doi.org/10.1007/s11136-012-0126-6>
- Auwaerter, P. G. (2011). *Borrelia*: Molecular Biology, Host Interaction and Pathogenesis. *Clinical Infectious Diseases*, 52(7), 965. <https://doi.org/10.1093/cid/cir083>
- Barbour, A. G. (1984). Isolation and cultivation of Lyme disease spirochetes. *The Yale Journal of Biology and Medicine*, 57(4), 521–525.
<https://pmc.ncbi.nlm.nih.gov/articles/PMC2589996/>
- Biesiada, G., Czepiel, J., Leśniak, M. R., Garlicki, A., & Mach, T. (2012). Lyme disease: Review. *Archives of Medical Science : AMS*, 8(6), 978–982.
<https://doi.org/10.5114/aoms.2012.30948>
- Bista, S., Brangulis, K., Bhattachan, B., Foor, S. D., Ronzetti, M. H., Jain, S., Miller, J., Subramanion, J. L., Kitsou, C., Rana, V. S., Rai, G., Zakharov, A. V., Simeonov, A., Baljinnyam, B., & Pal, U. (2026). Targeting of interaction between BB0323-BB0238 informs new paradigms in Lyme disease therapeutics. *PLOS Pathogens*, 22(1), e1013805.
<https://doi.org/10.1371/journal.ppat.1013805>
- Bitar, I., & Lally, E. V. (2008). Musculoskeletal manifestations of Lyme disease. *Medicine and Health, Rhode Island*, 91(7), 213–215.

- Blatch, G. L., & Lässle, M. (1999). The tetratricopeptide repeat: A structural motif mediating protein-protein interactions. *BioEssays*, 21(11), 932–939.
[https://doi.org/10.1002/\(SICI\)1521-1878\(199911\)21:11%3C932::AID-BIES5%3E3.0.CO;2-N](https://doi.org/10.1002/(SICI)1521-1878(199911)21:11%3C932::AID-BIES5%3E3.0.CO;2-N)
- Bobe, J. R., Jutras, B. L., Horn, E. J., Embers, M. E., Bailey, A., Moritz, R. L., Zhang, Y., Soloski, M. J., Ostfeld, R. S., Marconi, R. T., Aucott, J., Ma'ayan, A., Keesing, F., Lewis, K., Ben Mamoun, C., Rebman, A. W., McClune, M. E., Breitschwerdt, E. B., Reddy, P. J., ... Fallon, B. A. (2021). Recent Progress in Lyme Disease and Remaining Challenges. *Frontiers in Medicine*, 8. <https://doi.org/10.3389/fmed.2021.666554>
- Branda, J. A., Strle, K., Nigrovic, L. E., Lantos, P. M., Lepore, T. J., Damle, N. S., Ferraro, M. J., & Steere, A. C. (2017). Evaluation of Modified 2-Tiered Serodiagnostic Testing Algorithms for Early Lyme Disease. *Clinical Infectious Diseases*, 64(8), 1074–1080.
<https://doi.org/10.1093/cid/cix043>
- Brangulis, K. (2026a). Structural architecture of a hybrid β -barrel assembly machinery BAM-TAM complex in *Borrelia burgdorferi*. *The Journal of Biological Chemistry*, 302(8), 113267. <https://doi.org/10.1016/j.jbc.2026.113267>
- Brangulis, K. (2026b). Structural architecture of a hybrid β -barrel assembly machinery BAM-TAM complex in *Borrelia burgdorferi*. *The Journal of Biological Chemistry*, 302(8), 113267. <https://doi.org/10.1016/j.jbc.2026.113267>
- Brissette, C. A., & Gaultney, R. A. (2014). That's my story, and I'm sticking to it—An update on *B. burgdorferi* adhesins. *Frontiers in Cellular and Infection Microbiology*, 4.
<https://doi.org/10.3389/fcimb.2014.00041>

- Brooks, C. S., Vuppala, S. R., Jett, A. M., & Akins, D. R. (2006). Identification of *Borrelia burgdorferi* Outer Surface Proteins. *Infection and Immunity*, 74(1), 296–304.
<https://doi.org/10.1128/IAI.74.1.296-304.2006>
- Byram, R., Stewart, P. E., & Rosa, P. (2004). The Essential Nature of the Ubiquitous 26-Kilobase Circular Replicon of *Borrelia burgdorferi*. *Journal of Bacteriology*, 186(11), 3561–3569. <https://doi.org/10.1128/JB.186.11.3561-3569.2004>
- Caimano, M. J., Iyer, R., Eggers, C. H., Gonzalez, C., Morton, E. A., Gilbert, M. A., Schwartz, I., & Radolf, J. D. (2007). Analysis of the RpoS regulon in *Borrelia burgdorferi* in response to mammalian host signals provides insight into RpoS function during the enzootic cycle. *Molecular Microbiology*, 65(5), 1193–1217.
<https://doi.org/10.1111/j.1365-2958.2007.05860.x>
- Casjens, S., Palmer, N., Van Vugt, R., Mun Huang, W., Stevenson, B., Rosa, P., Lathigra, R., Sutton, G., Peterson, J., Dodson, R. J., Haft, D., Hickey, E., Gwinn, M., White, O., & M. Fraser, C. (2000). A bacterial genome in flux: The twelve linear and nine circular extrachromosomal DNAs in an infectious isolate of the Lyme disease spirochete *Borrelia burgdorferi*. *Molecular Microbiology*, 35(3), 490–516. <https://doi.org/10.1046/j.1365-2958.2000.01698.x>
- Cerveny, L., Straskova, A., Dankova, V., Hartlova, A., Ceckova, M., Staud, F., & Stulik, J. (2013). Tetratricopeptide Repeat Motifs in the World of Bacterial Pathogens: Role in Virulence Mechanisms. *Infection and Immunity*, 81(3), 629–635.
<https://doi.org/10.1128/iai.01035-12>

- Chang, Y., Xu, H., Motaleb, M. A., & Liu, J. (2021). Characterization of the Flagellar Collar Reveals Structural Plasticity Essential for Spirochete Motility. *mBio*, 12(6), e0249421. <https://doi.org/10.1128/mBio.02494-21>
- Coburn, J., Garcia, B., Hu, L. T., Jewett, M. W., Kraiczy, P., Norris, S. J., & Skare, J. (2021). Lyme Disease Pathogenesis. *Current Issues in Molecular Biology*, 42, 473–518. <https://doi.org/10.21775/cimb.042.473>
- Coleman, J. L., Crowley, J. T., Toledo, A. M., & Benach, J. L. (2013). The HtrA protease of *Borrelia burgdorferi* degrades outer membrane protein BmpD and chemotaxis phosphatase CheX. *Molecular Microbiology*, 88(3), 619–633. <https://doi.org/10.1111/mmi.12213>
- D’Andrea, L. D., & Regan, L. (2003). TPR proteins: The versatile helix. *Trends in Biochemical Sciences*, 28(12), 655–662. <https://doi.org/10.1016/j.tibs.2003.10.007>
- Das, A. K., Cohen, P. T. W., & Barford, D. (1998). The structure of the tetratricopeptide repeats of protein phosphatase 5: Implications for TPR-mediated protein–protein interactions. *The EMBO Journal*, 17(5), 1192–1199. <https://doi.org/10.1093/emboj/17.5.1192>
- de Boer, M., Hilarius-Stokman, P., Hossle, J., Verhoeven, A., Graf, N., Kenney, R., Seger, R., & Roos, D. (1994). Autosomal recessive chronic granulomatous disease with absence of the 67-kD cytosolic NADPH oxidase component: Identification of mutation and detection of carriers. *Blood*, 83(2), 531–536. <https://doi.org/10.1182/blood.V83.2.531.531>
- Dieppedale, J., Sobral, D., Dupuis, M., Dubail, I., Klimentova, J., Stulik, J., Postic, G., Frapy, E., Meibom, K. L., Barel, M., & Charbit, A. (2011). Identification of a Putative Chaperone Involved in Stress Resistance and Virulence in *Francisella tularensis*. *Infection and Immunity*, 79(4), 1428–1439. <https://doi.org/10.1128/iai.01012-10>

- Dong, Q., Yan, X., Zheng, M., & Yang, Z. (2013). Comparison of two type IV hyperthermophilic adenyl cyclases characterizations from the archaeon *Pyrococcus furiosus*. *Journal of Molecular Catalysis B: Enzymatic*, 88, 7–13.
<https://doi.org/10.1016/j.molcatb.2012.10.017>
- Dumic, I., & Severnini, E. (2018). “Ticking Bomb”: The Impact of Climate Change on the Incidence of Lyme Disease. *The Canadian Journal of Infectious Diseases & Medical Microbiology = Journal Canadien Des Maladies Infectieuses Et De La Microbiologie Medicale*, 2018, 5719081. <https://doi.org/10.1155/2018/5719081>
- Dunn, J. P., Kenedy, M. R., Iqbal, H., & Akins, D. R. (2015a). Characterization of the β -barrel assembly machine accessory lipoproteins from *Borrelia burgdorferi*. *BMC Microbiology*, 15(1), 70. <https://doi.org/10.1186/s12866-015-0411-y>
- Dunn, J. P., Kenedy, M. R., Iqbal, H., & Akins, D. R. (2015b). Characterization of the β -barrel assembly machine accessory lipoproteins from *Borrelia burgdorferi*. *BMC Microbiology*, 15(1), 70. <https://doi.org/10.1186/s12866-015-0411-y>
- Edqvist, P. J., Bröms, J. E., Betts, H. J., Forsberg, Å., Pallen, M. J., & Francis, M. S. (2006a). Tetratricopeptide repeats in the type III secretion chaperone, LcrH: Their role in substrate binding and secretion. *Molecular Microbiology*, 59(1), 31–44.
<https://doi.org/10.1111/j.1365-2958.2005.04923.x>
- Edqvist, P. J., Bröms, J. E., Betts, H. J., Forsberg, Å., Pallen, M. J., & Francis, M. S. (2006b). Tetratricopeptide repeats in the type III secretion chaperone, LcrH: Their role in substrate binding and secretion. *Molecular Microbiology*, 59(1), 31–44.
<https://doi.org/10.1111/j.1365-2958.2005.04923.x>

- Elias, A. F., Stewart, P. E., Grimm, D., Caimano, M. J., Eggers, C. H., Tilly, K., Bono, J. L., Akins, D. R., Radolf, J. D., Schwan, T. G., & Rosa, P. (2002). Clonal Polymorphism of *Borrelia burgdorferi* Strain B31 MI: Implications for Mutagenesis in an Infectious Strain Background. *Infection and Immunity*, 70(4), 2139–2150.
<https://doi.org/10.1128/IAI.70.4.2139-2150.2002>
- Fischer, J. R., Parveen, N., Magoun, L., & Leong, J. M. (2003). Decorin-binding proteins A and B confer distinct mammalian cell type-specific attachment by *Borrelia burgdorferi*, the Lyme disease spirochete. *Proceedings of the National Academy of Sciences of the United States of America*, 100(12), 7307–7312. <https://doi.org/10.1073/pnas.1231043100>
- Foor, S. D., Brangulis, K., Shakya, A. K., Rana, V. S., Bista, S., Kitsou, C., Ronzetti, M., Alreja, A. B., Linden, S. B., Altieri, A. S., Baljinnyam, B., Akopjana, I., Nelson, D. C., Simeonov, A., Herzberg, O., Caimano, M. J., & Pal, U. (2023). A unique borrelial protein facilitates microbial immune evasion. *mBio*, 14(5), e02135-23.
<https://doi.org/10.1128/mbio.02135-23>
- Fraser, C. M., Casjens, S., Huang, W. M., Sutton, G. G., Clayton, R., Lathigra, R., White, O., Ketchum, K. A., Dodson, R., Hickey, E. K., Gwinn, M., Dougherty, B., Tomb, J.-F., Fleischmann, R. D., Richardson, D., Peterson, J., Kerlavage, A. R., Quackenbush, J., Salzberg, S., ... Venter, J. C. (1997). Genomic sequence of a Lyme disease spirochaete, *Borrelia burgdorferi*. *Nature*, 390(6660), 580–586. <https://doi.org/10.1038/37551>
- Gallagher, D. T., Smith, N. N., Kim, S.-K., Heroux, A., Robinson, H., & Reddy, P. T. (2006). Structure of the class IV adenylyl cyclase reveals a novel fold. *Journal of Molecular Biology*, 362(1), 114–122. <https://doi.org/10.1016/j.jmb.2006.07.008>

- Gatsos, X., Perry, A. J., Anwari, K., Dolezal, P., Woly nec, P. P., Likić, V. A., Purcell, A. W., Buchanan, S. K., & Lithgow, T. (2008). Protein secretion and outer membrane assembly in Alphaproteobacteria. *FEMS Microbiology Reviews*, 32(6), 995–1009.
<https://doi.org/10.1111/j.1574-6976.2008.00130.x>
- Grassmann, A. A., McLain, M. A., Freeman, M. R., Caimano, M. J., & Radolf, J. D. (2026). DNA Binding by BosR Controls RpoS-Dependent and -Independent Gene Expression in *Borrelia burgdorferi*. *Molecular Microbiology*, 125(2), 85–107.
<https://doi.org/10.1111/mmi.70036>
- Gray, J. S., Kahl, O., Lane, R. S., & Stanek, G. (2002). *Lyme Borreliosis: Biology, Epidemiology and Control*. CABI.
<http://ebookcentral.proquest.com/lib/ksu/detail.action?docID=295062>
- Groshong, A. M., Fortune, D. E., Moore, B. P., Spencer, H. J., Skinner, R. A., Bellamy, W. T., & Blevins, J. S. (2014). BB0238, a Presumed Tetratricopeptide Repeat-Containing Protein, Is Required during *Borrelia burgdorferi* Mammalian Infection. *Infection and Immunity*, 82(10), 4292–4306. <https://doi.org/10.1128/IAI.01977-14>
- Hallgren, J., Tsirigos, K. D., Pedersen, M. D., Almagro Armenteros, J. J., Marcatili, P., Nielsen, H., Krogh, A., & Winther, O. (2022). *DeepTMHMM predicts alpha and beta transmembrane proteins using deep neural networks*. *Bioinformatics*.
<https://doi.org/10.1101/2022.04.08.487609>
- Helble, J. D., McCarthy, J. E., & Hu, L. T. (2021a). Interactions between *Borrelia burgdorferi* and its hosts across the enzootic cycle. *Parasite Immunology*, 43(5), e12816.
<https://doi.org/10.1111/pim.12816>

- Helble, J. D., McCarthy, J. E., & Hu, L. T. (2021b). Interactions between *Borrelia burgdorferi* and its hosts across the enzootic cycle. *Parasite Immunology*, 43(5), e12816.
<https://doi.org/10.1111/pim.12816>
- Hyde, J. A. (2017). *Borrelia burgdorferi* Keeps Moving and Carries on: A Review of Borrelial Dissemination and Invasion. *Frontiers in Immunology*, 8.
<https://doi.org/10.3389/fimmu.2017.00114>
- Jones, P., Binns, D., Chang, H.-Y., Fraser, M., Li, W., McAnulla, C., McWilliam, H., Maslen, J., Mitchell, A., Nuka, G., Pesseat, S., Quinn, A. F., Sangrador-Vegas, A., Scheremetjew, M., Yong, S.-Y., Lopez, R., & Hunter, S. (2014). InterProScan 5: Genome-scale protein function classification. *Bioinformatics*, 30(9), 1236–1240.
<https://doi.org/10.1093/bioinformatics/btu031>
- Kariu, T., Sharma, K., Singh, P., Smith, A. A., Backstedt, B., Buyuktanir, O., & Pal, U. (2015). BB0323 and Novel Virulence Determinant BB0238: *Borrelia burgdorferi* Proteins That Interact With and Stabilize Each Other and Are Critical for Infectivity. *The Journal of Infectious Diseases*, 211(3), 462–471. <https://doi.org/10.1093/infdis/jiu460>
- Karpenahalli, M. R., Lupas, A. N., & Söding, J. (2007). TPRpred: A tool for prediction of TPR-, PPR- and SEL1-like repeats from protein sequences. *BMC Bioinformatics*, 8, 2.
<https://doi.org/10.1186/1471-2105-8-2>
- Kawabata, H., Norris, S. J., & Watanabe, H. (2004). BBE02 Disruption Mutants of *Borrelia burgdorferi* B31 Have a Highly Transformable, Infectious Phenotype. *Infection and Immunity*, 72(12), 7147–7154. <https://doi.org/10.1128/IAI.72.12.7147-7154.2004>

- Knowles, T. J., Scott-Tucker, A., Overduin, M., & Henderson, I. R. (2009). Membrane protein architects: The role of the BAM complex in outer membrane protein assembly. *Nature Reviews. Microbiology*, 7(3), 206–214. <https://doi.org/10.1038/nrmicro2069>
- Koci, J., Bernard, Q., Yang, X., & Pal, U. (2018). *Borrelia burgdorferi* surface protein Lmp1 facilitates pathogen dissemination through ticks as studied by an artificial membrane feeding system. *Scientific Reports*, 8, 1910. <https://doi.org/10.1038/s41598-018-20208-4>
- Kondo, Y., Ohara, N., Sato, K., Yoshimura, M., Yukitake, H., Naito, M., Fujiwara, T., & Nakayama, K. (2010). Tetratricopeptide Repeat Protein-Associated Proteins Contribute to the Virulence of *Porphyromonas gingivalis*. *Infection and Immunity*, 78(6), 2846–2856. <https://doi.org/10.1128/iai.01448-09>
- Kugeler, K. J., Schwartz, A. M., Delorey, M. J., Mead, P. S., & Hinckley, A. F. (2021). *Estimating the Frequency of Lyme Disease Diagnoses, United States, 2010–2018—Volume 27, Number 2—February 2021—Emerging Infectious Diseases journal—CDC*. <https://doi.org/10.3201/eid2702.202731>
- Kurgan, L., & Yang, J. (2025). AlphaFold in protein structure and function prediction. *Computational and Structural Biotechnology Journal*, 27, 5521–5522. <https://doi.org/10.1016/j.csbj.2025.11.064>
- Kurtenbach, K., Hanincová, K., Tsao, J. I., Margos, G., Fish, D., & Ogden, N. H. (2006). Fundamental processes in the evolutionary ecology of Lyme borreliosis. *Nature Reviews. Microbiology*, 4(9), 660–669. <https://doi.org/10.1038/nrmicro1475>
- Lamb, J. R., Tugendreich, S., & Hieter, P. (1995). Tetratrico peptide repeat interactions: To TPR or not to TPR? *Trends in Biochemical Sciences*, 20(7), 257–259. [https://doi.org/10.1016/S0968-0004\(00\)89037-4](https://doi.org/10.1016/S0968-0004(00)89037-4)

- Lenhart, T. R., Kenedy, M. R., Yang, X., Pal, U., & Akins, D. R. (2012). BB0324 and BB0028 are constituents of the *Borrelia burgdorferi* β -barrel assembly machine (BAM) complex. *BMC Microbiology*, 12(1), 60. <https://doi.org/10.1186/1471-2180-12-60>
- Livak, K. J., & Schmittgen, T. D. (2001). Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the $2^{-\Delta\Delta CT}$ Method. *Methods*, 25(4), 402–408. <https://doi.org/10.1006/meth.2001.1262>
- Main, E. R. G., Xiong, Y., Cocco, M. J., D’Andrea, L., & Regan, L. (2003). Design of Stable α -Helical Arrays from an Idealized TPR Motif. *Structure*, 11(5), 497–508. [https://doi.org/10.1016/S0969-2126\(03\)00076-5](https://doi.org/10.1016/S0969-2126(03)00076-5)
- Mallory, K. L., Miller, D. P., Oliver, L. D., Jr, Freedman, J. C., Kostick-Dunn, J. L., Carlyon, J. A., Marion, J. D., Bell, J. K., & Marconi, R. T. (2016). Cyclic-di-GMP binding induces structural rearrangements in the PlzA and PlzC proteins of the Lyme disease and relapsing fever spirochetes: A possible switch mechanism for c-di-GMP-mediated effector functions. *Pathogens and Disease*, 74(8), ftw105. <https://doi.org/10.1093/femspd/ftw105>
- Marques, A. (2008). Chronic Lyme Disease: A Review. *Infectious Disease Clinics of North America, Tick-Borne Diseases, Part I: Lyme Disease*, 22(2), 341–360. <https://doi.org/10.1016/j.idc.2007.12.011>
- McDonough, K. A., & Rodriguez, A. (2011). The myriad roles of cyclic AMP in microbial pathogens, from signal to sword. *Nature Reviews. Microbiology*, 10(1), 27–38. <https://doi.org/10.1038/nrmicro2688>
- Mead, P. S. (2015). Epidemiology of Lyme Disease. *Infectious Disease Clinics of North America*, 29(2), 187–210. <https://doi.org/10.1016/j.idc.2015.02.010>

- Moon, K. H., Zhao, X., Xu, H., Liu, J., & Motaleb, M. A. (2018). A tetratricopeptide repeat domain protein has profound effects on assembly of periplasmic flagella, morphology and motility of the lyme disease spirochete *Borrelia burgdorferi*. *Molecular Microbiology*, 110(4), 634–647. <https://doi.org/10.1111/mmi.14121>
- Newton, C. A., Perkins, I., Widen, R. H., Friedman, H., & Klein, T. W. (2007). Role of Toll-Like Receptor 9 in *Legionella pneumophila*-Induced Interleukin-12 p40 Production in Bone Marrow-Derived Dendritic Cells and Macrophages from Permissive and Nonpermissive Mice. *Infection and Immunity*, 75(1), 146–151. <https://doi.org/10.1128/iai.01011-06>
- Nixon, A., Soliman, E., Bryk, J., Carr, M., Cooper, C. D. O., & Bingham, R. J. (2026). *Borrelia* BBJ25 is a plasmid-encoded Omp85-family protein associated with a potential export system. *Bioscience Reports*, 46(2), BSR20254082. <https://doi.org/10.1042/BSR20254082>
- Novak, E. A., Sultan, S. Z., & Motaleb, Md. A. (2014). The cyclic-di-GMP signaling pathway in the Lyme disease spirochete, *Borrelia burgdorferi*. *Frontiers in Cellular and Infection Microbiology*, 4, 56. <https://doi.org/10.3389/fcimb.2014.00056>
- Ouyang, Z., Deka, R. K., & Norgard, M. V. (2011). BosR (BB0647) Controls the RpoN-RpoS Regulatory Pathway and Virulence Expression in *Borrelia burgdorferi* by a Novel DNA-Binding Mechanism. *PLOS Pathogens*, 7(2), e1001272. <https://doi.org/10.1371/journal.ppat.1001272>
- Ouyang, Z., Kumar, M., Kariu, T., Haq, S., Goldberg, M., Pal, U., & Norgard, M. V. (2009). BosR (BB0647) governs virulence expression in *Borrelia burgdorferi*. *Molecular Microbiology*, 74(6), 1331–1343. <https://doi.org/10.1111/j.1365-2958.2009.06945.x>

- Probert, W. S., & Johnson, B. J. B. (1998). Identification of a 47 kDa fibronectin-binding protein expressed by *Borrelia burgdorferi* isolate B31. *Molecular Microbiology*, 30(5), 1003–1015. <https://doi.org/10.1046/j.1365-2958.1998.01127.x>
- Purser, J. E., Lawrenz, M. B., Caimano, M. J., Howell, J. K., Radolf, J. D., & Norris, S. J. (2003). A plasmid-encoded nicotinamidase (PncA) is essential for infectivity of *Borrelia burgdorferi* in a mammalian host. *Molecular Microbiology*, 48(3), 753–764. <https://doi.org/10.1046/j.1365-2958.2003.03452.x>
- Radolf, J. D., Caimano, M. J., Stevenson, B., & Hu, L. T. (2012). Of ticks, mice and men: Understanding the dual-host lifestyle of Lyme disease spirochaetes. *Nature Reviews Microbiology*, 10(2), 87–99. <https://doi.org/10.1038/nrmicro2714>
- Rebman, A. W., & Aucott, J. N. (2020). Post-treatment Lyme Disease as a Model for Persistent Symptoms in Lyme Disease. *Frontiers in Medicine*, 7. <https://doi.org/10.3389/fmed.2020.00057>
- Sader, H. S., Mendes, R. E., Kimbrough, J. H., Kantro, V., & Castanheira, M. (2023). Impact of the Recent Clinical and Laboratory Standards Institute Breakpoint Changes on the Antimicrobial Spectrum of Aminoglycosides and the Activity of Plazomicin Against Multidrug-Resistant and Carbapenem-Resistant Enterobacterales From United States Medical Centers. *Open Forum Infectious Diseases*, 10(2), ofad058. <https://doi.org/10.1093/ofid/ofad058>
- Scheufler, C., Brinker, A., Bourenkov, G., Pegoraro, S., Moroder, L., Bartunik, H., Hartl, F. U., & Moarefi, I. (2000a). Structure of TPR Domain–Peptide Complexes: Critical Elements in the Assembly of the Hsp70–Hsp90 Multichaperone Machine. *Cell*, 101(2), 199–210. [https://doi.org/10.1016/S0092-8674\(00\)80830-2](https://doi.org/10.1016/S0092-8674(00)80830-2)

- Scheufler, C., Brinker, A., Bourenkov, G., Pegoraro, S., Moroder, L., Bartunik, H., Hartl, F. U., & Moarefi, I. (2000b). Structure of TPR Domain–Peptide Complexes: Critical Elements in the Assembly of the Hsp70–Hsp90 Multichaperone Machine. *Cell*, *101*(2), 199–210. [https://doi.org/10.1016/S0092-8674\(00\)80830-2](https://doi.org/10.1016/S0092-8674(00)80830-2)
- Schwan, T. G., Burgdorfer, W., & Garon, C. F. (1988). Changes in infectivity and plasmid profile of the Lyme disease spirochete, *Borrelia burgdorferi*, as a result of in vitro cultivation. *Infection and Immunity*, *56*(8), 1831–1836. <https://doi.org/10.1128/iai.56.8.1831-1836.1988>
- Sikorski, R. S., Boguski, M. S., Goebel, M., & Hieter, P. (1990). A repeating amino acid motif in CDC23 defines a family of proteins and a new relationship among genes required for mitosis and RNA synthesis. *Cell*, *60*(2), 307–317. [https://doi.org/10.1016/0092-8674\(90\)90745-Z](https://doi.org/10.1016/0092-8674(90)90745-Z)
- Smith, N., Kim, S.-K., Reddy, P. T., & Gallagher, D. T. (2006). Crystallization of the class IV adenylyl cyclase from *Yersinia pestis*. *Acta Crystallographica Section F: Structural Biology and Crystallization Communications*, *62*(Pt 3), 200–204. <https://doi.org/10.1107/S1744309106002855>
- Smith, R. P., Schoen, R. T., Rahn, D. W., Sikand, V. K., Nowakowski, J., Parenti, D. L., Holman, M. S., Persing, D. H., & Steere, A. C. (2002). Clinical Characteristics and Treatment Outcome of Early Lyme Disease in Patients with Microbiologically Confirmed Erythema Migrans. *Annals of Internal Medicine*, *136*(6), 421–428. <https://doi.org/10.7326/0003-4819-136-6-200203190-00005>

- Song, L., Liu, L., Wu, Z., Li, Y., Ying, Z., Lin, C., Wu, J., Hu, B., Cheng, S.-Y., Li, M., & Li, J. (2012). TGF- β induces miR-182 to sustain NF- κ B activation in glioma subsets. *The Journal of Clinical Investigation*, 122(10), 3563–3578. <https://doi.org/10.1172/JCI62339>
- Steere, A. C., Bartenhagen, N. H., Craft, J. E., Hutchinson, G. J., Newman, J. H., Rahn, D. W., Sigal, L. H., Spieler, P. N., Stenn, K. S., & Malawista, S. E. (1983). The early clinical manifestations of Lyme disease. *Annals of Internal Medicine*, 99(1), 76–82. <https://doi.org/10.7326/0003-4819-99-1-76>
- Steere, A. C., Coburn, J., & Glickstein, L. (2004). The emergence of Lyme disease. *Journal of Clinical Investigation*, 113(8), 1093–1101. <https://doi.org/10.1172/JCI21681>
- Strnad, M., Rudenko, N., & Rego, R. O. M. (2023). Pathogenicity and virulence of *Borrelia burgdorferi*. *Virulence*, 14(1), 2265015. <https://doi.org/10.1080/21505594.2023.2265015>
- Sultan, S. Z., Pitzer, J. E., Miller, M. R., & Motaleb, Md. A. (2010). Analysis of a *Borrelia burgdorferi* phosphodiesterase demonstrates a role for cyclic-di-GMP in motility and virulence. *Molecular Microbiology*, 77(1), 128–142. <https://doi.org/10.1111/j.1365-2958.2010.07191.x>
- Teufel, F., Almagro Armenteros, J. J., Johansen, A. R., Gíslason, M. H., Pihl, S. I., Tsirigos, K. D., Winther, O., Brunak, S., von Heijne, G., & Nielsen, H. (2022). SignalP 6.0 predicts all five types of signal peptides using protein language models. *Nature Biotechnology*, 40(7), 1023–1025. <https://doi.org/10.1038/s41587-021-01156-3>
- Thakur, M., Sharma, K., Chao, K., Smith, A. A., Herzberg, O., & Pal, U. (2017). A protein-protein interaction dictates *Borrelia* infectivity. *Scientific Reports*, 7(1), 2932. <https://doi.org/10.1038/s41598-017-03279-7>

- Volokhina, E. B., Beckers, F., Tommassen, J., & Bos, M. P. (2009). The beta-barrel outer membrane protein assembly complex of *Neisseria meningitidis*. *Journal of Bacteriology*, *191*(22), 7074–7085. <https://doi.org/10.1128/JB.00737-09>
- Waddell, L. A., Greig, J., Mascarenhas, M., Harding, S., Lindsay, R., & Ogden, N. (2016). The Accuracy of Diagnostic Tests for Lyme Disease in Humans, A Systematic Review and Meta-Analysis of North American Research. *PLOS ONE*, *11*(12), e0168613. <https://doi.org/10.1371/journal.pone.0168613>
- Wright, W. F., Riedel, D. J., Talwani, R., & Gilliam, B. L. (2012). *Diagnosis and Management of Lyme Disease*. <https://www.aafp.org/pubs/afp/issues/2012/0601/p1086.html>
- Xu, H., He, J., Liu, J., & Motaleb, M. A. (2020). BB0326 is responsible for the formation of periplasmic flagellar collar and assembly of the stator complex in *Borrelia burgdorferi*. *Molecular Microbiology*, *113*(2), 418–429. <https://doi.org/10.1111/mmi.14428>
- Yang, X., Coleman, A. S., Anguita, J., & Pal, U. (2009). A Chromosomally Encoded Virulence Factor Protects the Lyme Disease Pathogen against Host-Adaptive Immunity. *PLOS Pathogens*, *5*(3), e1000326. <https://doi.org/10.1371/journal.ppat.1000326>
- Yang, X., Lin, Y.-P., Heselpoth, R. D., Buyuktanir, O., Qin, J., Kung, F., Nelson, D. C., Leong, J. M., & Pal, U. (2016a). Middle region of the *Borrelia burgdorferi* surface-located protein 1 (Lmp1) interacts with host chondroitin-6-sulfate and independently facilitates infection. *Cellular Microbiology*, *18*(1), 97–110. <https://doi.org/10.1111/cmi.12487>
- Yang, X., Lin, Y.-P., Heselpoth, R. D., Buyuktanir, O., Qin, J., Kung, F., Nelson, D. C., Leong, J. M., & Pal, U. (2016b). Middle region of the *Borrelia burgdorferi* surface-located protein 1 (Lmp1) interacts with host chondroitin-6-sulfate and independently facilitates infection. *Cellular Microbiology*, *18*(1), 97–110. <https://doi.org/10.1111/cmi.12487>

- Yu, N. Y., Wagner, J. R., Laird, M. R., Melli, G., Rey, S., Lo, R., Dao, P., Sahinalp, S. C., Ester, M., Foster, L. J., & Brinkman, F. S. L. (2010). PSORTb 3.0: Improved protein subcellular localization prediction with refined localization subcategories and predictive capabilities for all prokaryotes. *Bioinformatics*, 26(13), 1608–1615.
<https://doi.org/10.1093/bioinformatics/btq249>
- Zhang, J.-J., Chen, T., Yang, Y., Du, J., Li, H., Troxell, B., He, M., Carrasco, S. E., Gomelsky, M., & Yang, X. F. (2018). Positive and Negative Regulation of Glycerol Utilization by the c-di-GMP Binding Protein PlzA in *Borrelia burgdorferi*. *Journal of Bacteriology*, 200(22), e00243-18. <https://doi.org/10.1128/JB.00243-18>
- Zhuang, X., Yang, X., Altieri, A. S., Nelson, D. C., & Pal, U. (2018a). *Borrelia burgdorferi* surface-located Lmp1 protein processed into region-specific polypeptides that are critical for microbial persistence. *Cellular Microbiology*, 20(9), e12855.
<https://doi.org/10.1111/cmi.12855>
- Zhuang, X., Yang, X., Altieri, A. S., Nelson, D. C., & Pal, U. (2018b). *Borrelia burgdorferi* surface-located Lmp1 protein processed into region-specific polypeptides that are critical for microbial persistence. *Cellular Microbiology*, 20(9), e12855.
<https://doi.org/10.1111/cmi.12855>

Appendix A - Impact of *B. burgdorferi cyaB* at the host-pathogen interface

Introduction

B. burgdorferi uses second messenger signaling as one of the methods to coordinate its dual-host lifestyle. Cyclic di-guanosine monophosphate (c-di-GMP) is synthesized by the diguanylate cyclase Rrp1 as part of the Hk1-Rrp1 two component system. It governs adaptation to the tick by supporting mammal to tick transmission, midgut survival, motility, and glycerol utilization (Dunn et al., 2015b; Novak et al., 2014; Sultan et al., 2010). Once c-di-GMP is formed, it is bound by the effector protein PlzA, which positively regulates glycerol metabolism (Mallory et al., 2016; Zhang et al., 2018). Cyclic di-adenosine monophosphate (c-di-AMP) is required for normal growth *in vitro* and the production of mammalian virulence factors (Ye et al., 2014; Savage et al., 2015). The third second messenger, cyclic adenosine monophosphate (cAMP) supports mammalian virulence factor production (Ante et al., 2021).

cAMP is a bacterial second messenger generated by adenylate cyclases. In many bacterial species, cAMP regulates transcription of genes involved in metabolism, stress responses, and virulence (McDonough & Rodriguez, 2011). Adenylate cyclases are classified into six distinct classes (I-VI) based on their amino acid sequence, structural organization, and catalytic mechanism. *B. burgdorferi* encodes a single class IV adenylate cyclase, *cyaB* (Dong et al., 2013; Gallagher et al., 2006; N. Smith et al., 2006). Expression of *cyaB* was specifically induced during co-incubation with human epithelial-like cells compared to tick cells, suggesting that *cyaB* expression is influenced by cellular factors specific to mammalian cell lines (Ante et al., 2021).

Given that *cyaB* expression is induced in the presence of human epithelial-like cells, *cyaB* may play an important role during the interaction between *B. burgdorferi* and the mammalian host.

This study aims to determine the impact of *B. burgdorferi cyaB* on both the host and pathogen transcriptional responses using RNA sequencing. By comparing host cells co-cultivated with the *cyaB* mutant to those co-cultivated with the wild type *B. burgdorferi* strain, this approach can identify host and bacterial genes whose expression is altered in a *cyaB*-dependent manner. This study will provide us with a better understanding of how *cyaB* contributes to the host response and genetic regulation of *B. burgdorferi*.

Methods

Strains and culture conditions

B. burgdorferi strains used in this study are wild type strain 5A4-NP1, *cyaB* mutant JH522, and *cyaB* complement VA200 (Kawabata et al., 2004; Ante et al., 2021). These strains were grown in Barbour-Stoenner-Kelly II (BSKII) medium supplemented with 6% normal rabbit serum (NRS) at 37°C 5%CO₂ (Barbour, 1984). Human epithelial-like neuroglial H4 cells were grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS) at 37°C 5%CO₂ temperature.

***B. burgdorferi* co-cultivation assay**

B. burgdorferi was co-cultivated with the human epithelial-like neuroglial H4 cell line to evaluate transcriptional changes in both the host and the bacterium. H4 cells were seeded at 1×10^7 cells per 15-cm tissue-culture dish in 30mL DMEM supplemented with 10% FBS and incubated for 17 hours at 37°C 5%CO₂. In parallel, *B. burgdorferi* wild type, *cyaB* mutant, and *cyaB* complement strains were grown to mid logarithmic phase ($5-8 \times 10^7$ *B. burgdorferi*/mL) in BSKII supplemented with 6% NRS at 37°C 5%CO₂. All three strains of *B. burgdorferi* were confirmed to be motile and healthy by dark field microscopy and then harvested by centrifugation at 4600 xg for 20 minutes at room temperature. Then the samples were washed in 1X PBS and centrifuged at

6600 xg for 10 minutes at room temperature, this step was repeated one more time. The supernatant was then removed and pellets were resuspended in 1mL of pre-warmed DMEM. *B. burgdorferi* was counted using dark field microscopy and added directly to H4 cells at a multiplicity of infection (MOI) of 40:1 (4×10^8 *B. burgdorferi* per 1×10^7 H4 cells). In parallel, H4 cells were incubated in DMEM alone as an uninfected control and each *B. burgdorferi* strain was cultivated in DMEM alone. The co-cultivation samples along with the controls were incubated for 24 hours at 37°C 5%CO₂. After incubation, the supernatant containing *B. burgdorferi* was collected from each cell culture plate and pellets were collected by centrifugation at 4600 xg for 20 minutes at 4°C. The resulting pellets were lysed in 1mL TRIzol. The adherent H4 cells were washed three times in 1X PBS and lysed with 6mL TRIzol. All the lysates were stored at -80°C for future RNA isolation.

Polymerase chain reaction (PCR)

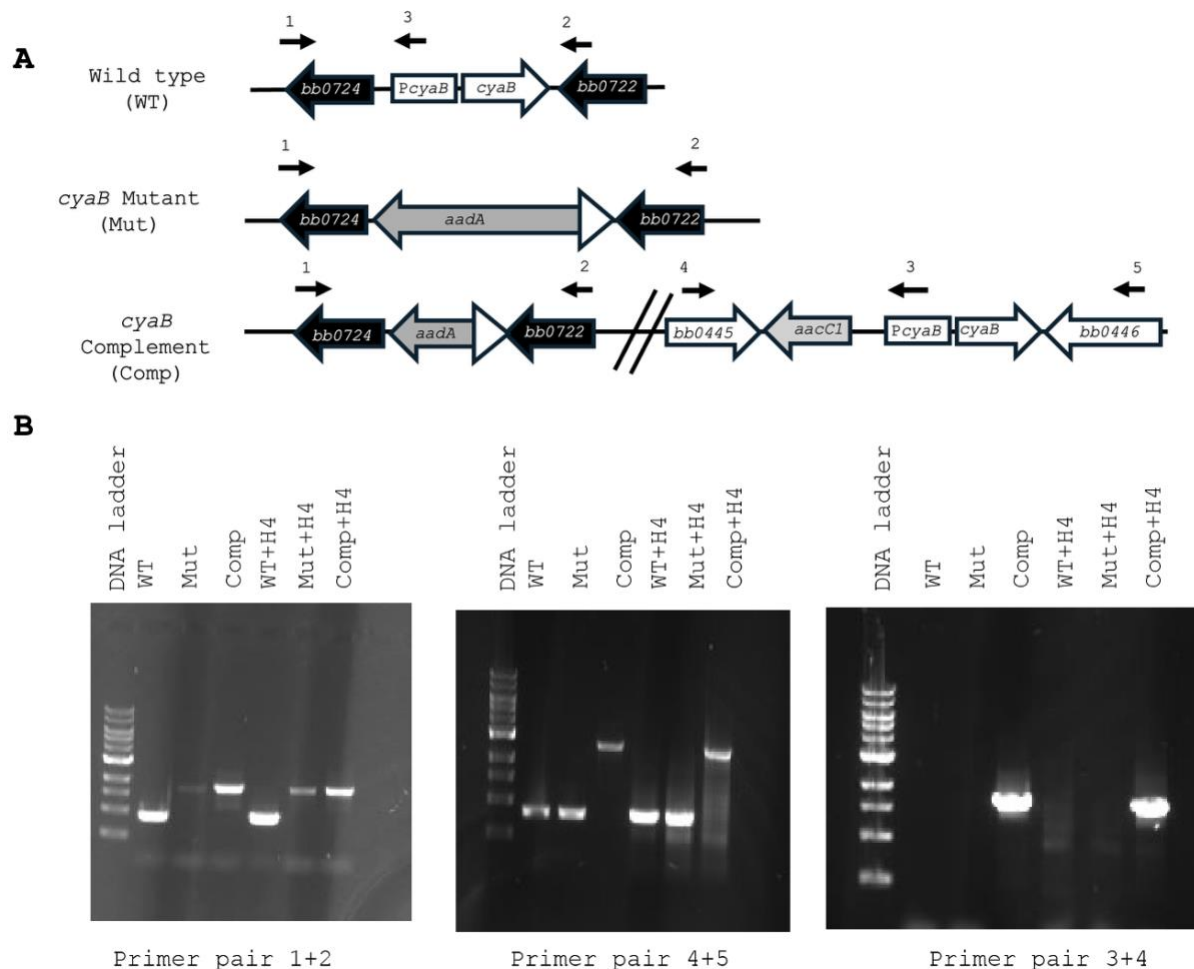
1x10⁷ *B. burgdorferi* co-cultivation sample was collected after 24 hours of incubation to screen for strain confirmation and plasmid loss. *B. burgdorferi* wild type, *cyaB* mutant, and *cyaB* complement strains cultivated with and without H4 cells were screened by PCR using primers listed in Appendix Table 1.

Appendix Table 1. List of primers used in this study.

Primer name	Sequence (5' to 3')
cp9-F	GAACTATTTATAATAAAAAGGAGAGC
cp9-R	ATCTTCTTCAAGATATTTTATTATAC
cp26-F	AATAATTCAGGGAAAGATGGG
cp26-R	AGGTTTTTTTGGACTTTCTGCC
lp17-F	CAAACCTATCAAATAGCTTATC
lp17-R	ACTGCCACCAAGTAATTTAAC
lp25-F	ATGGGTAAAATATTATTTTTTGGG
lp25-R	AAGATTGTATTTTGGCAAAAAATTTTC
lp28-1-F	ATGAACAAAAAATTTTCTATTTC
lp28-1-R	GTTGCTTTTGCAATATGAATAGG
lp28-2-F	TCCCTAGTTCTAGTATCTACTAGACC
lp28-2-R	TTTTTTTTGTATGCCAATTGTATAATG
lp28-3-F	GATGTTAGTAGATTAAATCAG
lp28-3-R	TAATAAAGTTTGCTTAATAGC
lp28-4-F	ATGTGGGATGCTATAATCGTGCC
lp28-4-R	TTGGTGGTGGCGCAGTGC
lp36-F	AAGTTTATGTTTATTATTGC
lp36-R	ATTGTTAGGTTTTTCTTTTCC
lp38-F	AAATTCTATGGAAGTGAT
lp38-R	TTTCTATTTATTTTATAGGC
lp54-F	GCACAAAAGGTGCTGAG
lp54-R	TTTTAAAGCGTTTTTAAGC
lp56-F	AAGATTGATGCAACTGGTAAAG
lp56-R	CTGACTGTAAGTATGTATCC
Primer 1	GTGCTGACATTGGGCTTTAT
Primer 2	AGAGAGCCTATAAACATAAT
Primer 3	GCTAGCAAATAAAAAATTTAAG
Primer 4	ATGAGGCGCTTTCTTATGGC
Primer 5	CCTAATCATGGTGGCATTGC

Result

Confirmation of experimental strains



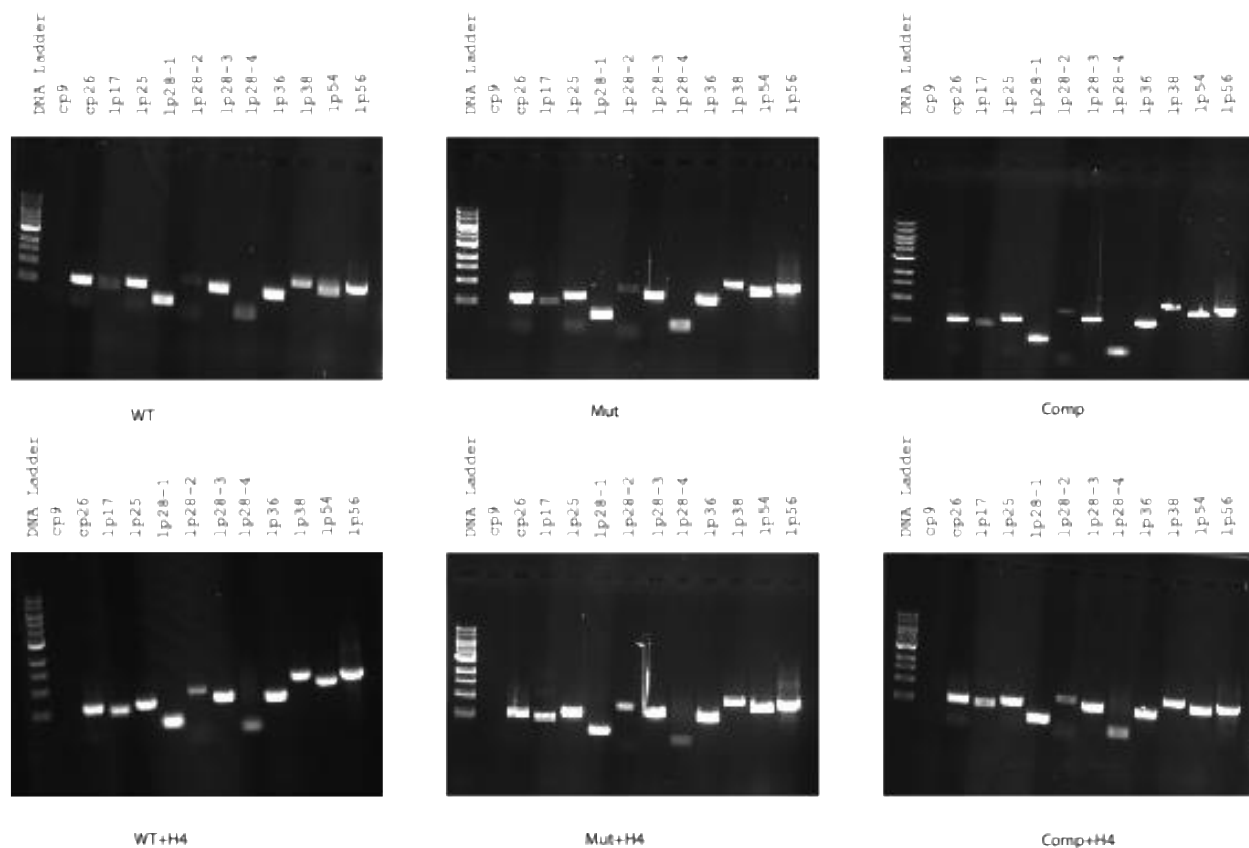
Appendix Figure 1. Confirmation of *B. burgdorferi* wild type, *cyaB* mutant, and *cyaB* complement strains by PCR.

(A) Schematic diagram of the *B. burgdorferi* wild type (WT), *cyaB* mutant (Mut), and *cyaB* complement (Comp) strains. Numbered arrows above each schematic shows the binding position and orientation of Primers 1–5. (B) PCR was used to confirm each of the strains and products were resolved on a 1% agarose gel. Samples assigned to each lane are labeled on top and primer pairs used for each PCR are labeled below each gel image.

B. burgdorferi WT, *cyaB* mutant, and *cyaB* complement strain exposed to H4 cells or to DMEM alone were isolated after 24 hours of incubation and PCR amplified to confirm the *B. burgdorferi* strain prior to downstream analysis. As shown in Appendix Figure 1B, there should be a difference in PCR amplicon sizes across the strains. Primer 1 and Primer 2 anneal to the flanking regions of *cyaB* which produce a 907 bp product in the wild type strain compared to a 1760 bp product in the mutant and complement strains. Primer 4 and Primer 5 anneal to *bb0445* and *bb0446*, respectively, which produces a 904 bp product in the wild type and mutant strains. In

the *cyaB* complement strain, the *cyaB* gene was reintroduced by allelic exchange between *bb0445* and *bb0446* as shown in Appendix Figure 1A. As a result, the Primer 4 and Primer 5 PCR product is 2636 bp as shown in Appendix Figure 1B. Primer 3 anneals to the *cyaB* promoter and Primer 4 anneals to *bb0445*, which can only amplify a product in the complement strain giving a product of 1895 bp. Both the H4-exposed *B. burgdorferi* samples and the DMEM-only control samples produced amplicons of the expected sizes, confirming the wild type, *cyaB* mutant, and *cyaB* complement strains are the correct strains and are not cross contaminated.

Screening for plasmid loss



Appendix Figure 2. Plasmid profiles of *B. burgdorferi* wild type, *cyaB* mutant, and *cyaB* complement strains from co-culture experiments.

Plasmid-specific primers were used to screen all three strains exposed in DMEM or H4 (labeled below each gel image). PCR products were resolved on a 1% agarose gel. WT= wild type, Mut= *cyaB* mutant, Comp= *cyaB* complement.

The *B. burgdorferi* genome consists of one linear chromosome and around 12 linear and 9 circular plasmids that are required during different phases of the enzootic cycle or *in vitro* growth

(Casjens et al., 2000; Fraser et al., 1997). During genetic modification or extensive *in vitro* culture, *B. burgdorferi* endogenous plasmids can be easily lost. Therefore, since this is an RNA sequencing experiment, we wanted to ensure that the entire starting genome is present before undergoing transcriptional analysis. To screen for the plasmid content from the co-culture experiment, plasmid profiles of the recovered strains were examined by PCR using plasmid-specific primers found in Appendix Table 1. The wild type strain lacks plasmid cp9 and the *cyaB* mutant and complement strains derived from the wild type strain also lack cp9 as shown in Appendix Figure 2. However, the *B. burgdorferi* wild type, *cyaB* mutant, and *cyaB* complement strains each retained the rest of the expected plasmid content assessed over the co-culture period. These results confirm that all three strains maintained a consistent plasmid profile, indicating that the samples are suitable for downstream transcriptional analysis.

Conclusion

Following co-cultivation, the identity of all three strains: the wild type, the *cyaB* mutant, and the *cyaB* complement, were confirmed by PCR (Appendix Figure 1). Moreover, plasmid profiling PCR for all these strains verified that each strain retained its expected plasmid content over the co-culture period (Appendix Figure 2). Together, this work provides an initial screening for downstream experiments to investigate the impact of *B. burgdorferi cyaB* at the host–pathogen interface.

Appendix B - Testing antimicrobial properties of polymers and microgel compounds

Polymer and microgel compounds evaluated in this Appendix were synthesized in Dr. Patricia Calvo's lab in the Department of Chemistry. These compounds were designed to exhibit antimicrobial activity and properties that support wound healing. We have used a variety of methods to test the antimicrobial properties of these polymer and microgel compounds.

Methods

Strains and growth conditions

Escherichia coli ATCC 25922, *Staphylococcus aureus* ATCC 29213, and *Pseudomonas aeruginosa* ATCC 27853 were grown from a single colony in 5mL Luria-Bertani (LB) broth at 37°C with shaking at 250 rpm overnight.

Broth microdilution method

The minimum inhibitory concentration (MIC) of the polymer compounds was determined using the broth microdilution method according to Clinical and Laboratory Standards Institute (CLSI) guidelines adapted from Wiegand et al., 2008. Four polymer compounds were resuspended with autoclaved ultrapure H₂O and tested at the range of 0.001-1mg/mL. The OD₆₀₀ of an overnight culture was measured and the culture was diluted in LB broth to an OD₆₀₀ of 0.002 (1×10^6 cells/mL). 50µL of polymer solution was added into 50 µL of 2X LB broth in the first well of a 96-well plate and a two-fold serial dilution in LB was done to test multiple concentration of the polymer. 50µl of 1×10^6 cells/mL bacterial culture was added to each well of the 96-well plate after serial dilution. Gentamicin was used as a control antibiotic at a range of 0.125-32µg/mL. The 96 well plates were incubated static at 37°C for 20 hours and the absorbance was measured at 600nm using a multiplate reader (BioTek Synergy LX from Agilent). The MIC was defined as the lowest compound concentration that completely inhibited visible bacterial growth and was determined for three biological replicates.

Agar direct diffusion method

Firstly, an LB agar plate was prepared with a uniform 25mL volume in each 10cm petri dish. Microgel compounds were freshly resuspended in autoclaved ultrapure water at a concentration of 60mg/mL. The OD₆₀₀ of an overnight culture was measured and the bacterial strain diluted in LB broth to an OD₆₀₀ of 0.1 (1×10^8 cells/mL). A cotton swab was used to streak the bacteria onto LB agar plates and was allowed to dry for 5-10 minutes. The microgels at

60mg/mL concentration were added directly on top of the agar plate at 2cm apart. Gentamicin is used as a control antibiotic at a concentration of 10µg. The agar plate with microgel was allowed to dry for around 10-15 minutes before incubating at 37°C overnight.

Result

Polymer compounds inhibit bacterial growth

The MIC of the four polymer compounds was determined by the broth microdilution method against *E. coli*, *P. aeruginosa*, and *S. aureus*, with gentamicin as a positive control (Appendix Table 2). Gentamicin inhibited *E. coli* at 0.008mg/mL and both *P. aeruginosa* and *S. aureus* at 0.004 mg/mL that follows the standard range of MIC according to CLSI (Sader et al., 2023). All four polymer compounds inhibited bacterial growth at 0.5mg/mL in the gram-negative *P. aeruginosa* and gram-positive *S. aureus* strains. Polymer compounds 1, 2, and 3 showed inhibition of *E. coli* growth at 1mg/mL, while polymer compound 4 failed to inhibit growth at the highest concentration tested. Together, these results indicate that all four polymers exhibit antimicrobial activity against *P. aeruginosa* and *S. aureus*, while *E. coli* was comparatively less susceptible and Polymer 4 was ineffective against this strain within the tested concentration range.

Appendix Table 2. Antimicrobial activity of polymer compounds.

Polymer	MIC (mg/mL)		
	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>
Gentamicin	0.008	0.004	0.004
Polymer 1	1	0.5	0.5
Polymer 2	1	0.5	0.5
Polymer 3	1	0.5	0.5
Polymer 4	>1	0.5	0.5

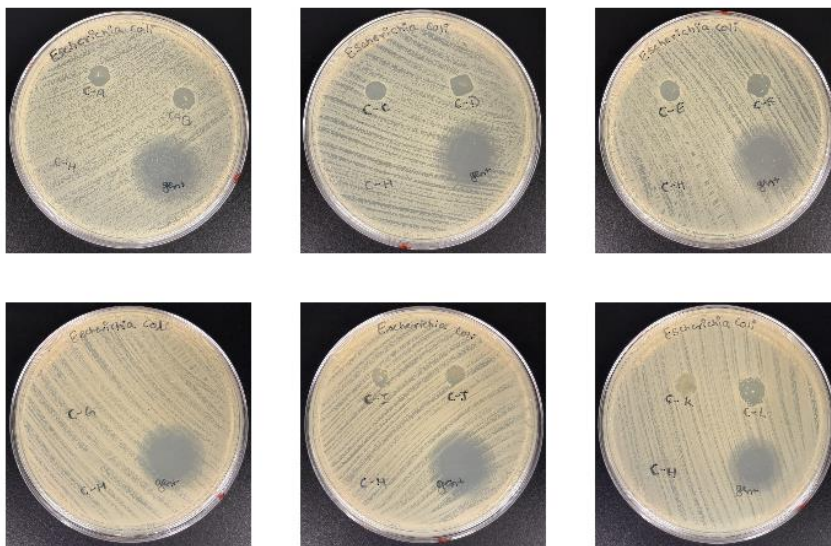
Standard deviation is ±1 two-fold change.

Microgel compounds inhibit bacterial growth

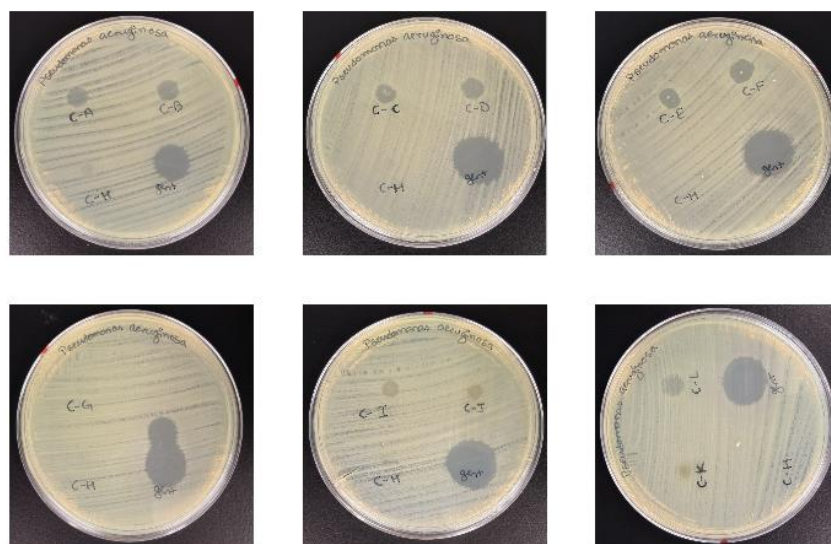
Appendix Table 3. Antimicrobial activity of microgel compounds.

Microgel	ZOI (mm)		
	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>
Gentamycin	16.1 (± 0.3)	16.6 (± 1.4)	18.81 (± 2.5)
C-G	-	-	-
C-H	-	-	-
C-A	+	+	+
C-B	+	+	+
C-C	+	+	+
C-D	+	+	+
C-E	+	+	+
C-F	+	+	+
C-I	+	+	+
C-J	+	+	+
C-K	+	+	+
C-L	+	+	+

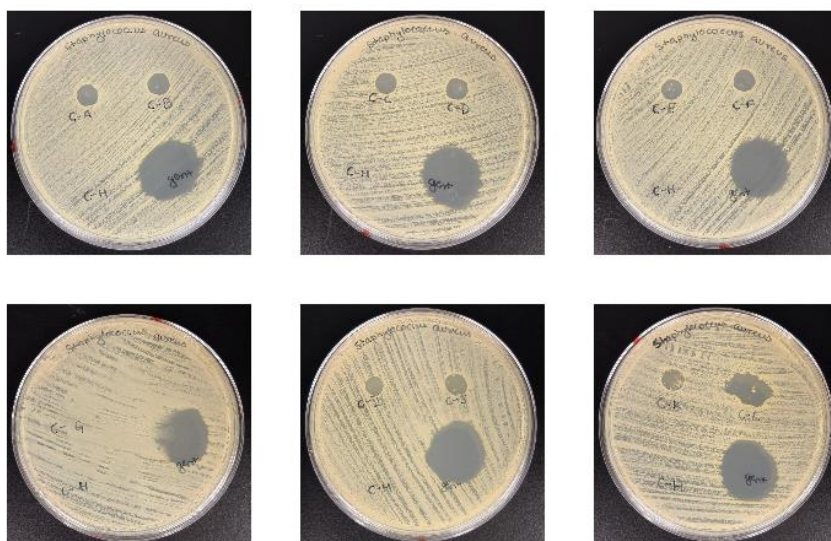
A



B



C



Appendix Figure 3. Antimicrobial activity of microgels evaluated by direct diffusion method.

Twelve microgel compounds were tested across bacterial strains (A) *E. coli*, (B) *P. aeruginosa*, and (C) *S. aureus*. Microgel compounds were added directly on the top of the LB agar plate swabbed with bacterial strains. Gentamycin at 10 μ g was used as an antibiotic control. Microgel compound C-H was used as a microgel control that doesn't show inhibition of bacterial growth.

The antimicrobial activity of twelve microgel compounds (C-A through C-L) was evaluated against *E. coli*, *P. aeruginosa*, and *S. aureus* using the agar direct diffusion method with gentamicin used as a control. Microgel C-H was used as a microgel control that didn't show inhibition in bacterial growth (Appendix Table 3). Gentamycin at 10 μ g produced clear zones of inhibition against all three strains measuring 16.1 ± 0.3 mm against *E. coli*, 16.6 ± 1.4 mm against *P. aeruginosa*, and 18.81 ± 2.5 mm against *S. aureus*. Ten of the twelve microgel compounds (C-A, C-B, C-C, C-D, C-E, C-F, C-I, C-J, C-K, and C-L) produced visible zones of inhibition against all three bacterial strains, indicating broad-spectrum activity against both the gram-negative strains *E. coli* and *P. aeruginosa* and the gram-positive strain *S. aureus* as shown in Appendix Figure 3. In contrast, the remaining two compounds, C-G and C-H, showed no detectable zones of inhibition against any of the three strains (Appendix Figure 3).

Conclusion

In this appendix, the antimicrobial properties of four polymers and twelve microgels were evaluated against the clinically relevant gram-negative and gram-positive bacteria *E. coli*, *P. aeruginosa*, and *S. aureus*. Using the broth microdilution method, all four polymers showed inhibition of bacterial growth for *P. aeruginosa* and *S. aureus* at a MIC of 0.5mg/mL, while *E. coli* was comparatively less susceptible. Polymer 4 showed no activity at the highest concentration tested for *E. coli*. Agar diffusion screening of the microgels showed that ten of the twelve compounds, C-A through C-F and C-I through C-L, produced clear zones of inhibition against all three bacterial strains tested, whereas compounds C-G and C-H were inactive. Taken

together, these results demonstrate that both the polymer and microgel formulations tested possess broad-spectrum antimicrobial activity, with the majority of compounds effective against both gram-negative and gram-positive bacteria. These findings support the idea that these compounds possess antimicrobial properties.

Appendix C - Supplementary documents for TPR-containing proteins

TPR consensus sequence comparison

BB0126		
Begin	--- w --- --- LG --- --- Y --- --- --- --- --- A --- --- F --- --- A --- --- P ---	End
82	SRFY L AR A VY F Q S Q A Q Y DE A I K D L D I V I K A K G I E	115
117	E I A F L N K A A V Y E K M G L K E D A L L V Y E D L I N S T S L D	150
BB0236		
Begin	--- w --- --- LG --- --- Y --- --- --- --- --- A --- --- F --- --- A --- --- P ---	End
42	F L I I D R V V N I Y I A N K N Y E D A L E I V N N G I I D D E S R	75
79	P L Y L Y L M G N I Y D S M G E D F V A F N I Y K R V D N F D D Y	112
158	G N Y F Y N L A L S L E D V Q D Y D E S Y F Y Y K K F L S I P R A H	191
BB0133		
Begin	--- w --- --- LG --- --- Y --- --- --- --- --- A --- --- F --- --- A --- --- P ---	End
6	I V E L I K E I Y L D F R K G N F K E A L V K S E E A H S L D F D N	39
49	S V Y W N G Q V E S L D R I G Q D Y E K A E F L I R E W N N F A G R	82
87	M G C N F L Q G R N S I K Y F V F Q T C L Y I Y K N I Y K M H P E N	120
121	L D L L I K I A K S Y K G M G N Y E R A I N V F L Q I L G D F K D N	154
155	S D V V A E L A D S Y A L V D E I K E A K V L F R E A F F I N P Q K	188
BB0134		
Begin	--- w --- --- LG --- --- Y --- --- --- --- --- A --- --- F --- --- A --- --- P ---	End
27	S N I K Q A I A I L L V S Q G K E E K A I Q I W L K M I E E N Q E V	60
BB0171		
Begin	--- w --- --- LG --- --- Y --- --- --- --- --- A --- --- F --- --- A --- --- P ---	End
34	A W G Y Y N L G V V Y S L G E Y E S S L R I F S Y A K K T N D I F	67
70	F N V N Y N E G V I Y Y N Q G L Y H K A E V A F K E A L K I N P G S	103
BB0195		
Begin	--- w --- --- LG --- --- Y --- --- --- --- --- A --- --- F --- --- A --- --- P ---	End
24	V T E K S K R G Y Q L I K E E R L S E A E S L F S D I L E K D N E N	57
58	N Y A L V G L G D I E R K K N N Y D K A I V Y Y Q K C L V K H P S N	91
92	N Y A L F G L G D C Y R N L D N Y K K A T D I W E E Y L K Y D P E N	125
126	I T V L T R V A A S Y R K L K N F Q K S K Q T Y L K V M E L I P D N	159
160	D Y A L V G I G H L Y Y D F K E Y K E A L K Y W L K M Y E L N Q S K	193
196	V R V L T S I G N C Y R K L R E F T R G I Y F F K K A L E I S P S N	229
230	F Y A V F G L A D C Y R G N K E Y K E A L K Y W L D I E K D P K N	263
297	M F A I L G L A L I Q K E Q G K Y E E A L I A I K S L I K N N P K N	330
331	S A L Y V N A A E C Y E A L G O I G N A V D I L S S F L Q L G M K N	367

BB0209

Begin	---w---LG---Y-----A---F---A---P--- ---L---IA---L-----S---Y---S---K--- ---Y---MS---F-----E---L---L---E---	End
29	SQR L IRIGKEEMKNKNYIQ A IET L SD A IKKY P KV	62
63	QLG Y Y F L S I A Y RENNQLTE A EG A L LDGIAVGGE I	96
100	LYYELGNIMFNRGEGYYPL A IK Y SN S IKSR P NY	133
141	AN A Y VQQ G KITSKEKEYQ K AWDS Y T M A IHDYSQ F	174

BB0261

Begin	---w---LG---Y-----A---F---A---P--- ---L---IA---L-----S---Y---S---K--- ---Y---MS---F-----E---L---L---E---	End
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61	IG A L E I L NN Y YK N KDHENG I KYAK K L CQL I E DN	94
103	FKAFLSY G FYNLKRNFN R E A LE Y L R K A FL I K K TD	136
137	EDAN Y L G V A F LKNEMYKE A LY Y L KKVHN F N K NN	170
171	KDV L K H L G I TLFNLESY R Q A V I I F NNIKK N IQGD	204
205	IE A L L A Y A KSLSKM N QDHL A LE I ANK I K QKDGM I	238
239	YE A L L I T S E I HSKNKELE K LEQNIKE I I K V K P DL	272
278	LELFYN L GELQISVENYQ K ATE A F TKVEE I D P NY	311

BB0298

Begin	---w---LG---Y-----A---F---A---P--- ---L---IA---L-----S---Y---S---K--- ---Y---MS---F-----E---L---L---E---	End
69	ARFFNL I GLEFFKLGQYGP A IE Y F AKNLEIN P NN	102
183	FE F L MLRG A NY S SLGDLGN A IL F Y DK A SKKASTE	216

BB0315

Begin	---w---LG---Y-----A---F---A---P--- ---L---IA---L-----S---Y---S---K--- ---Y---MS---F-----E---L---L---E---	End
37	IE K L YQKS M LLKDSK K YN K A IES L TK I INMDQ N Q	70
71	AD A HL L L SELE Y LNKNW K K A IIKSQD Y L K IID F K	104
106	KKNFLD I SWAYFLIGEV K N S MD Y I I K FFQSG K EL	139

BB0398

Begin	---w---LG---Y-----A---F---A---P--- ---L---IA---L-----S---Y---S---K--- ---Y---MS---F-----E---L---L---E---	End
42	FL I IDRVV N I Y IANKN Y ED A LEIVN N GIID D E SR	75
79	PL Y L Y L M G N I Y DSMGED F V A F N I Y KRVVD N FDD Y	112
158	G N YFYN L A L S L EDVQD Y DE S Y F Y Y KK F LSIP R A H	191

BB0459

Begin	---w---LG---Y-----A---F---A---P--- ---L---IA---L-----S---Y---S---K--- ---Y---MS---F-----E---L---L---E---	End
86	PFENYNLGVFYFNLKEYKLALSCFLKAKEQKSDF	119
120	VQALNNSAVLFEMLDNKDEALKLFFEARDLDQNN	153
187	ALVVNYLMYYFYSIGEISR A IRLSEKFLTDSHYS	220
221	KYIWHNRATI F HKIGNMTQATTSYVKA ILNFPNI	254
287	DLIYLYATLFFLRNRDLEDAYFYMKKLCELNPEP	320

BB0537

Begin	---w---LG---Y-----A---F---A---P--- ---L---IA---L-----S---Y---S---K--- ---Y---MS---F-----E---L---L---E---	End
25	PPKEKEDSLLLYKEGKFKEA ILNTLEEIRLNPSN	58
59	LDARTILIWSLIAIGEYKRAEKEAII GLGIKKHD	92
93	IRIIQALGEAYFFQKNYDNALKYFQEYISLDSKG	126
130	IKVYNLIADS FYELKRYNEADFA YEHALRFS PNN	163
164	QNL L I K L A R S R I N A K N K I L A E E A L I K I L T I S P N N	197

BB0542

Begin	---w---LG---Y-----A---F---A---P--- ---L---IA---L-----S---Y---S---K--- ---Y---MS---F-----E---L---L---E---	End
42	SGEYYKLA KLNEELGNDET SVLLYEK SIKFNINA	75
77	DDSSYNFILACINLKKYVEAELK LNSIIKEDPEN	110
111	ILLINLKGYL LFKKNDLDN ALIYYLKTLEFAPAN	144
145	KEA LFN IFYIYHLKSDKKN AKKYISKYKELNHPT	178

BB0714

Begin	---w---LG---Y-----A---F---A---P--- ---L---IA---L-----S---Y---S---K--- ---Y---MS---F-----E---L---L---E---	End
120	SPLYYI LGKAYSHKGEFYSELAVKFLKKALNADN	153
161	EDIFEYLGYSYQLLRDYKSSLKFFEKAFNENKSD	194
240	QKVYLLY GDI LLDKGDYDS AFNYYNKVL E INSSN	273
275	SVYVKIGDIYRKKDKDYPK ARKYWRE ALNLN PYL	308

BBJ23

Begin	---w---LG---Y-----A---F---A---P--- ---L---IA---L-----S---Y---S---K--- ---Y---MS---F-----E---L---L---E---	End
137	NLM L L R Y A G G A A L S K L A N E A R K Y F E K S L K I N S K N	170
213	YFANIW I S Q A H F L L K N E K E S L K Y I L K A G E I F P N G	246

BBJ24

Begin	---w---LG---Y-----A---F---A---P--- ---L---IA---L-----S---Y---S---K--- ---Y---MS---F-----E---L---L---E---	End
209	YLKELVKSSFL IRTNRRLEAIECLKKATAIFPNG	242

BB0459

Begin	---w--LG--Y-----A---F--A---P-- ---L--IA--L-----S---Y--S---K--- ---Y--MS--F-----E---L--L---E---	End
86	PFENYNLGVPFYFNLKEYKLALSCFLKAKEQKSDF	119
120	VQALNNSAVLFEMLDNKDEALKLFFEARDLDQNN	153
187	ALVVNYLMYYFYSIGEISRAIRLSEKFLTDSHYS	220
221	KYIWHNRATIFHKIGNMTQATTSYVKAILNFPNI	254
287	DLIYLYATLFFLRNRDLEDAYFYMKKLCELNPEP	320

BB0537

Begin	---w--LG--Y-----A---F--A---P-- ---L--IA--L-----S---Y--S---K--- ---Y--MS--F-----E---L--L---E---	End
25	PPKEKEDSLLLYKEGKFKEAILNTLEEIRLNP SN	58
59	LDARTILIWSLIAIGEYKRAEKEAIIGLGIKKHD	92
93	IRIIQALGEAYFFQKNYDNALKYFQEYISLDSKG	126
130	IKVYNLIADSFYELKRYNEADFAYEHALRFS PNN	163
164	QNLLIK LARS RINAKNKILAEELIKILTIS PNN	197

BB0542

Begin	---w--LG--Y-----A---F--A---P-- ---L--IA--L-----S---Y--S---K--- ---Y--MS--F-----E---L--L---E---	End
42	SGEYK LA KLNEELGNDET SVLLYEKSIKFNINA	75
77	DDSSYNFILACINLK KYVEAELK LNSIIKED PEN	110
111	ILLINLKGYL LFKKNDLDN ALIYYLKTLEFA PAN	144
145	KEALFNIFYIYHLKSDKKNAKKYISKYKELNHPI	178

BB0714

Begin	---w--LG--Y-----A---F--A---P-- ---L--IA--L-----S---Y--S---K--- ---Y--MS--F-----E---L--L---E---	End
120	SPLYYILGKAYSHKGEFYSELAVKFLKKALNADN	153
161	EDIFEYLGYSYQLLRDYKSSLKF FEKAFNENKSD	194
240	QKVYLLYGDI LLDKGDYDS AFNYYNKVLEINSSN	273
275	SVYVKIGDIYRKDKDYPKARKYWREALNLN PYL	308

BBJ23

Begin	---w--LG--Y-----A---F--A---P-- ---L--IA--L-----S---Y--S---K--- ---Y--MS--F-----E---L--L---E---	End
137	NLM L LRYAGGAALSKLANEAR KYFEKSLKINSKN	170
213	YFANIWISQAHFLLKNEKESLKYILKAGEIF PNG	246

BBJ24

Begin	---w--LG--Y-----A---F--A---P-- ---L--IA--L-----S---Y--S---K--- ---Y--MS--F-----E---L--L---E---	End
209	YLKELVKSSFLIRTNRRLAEIECLKKATAIF PNG	242

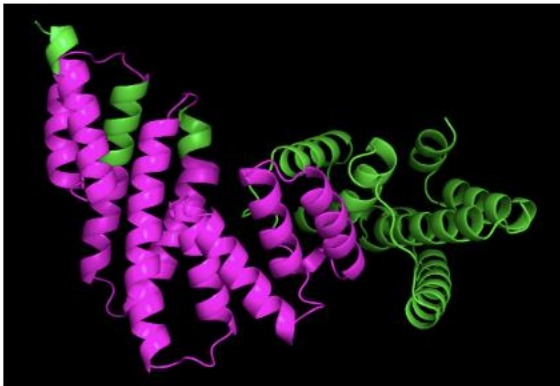
Appendix Figure 4. TPR consensus sequence comparison to *B. burgdorferi* putative TPR-containing proteins.

The identified TPR sequences of each of the 17 putative TPR-containing proteins of *B. burgdorferi* were predicted using TPRpred and aligned with the TPR consensus sequence. TPR consensus residues are placed on top of the identified TPR sequence for each TPR-containing protein and shown in magenta. The conserved residues that match the consensus residues are also highlighted in magenta. The numbers flanking each TPR sequence indicate the amino acid positions at which the sequence begins and ends as predicted by TPRpred.

B. burgdorferi TPR-containing protein structures

Cytoplasmic

BB0133



BB0459

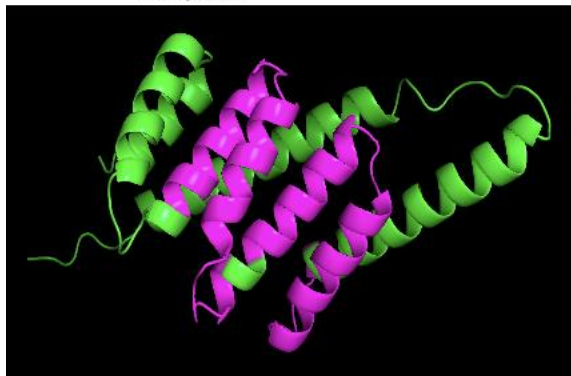


BB0195

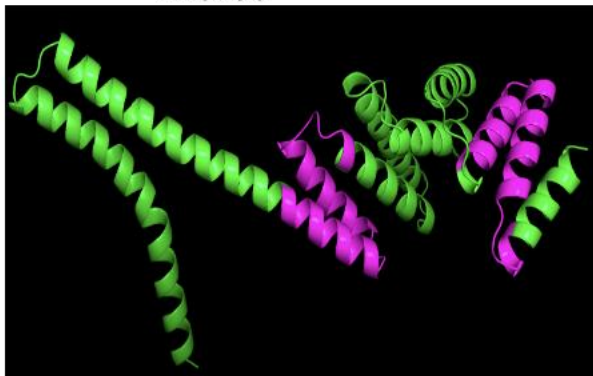


Lipoprotein

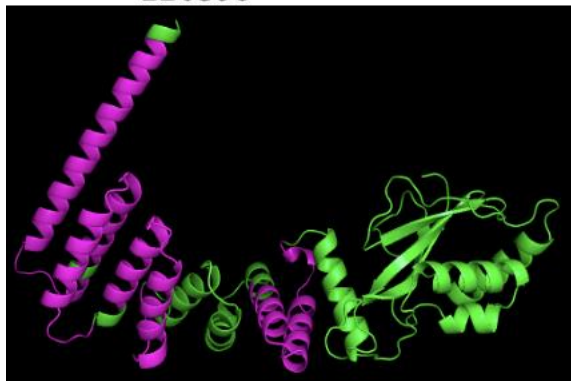
BB0171



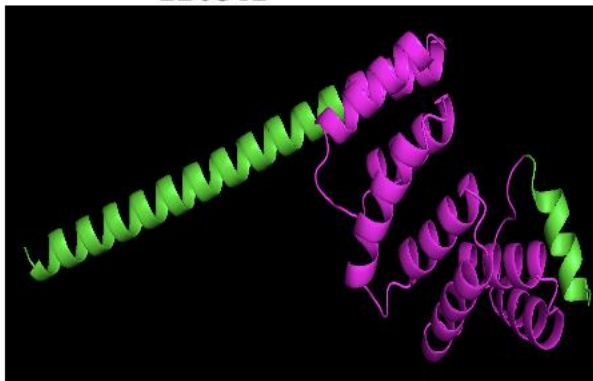
BB0298



BB0398

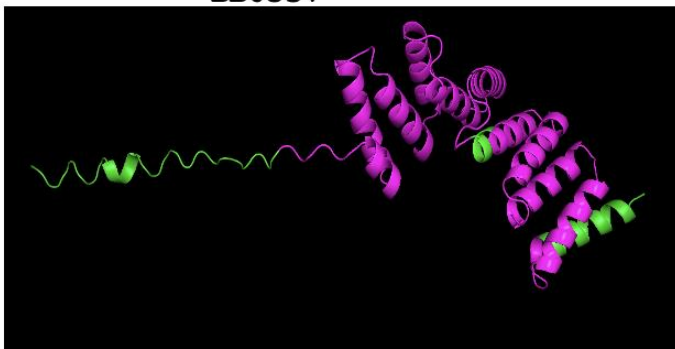


BB0542

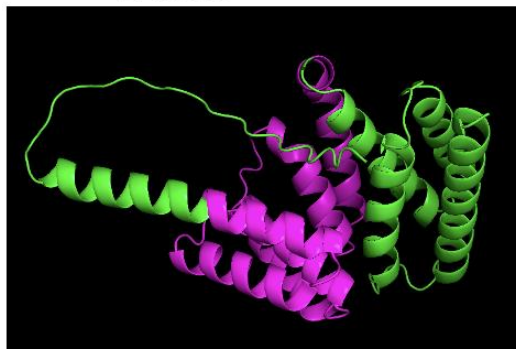


Signal sequence

BB0537



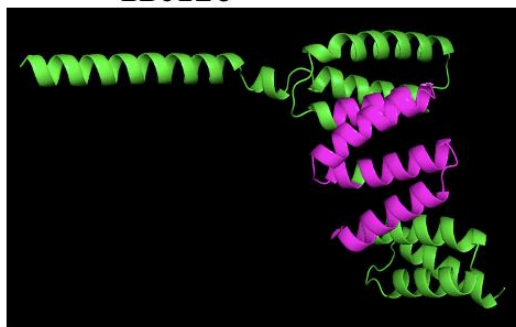
BB0315



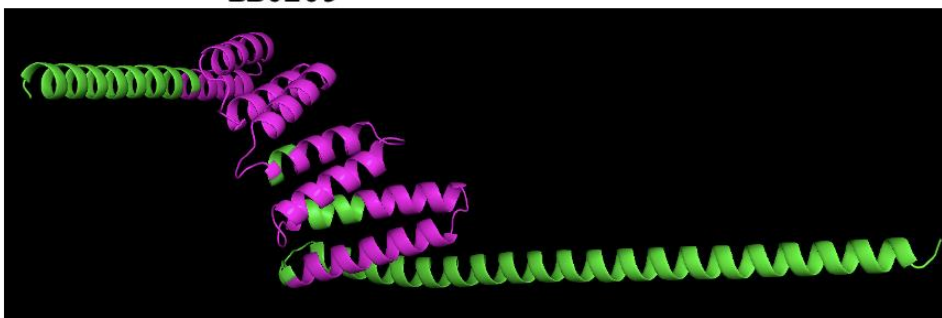
BB0106



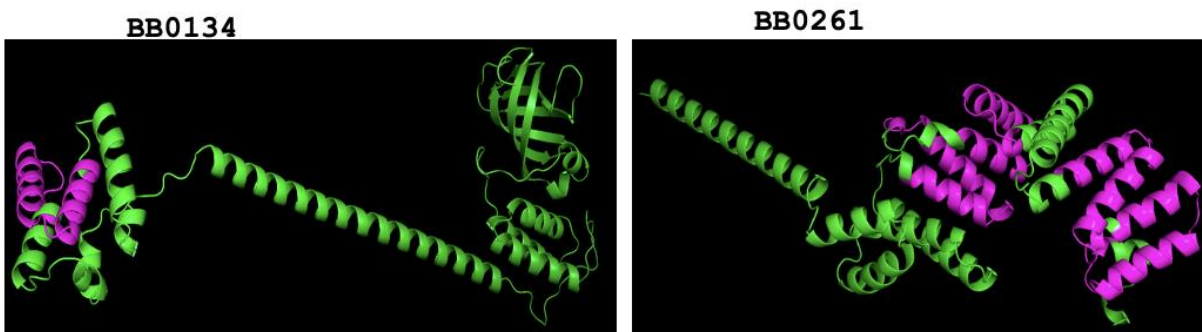
BB0126



BB0209



Transmembrane



Appendix Figure 5. Mapping of identified TPR motifs into the *B. burgdorferi* predicted protein structure.

TPR sequences generated by TPRpred have been mapped across the proteins structure predicted by AlphaFold3 in PyMOL and are highlighted in magenta. These proteins are categorized according to their predicted location given by PSORTb.