University of South Bohemia in České Budějovice

Faculty of Science

Differentiating the Adhesion Properties of Surface Proteins between Various *Borrelia* Species

Master's Thesis

Laboratory of Molecular Ecology of Vectors and Pathogens

INSTITUTE OF PARASITOLOGY

Biology Center, ASCR

Bc. Anna-Celine Danklmaier, Bsc.

Supervision by Ryan O. M. Rego, PhD

České Budějovice 2021

Danklmaier, A-C., 2021: Differentiation of the Adhesion Properties of Surface Proteins of Various *Borrelia* Species. Mgr. Thesis, in English. – 49 p., Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic.

Annotation: The aim of the thesis was to gain a better understanding of decorin binding proteins A and B (DbpAB) of various *Borrelia* species my different means. Overexpression of DbpA or DbpB in *B. afzelii* was addressed, examining phenotypic changes in mutants. Also, it was aimed to create shuttle vectors to complement DbpAB of different European *Borrelia* species as well as suicide vectors.

České Budějovice, 13.12.2021   Anna-Celine Danklmaier

Acknowledgement

1. Introduction

1.1. Lyme Disease

Lyme disease is considered as the most common arthropod-borne disease in the Northern Hemisphere, including North America, Europe and Asia [1,2]. In North America there are more than 476,000 cases reported annually [3] and in Europe the number of documented cases exceeds 200,000 [4]. Also referred to as Lyme borreliosis, it is defined as a multisystem inflammatory disorder caused by spirochetes of the *Borrelia burgdorferi* sensu lato (s.l.) complex [5]. The transmitting vector is the hard tick of the genus *Ixodes* [6].

1.2. *Borrelia*

The species of *Borrelia* belong to the phylum *Spirochaetes*. Together with other members of this phylum like *Leptospira* and *Treponema* they cause pathogenic disease in humans. The latter two genera are causative agents of, respectively, leptospirosis (*Leptospira interrogans*) and either syphilis (*Treponema pallidum*) or periodontal disease (*Treponema denticola*) [7–9]. *Borrelia* species are subdivided into two major phylogenetic groups, one related to relapsing fever and the other to Lyme disease, which is also called Lyme borreliosis. Its causative agents constitute the *Borrelia burgdorferi* sensu lato (s.l.) complex, consisting of more than 20 species [5,10] that are transmitted by ticks of the genus *Ixodes* [2,11]. However, primarily *B. burgdorferi* sensu stricto (s.s.), *B. afzelii*, *B. garinii* and *B. bavariensis* are known to be pathogenic in humans. Though there are other genospecies that occasionally cause human Lyme disease: *B. bissettii*, *B. mayonii*, *B. lusitaniae*, *B. spielmanii* and *B. valaisiana* [4,12–14]. Also, there are differences concerning the geographical distribution of the species. While *B. afzelii*, *B. garinii* and *B. bavariensis* cause human disease in Europe and Asia, *B. burgdorferi* is the predominant agent in North America [12].

1.3. Genome Complexity of *Borrelia*

The borrelial genome is one of the most complex genomes among bacteria due to its unique segmentation. It consists of a linear chromosome and a set of circular and linear plasmids (denoted cp and lp respectively). *B. burgdorferi* B31 was the first sequenced *Borrelia* strain, which genome comprises one large linear chromosome of about 910 kilo base pairs (kbp) and 21 extrachromosomal plasmids (12 lps and 9 cps) which range in size from 5 to 56 kpb

1

approximately, with a total of around 610 bp [15,16]. Two additional plasmids were found to be lost from the sequenced strain due to *in-vitro* cultivation [17].

In contrast to other bacteria, the genome appears to be rather small what corresponds to the parasitic lifestyle of *B. burgdorferi*, relying mainly on the biosynthetic machinery of its host. Genes necessary for the synthesis of amino acids, fatty acids, enzyme cofactors and nucleotide are almost completely absent [15,18,19]. Also, even though the structure of the borrelial genome is unusual, most genes encoded on the linear chromosome of *Borrelia* are also found in other bacterial genomes [15]. Reversely, only a fraction of plasmid-encoded genes shows similarities to genes in other genera [16]. The majority of housekeeping genes of *Borrelia* are located on the chromosome, while the plasmids carry most genes that encode surface lipoproteins that are necessary for the transition through the enzootic cycle. However, both, chromosome and plasmids carry genes that are important for infectivity or the maintenance of the enzootic cycle. The exact functions of these genes differ; some encode surface proteins necessary for host cell interactions, immune evasion, or basic cellular and regulatory functions [16,19–22]. For example, the plasmid-encoded nicotinamidase PncA, which is involved in NAD biosynthesis, was found to be essential for mammalian infection. In *B. burgdorferi* B31 *pncA* is found on lp25, which absence is associated with a loss of infectivity in mice [23–27] and ticks [28]. Another virulence gene is present on lp25, namely *bptA*, which is essential for the survival of *B. burgdorferi* in its tick vector [29]. Also, *adeC*, encoding for an adenine deaminase, was shown to be required for infectivity in mice, and is located on lp36 in B31 [30]. An essential mechanism for immune evasion of is the recombination at the antigenic variation locus *vls*, located on lp28-1in B31. It consists of 15 silent cassettes and the expression site *vlsE*, encoding for the variable surface lipoprotein VlsE [31]. It was confirmed that mutants lacking lp28-1 do not cause a persistent infection in mice [23,25,32].

Since a complete genome sequence was only available for *B. burgdorferi* B31 for a long time, most of the research done concerning genetic manipulations of *Borrelia* has been conducted with *B. burgdorferi* s.s. strains. With the release of the genome sequences of *B. afzelii* and *B. garinii* [33], the study of European species had become feasible. Also, the sequencing methods have been improved, so that more genome sequences had been published during the last years [21,22,34]. However, there is still only a moderate number of genetic studies carried out for European *Borrelia* species [35]. Additionally, the research involving genetic manipulations can be difficult, since *B. burgdorferi* s.l. species exhibit heterogenicity in their gene content, but also in number and types of plasmids they possess. The linear chromosomes were found to be relatively constant in gene content and organization throughout the species

[16,19–22,36]. Some plasmids also show a high degree of conversation among the sequenced isolates. Particularly cp26 and lp54 are present in all genomes with high sequence identities [16,21,22,37–39]. Nevertheless, these plasmids carry genes important for infection and host adaption, which are highly variable. The outer surface lipoprotein OspC, encoded exclusively on cp26, is expressed during early stage of a mammalian infection and is essential for establishment of an infection [26,40]. It is one of the most variable single-copy genes in the *B. burgdorferi* s.l. genome [41] and seems to have experienced several horizontal exchange events [42–45]. Similarly, decorin binding proteins A and B (DbpAB), encoded on lp54, show low sequence identity across *Borrelia* species [46]. The surface-expressed proteins facilitate adhesion to host tissues and are found to be important for virulence in mice [47–51]. The variations of the sequences contribute to differences in tissue tropism [52].

Since the number and types of plasmids is not identical across the species, certain genes are located on different plasmids. For instance, the previously mentioned virulence genes *pncA* and *bptA* are found on lp28-2 in *B. afzelii* strains, instead of partially related lp25 in *B. burgdorferi* [36] and *B. garinii* [22]. Similarly, *B. afzelii* does not possess lp36, which encodes for *adeC*, as in *B. burgdorferi*. However, the *adeC* homolog is located on lp38 [22,36] and on lp25 in *B. garinii* [22]. In Table 1.3.1 a summary of important virulence genes in different *B. burgdorferi* s.l. species is shown.

Table 1.3.1: Important genes for infectivity of chosen *B. burgdorferi* s.l. species and their respective location in the genome (adapted from Casjens *et al.* 2018 [22] for all strains except PBi which information was retrieved from NCBI). *In *B. bavariensis, vlsE* was found to be present on lp28, but it is unclear which one exactly [38].

Strain	*adeC*	*dbpAB*	*ospC*	*pncA*	*vlsE*
B. burgdorferi **B31**	lp36	lp54	cp26	lp25	lp28-1
B. afzelii **PKo**	lp38	lp54	cp26	lp28-2	lp28-8
B. garinii **PBr**	lp25	lp54	cp26	lp25	lp28-3
B. bavariensis **PBi**	lp25	lp54	cp26	lp25	lp28*

The described uniqueness of the borrelial genome (as well as the variations in genome content between the species) makes genetic manipulation a challenging task. In general, the lack of biosynthetic pathways requires a complex growth medium for *in-vitro* cultivation of *Borrelia*, like for example the addition of mammalian serum to the culture medium [15,20]. Also, transformations of *Borrelia* can be difficult due to low transformation efficiencies. In contrast to other bacteria which only need nanogram amounts of DNA to be transformed, *Borrelia*

require larger quantities in the microgram region [53]. This arises from the plasmid-encoded restriction modification enzymes which degrade foreign unmethylated DNA. It was observed that the loss of plasmids lp25 and lp56, which carry the putative restriction modification genes, leads to an increased transformation efficiency of *B. burgdorferi* [54]. Furthermore, it is essential to monitor the segmented borrelial genome during genetic manipulations, importantly after the selection of mutants, to ensure that wild type and mutants remain isogenic except for directed genetic changes [55]. Certain plasmids of the potentially unstable genome are frequently lost during *in-vitro* studies. For example, the plasmids lp25 and lp28-1 of *B. burgdorferi*, which are essential for the enzootic cycle, are commonly lost [56–58]. In general, passaging of cultures as well as multiple thawing and freezing of glycerol stocks can lead to loss of multiple plasmids, hence heterogeneity in the outgrowth population [59]. One exception is the circular plasmid cp26, which is never lost during genetic studies like *in-vitro* propagation [60,61].

1.4.   Transmission Dynamics

B. burgdorferi s.l. species are maintained in their natural enzootic cycles through the transmission by tick vectors to their vertebrate reservoir hosts and vice versa. Hard ticks of the genus *Ixodes* can acquire and transmit the bacteria. There are four principal species that are vector competent: *Ixodes scupularis* and *I. pacificus* in eastern and western North America, *I. ricinus* in Europe and *I. persulcatus* in Asia [6,62]. The ticks tend to feed on variable vertebrate hosts, which include rodents, birds, and lizards, but also larger vertebrates like deer. These host species differ in their capability to acquire *B. burgdorferi* s.l. from infected ticks and to further transmit it to uninfected ticks. It depends on the *Borrelia* species whether a host is reservoir competent [63]. Consequently, this leads to the interconnection of the ecological niches of *Borrelia* [6]. Rodents like mice and voles serve as reservoir for *B. afzelii*, whereas *B. garinii* depends on birds. In contrast *B. burgdorferi* relies on both, rodents, and birds. *B. bavariensis* is associated with mice as reservoir hosts [6,64].

During the infectious cycle of *B. burgdorferi* s.l. the hard tick vectors undergo three developmental stages (larva, nymph, adult) over the course of a two-year life cycle. As depicted in Figure 1.4.1, one blood meal is obtained per life stage of the ticks. Larvae must acquire *B. burgdorferi* via the first blood meal from an infected reservoir host, like small rodents or birds. *B. burgdorferi* is retained during the subsequent life stages. Infected nymphal ticks, which have a similar host range as larvae, transmit the spirochetes during the second

blood meal to a naïve host [65]. That larvae and nymphs feed on the same animal species is essential for the persistence of the spirochetes throughout different tick generations [5]. Since adult females feed primarily on large animals like deer, which are non-competent host, they are not important for the maintenance of *B. burgdorferi* [6]. Still, adults generally mate on deer what makes them essential for sustaining the tick populations. Mostly infected by nymphal ticks, humans are considered as incidental, suspectedly dead-end hosts, and are not part of the enzootic cycle [65].
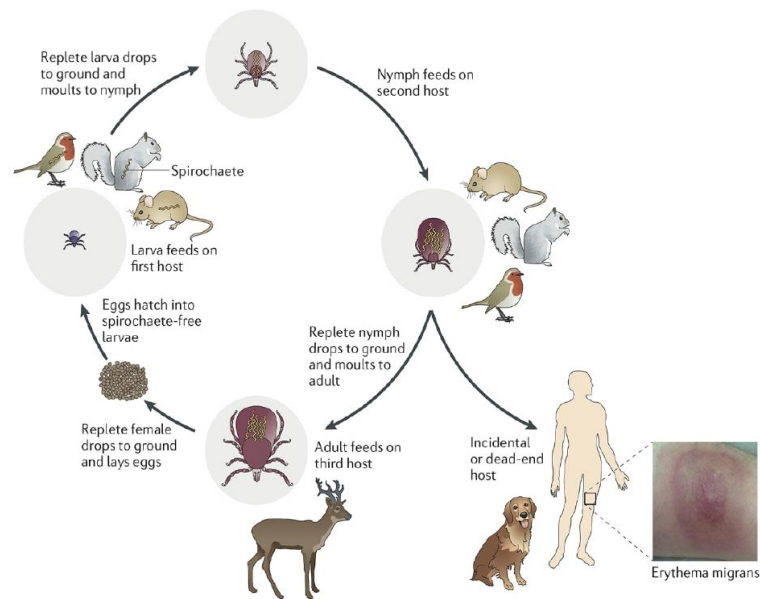

Figure 1.4.1: Graphical depiction of the enzootic life cycle of *B. burgdorferi* (from Radolf *et al.* 2020 [66]).

1.5. Morphology

As *Borrelia* belong to the phylum of spirochetes, they show similar morphology, like the distinctive helical cell shape and flat-wave morphology (see Figure 1.5.1). Instead of external flagellar filaments that are found in most motile bacteria, spirochetes possess periplasmic flagella. These are located within the periplasmic space between the outer membrane and the peptidoglycan layer which surrounds the protoplasmic cylinder that is enclosed by a cytoplasmic membrane [67,68]. Also, the outer membrane possesses a number of different surface-exposed lipoproteins [68]. Often, *B. burgdorferi* is referred to as gram-negative-like bacterium, however it lacks lipopolysaccharides on the outer membrane [15]. The periplasmic flagella are attached via the flagella motors to both terminal ends of the protoplasmic cylinder, the innermost compartment of spirochetes [68,69]. Depending on the species, the morphology can differ in terms of size, number of flagella and if these overlap in the middle of the cell

[68]. In the case of *B. burgdorferi* there are 7-11 periplasmic filaments inserted at the cell poles which wind around the protoplasmic cylinder, in a ribbon-like structure, and overlap in the middle [67,70]. Also, *B. burgdorferi* is considered as a comparatively large spirochete with a length of 10-20 µm and a diameter of 0.33 µm [71]. Its shape is characterized to be a planar flat wave with an amplitude of 0.78 µm and a wavelength of 2.83 µm [71,72]. Not only are periplasmic flagella essential for the motility, but also partly for the distinct morphology of the spirochetes. It was found that *B. burgdorferi* mutants which are deficient in *flaB*, encoding for the major filament forming protein FlaB, are non-motile and rod-shaped [73]. However, it was also shown that FlaB alone is not leading to the characteristic flat-wave morphology, but also motor rotation. Upon inactivation of *motB*, which codes for the flagellar motor stator MotB, *B. burgdorferi* loses its motility as well as the characteristic morphology, while retaining periplasmic flagella. Complementation restored the phenotype of the wild type [74]. Additionally, both mutants are unable to establish a murine infection by needle inoculation, but also via transmission from infected ticks. A reduced viability in fed ticks was observed as well [74,75].
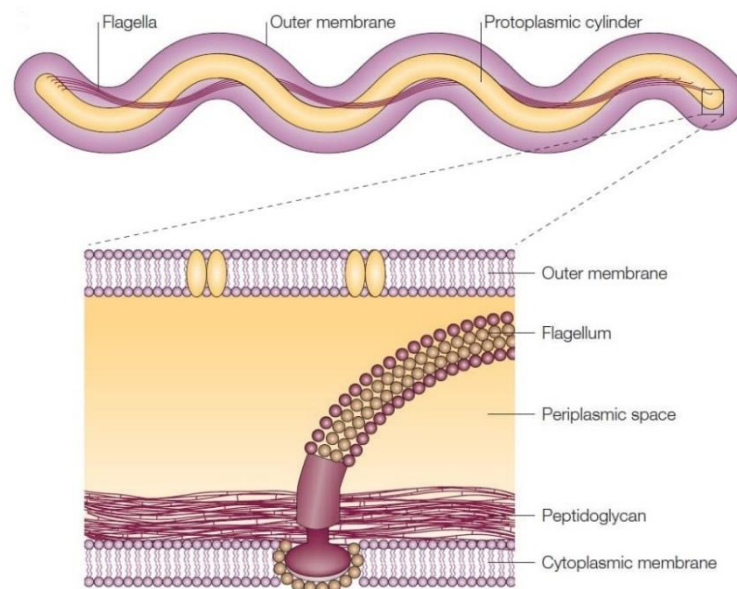

Figure 1.5.1: Structure and morphology of *B. burgdorferi*. (Adapted from Rosa *et al.* 2005 [76])

1.6. Clinical Manifestations

Lyme disease (or Lyme borreliosis) is a multisystem inflammatory disorder with diverse clinical presentations depending on the *B. burgdorferi* s.l. species [5,77]. In general, the course of infection can be divided into three stages: early localized, disseminated, and late infection [5,78]. However, this division is rather theoretical and is in many patient cases not strictly observed [79]. The transmission of the spirochetes by a tick bite leads to a localized infection, that may result in the characteristic skin lesion erythema migrans (EM) after days to weeks [5,80–82]. It is conventionally described as the so-called bull's eye rash: an expanding, annular erythematous skin lesion with or without central clearing [78,82]. Besides EM, *B. burgdorferi* s.s. might cause flu-like symptoms as for example malaise, fatigue, headache, and fever during the early stage of infection in the United States [78,83]. In Europe, EM caused by species of *B. burgdorferi* s.l. is usually not accompanied by other symptoms [5]. Infection of *B. garinii*, in contrast to *B. afzelii* and *B. burgdorferi* s.s., leads to a faster expanding skin lesion as well as more symptomatic lesions, commonly causing burning, itching and pain [84,85]. In rare cases, the skin manifestation borrelial lymphocytoma is observed in Europe, which is mostly caused by *B. afzelii* [5,80,86].

If the infection has not been treated with antibiotics, the spirochetes will migrate from the site of the tick bite within days to weeks. Depending on the species, different but sometimes overlapping clinical manifestations, develop during the early and late disseminated phase. Also, the phases must not be strictly separated from each other [2,5,80]. *B. burgdorferi* s.s. is commonly associated with arthritis and neuroborreliosis, but also carditis [78]. In contrast, *B. afzelii* is known to mainly cause skin conditions like acrodermatitis chronica atrophicans [79,87,88]. However, an infection with *B. garinii* or *B. bavariensis* leads to neuroborreliosis in most cases [79]. Additionally, even the different strains within a single species display variations in symptom severity and dissemination activity [89,90].

The mechanisms behind the observed species-specific differences in tissue tropism are not well understood yet. However, the adhesion of *Borrelia* via surface-expressed proteins to host tissues is known to be an important step for dissemination and colonization [91]. Even though the borrelial genome is overall rather similar, there are certain virulence genes encoding for such surface proteins which are highly variable between the species. Hence, the sequence heterogenicity is potentially contributing to the specific tissue tropisms.

1.7. Borrelial Adhesion and Dissemination

For *Borrelia* it is essential to cause a disseminated, persistent infection in a vertebrate host to maintain its enzootic cycle [92]. The crucial step in the infection process is the adhesion to host tissues via surface expressed proteins. It is necessary that binding occurs with sufficient strength: too extensive binding hinders the dissemination through the host which results in the clearance of the infection. Reversely, the inability to bind prevents the infection to happen in the first place [91]. Therefore, *Borrelia* possesses a wide range of adhesive proteins that are expressed on the surface to interact with specific host molecules [91–96].

The extracellular matrix (ECM) on the host cell surface is a major adhesive target of the spirochete. The ECM consists of different types of molecules which *Borrelia* can utilize to interact with the host tissues, like proteoglycans, glycosaminoglycans (GAGs), fibronectin, laminin and collagen. Additionally, *Borrelia* can bind to integrins of host cells and to components of host serum and extracellular fluids, like plasminogen and complement regulatory proteins [91–96]. Essentially important in the adhesion process appear to be the GAGs, which are large, linear, and negatively charged polysaccharide chains. The negative charge arises from sulfurylation of the repeating disaccharide units, the most common being chondroitin-4-sulfate (type A), dermatan sulfate (type B) and chondroitin-6-sulfate (type C). The GAGs decorate certain ECM proteins to form together the so-called proteoglycans (e.g. decorin, biglycan, aggrecan), which are either inserted into the cell membrane or localized to the cell surface ECM [97]. The proteoglycan decorin associates with collagen via its collagen binding core and its amino acid sequence is rich in leucine. Also, a single GAG side chain is bound to it, being either dermatan sulfate or chondroitin-6-sulfate [98–100]. Decorin has not only an important function as structural molecule of the ECM, but also acts as a cell signaling molecule [101]. Another relevant ECM component is fibronectin which is a large plasma glycoprotein that is involved in various processes like cell adhesion and migration, cell-cell signaling, ECM remodeling and more [102]. Laminin is also a glycoprotein component of the ECM, consisting of three subunits. It serves a cellular scaffolding function and interacts with integrins and other ECM constituents [103]. Finally, there are also integrins involved which are glycosylated transmembrane signaling molecules which possess a heterodimeric structure. They mediate cell-cell as well as cell-matrix interactions [104].

Lyme disease *Borrelia* encode a range of various surface-expressed adhesion proteins that participate during the multi-stage infectious process of mammalian hosts. The specific functions of all yet to known proteins are not perfectly understood still and some even seem multifunctional [105]. An outline of borrelial surface proteins is given in Table 1.7.1 with

8

respective substrate molecules in the mammalian host. Some of the adhesion proteins were found to play a role in borrelial motility and dissemination in the host. For example, BBK32 which binds fibronectin was determined to mediate three different types of interaction during vascular dissemination [106,107]. DbpA promotes only transient interactions with endothelial cells [108]. In contrast, P66 and OspC were found to be necessary for the transmigration through the vessel wall [109,110].

Table 1.7.1: Summary of important adhesion proteins during mammalian infection and their respective ligands in the host.

Borrelial adhesin	Host substrate molecule	Reference
BBK32	Fibronectin, GAGs	[111,112]
Bgp	Dermatan sulfate, GAGs, aggrecan	[113,114]
CspA/Z	Fibronectin, laminin, plasminogen, complement regulatory proteins	[115]
DbpA/B	Decorin, biglycan dermatan sulfate, GAGs, laminin	[108,116–119]
ErpA/C/P	Complement regulatory proteins, plasminogen	[120,121]
ErpX	laminin	[122]
OspC	Plasminogen, fibrinogen, dermatan sulfate	[110,123]
P66	integrins	[124]
RevA/B	Fibronectin, laminin (RevA)	[107,125]

1.7.1. Decorin binding proteins A and B

The decorin binding proteins A and B (DbpAB) were first identified by Guo and colleagues, who demonstrated that *B. burgdorferi* can bind decorin, a proteoglycan found in the ECM in mammals. The approximately 20 kDa large proteins were shown to be encoded in a bicistronic operon on lp54 [116,117]. It was shown that DbpA is a helical bundle protein consisting of five helices held together by a strong hydrophobic core. Portions of two helices form a basic patch together with two flexible linkers. Upon solving the DbpA structure of *B. burgdorferi* B31, insight into the biophysical interactions with GAGs has been gained [126]. Although dermatan sulfate is the GAG mostly associated with decorin, DbpA is able to bind

also other GAGs like heparin and heparan sulfate [127]. Additionally, DbpA shows even a higher binding affinity for heparin than dermatan sulfate [126]. Further, it can be stated that DbpA is a bifunctional protein, able to interact with both, the protein core of decorin as well as with the GAG side chain, dermatan sulfate, however with different affinities [117,128,129].

Later on, the DbpAB structures of *B. burgdorferi* N40 and *B. garinii* PBr had been solved (see **Fehler! Verweisquelle konnte nicht gefunden werden.**). Interestingly, the DbpAs of different *B. burgdorferi* s.l. species are highly variable up to approximately 40-60%, while DbpB is conserved between the species [46,128,130]. Accordingly, the structures of DbpA display conformational differences as well as varying substrate binding affinities. Specifically, the C-terminus of the PBr DbpA has a different orientation as found in the DbpAs of B31 and N40. Also, the flexible linker between helices one and two of the latter is mostly unstructured, while the linker in PBr DbpA adopts a helical conformation [131].
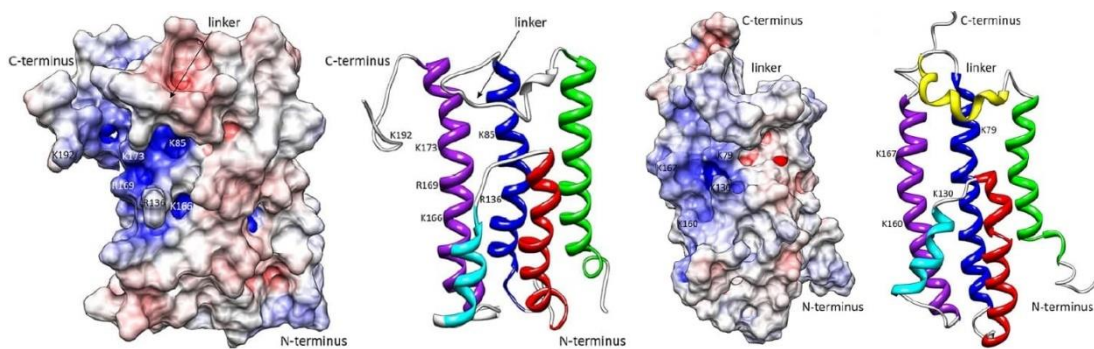

Figure 1.7.1: Electrostatic potential surface and ribbon diagram for N40 DbpA (left) and PBr (right).

As a functional consequence the PBr DbpA contains at least two independent GAG-binding epitopes, instead of only one found in the DbpAs of B31 and N40 and the retracted helical linker in PBr DbpA provides more space for the GAG ligands to bind at the epitopes [131]. Accordingly, the binding affinities of the PBr DbpA for decorin and dermatan sulfate are the highest, followed by B31 and N40 DbpA. The DbpAs of other strains were also assessed, including *B. afzelii* VS461. In contrast to the others, DbpA of VS461 promoted differential binding to the two substrates, the dermatan sulfate affinity laying between B31 and N40 DbpA, while the decorin affinity being greater than both [128]. Also, the group of Salo examined decorin binding of *B. burgdorferi*, *B. garinii* and *B. afzelii*. DbpA of *B. garinii* and DbpB of *B. garinii* and *B. burgdorferi* facilitate strong binding to decorin. Lower binding affinities were observed for DbpA of *B. burgdorferi* and both individual Dbps of *B. afzelii*. Whole spirochetes expressing *B. afzelii* Dbps were not able to bind decorin at all, in contrast to *B. garinii* and

B. burgdorferi. It was suggested that the Dbps of *B. afzelii* have different ligands and biological functions compared to the *B. garinii* and *B. burgdorferi* Dbps [132].

A different approach used to investigate DbpAB binding with certain ligands was used by Strnad and colleagues [119]. Single-molecule force spectroscopy was utilized to measure bond dissociation forces by mechanically pulling on the bound molecules. DbpA and DbpB of *B. afzelii* A91 were examined individually for decorin and laminin binding. Both proteins were found to exhibit stronger interactions with decroin than laminin. Dynamic force spectroscopy experiments with varying pulling speed demonstrated that bond lifetimes of DbpA and decorin are by far the longest, while DbpB and laminin show the shortest lifetime. Binding probabilities were largest for DbpA and DbpB interacting with decorin compared to lammin. Also, it was observed that the DbpA-decorin bond becomes highly stabilized under force load. Apparently, the complexes formed between Dbps and ECM components do not have the same dissociation behavior in solution and when force is applied [119].

Several different studies have demonstrated that DbpAB have an impact on the virulence and infectious phenotype in a mammalian host. Already in 2001 it was shown that *B. burgdorferi* experiences colonization defects in most tissues of decorin-deficient mice at low doses. At higher inocula, less spirochetes were detected specifically in joint tissue and decreases arthritis severity was observed [133]. The infectivity of *B. burgdorferi* mutants deficient in DbpAB was shown to be reduced [47–49,51,134,135], what correlates with the decreased ability to colonize various tissues [48,49,135] and to cause a persistent infection [48]. Upon complementation of DbpA [51] or DbpAB [47] into the knock-out mutant, the infectivity of the wild type was restored. Also, it was found that either DbpA or DbpB is reducing the infectivity significantly, demonstrating the importance of both proteins in mammalian infection. In comparison, though, DbpA is more essential for infectivity than DbpB [49].

As a fact, most genetic manipulations are carried out in *B. burgdorferi*. Since it has not been achieved yet to create a knock-out in European strains, comparative studies are only conducted using a gain of function approach in *B. burgdorferi*, lacking respective genes. Still there is only a minor amount of *in-vivo* studies exist that demonstrate differences of allelic variants of virulence genes in an isogenic strain background. To gain a better understanding of the variant Dbps, Lin and colleagues created isogenic *B. burgdorferi* mutants expressing DbpA of either *B. burgdorferi*, *B. garinii* or *B. afzelii* [81]. The mutants were tested in murine model to assess differences in tissue tropism. Also, recombinant DbpA protein of each species was produced and examined for binding activity against decorin and dermatan sulfate. It was found that DbpA of *B. garinii* causes the greatest colonization at the inoculation site and heart, leading

also to most severe carditis. Also, the greatest binding activity for both tested substrates was observed. In contrast, *B. burgdorferi* DbpA showed the weakest binding activities, however leading to the most stable joint colonization, probably due to other ligands (dermatan epimers, biglycan) present in mouse joints [52]. The group of Salo also demonstrated that not only DbpA, but also DbpA of *B. burgdorferi* is essential for early and late arthritis development [132].

1.7.2. Dissemination and Motility

Dissemination from the initial site of infection (site of the tick bite) to secondary colonization sites in the vertebrate host is an important step for the spirochetes to cause a persistent infection [107]. In mammals the migration occurs via hematogenous and non-hematogenous routes, like through the lymphatic system or directly through tissues [5,136]. Even though migration through the vasculature is the best understood pathway, the mechanism is not perfectly clarified due to difficulties to evaluate the spirochetes under shear stress conditions of the blood stream. However, in 2008, Moriarty and colleagues visualized disseminating *B. burgdorferi* within the vasculature of a mammalian host [137]. They engineered an infectious, green-fluorescing strain of *B. burgdorferi* and monitored it in real time using conventional and spinning disc confocal intravital microscopy (IVM). It was found that dissemination by the hematogenous route is a multi-stage process. First, the spirochetes transiently tether to the endothelium, followed by dragging along the vessel wall. Stationary adhesion, mostly observed at endothelial junctions, is the last step before transmigration through the vessel wall happens [137].

It was found that the fibronectin- and GAG-binding protein BBK32 plays a major role in the initiation events of vascular dissemination, namely transient and dragging interactions. Also, BBK32 might contribute to stationary adhesion, but additional adhesion proteins are likely necessary too [106]. Later, the group of Moriarty showed that BBK32-mediated adhesion is a two-step process: the initial fibronectin interactions during tethering permit the formation of more stable GAG interactions in the dragging phase [107]. Additionally, BBK32-deletion mutants do not exhibit a decrease in transmigration through the vessel wall *in vivo*, indicating that BBK32 is not required for extravasation. However, in absence of the integrin-binding protein P66 almost no transmigration occurred [109]. Also using IVM, OspC-mediated extravasation was observed facilitated by interactions with dermatan sulfate [110]. In contrast, the ability of DbpAB to aid in vascular adhesion was not yet observed *in vivo*. Salo and colleagues used an *in vitro* flow chamber to mimic shear stress of the blood stream. The Dbps

of *B. burgdorferi*, *B. garinii* and *B. afzelii* were each complemented into non-infectious *B. burgdorferi* B313 that is missing multiple plasmids. Apparently, only DbpAB of *B. garinii* mediate tethering interactions and DbpA was even found to be flow dependent. The Dbps of the different species were binding to biglycan, expressed by human endothelial cells, with different affinities [108]. Furthermore, DbpAB-deficient *B. burgdorferi* was observed to be attenuated in early hematogenous dissemination, leading to a colonization defect [47,48].

Similarly, the force dependence of DbpA of *B. afzelii* A91 interacting with decorin was demonstrated using atomic force microscopy. The bond between DbpA and decorin was shown to become greatly stabilized under force load, indicating the importance of DbpA to resist the shear stresses when adhering to blood vessels. In contrast, other bonds between Dbps and ECM components, especially DbpB and laminin, seem to facilitate long-term stationary adhesions in equilibrium and short term-binding under force, which allows *Borrelia* to detach under flow [119].

The same group also created recombinant *B. burgdorferi* 313, expressing either DbpAB, BBK32 or RevA of *B. afzelii* A91, to assess the motility in a near natural environment. A feeding set-up was created which should mimic the natural tick feeding on an infected host. First, *I. ricinus* ticks were put on fresh rabbit blood for 24 hours to induce feeding. This was followed by 4 hours of feeding on rabbit serum covering an ECM analog, wherein the *Borrelia* were embedded. Every hour rabbit serum samples were collected, and quantitative PCR was conducted to assess spirochete numbers. A significant increase in numbers and hence in motility was shown for DbpAB and BBK32 [119].

Seemingly, the initial adherence followed by dissemination and invasion of tissues of *Borrelia* in the mammalian host is not a straightforward process with many different adhesion proteins participating. Additionally, some of the binding activities may overlap partially or are correlated [92]. Also, considering *Borrelia* as a challenging experimental system with limited genetic tools, it is difficult to systemically characterize all adhesion pathways with respect to their biochemical functions, their impacts on host cell biology and their roles during infection [105].

The following passage ranging from page 14-43 contain classified information and is contained in the full version of the thesis only, which is stored at Faculty of Science, USB.

2. References

1. Dennis DT, Hayes EB. Epidemiology of Lyme Borreliosis. In: Kahl O, Gray JS, Lane R S, Stanek G, eds. Lyme borreliosis: biology, epidemiology and control. CABI Publishing, 2002: 251–280.
2. Stanek G, Wormser GP, Gray J, Strle F. Lyme borreliosis. The Lancet **2012**; 379:461–473.
3. Kuehn BM. CDC Estimates 300000 US Cases of Lyme Disease Annually. JAMA **2013**; 30:1110.
4. Rizzoli A, Hauffe HC, Carpi G, Vourc'h GI, Neteler M, Rosà R. Lyme borreliosis in Europe. Eurosurveillance **2011**; 16:1–8.
5. Steere AC, Strle F, Wormser GP, et al. Lyme borreliosis. Nature Reviews Disease Primers **2016**; 2:1–18.
6. Kurtenbach K, Hanincová K, Tsao JI, Margos G, Fish D, Ogden NH. Fundamental processes in the evolutionary ecology of Lyme borreliosis. Nature Reviews Microbiology **2006**; 4:660–669.
7. Bharti A, Nally J, Ricaldi J, et al. Leptospirosis: a zoonotic disease of global importance. Lancet Infect Dis **2003**; 3:757–71.
8. Radolf JD, Ranjit KD, Anand A, Smajs D, Norgard M v., Yang XF. Treponema pallidum, the syphilis spirochete: making a living as a stealth pathogen Justin. Nat Rev Microbiol **2011**; 14:744–759.
9. Yousefi L, Leylabadlo HE, Pourlak T, et al. Oral spirochetes: Pathogenic mechanisms in periodontal disease. Microbial Pathogenesis **2020**; 144.
10. Margos G, Vollmer SA, Cornet M, et al. A new Borrelia species defined by multilocus sequence analysis of housekeeping genes. Applied and Environmental Microbiology **2009**; 75:5410–5416.
11. Burgdorfer W, Barbour AG, Hayes SF, Benach JL, Jannasch HW. Lyme Disease--A Tick-Borne Spirochetosis ? Science **1982**; 216:1317–1319.
12. Mannelli A, Bertolotti L, Gern L, Gray J. Ecology of Borrelia burgdorferi sensu lato in Europe: Transmission dynamics in multi-host systems, influence of molecular processes and effects of climate change. FEMS Microbiology Reviews **2012**; 36:837–861.
13. Strnad M, Hönig V, Ružek D, Grubhoffer L, Rego ROM. Europe-Wide Meta-Analysis of Borrelia burgdorferi Sensu Lato Prevalence in Questing Ixodes ricinus Ticks. Applied and Environmental Microbiology **2017**; 83:1–16.
14. Radolf JD, Strle K, Lemieux JE, Strle F. Lyme disease in humans. Current Issues in Molecular Biology **2020**; 42:333–384.
15. Fraser CM, Casjens S, Huang WM, et al. Genomic sequence of a Lyme disease spirochaete, Borrelia burgdorferi. Nature **1997**; 390:580–586.
16. Casjens S, Palmer N, van Vugt R, et al. 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 **2000**; 35:490–516.
17. Miller JC, Bono JL, Babb K, El-Hage N, Casjens S, Stevenson B. A second allele of eppA in Borrelia burgdorferi strain B31 is located on the previously undetected circular plasmid cp9-2. Journal of Bacteriology **2000**; 182:6254–6258.
18. Gherardini F, Boylan J, Lawrence K, Skare J. Metabolism and Physiology of Borrelia. In: Samuels D, Radolf JD, eds. Borrelia: Molecular Biology, Host Interactions and Pathogenesis. Caister Academic Press, 2010: 103–138.
19. Casjens SR, Eggers CH, Schwartz I. Borrelia Genomics: Chromosome, Plasmids, Bacteriophages and Genetic Variation. In: Samuels SD, Radolf J D, eds. Borrelia: Molecular Biology, Host Ínteractions and Pathogenesis. Caister Academic Press, 2010: 21–47.
20. Tilly K, Rosa PA, Stewart PE. Biology of Infection with Borrelia burgdorferi. Infectious Disease Clinics of North America **2008**; 22:217–234.
21. Casjens SR, Gilcrease EB, Vujadinovic M, et al. Plasmid diversity and phylogenetic consistency in the Lyme disease agent Borrelia burgdorferi. BMC Genomics **2017**; 18:1–18.
22. Casjens SR, Di L, Akther S, et al. Primordial origin and diversification of plasmids in Lyme disease agent bacteria. BMC Genomics **2018**; 19:1–24.
23. Purser JE, Norris SJ. Correlation between plasmid content and infectivity in Borrelia burgdorferi. PNAS **2000**; 97:13865–13870.
24. Purser JE, Lawrenz MB, Caimano MJ, Howell JK, Radolf JD, Norris SJ. A plasmid-encoded nicotinamidase (PncA) is essential for infectivity of Borrelia burgdorferi in a mammalian host. Molecular Microbiology **2003**; 48:753–764.
25. Labandeira-Rey M, Seshu J, Skare JT. The absence of linear plasmid 25 or 28-1 of Borrelia burgdorferi dramatically alters the kinetics of experimental infection via distinct mechanisms. Infection and Immunity **2003**; 71:4608–4613.
26. Grimm D, Tilly K, Byram R, et al. Outer-surface protein C of the Lyme disease spirochete: A protein induced in ticks for infection of mammals. PNAS **2004**; 101:3142–3147.

27. Jewett MW, Jain S, Linowski AK, Sarkar A, Rosa PA. Molecular characterization of the Borrelia burgdorferi in vivo-essential protein PncA. Microbiology **2011**; 157:2831–2840.

28. Grimm D, Tilly K, Bueschel DM, et al. Defining plasmids required by Borrelia burgdorferi for colonization of tick vector Ixodes scapularis (Acari: Ixodidae). Journal of Medical Entomology **2005**; 42:676–684.

29. Revel AT, Blevins JS, Almazán C, et al. bptA (bbe16) is essential for the persistence of the Lyme disease spirochete, Borrelia burgdorferi, in its natural tick vector. PNAS **2005**; 102:6972–6977.

30. Jewett MW, Lawrence K, Bestor AC, et al. The critical role of the linear plasmid lp36 in the infectious cycle of Borrelia burgdorferi. Molecular Microbiology **2007**; 64:1358–1374.

31. Norris SJ. vls antigenic variation systems of lyme disease Borrelia: Eluding host immunity through both random, segmental gene conversion and framework heterogeneity. Mobile DNA III **2015**; 2:471–489.

32. Bankhead T, Chaconas G. The role of VlsE antigenic variation in the Lyme disease spirochete: Persistence through a mechanism that differs from other pathogens. Molecular Microbiology **2007**; 65:1547–1558.

33. Casjens SR, Fraser-Liggett CM, Mongodin EF, et al. Whole genome sequence of an unusual Borrelia burgdorferi sensu lato isolate. Journal of Bacteriology **2011**; 193:1489–1490.

34. Margos G, Hepner S, Mang C, et al. Lost in plasmids: Next generation sequencing and the complex genome of the tick-borne pathogen Borrelia burgdorferi. BMC Genomics **2017**; 18:1–15.

35. Strnad M, Rego ROM. The need to unravel the twisted nature of the Borrelia burgdorferi sensu lato complex across Europe. Microbiology **2020**;

36. Schüler W, Bunikis I, Weber-Lehman J, et al. Complete genome sequence of Borrelia afzelii K78 and comparative genome analysis. PLoS ONE **2015**; 10:1–27.

37. Glöckner G, Lehmann R, Romualdi A, et al. Comparative analysis of the Borrelia garinii genome. Nucleic Acids Research **2004**; 32:6038–6046.

38. Glöckner G, Schulte-Spechtel U, Schilhabel M, et al. Comparative genome analysis: Selection pressure on the Borrelia vls cassettes is essential for infectivity. BMC Genomics **2006**; 7:1–10.

39. Casjens SR, Mongodin EF, Qiu WG, et al. Genome stability of lyme disease spirochetes: Comparative genomics of borrelia burgdorferi plasmids. PLoS ONE **2012**; 7.

40. Tilly K, Krum JG, Bestor A, et al. Borrelia burgdorferi OspC protein required exclusively in a crucial early stage of mammalian infection. Infection and Immunity **2006**; 74:3554–3564.

41. Mongodin EF, Casjens SR, Bruno JF, et al. Inter- and intra-specific pan-genomes of Borrelia burgdorferi sensu lato: Genome stability and adaptive radiation. BMC Genomics **2013**; 14.

42. Wang IN, Dykhuizen DE, Qiu W, Dunn JJ, Bosler EM, Luft BJ. Genetic diversity of ospC in a local population of Borrelia burgdorferi sensu stricto. Genetics **1999**; 151:15–30.

43. Livey I, Gibbs CP, Schuster R, Dorner F. Evidence for lateral transfer and recombination in OspC variation in Lyme disease Borrelia. Molecular Microbiology **1995**; 18:257–269.

44. Barbour AG, Travinsky B. Evolution and distribution of the ospC gene, a transferable serotype determinant of Borrelia burgdorferi. mBio **2010**; 1.

45. Brisson D, Vandermause MF, Meece JK, Reed KD, Dykhuizen DE. Evolution of northeastern and midwestern Borrelia burgdorferi, United States. Emerging Infectious Diseases **2010**; 16:911–917.

46. Roberts WC, Mullikin BA, Lathigra R, Hanson MS. Molecular Analysis of Sequence Heterogeneity among Genes Encoding Decorin Binding Proteins A and B of Borrelia burgdorferi Sensu Lato. INFECTION AND IMMUNITY **1998**; 66:5275–5285.

47. Imai DM, Scott Samuels D, Feng S, Hodzic E, Olsen K, Barthold SW. The early dissemination defect attributed to disruption of decorin-binding proteins is abolished in chronic murine lyme borreliosis. Infection and Immunity **2013**; 81:1663–1673.

48. Weening EH, Parveen N, Trzeciakowski JP, Leong JM, Höök M, Skare JT. Borrelia burgdorferi lacking DbpBA exhibits an early survival defect during experimental infection. Infection and Immunity **2008**; 76:5694–5705.

49. Shi Y, Xu Q, McShan K, Fang TL. Both decorin-binding proteins A and B are critical for the overall virulence of Borrelia burgdorferi. Infection and Immunity **2008**; 76:1239–1246.

50. Salo J, Jaatinen A, Söderström M, Viljanen MK, Hytönen J. Decorin binding proteins of Borrelia burgdorferi promote arthritis development and joint specific post-treatment DNA persistence in mice. PLoS ONE **2015**; 10.

51. Blevins JS, Hagman KE, Norgard M v. Assessment of decorin-binding protein A to the infectivity of Borrelia burgdorferi in the murine models of needle and tick infection. BMC Microbiology **2008**; 8:1–13.

52. Lin YP, Benoit V, Yang X, Martínez-Herranz R, Pal U, Leong JM. Strain-Specific Variation of the Decorin-Binding Adhesin DbpA Influences the Tissue Tropism of the Lyme Disease Spirochete. PLoS Pathogens **2014**; 10:1–14.

53. Tilly K, Elias AF, Bono JL, Stewart P, Rosa P. DNA exchange and insertional inactivation in spirochetes. Journal of Molecular Microbiology and Biotechnology **2000**; 2:433–442.

54. Lawrenz MB, Kawabata H, Purser JE, Norris SJ. Decreased electroporation efficiency in Borrelia burgdorferi containing linear plasmids lp25 and lp56: Impact on transformation of infectious B. burgdorferi. Infection and Immunity **2002**; 70:4798–4804.

55. Elias AF, Stewart PE, Grimm D, et al. Clonal polymorphism of Borrelia burgdorferi strain B31 MI: Implications for mutagenesis in an infectious strain background. Infection and Immunity **2002**; 70:2139–2150.

56. Labandeira-Rey M, Skare JT. Decreased infectivity in Borrelia burgdorferi strain B31 is associated with loss of linear plasmid 25 or 28-1. Infection and Immunity **2001**; 69:446–455.

57. Purser JE, Lawrenz MB, Caimano MJ, Howell JK, Radolf JD, Norris SJ. A plasmid-encoded nicotinamidase (PncA) is essential for infectivity of Borrelia burgdorferi in a mammalian host. Molecular Microbiology **2003**; 48:753–764.

58. Zhang J-R, Hardham JM. Antigenic Variation in Lyme Disease Borreliae by Promiscuous Recombination of VMP-like Sequence Cassettes. Cell **1997**; 89:275–285.

59. Grimm D, Elias AF, Tilly K, Rosa PA. Plasmid stability during in vitro propagation of Borrelia burgdorferi assessed at a clonal level. Infection and Immunity **2003**; 71:3138–3145.

60. Byram R, Stewart PE, Rosa P. The essential nature of the ubiquitous 26-kilobase circular replicon of Borrelia burgdorferi. Journal of Bacteriology **2004**; 186:3561–3569.

61. Jewett MW, Lawrence K, Bestor AC, et al. The critical role of the linear plasmid lp36 in the infectious cycle of Borrelia burgdorferi. Molecular Microbiology **2007**; 64:1358–1374.

62. Piesman J, Schwan T. Ecology of Borreliae and their arthropod vectors. In: Samuels D, Radolf J, eds. Borrelia: Molecular Biology, Host Interactions and Pathogenesis. Caister Academic Press, 2010: 251–278.

63. Gern L. Life Cycle of Borrelia burgdorferi sensu lato and Transmission to Humans. Current Problems in Dermatology **2009**; 37:18–30.

64. Mannelli A, Bertolotti L, Gern L, Gray J. Ecology of Borrelia burgdorferi sensu lato in Europe: Transmission dynamics in multi-host systems, influence of molecular processes and effects of climate change. FEMS Microbiology Reviews **2012**; 36:837–861.

65. Radolf JD, Caimano MJ, Stevenson B, T HL. Of Ticks, mice and men. Nature reviews Microbiology **2012**; 10:87–99.

66. Radolf JD, Strle K, Lemieux JE, Strle F. Lyme disease in humans. Current Issues in Molecular Biology **2020**; 42:333–384.

67. Barbour AG, Hayes SF. Biology of Borrelia species. Microbiological Reviews **1986**; 50:381–400.

68. Charon NW, Cockburn A, Li C, et al. The unique paradigm of spirochete motility and chemotaxis. Annual Review of Microbiology **2012**; 66:349–370.

69. Zhao X, Norris SJ, Liu J. Molecular architecture of the bacterial flagellar motor in cells. Biochemistry **2014**; 53:4323–4333.

70. Hyde JA. Borrelia burgdorferi keeps moving and carries on: A review of borrelial dissemination and invasion. Frontiers in Immunology **2017**; 8:1–16.

71. Goldstein SF, Buttle KF, Charon NW. Structural analysis of the Leptospiraceae and Borrelia burgdorferi by high-voltage electron microscopy. Journal of Bacteriology **1996**; 178:6539–6545.

72. Goldstein SF, Charon NW, Kreiling JA. Borrelia burgdorferi swims with a planar waveform similar to that of eukaryotic flagella. Proc Natl Acad Sci USA **1994**; 91:3433–3437.

73. Motaleb MA, Corum L, Bono JL, et al. Borrelia burgdorferi periplasmic flagella have both skeletal and motility functions. PNAS **2000**; 97:10899–10904.

74. Sultan SZ, Sekar P, Zhao X, et al. Motor rotation is essential for the formation of the periplasmic flagellar ribbon, cellular morphology, and Borrelia burgdorferi persistence within Ixodes scapularis tick and murine hosts. Infection and Immunity **2015**; 83:1765–1777.

75. Sultan SZ, Manne A, Stewart PE, et al. Motility is crucial for the infectious life cycle of borrelia burgdorferi. Infection and Immunity **2013**; 81:2012–2021.

76. Rosa PA, Tilly K, Stewart PE. The Burgeoning Molecular Genetics of the Lyme Disease Spirochaete. Nature Reviews Microbiology **2005**; 3:129–143.

77. Stanek G, Strle F. Lyme borreliosis-from tick bite to diagnosis and treatment. FEMS Microbiology Reviews **2018**; 42:233–258.

78. Steere AC. Lyme Disease. N Engl J Med **2001**; 345:115–125.

79. Wormser GP, Dattwyler RJ, Shapiro ED, et al. The Clinical Assessment, Treatment, and Prevention of Lyme Disease, Human Granulocytic Anaplasmosis, and Babesiosis: Clinical Practice Guidelines by the Infectious Diseases Society of America. CID **2006**; 43:1089–1134.

80. Stanek G, Strle F. Lyme borreliosis-from tick bite to diagnosis and treatment. FEMS Microbiology Reviews. 2018; 42:233–258.

81. Lin YP, Benoit V, Yang X, Martínez-Herranz R, Pal U, Leong JM. Strain-Specific Variation of the Decorin-Binding Adhesin DbpA Influences the Tissue Tropism of the Lyme Disease Spirochete. PLoS Pathogens **2014**; 10.

82. Stanek G, Fingerle V, Hunfeld KP, et al. Lyme borreliosis: Clinical case definitions for diagnosis and management in Europe. Clinical Microbiology and Infection **2011**; 17:69–79.
83. Wormser GP. Early Lyme Disease. N Engl J Med **2006**; 354:2794–2801.
84. Strle F, Ružic E, Ružic´- R, et al. Comparison of Findings for Patients with Borrelia garinii and Borrelia afzelii Isolated from Cerebrospinal Fluid. CID **2006**; 43:704–710.
85. Strle F, Ružić-Sabljić E, Logar M, et al. Comparison of erythema migrans caused by Borrelia burgdorferi and Borrelia garinii. Vector-Borne and Zoonotic Diseases **2011**; 11:1253–1258.
86. Maraspin V, Nahtigal Klevišar M, Ružić-Sabljić E, Lusa L, Strle F. Borrelial Lymphocytoma in Adult Patients. Clinical Infectious Diseases **2016**; 63:914–921.
87. Ohlenbusch A, Matuschka F, Richter D, et al. Etiology of the acrodermatitis chronica atrophicans lesion in Lyme disease. JID **1996**; 174:421–423.
88. Canica MM, Nato F, du Merle L, et al. Monoclonal Antibodies for Identification of Borrelia afzelii sp. nov. Associated with Late Cutaneous Manifestations of Lyme Borreliosis. Scand J Infect Dis **1993**; 25:441–448.
89. Wang G, Ojaimi C, Iyer R, et al. Impact of genotypic variation of Borrelia burgdorferi sensu stricto on kinetics of dissemination and severity of disease in C3H/HeJ mice. Infection and Immunity **2001**; 69:4303–4312.
90. Wang G, Ojaimi C, Wu H, et al. Disease severity in a murine model of Lyme borreliosis is associated with the genotype of the infecting Borrelia burgdorferi sensu stricto strain. Journal of Infectious Diseases **2002**; 186:782–791.
91. Brissette CA, Gaultney RA. That's my story, and I'm sticking to it-an update on B. burgdorferi adhesins. Frontiers in Cellular and Infection Microbiology **2014**; 4.
92. Coburn J, Leong J, Chaconas G. Illuminating the roles of the Borrelia burgdorferi adhesins. Trends in Microbiology **2013**; 21:372–379.
93. Lin YP, Li L, Zhang F, Linhardt RJ. Borrelia burgdorferi glycosaminoglycan-binding proteins: A potential target for new therapeutics against Lyme disease. Microbiology (United Kingdom) **2017**; 163:1759–1766.
94. Hyde JA. Borrelia burgdorferi keeps moving and carries on: A review of borrelial dissemination and invasion. Frontiers in Immunology. 2017; 8.
95. Antonara S, Ristow L, Coburn J. Adhesion mechanisms of Borrelia burgdorferi. Advances in Experimental Medicine and Biology **2011**; 715:35–49.
96. Caine JA, Coburn J. Multifunctional and redundant roles of Borrelia burgdorferi outer surface proteins in tissue adhesion, colonization, and complement evasion. Frontiers in Immunology **2016**; 7.
97. Li L, Ly M, Linhardt RJ. Proteoglycan sequence. Molecular BioSystems **2012**; 8:1613–1625.
98. Danielson KG, Baribault H, Holmes DF, Graham H, Kadler KE, Iozzo R v. Targeted Disruption of Decorin Leads to Abnormal Collagen Fibril Morphology and Skin Fragility. The Journal of Cell Biology **1997**; 136:729–743.
99. Keene DR, San Antonio JD, Mayne R, et al. Decorin binds near the C terminus of type I collagen. Journal of Biological Chemistry **2000**; 275:21801–21804.
100. Zhang G, Ezura Y, Chervoneva I, et al. Decorin regulates assembly of collagen fibrils and acquisition of biomechanical properties during tendon development. Journal of Cellular Biochemistry **2006**; 98:1436–1449.
101. Dugan TA, Yang VWC, McQuillan DJ, Höök M. Decorin modulates fibrin assembly and structure. Journal of Biological Chemistry **2006**; 281:38208–38216.
102. Pankov R, Yamada KM. Fibronectin at a glance. Journal of Cell Science **2002**; 115:3861–3863.
103. Colognato H, Yurchenco PD. Form and Function: The Laminin Family of Heterotrimers. Developmental Dynamics **2000**; 218:213–234.
104. Takada Y, Ye X, Simon S. The integrins. Genome Biology **2007**; 8.
105. Coburn J, Garcia B, Hu LT, et al. Lyme disease pathogenesis. Current Issues in Molecular Biology **2021**; 42:473–518.
106. Norman UM, Moriarty TJ, Dresser AR, Millen B, Kubes P, Chaconas G. Molecular mechanisms involved in vascular interactions of the lyme disease pathogen in a living host. PLoS Pathogens **2008**; 4:1–11.
107. Moriarty TJ, Shi M, Lin Y, et al. Vascular binding of a pathogen under shear force through mechanistically distinct sequential interactions with host macromolecules. Molecular Microbiology **2012**; 86:1116–1131.
108. Salo J, Pietikäinen A, Söderström M, et al. Flow-Tolerant Adhesion of a Bacterial Pathogen to Human Endothelial Cells Through Interaction with Biglycan. Journal of Infectious Diseases **2016**; 213:1623–1631.
109. Kumar D, Ristow LC, Shi M, et al. Intravital Imaging of Vascular Transmigration by the Lyme Spirochete: Requirement for the Integrin Binding Residues of the B. burgdorferi P66 Protein. PLoS Pathogens **2015**; 11.

110.   Lin YP, Tan X, Caine JA, et al. Strain-specific joint invasion and colonization by Lyme disease spirochetes is promoted by outer surface protein C. PLoS Pathogens **2020**; 16.

111.   Probert WS, Johnson BJB. Identification of a 47 kDa fibronectin-binding protein expressed by Borrelia burgdorferi isolate B31. Molecular Microbiology **1998**; 30:1003–1015.

112.   Fischer JR, LeBlanc KT, Leong JM. Fibronectin binding protein BBK32 of the Lyme disease spirochete promotes bacterial attachment to glycosaminoglycans. Infection and Immunity **2006**; 74:435–441.

113.   Parveen N, Cornell KA, Bono JL, Chamberland C, Rosa P, Leong JM. Bgp, a secreted glycosaminoglycan-binding protein of Borrelia burgdorferi strain N40, displays nucleosidase activity and is not essential for infection of immunodeficient mice. Infection and Immunity **2006**; 74:3016–3020.

114.   Russell TM, Johnson BJB. Lyme disease spirochaetes possess an aggrecan-binding protease with aggrecanase activity. Molecular Microbiology **2013**; 90:228–240.

115.   Hallström T, Haupt K, Kraiczy P, et al. Complement regulator-acquiring surface protein 1 of borrelia burgdorferi binds to human bone morphogenic protein 2, several extracellular matrix proteins, and plasminogen. Journal of Infectious Diseases **2010**; 202:490–498.

116.   Guo BP, Norris SJ, Rosenberg LC, Hook M. Adherence of Borrelia burgdorferi to the proteoglycan decorin. Infection and Immunity **1995**; 63:3467–3472.

117.   Guo BP, Brown EL, Dorward DW, Rosenberg LC, Höök M. Decorin-binding adhesins from Borrelia burgdorferi. Molecular Microbiology **1998**; 30:711–723.

118.   Brown EL, Guo BP, O'neal P, Hö M. Adherence of Borrelia burgdorferi IDENTIFICATION OF CRITICAL LYSINE RESIDUES IN DbpA REQUIRED FOR DECORIN BINDING. Journal of Biological Chemistry **1999**; 274:26272–26278. Available at: http://www.jbc.org.

119.   Strnad M, Oh YJ, Vancová M, et al. Nanomechanical mechanisms of Lyme disease spirochete motility enhancement in extracellular matrix. Communications biology **2021**; 4:268.

120.   Kenedy MR, Vuppala SR, Siegel C, Kraiczy P, Akins DR. CspA-mediated binding of human factor H inhibits complement deposition and confers serum resistance in Borrelia burgdorferi. Infection and Immunity **2009**; 77:2773–2782.

121.   Brissette CA, Haupt K, Barthel D, et al. Borrelia burgdorferi infection-associated surface proteins ErpP, ErpA, and ErpC bind human plasminogen. Infection and Immunity **2009**; 77:300–306.

122.   Brissette CA, Verma A, Bowman A, Cooley AE, Stevenson B. The Borrelia burgdorferi outer-surface protein ErpX binds mammalian laminin. Microbiology **2009**; 155:863–872.

123.   Önder Ö, Humphrey PT, McOmber B, et al. OspC is potent plasminogen receptor on surface of borrelia burgdorferi. Journal of Biological Chemistry **2012**; 287:16860–16868.

124.   Coburn J, Chege W, Magoun L, Bodary SC, Leong JM. Characterization of a candidate Borrelia burgdorferi 3-chain integrin ligand identified using a phage display library. Molecular Microbiology **1999**; 34:926–940.

125.   Brissette CA, Bykowski T, Cooley AE, Bowman A, Stevenson B. Borrelia burgdorferi RevA antigen binds host fibronectin. Infection and Immunity **2009**; 77:2802–2812.

126.   Wang X. Solution structure of decorin-binding protein A from Borrelia burgdorferi. Biochemistry **2012**; 51:8353–8362.

127.   Leong JM, Robbins D, Rosenfeld L, Lahiri B, Parveen N. Structural Requirements for Glycosaminoglycan Recognition by the Lyme Disease Spirochete, Borrelia burgdorferi. Infection and Immunity **1998**; 66:6045–6048.

128.   Benoit VM, Fischer JR, Lin YP, Parveen N, Leong JM. Allelic variation of the Lyme disease spirochete adhesin DbpA influences spirochetal binding to decorin, dermatan sulfate, and mammalian cells. Infection and Immunity **2011**; 79:3501–3509.

129.   Fischer JR, Parveen N, Magoun L, Leong JM. Decorin-binding proteins A and B confer distinct mammalian cell type-specific attachment by Borrelia burgdorferi, the Lyme disease spirochete. PNAS **2003**; 100:7307–7312.

130.   Heikkilä T, Seppälä I, Saxen H, Panelius J, Yrjänäinen H, Lahdenne P. Species-specific serodiagnosis of lyme arthritis and neuroborreliosis due to Borrelia burgdorferi sensu stricto, B. afzelii, and B. garinii by using decorin binding protein A. Journal of Clinical Microbiology **2002**; 40:453–460.

131.   Morgan AM, Wang X. Structural mechanisms underlying sequence-dependent variations in GAG affinities of decorin binding protein A, a Borrelia burgdorferi adhesin. Biochemical Journal **2015**; 467:439–451.

132.   Salo J, Jaatinen A, Söderström M, Viljanen MK, Hytönen J. Decorin binding proteins of Borrelia burgdorferi promote arthritis development and joint specific post-treatment DNA persistence in mice. PLoS ONE **2015**; 10:1–17.

133.   Brown EL, Wooten RM, Johnson BJB, et al. Resistance to Lyme disease in decorin-deficient mice. Journal of Clinical Investigation **2001**; 107:845–852.

134.   Shi Y, Xu Q, Seemanapalli S v., McShan K, Liang FT. The dbpBA locus of Borrelia burgdorferi is not essential for infection of mice. Infection and Immunity **2006**; 74:6509–6512.

135. Hyde JA, Weening EH, Chang M, et al. Bioluminescent imaging of Borrelia burgdorferi in vivo demonstrates that the fibronectin-binding protein BBK32 is required for optimal infectivity. Molecular Microbiology **2011**; 82:99–113.

136. Wormser GP. Hematogenous dissemination in early Lyme disease. Wiener Klinische Wochenschrift **2006**; 118:634–637.

137. Moriarty TJ, Norman MU, Colarusso P, Bankhead T, Kubes P, Chaconas G. Real-time high resolution 3D imaging of the lyme disease spirochete adhering to and escaping from the vasculature of a living host. PLoS Pathogens **2008**; 4:17–19.

138. Salo J, Loimaranta V, Lahdenne P, Viljanen MK, Hytönen J. Decorin binding by DbpA and B of Borrelia garinii, Borrelia afzelii, and Borrelia burgdorferi sensu stricto. Journal of Infectious Diseases **2011**; 204:65–73.

139. Rego ROM, Bestor A, Rosa PA. Defining the plasmid-borne restriction-modification systems of the lyme disease spirochete Borrelia burgdorferi. Journal of Bacteriology **2011**; 193:1161–1171.

140. Rosa PA, Jewett MW. Genetic manipulation of Borrelia. Current Issues in Molecular Biology **2020**; 42:307–332.

141. Samuels DS, Mach KE, Garon CF. Genetic Transformation of the Lyme Disease Agent Borrelia burgdorferi with Coumarin-Resistant gyrB. JOURNAL OF BACTERIOLOGY **1994**; 176:6045–6049.

142. Fingerle V, Goettner G, Gern L, Wilske B, Schulte-Spechtel U. Complementation of a Borrelia afzelii OspC mutant highlights the crucial role of OspC for dissemination of Borrelia afzelii in Ixodes ricinus. International Journal of Medical Microbiology **2007**; 297:97–107.

143. Bontemps-Gallo S, Lawrence KA, Richards CL, Gherardini FC. Genomic and phenotypic characterization of Borrelia afzelii BO23 and Borrelia garinii CIP 103362. PLoS ONE **2018**; 13.

144. Siegel C, Schreiber J, Haupt K, et al. Deciphering the ligand-binding sites in the Borrelia burgdorferi complement regulator-acquiring surface protein 2 required for interactions with the human immune regulators factor H and factor H-like protein 1. Journal of Biological Chemistry **2008**; 283:34855–34863.

145. Barbour AG. Isolation and Cultivation of Lyme Disease Spirochetes. THE YALE JOURNAL OF BIOLOGY AND MEDICINE **1984**; 57:521–525.

146. Hyde JA, Weening EH, Skare JT. Genetic transformation of Borrelia burgdorferi. Current Protocols in Microbiology **2011**; 20:12C.4.1-12C.4.17.

147. Samuels DS. Electrotransformation of the spirochete Borrelia burgdorferi. Methods in molecular biology (Clifton, NJ) **1995**; 47:253–259.

148. Scott Samuels D, Drecktrah D, Hall LS. Genetic Transformation and Complementation. In: Methods in Molecular Biology. Humana Press Inc., 2018: 183–200.

149. Hübner A, Yang X, Nolen DM, Popova TG, Cabello FC, Norgard M v. Expression of Borrelia burgdorferi OspC and DbpA is controlled by a RpoN-RpoS regulatory pathway. PNAS **2001**; 98:12724–12729. Available at: www.pnas.org.

150. Eggers C, Caimano M, Clawson M, Miller W, Samuels D, Radolf J. Identification of loci critical for replication and compatibility of a Borrelia burgdorfericp32 plasmid and use of a cp32-based shuttle vector for theexpression of fluorescent reporters in the Lymedisease spirochaete. Molecular Microbiology **2002**; 43:281–295.