Structure of a human monoclonal antibody in complex with Outer surface protein C (OspC) of the Lyme disease spirochete, *Borreliella burgdorferi*

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Running title: Human antibody in complex with OspC

Keywords: antibody, human, epitope, infection, vaccine

Abstract

Lyme disease is a tick-borne, multisystem infection caused by the spirochete, *Borreliella burgdorferi*. Although antibodies have been implicated in the resolution of Lyme disease, the specific B cell epitopes targeted during human infections remain largely unknown. In this study, we characterized and defined the structural epitope of a patient-derived bactericidal monoclonal IgG ("B11") against Outer surface protein C (OspC), a homodimeric lipoprotein necessary for *B. burgdorferi* tick-mediated transmission and early-stage colonization of vertebrate hosts. High-resolution epitope mapping was accomplished through hydrogen deuterium exchange-mass spectrometry (HDX-MS) and X-ray crystallography. Structural analysis of B11 Fab-$OspC_A$ complexes revealed the B11 Fabs associated in a 1:1 stoichiometry with the lateral faces of $OspC_A$ homodimers such that the antibodies are essentially positioned perpendicular to the spirochete's outer surface. B11's primary contacts reside within the membrane proximal regions of α-helices 1 and 6 and adjacent loops 5 and 6 in one $OspC_A$ monomer. In addition, B11 spans the $OspC_A$ dimer interface, engaging opposing α-helix 1', α-helix 2', and loop 2-3' in the second $OspC_A$ monomer. The B11-$OspC_A$ structure is reminiscent of the recently solved mouse transmission blocking monoclonal IgG B5 in complex with $OspC_A$, indicating a mode of engagement with OspC that is conserved across species. In conclusion, we provide the first detailed insight into the interaction between a functional human antibody and an immunodominant Lyme disease antigen long considered an important vaccine target.

Author Summary

Lyme disease, the most common vector-borne disease in the United States, occurs as the result of an infection by the bacterium, *Borreliella burgdorferi*, which is transmitted to humans by the black-legged tick. The clinical manifestations, duration, and severity of Lyme disease can vary widely, even following antibiotic intervention. While it is postulated that disease resolution is associated with a robust immune response to *B. burgdorferi*, the specific details of such responses are not fully understood and are complicated by the fact that many of the immune targets of the surface of *B. burgdorferi* are highly variable. In this report, we describe the molecular interaction between a highly reactive *B. burgdorferi* surface protein, OspC, and a human monoclonal antibody ("B11") originally isolated from a Lyme disease patient. Our results demonstrate that antibody B11 associates with the lateral face of OspC in a side-on orientation, possibly signifying a unique mode of attack that renders *B. burgdorferi* susceptible to immune killing. This finding advances our understanding of how antibodies interact with *B. burgdorferi* and their possible roles in promoting disease resolution.

66 # Introduction

67 Lyme borreliosis or Lyme disease is the most common vector-borne infection in the
68 United States, with an estimated 450,000 cases per year [1]. The primary etiologic agent of Lyme
69 disease is the spirochete bacterium, *Borreliella burgdorferi* sensu latu (herein referred to as
70 simply *B. burgdorferi*). In North America, the spirochete is transmitted to humans by black
71 legged ticks, *Ixodes scapularis* and *Ixodes pacificus*, during the course of a blood meal. The
72 spirochete proliferates at the site of the tick bite, typically resulting in an expanding skin lesion
73 commonly referred to as a bull's eye rash or erythema migrans [2-4]. In the absence of antibiotic
74 intervention, *B. burgdorferi* disseminates to peripheral tissues, organs, large joints, and the
75 central nervous system, potentially resulting in severe complications including neuroborreliosis,
76 carditis and/or Lyme arthritis [2, 5]. A fraction of Lyme disease patients who receive a full
77 regimen of antibiotics will report persistent health issues (e.g., fatigue, cognitive issues,
78 musculoskeletal pain), a syndrome referred to as post-treatment Lyme disease (PTLD) [6-8].

79 In an effort to define the immunologic factors that drive resolution of Lyme disease,
80 Blum and colleagues characterized B cell responses in patients across the course of infection [9].
81 Blood plasmablasts from Lyme disease patients and a healthy cohort were subjected to bulk BCR
82 and single cell paired V_H and V_L sequencing [9]. The analysis revealed that robust plasmablast
83 responses correlated with a more rapid resolution of disease symptoms. Moreover, expression of
84 a panel of recombinant human monoclonal antibodies (MAbs) using paired V_H and V_L sequences
85 from representative clones were screened for reactivity with *B. burgdorferi* antigens. The "hits"
86 included a number of immunodominant *B. burgdorferi* surface lipoproteins previously known to
87 be reactive in convalescent human Lyme disease sera, including VlsE, DbpA, DbpB and OspC
88 [9-11]. The recombinant MAbs were further screened for the ability to inhibit growth of *B.*
89 *burgdorferi* strain B31 in culture. Of the six MAbs with borreliastatic activity (and therefore
90 implicated in disease resolution), two (B2 and B11) were directed against OspC.

91 Outer surface protein C (OspC) is a member of the small variable surface protein (Vsp)
92 family of immunodominant antigens unique to *Borrelia* and *Borreliella* including the relapsing
93 fever spirochetes (*B. turicatae*) [12]. In *B. burgdorferi*, OspC is expressed during tick
94 transmission and in the early stages of mammalian infection [13-15]. During this period, OspC
95 plays a multifaceted role in pathogenesis, including facilitating spirochete egress from the tick
96 during the course of a blood meal, enabling survival in the early stages of mammalian skin

infection, plasminogen interactions, and modulating transmigration across vascular walls [14-20]. The importance of OspC in *B. burgdorferi* pathogenesis is underscored by the fact that active vaccination with recombinant OspC or passive transfer of OspC antibodies completely prevent tick-mediated *B. burgdorferi* infections in mouse models [21-25]. However, while only a single copy of *ospC* is encoded on any *B. burgdorferi* genome, there are >26 *ospC* types and subtypes within *B. burgdorferi* isolates in North America, with multiple types often found in the same geographical proximity [26-28]. The polymorphic nature of OspC is proposed to constitute a means of *B. burgdorferi* immune evasion, as antibodies to one OspC type have limited cross-reactivity and cross-protection with other OspC types [28-31].

Structurally, OspC is ~21 kDa helical bundle that forms homodimers on the spirochete outer surface [32-36]. Despite a high degree of amino acid variability across the different OspC types, the tertiary and quaternary structures of OspC are virtually identical. As OspC's N-termini are anchored in the outer membrane via a lipid moiety, the molecule is generally depicted with the stem tethered to the spirochete surface and the dome, with more variable residues, projecting outward [35]. As such it is reasonable to expect that type-specific antibodies target the outward face of OspC, occluding the putative ligand binding cavities [35]. However, the X-ray crystal structure of OspC type A ($OspC_A$) complexed with the mouse MAb, B5, reveals a more complicated picture. B5 is the most well-characterized OspC monoclonal antibody originally isolated from *B. burgdorferi*-infected mice [23, 37]. In passive immunization studies, B5 blocks tick-mediated transmission of *B. burgdorferi*, presumably by entrapping the spirochetes in the tick midgut [23]. The X-ray crystal structure revealed that B5 Fabs associates at a nearly perpendicular angle with the lateral sides of homodimeric $OspC_A$ [38]. The side-on orientation of B5 with $OspC_A$ raises questions about the mechanisms by which the antibody interferes with spirochetal transmission as the putative ligand binding pockets on the outer most face of OspC are unobstructed. A limited insight into the nature of OspC-antibody interactions in mice (and none in humans) represents a gap in our understanding of a critical immune interaction with implications for both Lyme disease resolution and vaccine design. In this study, we report the structure of the human MAb B11 in complex with OspC and its impact on *B. burgdorferi* viability *in vitro*.

Results

128 **MAb B11 recognition of native and recombinant OspC**

129 The human MAb, B11, was originally isolated by Blum and colleagues as a recombinant IgG1
130 with paired V_H and V_L sequences from single-cell sorted, blood-derived plasmablasts from a
131 Lyme disease patient [9]. The B11 V_H and V_L sequences are assigned germline HV4-39 and
132 KV3-15, based on the IMGT database[39]. Based on that assignment, the B11 V_H and V_L coding
133 regions have as many as 16 amino acid mutations from V_H HV4-39 germline and 10 from the V_L
134 KV3-15 germline, suggesting the antibody has undergone significant somatic hypermutation.
135 Analysis of the available BioProject database indicate that one other B11 clone exists, alongside
136 three closely related clones all derived from the same patient (D. Vance, *unpublished*
137 *observations*).

138 The B11 V_H and V_L coding regions were cloned in-frame into pcDNA3.1-based human
139 IgG_1 Fc and kappa light chain expression plasmids, respectively, and then co-transfected into
140 Expi293 cells. Purified B11 IgG1 was assessed for the ability to recognize native OspC. Live *B.*
141 *burgdorferi* strains expressing $OspC_A$ (strain B313), $OspC_B$ (strain ZS7), or $OspC_K$ (strain 297)
142 were incubated with B11 IgG_1 or an isotype control (PB10 IgG_1), then subjected to flow
143 cytometry (**Figure 1**). A B31 derivative lacking *ospC* (B31A Δ*ospC*) was also included as a
144 control [40]. By flow cytometry, B11 was highly reactive with strain B313 expressing $OspC_A$
145 (MFI >3300), but not with the *B. burgdorferi* Δ*ospC* mutant. Moreover, MAb B11 did not react
146 with strain ZS7 expressing $OspC_B$ (MFI <35) or 297 expressing $OspC_K$ (MFI <35), indicating
147 that B11 recognition of OspC is type restricted. Indeed, we employed BLI to examine B11
148 reactivity with recombinant dimeric $OspC_A$. In that assay, B11 IgG had an apparent dissociation
149 constant (K_D) of ~39 nM for recombinant dimeric $OspC_A$ (**Figure S1**), but negligible reactivity
150 with $OspC_B$ or $OspC_K$ (**data not shown**). This OspC reactivity profile is reminiscent of MAb B5
151 [38].

152

153 **MAb B11 promotes *B. burgdorferi* agglutination and alterations in outer membrane**
154 **permeability.**

155 We recently reported that antibody-mediated agglutination of live *B. burgdorferi* spirochetes
156 results in flow cytometric events with increased size (FSC) and granularity (SSC) [41].
157 Furthermore, the addition of propidium iodide (PI) to the spirochete preparations just prior to
158 flow cytometry serves as indicator of antibody-induced changes to OM permeability [41]. We

159 have postulated that antibody-mediated agglutination explains, at least in part, how OspA and
160 possibly OspC antibodies compromise *B. burgdorferi* within the tick midgut and limit their
161 transmission to vertebrate hosts [23, 30, 38]. We therefore examined what impact that B11 had
162 on *B. burgdorferi* agglutination. Following B11 treatment, we observed significant *B.*
163 *burgdorferi* B313 agglutination (20-25% of the population) with a fraction of aggregated cells
164 also PI^+. This level of agglutination and PI^+ staining is similar to what we have observed
165 previously for MAb B5 (**Figure 1B**). Neither MAb B11 nor B5 influenced agglutination or PI
166 positivity of the *B. burgdorferi* Δ*ospC* mutant or *B. burgdorferi* strains ZS7 or 297 (**Figure 1B;**
167 **data not shown**). Thus, B11 recognizes $OspC_A$ on the spirochete surface and has the propensity
168 to promote both spirochete agglutination and alterations in outer membrane permeability in the
169 absence of human complement.
170
171 **Complement-dependent and independent impact of MAb B11 on *B. burgdorferi* motility**
172 Blum and colleagues reported that MAb B11 (20 μg/mL) had growth inhibitory (bacteriostatic)
173 activity on *B. burgdorferi* in standard culture conditions (BSK II) [9]. To confirm this
174 observation, we examined *B. burgdorferi* B31 motility (as a proxy for cell viability) in the
175 absence and presence of 20% human complement across a range of B11 doses (1-30 μg/mL). To
176 circumvent issues associated with intrinsically low OspC expression by *B. burgdorferi* B31 in
177 culture, we utilized a strain with an IPTG inducible *rpoS* allele, thereby activating native *ospC*
178 expression in *trans* (see Methods). In the absence of complement, B11 had a modest effect on
179 bacterial motility that but did not reach statistical significance as compared to an the isotype
180 control, PB10 (**Figure 2**). The addition of human complement resulted in significant reduction in
181 bacterial motility at B11 concentrations at 3 μg/ml and above. The transmission blocking MAb
182 B5 was significantly more potent than B11 at arresting *B. burgdorferi* motility in absence of
183 human complement and was similar to B11 in the presence of complement (**Figure 2**). Thus, in
184 our hands, B11's effects on spirochete motility arrest are complement-dependent.
185
186 **Localization of MAb B11's epitope on $OspC_A$ by HX-MS.**
187 We employed HX-MS as a means of identifying B11's epitope on $OspC_A$. A series of
188 preliminary quench and digestion experiments revealed that proteolysis of recombinant dimeric
189 $OspC_A$ with Nepenthesin II (without the addition of urea) generated the largest set of observable

190 peptides. Filtering out weak and overlapping signals resulted in 74 unique peptides with a
191 sequence coverage of 98.8% and redundancy of 5.3. The addition of MAb B11 IgG resulted in
192 strong protection at residues 49-57 and 186-196, corresponding to the N- and C-termini of
193 $OspC_A$, respectively (**Figure 3; Supplemental Excel file**). Moderate protection was also
194 observed elsewhere (e.g., peptide 157-162). The B11 IgG HX-MS protection profile is
195 reminiscent of the profile we reported for B5 IgG using slightly different HX-MS conditions
196 [38]. To enable a direct comparison between B11 and B5, we subjected $OspC_A$ to HX-MS with
197 B5 IgG under the same conditions as B11. The results revealed that HX-MS profiles were indeed
198 distinct with B5 causing notably stronger protection at residues 72-80, 157-176, and 186-196,
199 further from the N/C termini (**Figure 3**). These results suggest that B5 and B11 recognize
200 overlapping but distinct epitopes primarily focused on $OspC_A$'s α-helix 5 and 6. To test this
201 experimentally, we performed competitive binding assays between B5 and B11 using the BLI
202 platform in which $OspC_A$ was first saturated with B5 then probed with B11. The results
203 confirmed that B11's ability to associate with $OspC_A$ is abrogated by B5 occupancy (**Figure**
204 **S1C**).
205
206 **Structural analysis of the Fab B11-$OspC_A$ complex.**
207 To resolve B11's epitope on $OspC_A$ in greater detail, we solved the X-ray crystal structure of
208 B11 Fabs in complex with $OspC_A$ at 3.1 Å resolution in the P1 space group. The structure
209 revealed two B11 Fabs bound to a single $OspC_A$ homodimer (1:1 Fab:$OspC_A$ stoichiometry) in a
210 side-on fashion reminiscent (at first glance) of the B5 Fab-$OspC_A$ complex (**Figure 4**) . The B11
211 Fab fragments (Fab, Fab') made identical contacts on opposite sides of the $OspC_A$ homodimer
212 ($OspC_A$-$OspC_A$'), as described in detail below. Each B11 Fab assumed the canonical antibody
213 structure with two heavy chain immunoglobulin domains (V_H, C_H1) and two light
214 immunoglobulin domains (V_L, C_L) each containing 7-10 β-strands arranged in two β-sheets that
215 fold into a two-layer β-sandwich. The six CDRs (LCDR1-3, HCDR1-3) were situated on one
216 face of the molecule. The four B11 FAb-$OspC_A$ complexes within the asymmetric unit were
217 structurally similar, with Root-Mean-Square Deviations (RMSD) in the range of 0.8-1.8 Å upon
218 Cα-superpositioning. $OspC_A$ homodimers in the absence and presence of B11 Fabs were also
219 structurally nearly identical [RMSD of 0.6 Å], demonstrating that the B11 Fabs do not induce
220 any significant conformational changes $OspC_A$.

221 Each B11 Fab contacted α-helix 1, α-helix 6, loop 5 (located between α-helices 5 and 6)
222 and loop 6 (after α-helix 6) of their respective $OspC_A$ monomers. In addition, the Fabs each
223 bridged the $OspC_A$ dimer interface with residues from HCDR1, HCRD3, and LCDR2 engageing
224 with α-helix 1', α-helix 2', and loop 2' on the opposing $OspC_A$ molecule (**Figure 5A, B**),
225 thereby demonstrating that B11's epitope is quaternary in nature. The B11 Fab and $OspC_A$
226 interface buried a total surface area ranging from 2,406 $Å^2$ to 2,772 $Å^2$ (**Table 2**) Shape
227 complementarity (SC) scores ranged between 0.44-0.59 within the eight Fab-$OspC_A$ interfaces in
228 the asymmetric unit, while H-bonds ranged from 3 to 13 and salt bridges from 2 to 4.
229 While all six B11 CDR elements (L1-L3 and H1-H3) associated with $OspC_A$, the bulk of
230 the interaction was mediated by HCDR1-3. Specifically, the B11 V_H domain accounted for
231 ~75% of the total BSA within the complex and most of the H-bonds and salt bridges. Key H-
232 bonds include HCDR1 Ser-33 with $OspC_A$ Lys-60, and HCDR2 residue Tyr-54 with $OspC_A$ Glu-
233 63, with a notable salt bridge occurring between HCDR2 His-55 with $OspC_A$ Glu-63 (**Figure**
234 **5C**). In terms of the B11 V_L domain, H-bonds occurred between LCDR1 Asn-31 and $OspC_A$
235 Lys-196, and LCDR1 Asn-32 with $OspC_A$ Glu-189. The B11 V_L domain formed a single salt
236 bridge between LCDR2 Asp-50 with $OspC_A$ Lys-196 (**Figure 5D**).
237
238 **Structural comparisons between B11 and B5 Fab-$OspC_A$ complexes.**
239 As noted in the Introduction, we recently reported the structure of recombinant homodimeric
240 $OspC_A$ complexed with the Fab fragments of the transmission blocking murine MAb, B5 [**PDB**
241 **ID 7UIJ**] [26]. The B11 Fab-$OspC_A$ complex is reminiscent of that structure in that B5 and B11
242 Fabs engage with the lateral face of $OspC_A$ in a side-on orientation (**Figure 6A**). However, the
243 B5 and B11 Fabs are offset relative to each other, with B5 membrane-distal and B11 membrane-
244 proximal (**Figure 6A**). Moreover, while B5 Fabs are virtually perpendicular to $OspC_A$ (**Figure**
245 **6A**), B11 Fabs associate with $OspC_A$ at a slight angle, thereby positioning B11 Fabs closer to the
246 spirochete outer surface (**Figure 6A**). Nevertheless, there is sufficient distance between B11's
247 epitope on with $OspC_A$ and the spirochete membrane to accommodate an antibody as shown
248 when we super positioned the HIV-1 IgG b12 (PDB ID: **1HZH**) onto the B11 Fab (**Figure 6B**).
249 One possible orientation of OspC-B11 IgG relative to the bacterial outer surface is shown in
250 **Figure S4**.

251 B5 IgG and B11 IgG also resemble each other in terms of OspC type specificity: they
252 react with $OspC_A$, but not $OspC_B$ and $OspC_K$. To elucidate the structural basis of B11's
253 specificity for $OspC_A$, we superpositioned $OspC_A$ from within the B11 Fab-$OspC_A$ complex onto
254 the crystal structures of $OspC_B$ [**PDB ID 7UJ2**] and $OspC_K$ [**PDB ID 7UJ6**], which are
255 structurally similar to $OspC_A$ (RMSDs 0.9 Å to 1.1 Å; **Figure S2**). Although the unliganded form
256 of $OspC_A$ more closely resembles $OspC_K$ (RMSD of ~0.7 Å) than $OspC_B$ (RMSD of 1.0 Å),
257 several structural features of $OspC_B$ and $OspC_K$ may account for the lack of B11 IgG
258 recognition. The most notable is a Lys at position 113 in $OspC_B$ compared to a Gly at residue
259 114 in $OspC_A$. Lys-113 would be expected to sterically clash with B11 Ser-28 (**Figures S3A**,
260 **S4A**). Furthermore, a "deletion" of Ala-74 within α-helix 1 of $OspC_B$ relative to $OspC_A$ alters
261 the relative configuration of this region. The different conformation of this segment in $OspC_B$
262 would position residue Lys-74 within 4 Å of B11 residue Arg-77 creating an electrostatic clash
263 between these two positively charged residues (**Figures S3B, S5A**). In the case of $OspC_K$, the
264 most prominent structural feature damping B11 recognition involves the change of Lys-161 in
265 $OspC_A$, which H-bonds to Ser-56 of B11, to Ile-162 in $OspC_K$ thus precluding this interaction
266 (**Figures S3C, S5A**).
267 Understanding the molecular basis of OspC type-immunospecificity is a longstanding
268 challenge with relevance to vaccine design [42, 43]. We previously speculated that B5 IgG
269 reactivity is restricted to just three OspC types (A, C3 and I3), based the variability of key
270 paratope-epitope contacts [38]. By the same token, multiple sequence alignment (MSA) of 23
271 prominent *B. burgdorferi* OspC types suggest that B11 is similarly restricted. The primary
272 sequences of four of the 23 OspC Types (C3, I3, J, and M) examined contain the residues
273 ostensibly associated with B11 recognition on Type A (**Figure S5B**). OspC types C3, I3, J, and
274 M share 72-80% amino acid identity with $OspC_A$. More importantly, sequences within key B11-
275 binding residues within each of these four OspC types are identical $OspC_A$. The main-chain
276 structure around residue 74 is conserved (whereas a deletion exists in other types, as described
277 above) and OspC types C3, I3, J, and M contain a glycine or nonbulky amino acid at position
278 114 alleviating potential steric clash with B11 that appears in other OspC types (as described
279 above). Each of the four selected potential B11-binders possess a phenylalanine at position 177
280 which buries much of its bulky side chain in the interface with B11. The loss of this bulky side
281 chain would diminish the interaction with B11 by ~22 $Å^2$ based on buried surface area

282 calculations with Phe-177 $Å^2$ as a serine residue as found in many of the 23 OspC genotypes.
283 Finally, these four putative B11-binding OspC types also bear a lysine or arginine at residue 161,
284 which putatively H-bonds with B11. From this information, we speculate that B11 likely
285 recognizes four additional OspC types: C3, I3, J, and M (**Figure S5B**). This contrasts with B5
286 IgG, which we proposed is restricted to OspC types A, C3 and I3 [38]. Cross reactivity with I3 is
287 not surprising given that is a naturally occurring chimera between $OspC_F$ and $OspC_A$ with the C-
288 terminus (residues 128-199) derived from type A [44].
289

290 # Discussion

291 OspC plays a pivotal role in *B. burgdorferi* tick-to-mammalian transmission and in early
292 stages of dissemination. Although OspC may not be the sole determinant of spirochete
293 dissemination, its importance is underscored by the fact that certain *ospC* genotypes are
294 associated with more invasive *B. burgdorferi* clinical isolates [45-48]. From the host vantage
295 point, *B. burgdorferi* infection is accompanied by a robust OspC-specific antibody response,
296 which subjects the spirochetes to an enormous immune pressure that may contribute to bacterial
297 clearance [42, 49]. Despite the centrality of OspC in both infection and immunity, a high-
298 resolution molecular and structural picture OspC-antibody interactions is lacking in humans.
299 In this study, we solved the first structure of OspC complexed with Fabs from a human
300 monoclonal antibody. The antibody, B11, was originally isolated by Blum and colleagues as a
301 recombinant IgG1 with paired V_H and V_L sequences from single-cell sorted, blood-derived
302 plasmablasts from a Lyme disease patient [9]. Recombinant B11 was shown to be bacteriostatic
303 (without addition of human complement) and capable of promoting *B. burgdorferi*
304 opsonophagocytosis in culture [9, 50]. We have extended those observations by confirming that
305 B11 recognizes native OspC on the surface of *B. burgdorferi* strain B31 (but not strains
306 expressing OspC type B or K) and has both complement-independent and -dependent activities
307 against the spirochetes in culture. Based on these observations we predict that "B11-like"
308 antibodies contribute to the reduction and/or clearance of an active *B. burgdorferi* infection in
309 humans.
310 The crystal structure of B11 Fabs complexed with dimeric $OspC_A$ is remarkable in that it
311 demonstrates antibody association with the lateral faces ("stem") of $OspC_A$, rather than the more
312 surface exposed "dome". Considering that OspC is tethered to the spirochete outer membrane via

313 its lipidated N-terminus, the molecule is generally depicted as projecting perpendicularly from
314 the spirochete surface [33-35]. In such an orientation, the predominantly variable residues face
315 outwards, while conserved amino acids are membrane proximal [34, 35]. Thus, it is natural to
316 assume that type-specific antibodies engage the dome region of OspC via their Fab elements in a
317 top-down orientation [35]. That assumption was called into question by the structure of B5 Fab-
318 $OspC_A$ [38], and further undermined by the structure of B11 Fab-$OspC_A$.

319 Perhaps it is worth turning things on their side when rethinking the nature of OspC-
320 antibody interactions. Indeed, Lawson and colleagues pondered this exact possibility when
321 scrutinizing the structures of OspC and related Vsps from *B. turicatae* [35]. They cite evidence
322 to suggest that the N- and C-termini of OspC and Vsp1 dimers "…either adopt a mostly 'random
323 coil' conformation or are disordered." If correct, they argue, then OspC is essentially bound to
324 the spirochete by a flexible tail, thereby enabling the bulk of OspC to sit sideways or even
325 "upside down" on the cell membrane. Moreover, they note that such an unconventional
326 orientation would explain how IgM antibodies are able access the very C-terminal decapeptide of
327 OspC, a linear epitope known as C10 [51]. In such a configuration, the Fc regions of antibodies
328 like B11 IgG would protrude from the bacterial surface and be prime targets for components of
329 the complement system and Fc receptors on phagocytes [50].

330 The structural elucidation of the of B11 Fab-OspC complex has implications when
331 considering the design of OspC-based vaccine antigens. First, the B11 Fabs bridge the OspC-
332 OspC' dimer interface. This observation indicates that B11 underwent affinity maturation in the
333 context of dimeric (not monomeric) OspC. From the standpoint of vaccine design, preserving the
334 dimeric interface of OspC antigens is paramount for eliciting potent B11- and B5-like antibodies,
335 an observation that has been alluded to by others [52]. Second, a detailed view of B11 and B5's
336 epitopes reveal the molecular basis of OspC type-specificity, a factor that has limited OspC's
337 utility as a Lyme disease vaccine antigen [28, 42, 53]. However, considering the current
338 exponential advances in protein modeling and design, the availability of structural B cell
339 epitopes will prove invaluable. Finally, tt is worth noting that in separate efforts we have
340 identified human monoclonal and camelid-derived single-domain antibodies that have pan-OspC
341 reactivity (L. Cavacini, D. Vance, M. Rudolph, N. Mantis, *manuscripts in preparation*). High-
342 resolution epitope mapping studies of those pan-reactive OspC antibodies are ongoing, alongside
343 passive protection studies in mouse models of *B. burgdoferi* by tick-mediated infection.

344

345 Materials and Methods

346 **Cloning and expression of B11 IgG**

347 The B11 V_H and V_L coding regions were extracted from NCBI's BioProject database under
348 accession PRJNA470931 (GSE114310) [9]. The B11 V_H and V_L coding regions were
349 synthesized as gBlock™ dsDNA fragments (GenScript, Piscataway, NJ) and cloned in-frame
350 into pcDNA3.1-based human IgG_1 Fc and kappa light chain expression plasmids, respectively
351 [54, 55]. The two plasmids were co-transfected into Expi293 cells using ExpiFectamine293™
352 transfection reagents (Thermo Fisher Scientific, Waltham, MA), following manufacturer's
353 instructions. Five to six days later, supernatants containing the secreted antibodies were
354 harvested and clarified before being subjected to protein A chromatography. Affinity purified
355 IgG_1 B11 was then subjected to buffer exchange into PBS and stored at 4 °C. B11 Fabs were
356 generated as described [38]. Purification and specificity of MAbs B5 [38]and hPB10 [56]have
357 been previously described.

358

359 **Cloning, expression, and purification of OspC**

360 Recombinant $OspC_A$ (residues 38 to 201) from *B. burgdorferi* B31 [UniProt: accession Q07337]
361 was expressed and purified as a homodimer from *E. coli*, as described [38].

362

363 ***B. burgdorferi* strains and culture conditions**

364 *B. burgdorferi* strains expressing OspC types A (B313), B (ZS7), K (297), and the *ospC* deletion
365 strain B31A3ΔospC were cultured in BSK-II medium at 37°C with 5% CO_2 to mid-log phase
366 [57], collected by centrifugation (3,300 × *g*), washed, suspended in BSK II with 20% glycerol
367 and stored at -20 ºC until needed. Strains B313 [40], ZS7, and B31A3Δ*ospC* ("*ospC*K1") were
368 kindly provided by Dr. Yi-Pin Lin (Wadsworth Center). Strain 297 was obtained from the
369 American Type Culture Collection (ATCC; Manasas,VA).

370 A strain of *B. burgdorferi* B31 was engineered to deliver consistent ectopic expression of
371 *ospC* under *in vitro* culturing conditions (BSK-II at 37°C). An IPTG-inducible *rpoS* expression
372 plasmid was created through modification of the pBSV2G-derived *mCherry* plasmid, pGW163
373 [58]. Briefly, the *rpoS* open reading frame (ORF) was amplified from *B. burgdorferi* B31
374 [NCBI:txid224326] genomic DNA using Q5 DNA polymerase (NEB, Beverly, MA) and the

375 b31_rpoS_gibson_F (5'- agaattcattaaagaggagaaattacccatgaacatatttagtaatgaggatttaaacat -3') and
376 b31_rpoS_gibson_R (5'-gtaaaacgacggccagtgccaagcttaatttatttcttcttttaatttttttaagaactc-3') tailed
377 primer set. The *mCherry* ORF was excised from pGW163 using NcoI and HindIII (NEB) and
378 subsequently replaced with the tailed *rpoS* DNA fragment using the HiFi DNA assembly kit
379 (NEB). Following the assembly reaction, the DNA was transformed into NEB® 5-alpha F' I q
380 chemically competent cells (NEB). Recombinants were selected on LB agar supplemented with
381 10 µg/ml of gentamicin. Transformants that arose following overnight incubation at 30°C were
382 screened via PCR using the b31_rpoS_gibson_F and b31_rpoS_gibson_R primer set. The IPTG-
383 inducible *rpoS* expression plasmid, pGW181, was isolated from a single PCR-positive colony.
384 Following purification from a large batch culture, pGW181 was electrotransformed into *B.*
385 *burgdorferi* B31 as described [59], ultimately yielding the IPTG-inducible *rpoS* expression
386 strain, GGW941.
387 For routine culture, frozen aliquots of *B. burgdorferi* were thawed and used to inoculate
388 modified BSK-II lacking gelatin, supplemented with 6% rabbit serum (Pel-Freeze Biologicals,
389 Rogers, AR) [60]. Base BSK-II medium was prepared by the Wadsworth Center's Tissue and
390 Media Core Facility and filter sterilized (0.2 µm) prior to use. Cultures were maintained at 37°C
391 with 5% CO_2 and passaged by dilution (1:10,000) into fresh BSK-II medium. *B. burgdorferi*
392 cultures were routinely inspected for culture viability and motility during *in vitro* culture
393 maintenance prior to the initiation of any experiments.
394 To induce RpoS expression, GGW941 culture medium was spiked with 250 µM IPTG at
395 24 h before use. For flow cytometry and microscopy studies (as described below), *B. burgdorferi*
396 cells were collected in mid-logarithmic phase (~1-3 × 10^7 spirochetes per mL). As controls,
397 spirochetes were heat killed by incubation at 56°C for at least 30 min, as described [61].
398
399 ***B. burgdorferi* motility determinations by dark field microscopy**
400 Mid-log-phase cultures of GGW941 treated 24 h prior with IPTG to induce *rpoS* expression,
401 were adjusted to 5 x 10^6 bacteria per 50 µL in PBS then treated with indicated OspC MAbs (1, 2,
402 3, 10, 20, 30 µg/mL) in the presence or absence of 20% human complement (Sigma-Aldrich) for
403 16 or 24 h. Cultures were examined in a double-blind fashion by dark-field microscopy for
404 motile spirochetes. 1:5 dilutions of the reactions were prepared by aspirating from the center of
405 the reaction and diluted into fresh BSK-II medium. Dark-field microscopy was performed

406 utilizing a Trinocular DF microscope (AmScope) equipped with a camera with reduction lens
407 (AmScope SKU: MU1603) using a 40× dry darkfield condenser (AmScope; DK-DRY200).
408 Spirochetes were considered dead when complete loss of motility and refractivity was observed.
409 Spirochetes were enumerated in 4 visual fields, and the percent viability was calculated as the
410 ratio of live spirochetes (mean of 4 fields) in treated samples to spirochetes in the untreated
411 control samples (mean of 4 fields). Polyclonal serum from *B. burgdorferi*-infected mice and
412 MAb B5 were used as positive controls; naive serum and the PB10 isotype were used as negative
413 controls. This experimental set up was conducted over the course of three independent sessions
414 and data is plotted as the means for the three days of counting. Statistical analysis was
415 determined using 2-way ANOVA with Dunnett's multiple comparisons test in which
416 experimental means (B11, B5) were compared to control mean (PB10) for each antibody
417 concentration.
418
419 **Flow Cytometry**
420 To examine the ability of B11 and B5 IgG1 to bind the spirochete surface and promote bacterial
421 agglutination, and membrane permeability, *B. burgdorferi* strains expressing OspC types A
422 (B313), B (ZS7), and K (297), were cultured, treated with 10 µg/ml IgG1, and analyzed by flow
423 cytometry as described [38]. Briefly, an Alexa Fluor 647-labeled goat anti-human IgG [H+L]
424 secondary antibody (Invitrogen) was used to detect bound IgG1 to the bacterial surface, and
425 propidium iodide staining was used to indicate bacterial membrane permeability. The *ospC*
426 deletion strain B31-A3*ospC*K1was used as a negative strain control, and the ricin toxin MAb,
427 PB10, was used as an IgG1 isotype control. Bacteria were analyzed on a BD FACSCalibur (BD
428 Biosciences). Voltage was set with untreated bacteria as a reference point (gMFI~ 5.0), and
429 bacteria were gated on forward scatter (FSC) and side scatter (SSC) to exclude debris and assess
430 aggregate size and granularity. Agglutination was calculated as the sum of events in the upper-
431 left, upper-right, and lower-right quadrants relative to the total event counted (20,000).
432
433 **Epitope mapping of B11 by HX-MS**
434 Stock concentrations of $OspC_A$ (8.5 µM) in PBS alone or in a complex with a 2-fold molar
435 excess of antibody B11 were diluted into 90 µL of deuterated PBS buffer (20 mM phosphate,
436 150 mM NaCl, 0.02% sodium azide, 1 mM EDTA pH* 7.54, 85%D final) containing 0.2 nM

437 bradykinin and incubated 3 sec on ice, or either 3 seconds, 1 minute, 30 minutes, or 20 hours at
438 21°C. Each starting stock also included a mixture of imidazolium compounds to serve as
439 exchange reference standards [62]. At the desired time point the sample was rapidly mixed with
440 an equal volume of ice cold 0.2% formic acid and 0.1% trifluoroacetic acid (TFA) for a final pH
441 of 2.5. Samples were then immediately frozen on ethanol/dry ice and stored at -80°C until LC-
442 MS analysis. Undeuterated samples were prepared the same way but with undeuterated buffer for
443 each step.
444 Samples were thawed at 5°C for 8 minutes and injected using a custom LEAP robot
445 integrated with an LC-MS system [63]. The protein was first passed over a Nepenthesin II
446 column (2.1 x 30 mm; AffiPro) at 400 µL/min for inline digestion with the protease column held
447 at 20°C. Peptides were then trapped on a Waters XSelect CSH C18 trap cartridge column (2.1 x
448 5 mm 2.5 µm) and resolved over a CSH C18 column (1 x 50 mm 1.7 µm 130Å) using linear
449 gradient of 5 to 35% B (A: 0.1% FA, 0.025% TFA, 5% ACN; B: ACN with 0.1% FA) over 10
450 minutes and analyzed on a Thermo Orbitrap Ascend mass spectrometer at a resolution setting of
451 120,000. A series of washes over the trap and pepsin columns was used between injections to
452 minimize carry-over as described [63]. Data dependent MS/MS acquisition was performed on an
453 undeuterated sample using rapid CID and HCD scans and processed in Byonic (Protein Metrics)
454 with a score cutoff of 150 to identify peptides. Deuterium incorporation was analyzed using
455 HDExaminer v3 (Sierra Analytics).
456
457 **Crystallization and data collection.**
458 Fab B11 was complexed with dimeric $OspC_A$ in a 1:1 stoichiometry, then concentrated to 10
459 mg/ml final for all crystallization trials. Crystals were grown by sitting drop vapor diffusion
460 using a protein to reservoir volume ratio of 1:1 with total drop volumes of 0.2 µl. Crystals of the
461 B11 Fab-$OspC_A$ complex were produced at 22°C using a crystallization solution containing 100
462 mM sodium HEPES [pH 6.5], 18.6% PEG 6K, and 6.6 mM prasseodymium acetate. All crystals
463 were flash frozen in liquid nitrogen after a short soak in the appropriate crystallization buffers
464 supplemented with 10% ethylene glycol. Data were collected at the 24-ID-E beamline at the
465 Advanced Photon Source, Argonne National Labs. All data was indexed, merged, and scaled
466 using HKL2000[64] then converted to structure factor amplitudes using CCP4 [65].
467

468 **Structure determination and refinement.**

469 The B11 Fab-OspC$_A$ complex structure was solved by molecular replacement using Phaser [64].
470 Molecular replacement calculations were performed using the V_L and C_L domain coordinates of
471 the human monoclonal mAb MAB4-5 (PDB ID: 5Y11) along with the V_H and C_H1 domains
472 from the human anti-HIV antibody (PDB ID: 5UBY) as the search model for the first two B11
473 Fabs in B11-OspC$_A$ complex within the asymmetric unit. The OspC$_A$ coordinates (PDB ID:
474 1GGQ) were then used as the search model for the OspC$_A$ dimer in the first OspC$_A$-B11
475 complex. The initial B11-OspC$_A$ complex detected was then used to find the additional three
476 B11-OspC$_A$ complexes present in the asymmetric unit. The resulting phase information from
477 molecular replacement was used for some manual model building of the B11-OspC$_A$ structure
478 using the graphics program COOT [66] and structural refinement employing the PHENIX
479 package [67]. Data collection and refinement statistics are listed in **Table S1**, as is the Protein
480 Data Bank (http://www.rcsb.org/pdb/) code for the B11-OspC$_A$ structure PDB ID 9BIF.
481 Molecular graphics were prepared using PyMOL (Schrodinger, DeLano Scientific LLC, Palo
482 Alto, CA).

483

484 **Data availability**

485 All results and data files associated with this article are included in the main text and
486 supplemental material.

487

488 **Supporting information**

489 This article contains supporting information, including supplemental tables with X-ray
490 crystallography conditions, supplemental figures related to epitope mapping, and HX-MS data
491 files.

492

493 **Acknowledgements**

494 We are grateful to Drs. Lisa Blum and William Robinson (Stanford University) for sharing the
495 B11 VH and VL sequences and for follow-up discussions. We thank Dr. Renjie Song at the
496 Wadsworth Center's Immunology Core facility for assistance with flow cytometry. We thank the
497 DNA sequencing core and members of the Wadsworth Center's bioinformatics core for useful

498 discussions. As always, we are grateful to Elizabeth Cavosie (Wadsworth Center) and Donna
499 DeLuca (Health Research, Inc) for administrative assistance and program overview.
500
501 **Funding sources**
502 This work was supported by the National Institute of Allergy and Infectious Diseases (NIAID),
503 National Institutes of Health (NIH), Department of Health and Human Services Contract No.
504 75N93019C00040. X-ray analysis as conducted at the Northeastern Collaborative Access Team
505 beamlines, which are funded by P30 GM124165 from the National Institute of General Medical
506 Sciences (NIGMS), NIH. The Eiger 16M detector on the 24-ID-E beam line is funded by a NIH-
507 ORIPHEI grant (S10OD021527). HDX-MS studies conducted at the University of Washington
508 was supported by award S10OD030237 from NIGMS. The content is solely the responsibility of
509 the authors and does not necessarily represent the official views of the NIH.
510
511 **Conflict of interest**
512 The authors have no conflicts of interest to declare.
513
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698

699 **Figure Legends**

700 **Figure 1. Reactivity of B11 and B5 with $OspC_A$ on live spirochetes.** Flow cytometric analysis
701 of B11 and B5 MAb reactivity with *B. burgdorferi* strains expressing OspC types A (B313), B
702 (ZS7), or K (297). A B. burgdorferi B31 OspC mutant (B31-A3Δ*ospC*) was included as a control.
703 (**A**) Representative fluorescence histograms where an Alexa Fluor 647-labeled anti-human IgG
704 secondary antibody was used to detect bacterial surface bound IgG. Panels show B11 (blue) or B5
705 (gray) compared to the isotype control (PB10, black). Events positive for 647 fluorescence fall to
706 the right of the isotype control. The percent and geometric mean fluorescence intensity (gMFI) of
707 positive events for bacteria incubated with B11 (blue) and B5 (gray) are indicated on each panel.
708 (**B**) Corresponding FSC/SSC dot plots. Events that are increased in size (FSC) and granularity
709 (SSC) represent multi-bacteria aggregates as the result of antibody-mediated agglutination of live
710 spirochetes. The percentage of events that are agglutinated is indicated (black) and was calculated
711 from the sum of events with increased FSC and SSC, (UL+UR+LR quadrants), relative to the total
712 events counted (20,000). The percent of events positive for propidium iodide staining, indicating
713 membrane permeability, is labeled, and highlighted in red.

714

715 **Figure 2. Complement-dependent and -independent effects of B5 and B11.** Mid-log-phase
716 cultures of *B. burgdorferi* strain GGW941 treated for 24 h with IPTG induce *rpoS* expression,
717 were adjusted to 5 x 10^6 bacteria per 50 µL in BSK II without (left columns) and with (right
718 columns) 20% human complement. Cultures were then treated with (**A**) B11, (**B**) B5 or (**C**)
719 PB10 at indicated concentrations (x-axis). Sixteen hours later, the cultures were examined in a
720 double-blind fashion by dark-field microscopy for motile spirochetes. Spirochetes were
721 enumerated in 4 visual fields, and the percent viability was calculated as the ratio of live
722 spirochetes (mean of 4 fields) in treated samples to spirochetes in the untreated control samples
723 (mean of 4 fields). The experimental set up was conducted over the course of three independent
724 sessions and data is plotted as the means for the three days of counting. Statistical analysis to
725 determined significance (*, $p<0.05$) was done with 2-way ANOVA with Dunnett's multiple
726 comparisons test for each B11 and B5 versus PB10 (isotype control) at each dose.

727

728

729 **Figure 3. Localization of the B11 epitope on $OspC_A$ using HDX-MS.** HDX changes upon the
730 addition of (**A**) B11 IgG and B5 IgG are plotted on the structure of $OspC_A$ [PDB ID 1GGQ].
731 Regions with reduced deuterium exchange (more protected) are colored blue, while more
732 deuterium exchange are colored red. (**B-G)** Deuterium uptake plots for unbound $OspC_A$ (black
733 liunes), B5-$OspC_A$ (purple lines) and B11-$OspC_A$ (green lines) are shown for selected regions,
734 with the panel letter corresponding to the labels in panel A. Error bars represent standard
735 deviations from triplicate measurements. Comprehensive exchange data is provided as an Excel
736 file as Supplemental material.

737

738 **Figure 4. Structure of B11 Fab-$OspC_A$.** (A) Side-on and (B) top-down ribbon diagrams of
739 $OspC_A$ homodimer ($OspC_A$, $OspC_A$') in complex with B11 Fabs (B11, B11'). $OspC_A$ is colored
740 in cyan and $OspC_A$' in green. The B11 Fab V_H and C_H1 elements are colored in salmon and V_L
741 and C_L in light gray. The B11' Fab V_H and C_H1 elements are colored in magenta and V_L and C_L
742 in yellow. The V_H and V_L domains (V_H,V_L) along with the N- and C-termini of $OspC_A$ and
743 $OspC_A$' are labelled accordingly.

744

745 **Figure 5. Detailed interactions between B11 and $OspC_A$ revealed from the co-crystal**
746 **structure.** (**A**) Ribbon structure (top-down view) of $OspC_A$ homodimer ($OspC_A$, cyan; $OspC_A$',
747 green) in complex with a single B11 Fab (V_H and C_H1 elements, magenta; V_L and C_L, yellow).
748 The $OspC_A$ residues that engage with B11 are colored blue. Key secondary structures (α-helices
749 1, 2, 3, 5, and 6) and all six CDRs are labeled; (**B**) Ribbon (left) and surface (right) depiction of
750 an $OspC_A$ homodimer ($OspC_A$, cyan; $OspC_A$', green) with B11-interacting residues shaded in
751 dark blue. $OspC_A$ N and C-termini are labelled N and C, respectively. Representations of key H-
752 bonds (red dashes) and salt bridges (yellow dashes) between (**C**) $OspC_A$ (green) and B11' V_H
753 domain (magenta) and (**D**) $OspC_A$ (green) and B11' V_L domain (yellow). Side chains are drawn
754 as sticks and color coordinated to the main chain color, with nitrogen atoms shaded blue and
755 oxygen atoms shaded red. CDR elements are labelled per convention: CDR-L1, -L3, -L3; CDR-
756 H1, -H2, -H3. $OspC_A$ N and C-termini are labelled N and C, respectively. All drawings are
757 depicted as ribbon diagrams except the molecular surface representation in panel B.

758

759 **Figure 6. Structural comparison of B11-OspC$_A$ with and B5-OspC$_A$.** (**A**) Superposition of
760 B11-OspC$_A$ and B5-OspC$_A$ drawn as Cα-traces depicting the similar side-on approach of each
761 Fab to OspC$_A$ while highlighting the greater distance of B5 from the N and C-termini of OspC$_A$
762 relative to the B11 position. OspC$_A$ is colored cyan and green while the B11 Fab V_H and C_H1
763 elements are colored in salmon red and V_L and C_L in light gray. The B11' Fab V_H and C_H1
764 elements are colored in magenta and V_L and C_L in yellow. B5 Fabs are colored dark gray. (**B**)
765 Superposition of the anti-HIV IgG1 mAb (PDB ID 1HZH) drawn as a gray Ca-trace onto B11
766 also depicted as a Ca-trace revealed no significant steric clash between the OspCA-bound IgG
767 and the spirochete membrane. The OspCA-B11 structure is colored identically to panel A in this
768 figure.
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770
771
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773
774

Table 1. Functional activity associated with MAbs B11 and B5

		B11				B5			
Strain	**type**	**Binding (%)[a]**	**Binding (gMFI)[a]**	**Agg (%)[b]**	**PI + (%)[c]**	**Binding (%)[a]**	**Binding (gMFI)[a]**	**Agg (%)[b]**	**PI + (%)[c]**
Δ*ospC*	-	-0.1 +/- 0.1	-13.8 +/- 10.5	0.1+/- 0.1	0.0 +/- 0.2	5.5 +/- 12.3	55.3 +/- 128.8	0.1 +/- 0.2	0.2 +/- 0.7
B313	A	92.8 +/- 2.0	3822.3 +/- 1516.1	22.5 +/- 8.6	9.3 +/- 5.3	94.5 +/- 2.7	6903 +/- 1023	33.4 +/- 6.1	15.2 +/- 10.4
ZS7	B	0.3 +/- 0.4	-7.7 +/- 16.3	-0.1 +/- 0.1	-0.1 +/- 0.2	0.0 +/- 0.1	-3.7 +/- 18.1	-0.1 +/- 0.1	-0.1 +/- 0.2
297	K	1.5 +/- 3.5	0.9 +/- 13.0	-0.2 +/- 0.3	0.01+/- 0.3	-0.2 +/- 0.2	3.6 +/- 11.1	-0.1 +/- 0.2	0.0 +/- 0.1

[a], *B. burgdorferi* surface binding; [b], Agglutination; [c], propidium iodide positive events. Flow cytometric analysis of OspC IgG mAbs B11 and B5 and their reactivity with live *B. burgdorferi* strains expressing different OspC types. An anti-human IgG Alexa 647-labeled secondary antibody was used to detect mAb binding and is displayed as percentage of events positive for 647 fluorescence, and geometric mean fluorescence intensity (gMFI). The percentage of events with increased size and granularity (FSC/SSC) demonstrating agglutination, and percentage of events with propidium iodide uptake are shown. Displayed are mean values, with background subtraction, +/- standard deviation, n=4.

775
776

Table 2. Summary of B11-OspC$_A$ binding data and interface information

Interface(s)[a]	H-bonds [b]	SB [c]	SC [d]	Total BSA (Å^2)
1 (2)	4 (9)	3 (3)	0.51 (0.44)	2706 (2772)
3 (4)	12 (13)	2(3)	0.48 (53)	2671 (2710)
5 (6)	3 (8)	3(4)	0.59 (0.47)	2636 (2814)
7 (8)	8 (11)	3(3)	0.51 (0.59)	2406 (2600)

[a], primary and secondary (in parentheses) interfaces; [b],hydrogen bonds; [c], salt bridges; [d], shape complementarity score

777
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780
781

Figure 1

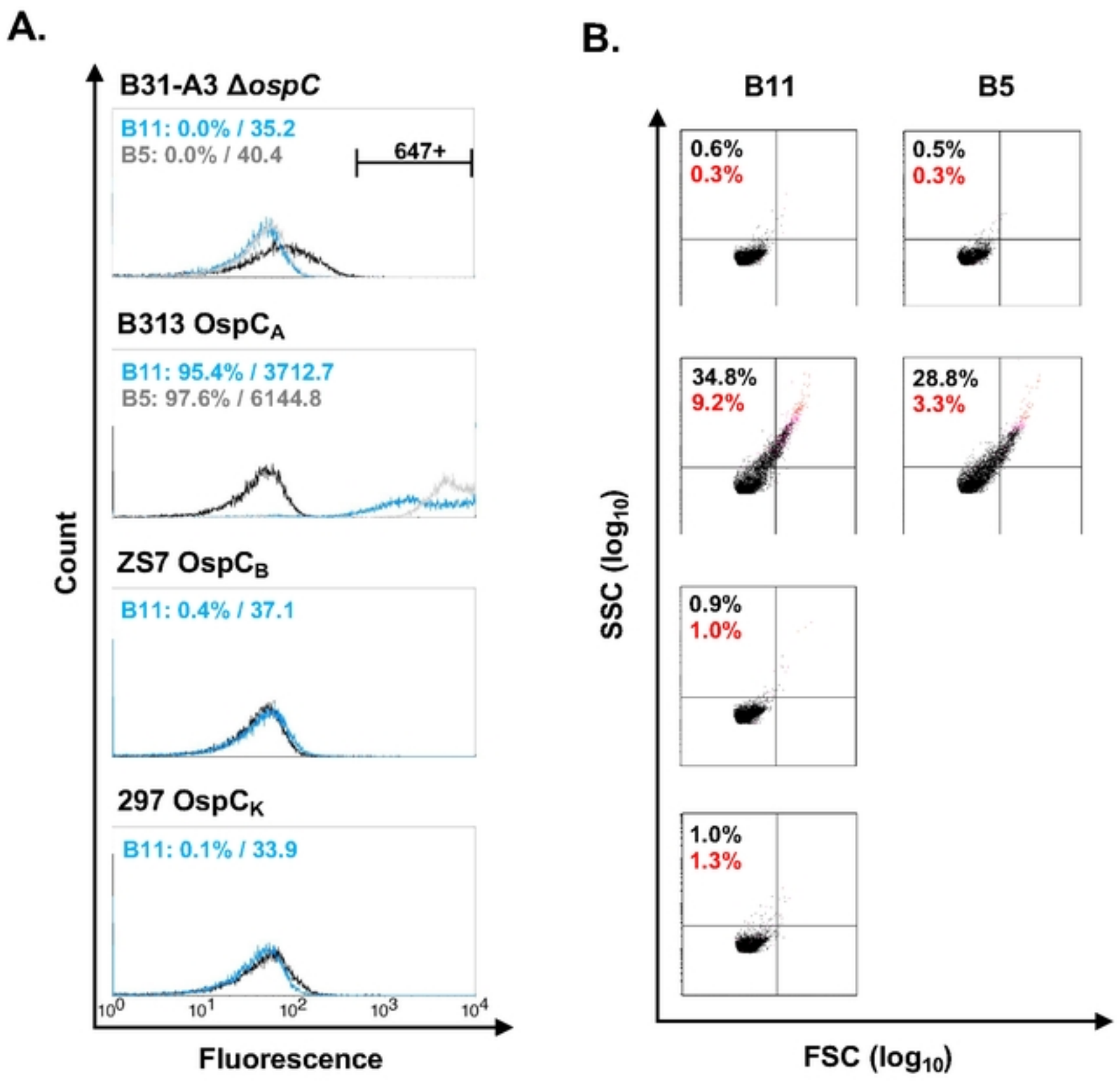

A.
B31-A3 ΔospC
B11: 0.0% / 35.2
B5: 0.0% / 40.4
647+
B313 OspC$_A$
B11: 95.4% / 3712.7
B5: 97.6% / 6144.8
ZS7 OspC$_B$
B11: 0.4% / 37.1
297 OspC$_K$
B11: 0.1% / 33.9
Count
10^0
10^1
10^2
10^3
10^4
Fluorescence
B.
B11
B5
0.6%
0.3%
0.5%
0.3%
34.8%
9.2%
28.8%
3.3%
0.9%
1.0%
1.0%
1.3%
SSC ($\log_{10}$)
FSC ($\log_{10}$)

Figure 2

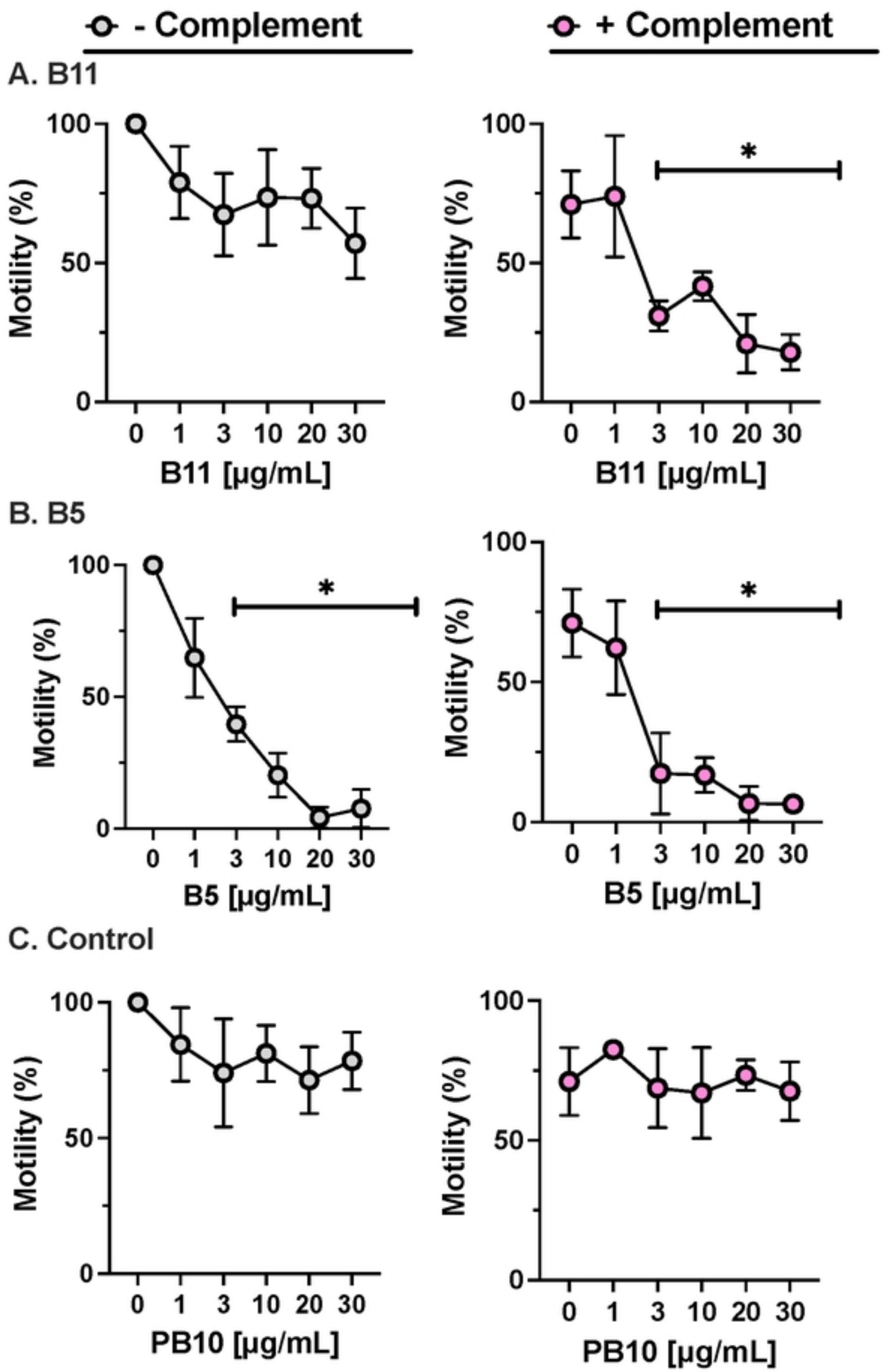

- Complement
+ Complement
A. B11
Motility (%)
100
50
0
0 1 3 10 20 30
B11 [µg/mL]
*
B. B5
B5 [µg/mL]
C. Control
PB10 [µg/mL]

Figure 3

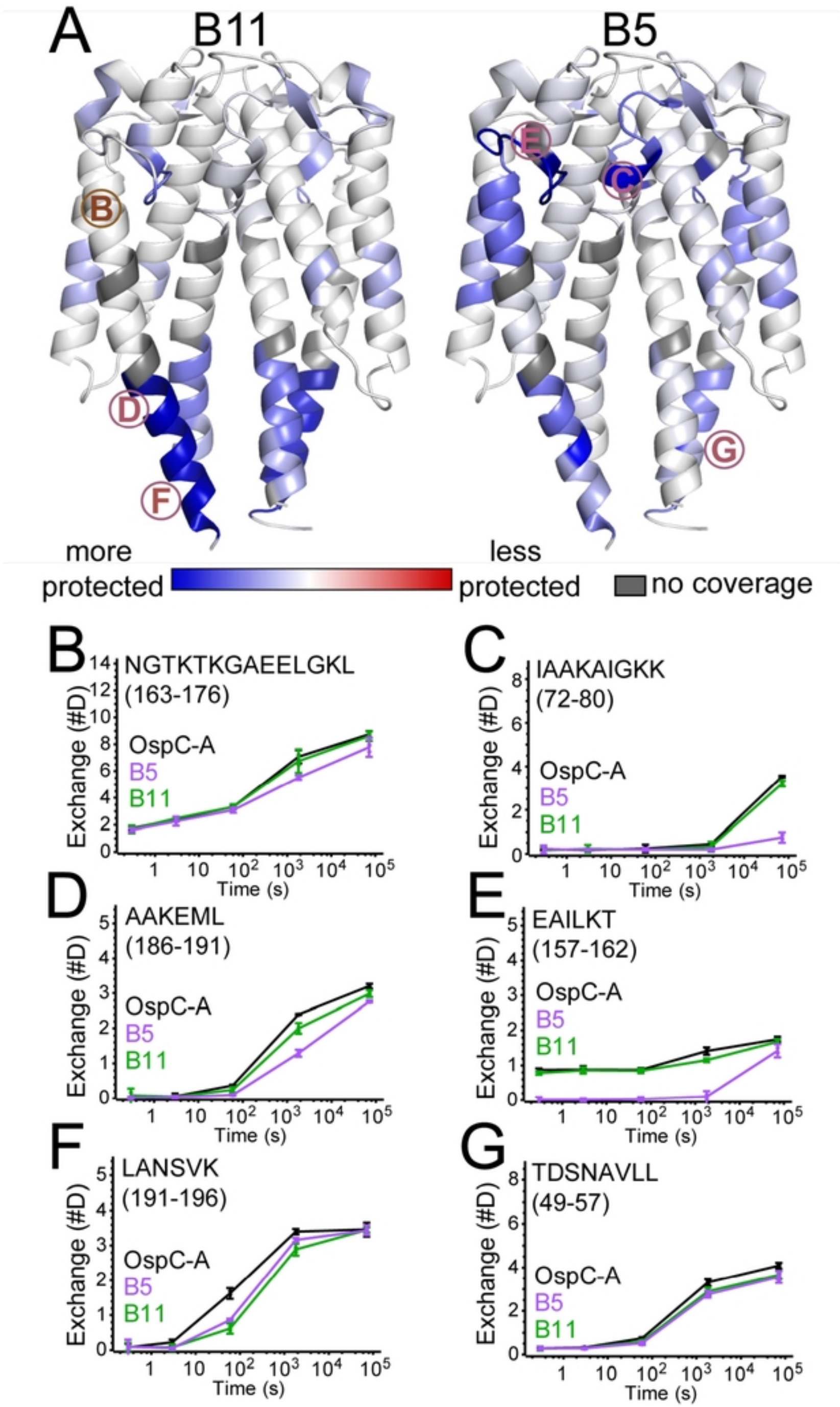
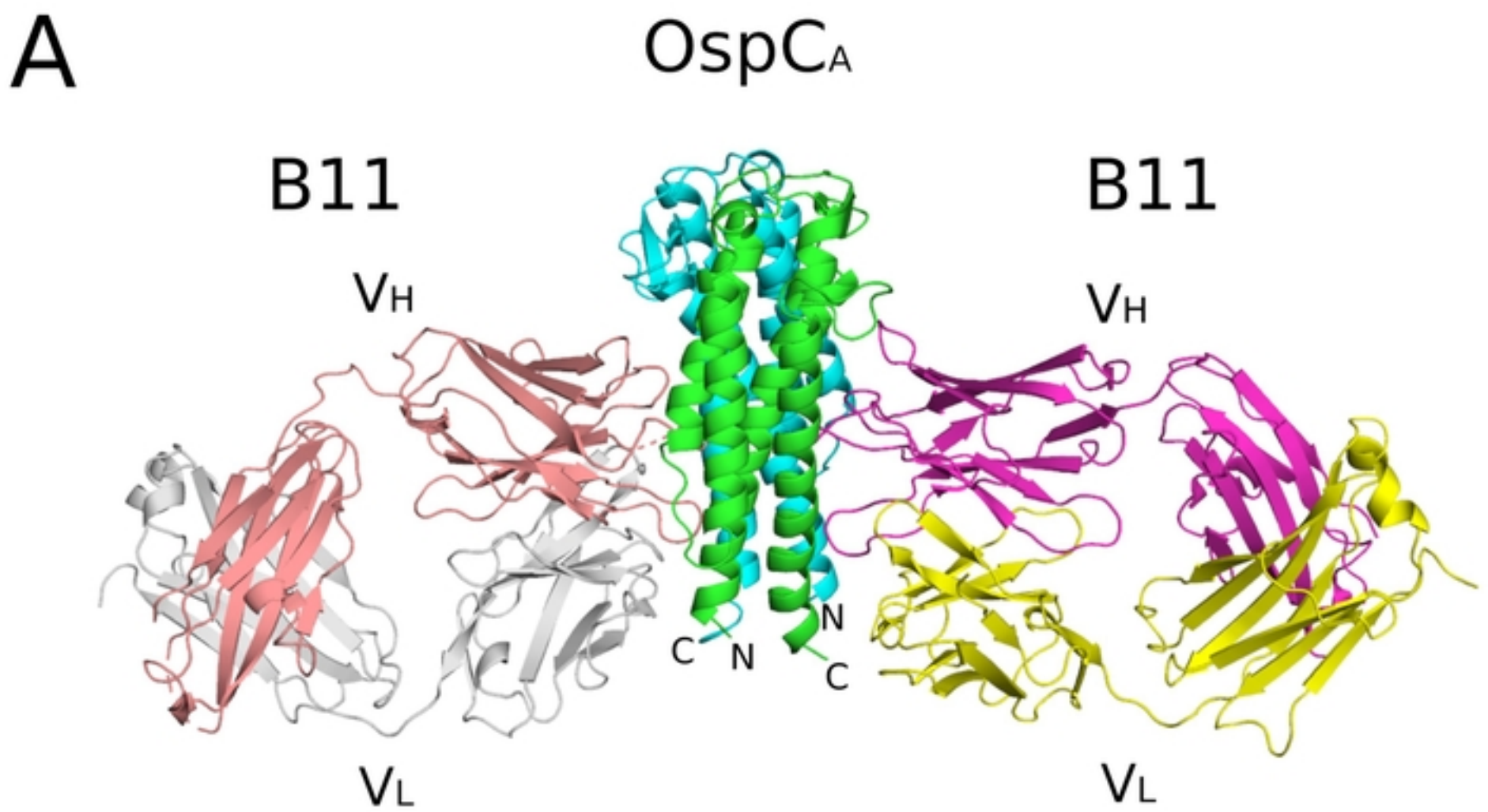

A
B11
B5
B
D
F
E
C
G
more protected
less protected
no coverage
B
NGTKTKGAEELGKL
(163-176)
OspC-A
B5
B11
Exchange (#D)
Time (s)
C
IAAKAIGKK
(72-80)
OspC-A
B5
B11
Exchange (#D)
Time (s)
D
AAKEML
(186-191)
OspC-A
B5
B11
Exchange (#D)
Time (s)
E
EAILKT
(157-162)
OspC-A
B5
B11
Exchange (#D)
Time (s)
F
LANSVK
(191-196)
OspC-A
B5
B11
Exchange (#D)
Time (s)
G
TDSNAVLL
(49-57)
OspC-A
B5
B11
Exchange (#D)
Time (s)

Figure 4



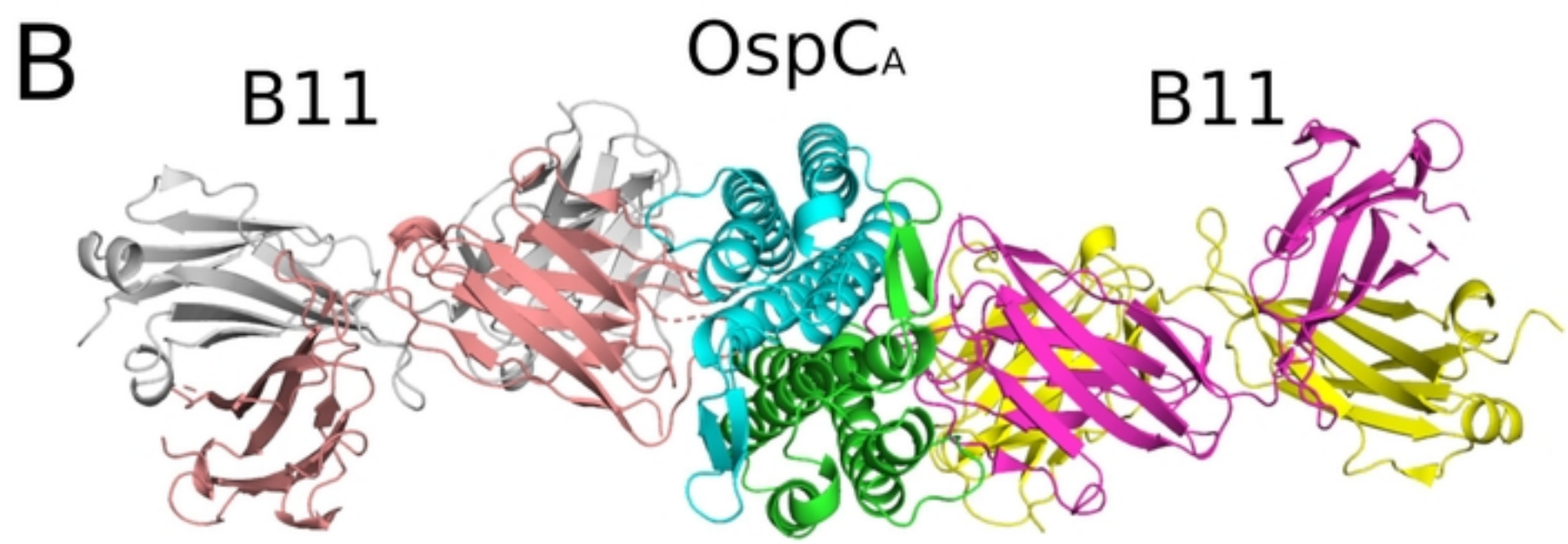
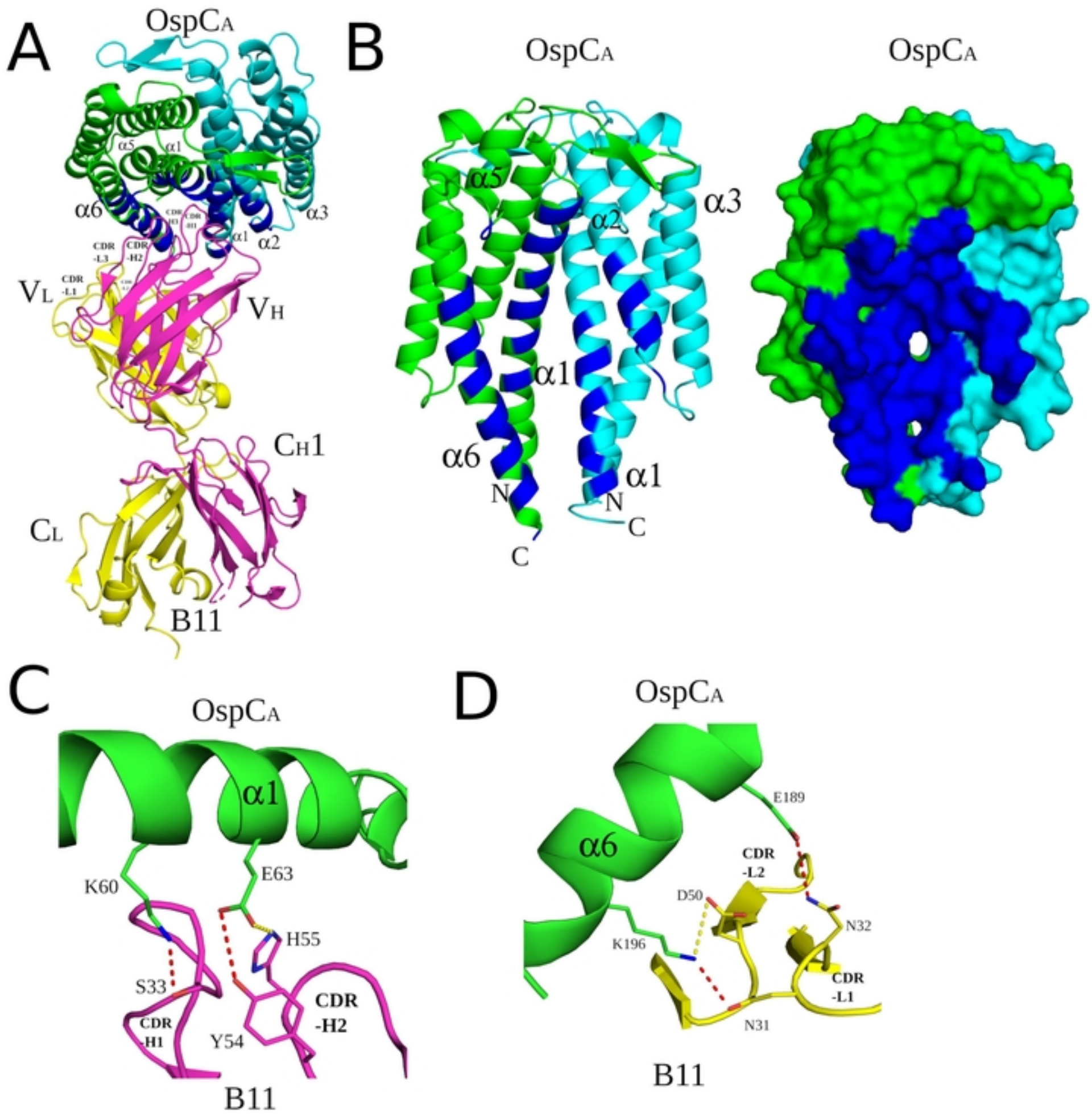


Figure 5


A
OspCA
α5
α1
α6
α3
α1
α2
CDR -L3
CDR -H2
CDR -L1
VL
VH
CH1
CL
B11
B
OspCA
OspCA
α5
α3
α2
α1
α6
α1
N
N
C
C
C
OspCA
α1
K60
E63
H55
S33
CDR -H1
Y54
CDR -H2
B11
D
OspCA
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α6
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N32
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CDR -L1
N31
B11
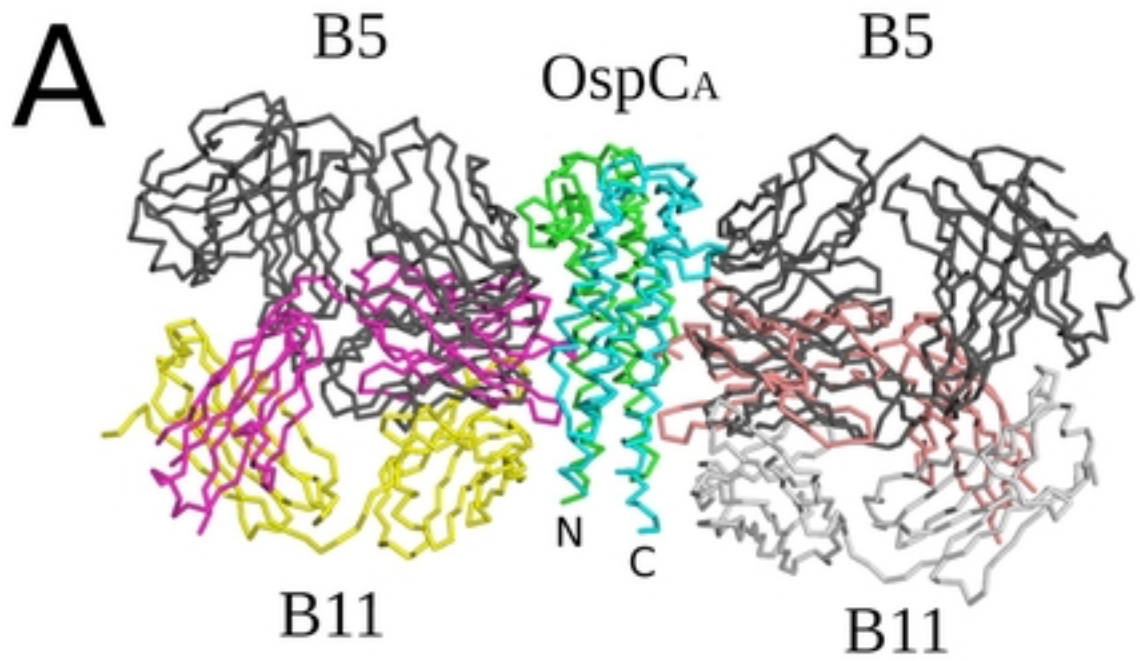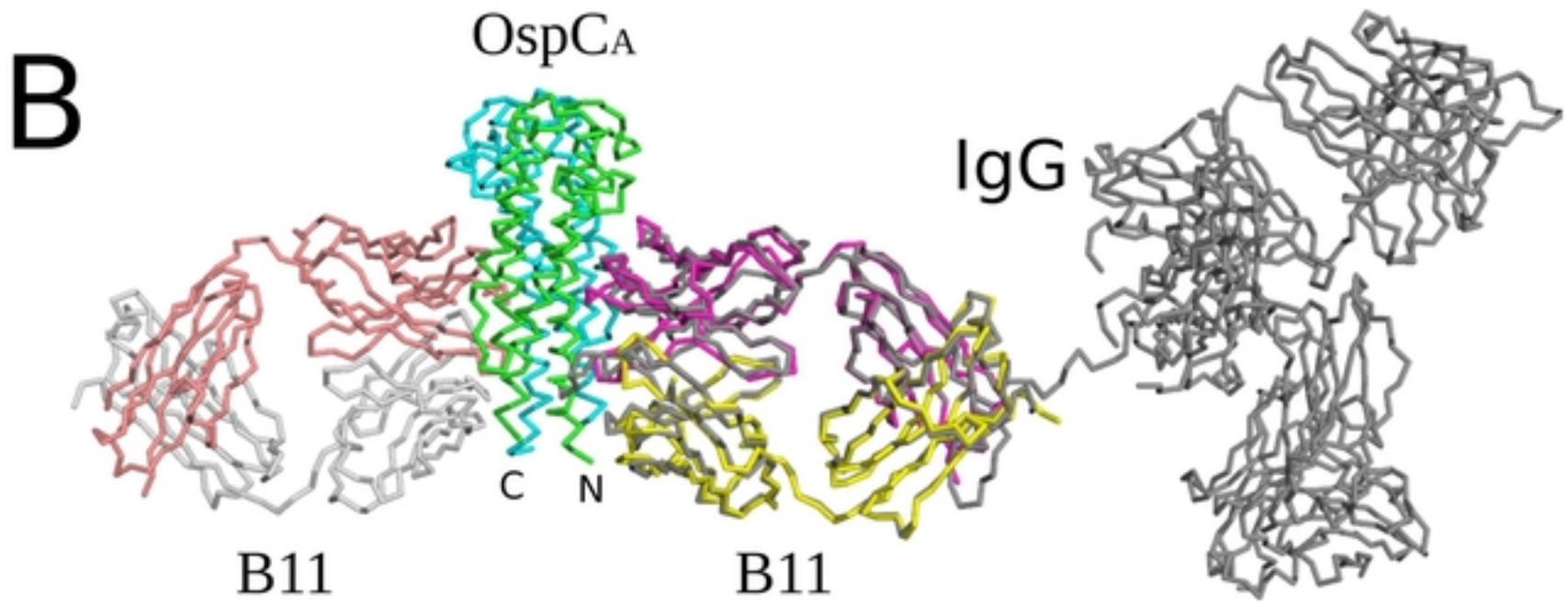
Figure 6