TipA, a *Bdellovibrio bacteriovorus* BPI-like double-TULIP is opened by its adapter protein, TipB.

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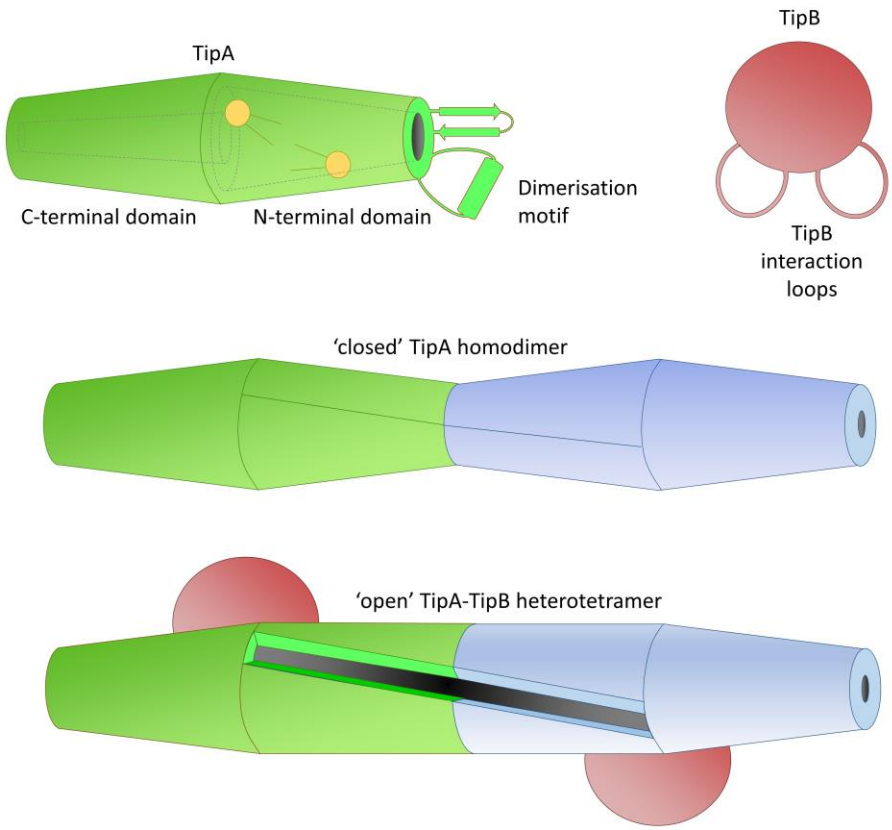
Abstract

Bdellovibrio bacteriovorus is a predatory bacterium that invades the periplasm of other Gram-negative bacteria and liberates prey biomolecules for replication. *Bdellovibrio* has a wealth of genes that encode unique proteins to enable this lifestyle. Using a series of x-ray structures, we show that the operonal pairing of *bd2538* and *bd2539,* encode a double TULIP (tubular lipid binding protein) invasion protein A (TipA) and a small beta sandwich (TipB), respectively. TipA has a specialised N-terminal TULIP domain with a beta hairpin and helix that mediate homodimerisation through hairpin interdigitation. This dimerisation creates a large, continuous, enclosed lumen that we demonstrate to contain multiple lipid molecules. In addition, we show that TipB functions as a small adapter protein that binds to TipA using specialised loops that bury into the TipA hydrophobic core. This binding forms a clamp on the edge of the N-terminal beta sheet and induces a large 20 Å conformational change, opening the TULIP fold to create an accessible interior. This study presents the first structural characterisation of lipid binding proteins in *Bdellovibrio*, and the first example of conformational change in TULIPs mediated by an adaptor protein.

Synopsis



Bdellovibrio bacteriovorus is a predatory bacterium that invades the periplasm of other Gram-negative bacteria in order to consume them from within. To enable this lifestyle, it has an arsenal of predation-associated genes, including the operonal pair *bd2538* and *bd2539*. We use structural and biophysical techniques to characterise two proteins produced by these genes, TipA and TipB.

- TipA is a double tubular lipid binding protein (TULIP) that forms a homodimer that sequesters lipids in its lumen and binds to lipid bilayers
- TipB is a small beta sandwich that interacts with TipA via two specialised loops
- Interaction of TipA with TipB opens the TULIP fold of TipA

Acronyms

ASU – Asymmetric unit
TULIP – Tubular lipid binding protein
SMP – Synaptotagmin-like mitochondrial-lipid-binding protein
LPT – Lipopolysaccharide transport
MLA - Maintenance of OM lipid asymmetry
BPI – Bacterial permeability-increasing protein
CETP – Cholesteryl ester transfer protein
LBP – LPS-binding protein
E-Syt – Extended Syntaptotagmin
ERMES – ER mitochondrial encounter structure
RMSD – Root mean square deviation
TLC – Thin layer chromatography
ITC – Isothermal titration calorimetry
QCM-D – Quartz crystal microbalance with dissipation
PDB – protein data bank
CL – cardiolipin
PE – Phosphatidylethanolamine
PG – phosphatidylglycerol
AFDB50 – AlphaFold Database 50

Introduction

Bdellovibrio bacteriovorus is a predatory bacterium that preys on other Gram-negative bacteria. Upon prey encounter, *B. bacteriovorus* attaches to its prey and invades its periplasm, where it remodels the host peptidoglycan to form a rounded bdelloplast. Here, it liberates prey biomolecules for its filamentous growth, followed by septation into progeny. Finally, when the prey cell is exhausted of nutrients, it becomes lysed to release free swimming *B. bacteriovorus* to repeat the cycle anew [1]. This remarkable prey-killing lifestyle, and its indifference to prey antibiotic-resistance status gives *Bdellovibrio* huge potential as a novel biological control agent [2]. However, this lifestyle also requires a wealth of specialised tools [3]. Indeed, *Bdellovibrio* utilizes a number of liberated biomolecules from its prey [4-7],

including both lipid A [8] and fatty acids [9]. These lipids can either remain in their original form or undergo metabolic alterations by *Bdellovibrio*.

Bdellovibrio's intricate association with membranes is central to its predatory lifestyle. Membrane and cell wall remodelling [10-12], as well as lipid uptake [8, 9] are critical facets of host cell penetration and subsequent intraperiplasmic survival. The mechanisms through which *Bdellovibrio* obtains lipids from its prey remain elusive, highlighting the complexity of lipid transport in bacteria, which typically necessitates dedicated machinery, such as the Lpt or Mla systems for lipid movement across the aqueous periplasmic environment [13]. However, these systems are responsible for intracellular transport of lipids across the periplasm of a cell, shuttling between inner and outer membranes. *Bdellovibrio* has the additional need for intercellular lipid transport, to extract and transfer lipids from prey cell membranes to self.

Tubular lipid-binding proteins (TULIPs) exhibit unique structural features comprising a cylindrical fold that surrounds a hydrophobic cavity, enabling them to sequester lipids from the surrounding environment. These include the extracellular bacterial permeability increasing protein-like (BPI-like) double-TULIP family, Takeout-like proteins and intracellular Synaptotagmin-like mitochondrial-lipid-binding protein (SMP) family [14, 15]. TULIPs have gained attention for their diverse roles in membrane tethering, lipid transfer, hormone binding and innate immunity in eukaryotes [15, 16]. Recently, widespread identification in prokaryotes has made it clear that TULIPs have important roles in prokaryote physiology [17, 18]. Structurally characterised prokaryotic TULIPS are few, and include *E. coli* YceB, and *Clostridium botulinum* neurotoxins/neurotoxin-associated proteins, P47 and OrfX proteins [19, 20]. Understanding the molecular mechanisms governing TULIP-lipid interactions is crucial for deciphering their role in prokaryotic organisms, inclusive of alternative lifestyles like the predator *Bdellovibrio*.

In this study, we provide X-ray crystal structures of two proteins encoded from the operonal gene cluster *bd2538* and *bd2539,* TULIP invasion protein A (TipA) and TULIP-associated invasion protein B (TipB), respectively. These proteins are upregulated during the invasion process [21] and secreted into prey [22]. We show that TipA forms a novel dimeric BPI-like double-TULIP, longer than classical characterized family representatives, and that TipB binds to TipA to induce a conformational opening of the TULIP fold. These results provide the first

115 example of a lipid-binding protein in *Bdellovibrio* and provide insight into the molecular
116 mechanisms of lipid trafficking during its predatory lifestyle.
117
118 **Results**
119
120 **Bdellovibrio TipA is a BPI-like double TULIP divergent from other family**
121 **members**
122
123 The operonal genes *bd2538* and *bd2539* (Fig. 1A) encode two proteins we term TULIP
124 invasion protein A (TipA) and TULIP-associated invasion protein B (TipB), respectively. To
125 identify the role of TipA in predation we sought to determine its structure (mature enzyme,
126 minus signal peptide) and elucidate its function. The crystal structure of selenomethionine-
127 incorporated TipA was solved in space group P 3_1 2 at 4 Å resolution with three molecules in
128 the asymmetric unit (ASU). The low resolution of diffraction was likely a consequence of the
129 87% solvent content caused by the end-to-end packing of the proteins in the unit cell.
130 Nevertheless, maps of sufficient quality were obtained (Fig. 1B) and in all three chains, at least
131 residues N35-A566 were traced with no unmodelled gaps in the model (Fig. 1C). TipA was
132 annotated to contain a DUF2785 domain, however the crystal structure confirms mis-
133 annotation, as the protein fold shows it to be of the lipid binding protein BPI/LBP superfamily
134 (IPR030675) [23].
135
136 TipA shows similar gross features to those of other BPI/LBP family structures: a double-
137 TULIP fold, comprising a central beta sheet sandwiched by two tubular domains each formed
138 by a beta sheet that wraps around an extended helix (Fig. 1C). Each TULIP domain has the
139 typical secondary structure characteristic of that domain: an N-terminal helix (helix A), an
140 elongated beta sheet, and a C-terminal helix (helix B). The single protomer forms a cylindrical
141 protein with dimensions of approximately 142 x 35 x 40 Å enveloping a central hydrophobic
142 cavity throughout the entire protein.
143
144 TipA was submitted to the DALI server [24] to identify proteins with similar structures. The
145 top hits were all double TULIP proteins: Bacterial permeability-increasing protein (BPI; Z-
146 score 16.5; 21% seq ID), Cholesteryl ester transfer protein (CETP; Z-scores 13.5-15) and LPS-
147 binding protein (LBP; Z-scores 10.4-11). Superposition of the individual N- and C-terminal

148 domains of TipA with those of BPI shows significant divergence of the protein fold, with
149 RMSD values of 5.7 Å over 176 residues for the N-terminal domain and 5.8 Å over 112
150 residues for the C-terminal domain (Fig. 1D-E). Hence, we conclude that TipA is a divergent
151 TULIP representing a grouping of the superfamily previously uncharacterised.
152
153 The N-terminal domain shows three inserts relative to that of BPI: a beta hairpin between the
154 last beta strand and helix B, a loop containing an additional helix between the first and second
155 strands, and a small helix between the second and third strands (Fig. 1D). The first two of these
156 inserts comprise the dimerisation interface of TipA, whereas the function of the latter is
157 unclear.
158
159 Compared to BPI, the C-terminal domain has a truncated loop between strands 1 and 2, a larger
160 triangular-shaped loop between strands 2 and 3, and two small helices inserted between the last
161 strand and helix B. Superposition reveals that the central beta sheet and helix A align well with
162 the BPI C-terminal domain, but upon exit from helix A, TipA diverges greatly (Fig. 1E). In
163 particular, both the sheet and helix B are much shorter than BPI, and this causes a relative
164 displacement.
165

166 **TipA forms a cylindrical homodimer**

167
168 A surprising finding of the crystal structure of TipA was that unlike any other characterised
169 TULIP, TipA forms a C2 symmetrical dimer (Fig. 2A), with one complete dimer in the ASU
170 and one protein forming a dimer with a symmetry copy. TipA has a novel beta hairpin and
171 helix on the N-terminal domain that form a dimer interface, producing an extended
172 hydrophobic tube through an end-on-end interaction (Fig. 2A-B). The channel throughout
173 dimeric TipA is almost continuous, containing a pinch point in each of the central sheet
174 domains, and has a total volume of approximately 25000 $Å^3$ as calculated by 3vee [25] (Fig.
175 2B). The dimerisation hairpins form a single antiparallel beta sheet and produce hydrophobic
176 and polar faces with two intermolecular electrostatic interactions formed by E251 and K256
177 (Fig. 2C). A sequence alignment of TipA shows a number of highly conserved residues that
178 are important structurally (Fig. EV1). The tip of the dimerisation hairpin contains a highly
179 conserved asparagine (N253) that hydrogen bonds with the backbone of F184. N260 is also
180 highly conserved and serves to propagate helix B following the hairpin. The highly conserved

181 P246 kinks the backbone to bend the hairpin away from the beta sheet and angle it parallel to
182 the protein tube, allowing interdigitation with the hairpin of the second molecule. The N-
183 terminus of the dimerisation helix packs against its own helix B and extends outwards to pack
184 its C-terminal tryptophan into the hydrophobic face of the other protomer's helix B (Fig. 2D).
185 The dimerisation helix makes hydrogen bonds between Q104 and Q104, and E263 and Q111.
186 The associated loop (L113-S117) forms backbone hydrophobic interactions through L113 and
187 F116 and backbone hydrogen bonds between V114 and Y258. A highly conserved glycine
188 (G115) allows the chain to form a sharp turn to thread back to the beta sheet of the N-terminal
189 domain.
190
191 Due to the spacious packing of the TipA dimer crystals, and consequent relatively low
192 resolution, we subsequently sought to obtain a higher resolution structure of TipA by altering
193 the packing of the crystal lattice. To do this we substituted the hairpin region (A249-F258) with
194 a triple glycine linker, termed $TipA_{\Delta Hairpin}$. We expected the loss of dimerisation to allow better
195 packing of the protein, reducing solvent content to produce higher resolution diffraction. This
196 substitution shifted the elution volume of the protein from 164 mL to 200 mL on a Superdex
197 200 pg 26/60 column, showing a drastic decrease in hydrodynamic radius of the protein, and
198 indicating that the protein is primarily monomeric in solution (Fig. 2E). As predicted, the
199 $TipA_{\Delta Hairpin}$ protein crystallised with different packing in space group P 2_1 2_1 2_1, with a solvent
200 content of 60% and higher resolution diffraction of 2.6 Å. The crystal structure of $TipA_{\Delta Hairpin}$
201 contains a single monomeric copy in the ASU (Fig. 2F). The lack of dimer interface introduced
202 disorder into the N-terminal domain and the regions of G107-M118 and the glycine linker
203 region P246-N253 (N260 in wild-type (WT)) so these residues could not be modelled. The C-
204 terminal domain and central sheet are very similar to that of the WT protein, but the N-terminal
205 TULIP sheet is pulled inward, mediated by a bend in Helix B at G277 (G284 in WT). This
206 suggests that dimerisation is integral to maintaining correct folding of the N-terminal domain.
207

208 **TULIP partner TipB is a β-sandwich with exposed hydrophobic loops**

209 Since the TipA-encoding *bd2538* gene is in a predicted operon with the downstream *bd2539*
210 gene, which it overlaps by 4 nucleotides and which we name *tipB*, we hypothesised that TipB
211 could be functionally linked to TipA. Following our structural studies of TipA, we purified and
212 crystalized the cryptic operonal TipB protein. TipB crystallised in space group C 2 2 2_1 with
213 three copies in the ASU and diffracted to 2.17 Å resolution. The structure reveals that TipB

214 forms a compact β-sandwich, in which one four-strand anti-parallel sheet forms the central
215 globular core of the protein and is packed on one side by a pair of short beta sheets and the
216 other side by an elongated capping-loop extending from β7 and β8 (Fig. 3A). This loop folds
217 back on the sheet burying Y120, W122 and M133 into the core. The protein has another pair
218 of loops that extend between β3 and β4, and β6 and β7, termed interaction loops 1 and 2,
219 respectively (Fig. 3A). Importantly, both loops have hydrophobic residues at the ends: F65 and
220 I66 in loop 1 and F103 in loop 2, and demonstrate a high degree of flexibility (as inferred by
221 electron density and B-factor values; Fig 3B). Finally, the protein contains a short helical turn
222 between strands 2 and 3 and an 11-residue helix between strands 8 and 9.

223 We used the DALI server [24] to identify structural homologues of TipB and the highest
224 scoring structure was *P. aeruginosa* ExsB [26] (PDB code 2YJL), with a Z-score of 7.3. Both
225 TipB and ExsB show a topology similar to that of type II C2 domains (Fig. 3C-E) [27], a
226 calcium-dependent membrane-localisation domain found in multiple eukaryotic signalling
227 proteins including kinases [28], lipases [29] and membrane trafficking proteins [30]. In
228 comparison, TipB lacks the 8th strand that runs antiparallel between the 1st and 7th strands,
229 instead packing its last strand parallel to the 2nd strand (the 1st strand is broken into two smaller
230 strands due to a broken beta sheet in TipB), forming a mixed sheet. The other top-scoring hits
231 all had the standard type II C2-domain topology, with varying additional loops and secondary
232 structure components. Most of the C2-domain proteins have an exposed face on the beta sheet,
233 however some homologues show extensions in the β7 and β8 loop that cap off this sheet in a
234 similar manner to TipB (Fig. 3E). None of the homologues had extensions between β3 and β4
235 or β6 and β7 strands where the interaction loops are observed in TipB.

236 ExsB has an exposed beta sheet with a basic face predicted to mediate binding to acidic lipids
237 (Fig. 3E). In TipB, this sheet face is not exposed and forms part of the hydrophobic core that
238 is enclosed by an extended loop between β7 and β8. To investigate whether TipB would adhere
239 to a lipid bilayer, we used quartz crystal microbalance with dissipation monitoring (QCM-D)
240 measurements (Fig. 3F). This surface-based technique monitors changes to the resonant
241 frequency of a surface (Δf) under an applied voltage. Δf is inversely proportional to the mass
242 deposited on the surface, and as such allows us to monitor interactions between biomolecules
243 [31]. In the case of TipB-lipid interactions, a stable POPC bilayer was deposited onto a silicon
244 QCM-D sensor, following well-established methods [32, 33]. Despite not being a constituent
245 of bacterial membranes, a POPC bilayer was used here due to its well-reported ability to form

246 highly stable bilayers when deposited onto a surface [34]. Once lipid bilayers were deposited,
247 TipB was introduced and its interaction with the lipid bilayer was assessed in a semi-
248 quantitative manner. Upon flow of TipB over the bilayer, negligible frequency (0.3 Hz) and
249 dissipation (0.48 ppm) changes occurred, thus confirming that TipB alone does not bind
250 directly to lipid membranes.

251

252 C2 domains commonly bind calcium, such as the high-scoring DALI hit *Homo sapiens*
253 phospholipase 2 [35]. We wanted to confirm that TipB doesn't bind calcium as it does not
254 contain obvious calcium-binding residues nor was calcium observed in the crystal structure.
255 After treatment with 5 mM EGTA and 5 mM EDTA, and subsequent gel filtration, no binding
256 was seen using isothermal titration calorimetry (ITC; Fig. 3G), confirming that TipB is a
257 calcium-independent C2-like β-sandwich.

258

259 **TipA and TipB form a TULIP:partner complex**

260

261 Since TipB did not display any phospholipid binding activity we next hypothesised that TipA
262 and TipB might interact with each other in a functional manner (whilst also being stable in their
263 isolated states, as inferred from our structures). Isothermal titration calorimetry (ITC) of TipB
264 into TipA resulted in a clear binding curve (Fig. 4A), and the -TΔS value suggests the
265 interaction is largely driven through entropy effects, possibly through hydrophobic
266 contributions. However, due to the low stoichiometry observed (0.25), the curve wasn't
267 suitable to calculate an accurate Kd. The relatively poor stability of the TipA protein would not
268 allow for higher concentrations to be used. The low stoichiometry could result from TipA
269 existing as multiple species in solution. Although TipA elutes as a single peak in gel filtration
270 experiments, this peak is broad (when compared with ΔHairpin TipA), potentially the result of
271 multiple TipA conformers (Fig. 2E). It is therefore likely that only a subpopulation of TipA is
272 competent for binding and may further demonstrate the flexible nature of the protein.

273

274 We subsequently desired to obtain a crystal structure of the complex of TipA and TipB. The
275 two proteins were purified and mixed with a molar excess of TipB in the crystallisation
276 conditions in order to ensure a complex of the two proteins. This complex crystallised in space
277 group P 2 2 2_1 and diffracted to 2.6 Å with one copy of each protein in the ASU. The TipA
278 component formed a dimer on a crystallographic 2-fold axis exhibiting the same hairpin-helix

279 dimer interface as the selenomethionine-labelled TipA crystals (Fig. 4B). TipB can be observed
280 binding centrally on TipA, straddling the unusually kinked helix A, which protrudes away from
281 the beta sheet, forming a triangular seat (Fig. 4C-E). The structure of complexed TipB is very
282 similar to that of TipB alone, with RMSDs ranging from 1.43 to 1.81 Å over 139 amino acids
283 for the three chains of the TipB crystal structure. Differences are mostly observed in interaction
284 loops 1 and 2, which fold down over the seat and bury residues F65, I66 and F103 into the
285 hydrophobic lumen of TipA (Fig. 4F-G). In addition to the hydrophobic components, there are
286 a number of hydrogen and electrostatic bonds from side chains and backbone atoms throughout
287 the 1346 $Å^2$ interface (Fig. 4H-I), as calculated by PISA [36].
288

289 **Partner TipB binds to open up the TULIP fold and central cavity**

290

291 The most striking observation of the complex structure is the conformational change seen in
292 TipA's N-terminal domain (Fig. 5A-B). While the superposition of the C-terminal domains of
293 the apo and complexed TipA structures show an RMSD of 1.26 Å over 261 residues, the N-
294 terminal domain shows an RMSD of 6.13 Å over 271 residues. The N-terminal domain of the
295 TipA apo structure comprises an almost completely closed tube, with very little access to
296 solvent (Fig. 5C). However, the N-terminal domain of the complex is in an open conformation,
297 with a gap of approximately 20 Å (from Y80-S282; Fig. 5D). This opening is mediated by an
298 approximate 45° rotation of the protruding TipA helix A (Fig. 5A), which acts like a lever,
299 pulling the N-terminal sheet open. This opening is also stabilised by the anchoring of
300 interaction loop 1 into the C-terminal domain, and the packing of interaction loop 2 F103 under
301 the lifted N-terminal sheet. TipB F103 acts as a clamp underneath the TipA beta sheet in
302 concert with K100 and R34, which form electrostatic interactions with E72 and E129,
303 respectively, on the top of the sheet (Fig. 5E). Analysis by DynDom [37] characterises this as
304 a hinge mechanism, comprising three moving domains (Fig 5F): the C-terminal domain and
305 most of the N-terminal helix B (Domain 1); most of the N-terminal sheet and dimerisation
306 hairpin (Domain 2); and the end of the N-terminal sheet and dimerisation helix (Domain 3).
307 The analysis identifies two hinge points between these domains which include the rotation
308 point of the N-terminal helix A seat. Domain 2 rotates approximately 29° relative to Domain 1
309 and Domain 3 rotates 36° relative to Domain 2, keeping it a similar relative position to Domain
310 1 in both structures (Fig. 5G). Hence, the function of TipB is to convert TipA to a more open
311 form and, to the best of our knowledge, represents a new type of TULIP binding-partner.

312

313 Using Foldseek [38] to search for homologous proteins, we observe double-TULIPs with
314 additional fused domains (Fig. EV2). These include beta-rich domains, helical bundles and
315 mixed domains including enzymes and entire double-TULIP repeats. Of particular interest is
316 the presence of a double-TULIP in the *Planctomyces sp*. SH-PL14 protein, Uniprot accession
317 A0A142WTQ2. This protein contains a fused N-terminal esterase-like enzyme in addition to a
318 C-terminal beta-sandwich. This beta-sandwich has similar topology to TipB, and is also
319 predicted to localise on the N-terminal Helix A and sheet, suggesting that it could have a similar
320 role to TipB in this system.

321

322 **TipA binds rapidly to membrane bilayer surfaces**

323

324 During the purification process of TipA, we observed that under conventional buffer conditions
325 (<500 mM NaCl) the protein remained largely in the pellet after lysis and centrifugation.
326 However, upon addition of high salt concentrations or detergents, the protein was released into
327 the supernatant. This led us to believe that the protein was adhering to the membrane fraction
328 (Fig. 6A).

329

330 To confirm this, we again used QCM-D (Fig. 6B-E). To investigate TipA-lipid interactions,
331 stable bilayers consisting of either *E. coli* polar lipids, POPC or mixed POPC:LPS lipids were
332 deposited onto a silicon QCM-D sensor. Upon introduction of TipA to all three bilayer
333 compositions monitored here, it exerted a dramatic negative Δf, suggesting it was rapidly
334 deposited into the bilayer systems. This interaction was most pronounced for TipA deposition
335 into POPC:LPS bilayers (Fig. 6D), with a Δf3 of -1106 Hz, compared to -1064 Hz and -686
336 Hz for POPC and polar lipid bilayers, respectively. In all bilayers tested, a critical point was
337 reached upon which further addition of TipA resulted in removal of mass from the surface.
338 This suggests an ability to first insert into lipid bilayers followed by membrane disruption.
339 Furthermore, in bilayers containing POPC a double-dip insertion of TULIP was observed (Fig.
340 6C-D), which may result from the ability of POPC to form more stable bilayers on silicon
341 surfaces than *E. coli* polar lipids. Interestingly, this distinctive mode of interaction was not
342 observed when TipA was introduced to an empty silicon surface generated through the inability
343 to deposit polar lipid:LPS bilayers, with no major mass deposition or removal observed (Fig.
344 6E).

345

346 **Lipids are observed in the TipA TULIP lumen in multiple states**

347

348 In order to confirm that TipA is a classic TULIP that binds lipids within its cavity, thin layer
349 chromatography (TLC) was performed (Fig. 7A). TipA clearly copurifies with all three
350 common *E. coli* polar lipids, cardiolipin (CL), phosphatidylethanolamine (PE) and
351 phosphatidylglycerol (PG). These have likely been extracted from *E. coli* during the expression
352 or extraction process. Density for lipids was observed in all three crystal structures, however
353 the low resolution of the selenomethionine form prevented their modelling (Fig. EV2A). In
354 both the 2.6 Å TipAB complex structure (Fig. 7B) and the 2.6 Å monomeric $TipA_{\Delta Hairpin}$
355 structure (Fig. 7C), clear densities are observed for two lipid head groups in the N-terminal
356 TULIP domain. It was also possible to trace the lipid tails in the $TipA_{\Delta Hairpin}$ structure but not
357 in the complex structure. The lipids were modelled as PE as this headgroup fit the density well
358 (Fig. 7D), and the density would not accommodate the glycerol moiety of PG, nor did the
359 density indicate the presence of the double headgroup of CL.

360

361 In both structures the lipid headgroups are seen in similar regions in the protein (as are the Fo-
362 Fc peaks in the selenomethionine TipA structure; Fig. EV2A), one underneath Helix A, and
363 the other between the sheet and Helix B (Fig. 7E-F). However, contributing amino acids that
364 bind the ligand are different between the structures. The placement of the inner lipid in the
365 TipAB structure means that the headgroup makes a H-bond and hydrophobic interactions with
366 G102 and F103. In the $TipA_{\Delta Hairpin}$ structure (where TipB is not present), it makes H-bonds
367 with Y132 and K69, but additional interactions are formed by V493 of the symmetry copy,
368 providing a backbone H-bond and sidechain hydrophobic interactions. These modelling
369 differences suggest that TipB affects lipid interactions with TipA.

370

371 Interestingly, in both structures one of the lipids makes contact with the C-terminal domain of
372 a symmetry copy (Fig. EV2B-D), with polar contributions from the symmetry copy K457 and
373 K503 to the phosphate moiety of the outer lipid in the TipAB structure. When visualising
374 higher order packing in all three TipA structures, this is a common occurrence – in three
375 different space groups the C-terminal hydrophobic residues M454, F455, L504 and F505 pack
376 into the N-terminal domain 'seam' between Helix B and the 1st beta strand (Fig. EV2E-G).

377 Further structures of close TipA homologues are needed to determine if this mode of interaction
378 is a conserved feature.
379

380 **Discussion**

381 Tubular lipid binding proteins (TULIP) proteins are a universal lipid-binding fold,
382 characterised by their ability to sequester lipids within their internal hydrophobic cavities.
383 Whilst these folds are ubiquitous to all domains of life, prokaryotic TULIPS present an under-
384 represented group within the literature [17]. We have structurally characterised the first
385 example of a lipid-binding protein from *Bdellovibrio bacteriovorus* HD100 - the predation-
386 associated TULIP, TipA, and identified an associated adapter protein TipB.
387

388 TipA is in the same superfamily as the eukaryotic bacterial permeability increasing protein
389 (BPI), which also forms a double-TULIP fold. The protein shows similar structural features to
390 that of other BPI-like proteins, such as the classical Helix-ββββ-Helix TULIP core secondary
391 structure, two TULIP domains back-to-back with an intervening central sheet, and longer N-
392 terminal than C-terminal TULIP domain. However, modifications to the protein have made the
393 overall fold divergent, with the addition of molecular features that enable the formation of a
394 dimeric double-TULIP, and for interaction with the TipB adapter protein. The dimerisation
395 motif includes an additional hairpin and helix that lock the homodimer together to form a
396 hugely elongated ~260 Å tube.
397

398 While no other double-TULIP has been observed in a dimeric form, the extended
399 synaptotagmins (E-Syts) and the eukaryotic ER-mitochondria encounter structure (ERMES)
400 proteins do form single-TULIP homodimers, but they pack using a different interface where
401 helix B packs against the beta sheet edge [39]. It is interesting that a heterotetrameric form of
402 TULIP is also seen between ERMES proteins Mdm12 and Mmm1, where heterodimers stack
403 head-to-head through an Mmm1-Mmm1 interface [40]. This creates an equivalent chain of four
404 TULIP domains, highlighting that higher-order arrangements of TULIPs are functionally
405 significant. It is possible that this increase in TULIP length is needed in order to efficiently
406 transport lipids effectively at some juncture between prey and *Bdellovibrio*. The length of the
407 dimer is between 250 and 260 Å which is equivalent to that of the periplasmic spanning
408 transporter complexes, Yeb and Pqi [41] as well as the predicted structure of the putative
409 transporter YhdP [42]. ERMES proteins and E-Syts tether membranes and transfer lipids

410 between them, which is dependent on the presence of their C2 domains for membrane tethering
411 [43], and therefore proximity of membranes to their single-TULIP is required. The increased
412 length of the TipA dimer could allow for lipid transfer between membranes without the need
413 for direct membrane tethering by additional domains.
414
415 It is also noteworthy that we consistently observe TipA packing C-terminal hydrophobic
416 residues into the N-terminal domain – which creates a network of TULIPs in the crystal lattice.
417 This could indicate that during predation TipA could form higher order structures that facilitate
418 transport of lipids from TULIP to TULIP. Higher order structures also occur in the ERMES
419 complex as the Mmm1-Mdm12 complex also interacts with another TULIP Mdm34 [40, 44],
420 but structural information and exact stoichiometry is unknown for these larger complexes.
421 Further work could confirm whether there is a physiological function for this consistent C-
422 terminal-N-terminal TULIP packing of TipA.
423
424 The *tipA* gene is in an operon with *tipB*, which encodes for a small C2-like sandwich domain.
425 The functional link between TULIPs and C2 domains is observed in ERMES and E-Syt
426 systems, where the C2 domains mediate membrane tethering. TipB, while C2-like, does not
427 bind calcium or membranes, and functions in an entirely different manner. The TipB sandwich
428 has been adorned with two extended loops that are used for interaction with TipA by insertion
429 into its lumen. The crystal structure of the complex of TipA and TipB shows that upon binding
430 of the two proteins there is a large conformational change in TipA, mediated by a clamping
431 mechanism that lifts its N-terminal beta sheet. This creates an opening of the TULIP seam,
432 creating an accessible core across both N-terminal domains of the TipA dimer. Previously, a
433 gate-and-latch mechanism has been proposed to account for lipid binding into the lumen of the
434 single-TULIP juvenile-hormone binding protein from the domestic silk moth [45]. Here, the
435 N-terminal helix A opens in the apo form and closes on the buried sesquiterpene molecule
436 when bound. As the N-terminal helix of double-TULIP proteins is locked into the central beta
437 sheet by an additional N-terminal beta strand, this latch mechanism is not possible. Instead, it
438 is possible that the opening of TipA by TipB is what facilitates loading or unloading of lipids;
439 observation of lipid-loaded recombinant TipA by TLC indicates that it would not be dependent
440 on TipB. The intriguing finding of a planctomycete double-TULIP with a fused beta-sandwich
441 that binds to the N-terminal domain Helix A and sheet proposes the idea that this functional
442 relationship is repeated across diverse bacteria. In addition, it is possible that TipA
443 homodimerisation is also required to ensure the stability of the overall fold during this opening

444 and closing process as we have observed a high degree of flexibility in the N-terminal sheet
445 (very pronounced in $TipA_{\Delta hairpin}$), and the lumen of the N-terminal domain is larger than
446 typically observed in other double-TULIPs.
447
448 TipA's ability to adhere to membranes and destabilise them as shown via QCM-D suggests
449 that it could be used to extract lipids directly from prey bacterial membranes. Binding and
450 membrane destabilisation by TipA could aid *Bdellovibrio*, and could work in a similar fashion
451 to BPI [46] by increasing membrane permeability. TipA is not the first example of a TULIP
452 protein adhering to membranes without C2 domain partners - tricalbin 2 (a yeast E-syt
453 homologue) and nucleus-vacuole junction protein 1 TULIP domains were shown to bind to
454 membranes *in vitro* using liposomes [47], and the tricalbins are known to transfer lipids
455 between membrane contact sites. Therefore, membrane adherence (as observed by both our
456 purification procedure and QCM-D experiments) is likely an effective way for TipA to uptake
457 lipids into its core ready for transfer to another protein or membrane.
458
459 In conclusion, our findings underscore the evolutionary significance of TULIPs as universal
460 lipid-binding folds across both eukaryotes and prokaryotes. The crystal structures of TipA,
461 TipB and their binary complex show that *B. bacteriovorus* has novel lipid-binding machinery
462 for its predatory lifestyle in the form of a modified dimeric double-TULIP protein. Through
463 the mechanistic interdependence of TipA and TipB, we give new insight into the
464 conformational changes possible in double-TULIP proteins and suggest an adaptor-mediated
465 mechanism for promoting lipid loading and unloading.
466

467 **Methods**

468

469 **Cloning, expression and purification**

470
471 TipA-His was cloned into a modified version of the pET41c overexpression vector using
472 restriction-free cloning methods. The final construct contained residues of 26-572 of TipA
473 (Uniprot accession Q6MK73) fused to a C-terminal octa-His-tag. TipB was cloned into pET26b
474 using NdeI and NotI. A tagless form of TipA was made by mutagenesis to remove the octa-
475 Histag. A monomeric form of tagless TipA ($TipA_{\Delta Hairpin}$) was created using Q5 mutagenesis,
476 replacing the dimer-hairpin residues A249-Y-258 (AVEVNGKKFY) with a triple glycine

477 linker. TipB was cloned into pET26b using NdeI and XhoI restriction sites. The final construct
478 contained an N-terminal MAHHHHHH tag and residues 29-171 of TipB (Uniprot accession
479 Q6MK72).
480
481 To express selenomethionine-labelled TipA, a 60 mL overnight culture of BL21 (DE3) cells in
482 LB was centrifuged and resuspended in minimal media supplemented with kanamycin and
483 chloramphenicol to a final volume of 1 L. At OD_{600} 0.4, 0.1 g of lysine, 0.1 g of threonine, 0.1
484 g of phenylalanine, 0.05 g leucine, 0.05 g of isoleucine, 0.05 g of valine and 0.06 g of
485 selenomethionine were added to the culture. After 15 minutes, IPTG was added to 1 mM. Cells
486 were harvested by centrifugation after 16 h at 18 ˚C and stored at -20 ˚C.
487
488 $TipA_{\Delta Hairpin}$, TipA-His and TipB constructs were expressed in *Escherichia coli* BL21 RIPL
489 (DE3). Cells were grown in LB supplemented with kanamycin to an OD_{600} of 0.5-0.7 and
490 induced with 0.5 mM IPTG at 18 °C for 16 h. Cells were harvested by centrifugation at 4000g
491 for 15 minutes. During trial purifications we noted that the majority of TipA protein sedimented
492 with insoluble cell components following lysis and centrifugation, but could be liberated from
493 the insoluble pellet by the addition of high (1M) NaCl. We exploited this characteristic of TipA
494 in our subsequent purification protocols. Cells expressing untagged selenomethionine-labelled
495 TipA and $TipA_{\Delta Hairpin}$ were resuspended in wash buffer (50 mM HEPES and 0.15 M NaCl, pH
496 7.5), sonicated and centrifuged at 48384 g for 1 h. The pellet was again resuspended, sonicated
497 and centrifuged to wash away soluble proteins. A third pellet wash was performed and finally
498 the pellet was resuspended in 50 mM HEPES and 1 M NaCl, pH 7.5. Cells expressing his-
499 tagged proteins were resuspended in buffer A (TipA-His - 50 mM HEPES and 1M NaCl, pH
500 7.5; TipB - 50 mM HEPES and 300 mM NaCl, pH 7.5). The cells were lysed by sonication on
501 ice and centrifuged at 48384 g for 1 hour. The pellet was resuspended. The lysates were loaded
502 onto a 5 mL Ni-NTA column (GE Healthcare) and washed with 10 CV of lysis buffer. The
503 protein was eluted with a gradient of 20–500 mM imidazole. All proteins were finally passed
504 through a Superdex 200 pg or 75 pg 26/60 column (GE Healthcare), which was pre-equilibrated
505 with 20 mM HEPES, 150 mM NaCl, pH 7.5. Fractions containing the protein of interest were
506 pooled, concentrated and stored frozen at -70 °C if not required for immediate use.
507
508 **Crystallisation**
509

510 Selenomethionine-lebelled TipA crystallisation screens were prepared by mixing a 1.5 µL of 8
511 mg/mL TipA and 1.5 µL precipitant solution. TipA crystallised in space group P 31 2 1 (a
512 =252.4, b=252.4, c=181.1, α=90, β=90, γ=120) in a precipitant solution from the Jena
513 Bioscience JBS Classic Screen consisting of 15% w/v PEG 4000; 100 mM trisosodium citrate,
514 pH 5.6; 200 mM ammonium sulphate. Diffraction data were collected at Diamond Light
515 Source. Data reduction and processing were performed using XDS.
516
517 $TipA_{\Delta Hairpin}$ crystallisation screens were prepared by mixing a 0.4 µL of 10 mg/mL TipA and
518 0.4 µL precipitant solution. TipA crystallised in space group P 21 21 21 (a =58.0, b=97.8,
519 c=132.0, α=90, β=90, γ=90) in a precipitant solution from the BCS screen consisting of 0.1 M
520 MES pH 6.5, 2% v/v PEG 400, 2% v/v PEG 500 MME, 2% v/v PEG 600, 2% w/v PEG 1000,
521 2% w/v PEG 2000, 2% w/v PEG 3350, 2% w/v PEG 4000, 2% w/v PEG 5000 MME, 2% w/v
522 PEG 6000, 2% w/v PEG 8000, 2% w/v PEG 10000. Diffraction data were collected at Diamond
523 Light Source. Data reduction and processing were performed through the Xia-2 Dials pipline,
524 and Aimless.
525
526 TipB crystallisation screens were prepared by mixing a 0.4 µL of 10 mg/mL TipB and 0.4 µL
527 precipitant solution. TipB crystallised in space group C 2 2 21 (a =106.6, b=126.2, c=78.0,
528 α=90, β=90, γ=90) in a precipitant solution from the Morpheus screen consisting of 0.03 M
529 Diethylene glycol; 0.03 M Triethylene glycol; 0.03 M Tetraethylene glycol; 0.03 M
530 Pentaethylene glycol, 0.1 M Bicine, 0.1 M Tris, pH 8.5, 20% v/v PEG 500 MME, 10 % PEG
531 20000. Diffraction data were collected at Diamond Light Source. Data reduction and
532 processing were performed through the Autoproc suite, and Aimless.
533
534 TipAB crystallisation screens were prepared by mixing a 0.5 µL of 8 mg/mL TipA and 9.5
535 mg/mL TipB (approx. 5X molar ratio) and 0.5 µL precipitant solution. The TipAB complex
536 crystallised in space group C 2 2 21 (a =89.3, b=230.7, c=131.6, α=90, β=90, γ=90) in a
537 precipitant solution from the Proplex screen consisting of 0.1 M Sodium acetate pH 5, 0 2 %
538 w/v PEG 4000 15 % v/v MPD. Diffraction data were collected at European Synchrotron
539 Radiation Facility. Data reduction and processing were performed through the Xia2-Dials
540 pipeline.
541

542 **Structure determination and model building**

543

544 Diffraction data processing and refinement statistics are given in Table EV1.

545

546 The crystal structure of Selenomethionine-labelled TipA was solved using Phenix, with manual
547 interpretation and improvement of non-crystallographic symmetry operators.

548

549 The crystal structure of TipB was solved by molecular replacement with a Colabfold model
550 [48] in Phenix MR [49] Likewise, $TipA_{\Delta hairpin}$ was solved by molecular replacement with the
551 C-terminal domain of the selenomethionine-labelled TipA and manual boot-strap building and
552 refinement of the N-terminal domain. The complex TipAB structure was solved using
553 molecular replacement of the TipB structure with the interaction loops removed, and the C-
554 terminal domain of the selenomethionine-labelled TipA structure. The N-terminal domain was
555 also manually built through boot-strap building and refinement. All structures were refined
556 through iterative cycles of real-space building in Coot [50] and refinement in Phenix Refine
557 [51].

558

559 **Pellet binding assays**

560

561 Untagged TipA was expressed in *Escherichia coli* BL21 RIPL (DE3). Cells were grown in 10
562 mL LB supplemented with kanamycin to an OD_{600} of 0.5-0.7 and induced with 0.5 mM IPTG
563 at 18 °C for 16 h. 5 X 1 mL of cells were centrifuged at 13000 RPM for 15 seconds and the
564 supernatant was removed. The cells were resuspended in 500 µL of either 0.15 M NaCl, 0.05
565 M HEPES pH 7.5; 0.5 M NaCl, 0.05 M HEPES pH 7.5; 1 M NaCl, 0.05 M HEPES pH 7.5;
566 0.15 M NaCl, 0.05 M HEPES pH 7.5, 0.05% Triton X100; 0.15 M NaCl, 0.05 M HEPES pH
567 7.5, 0.05% Tween 20. sonicated for 15 seconds and centrifuged at 13000 RPM for 15 minutes.
568 The pellet obtained from the 0.15 M NaCl buffer was resuspended in 500 µL SDS-PAGE
569 loading buffer for the pellet control. The pellet control and supernatant of each buffer was run
570 on SDS-PAGE.

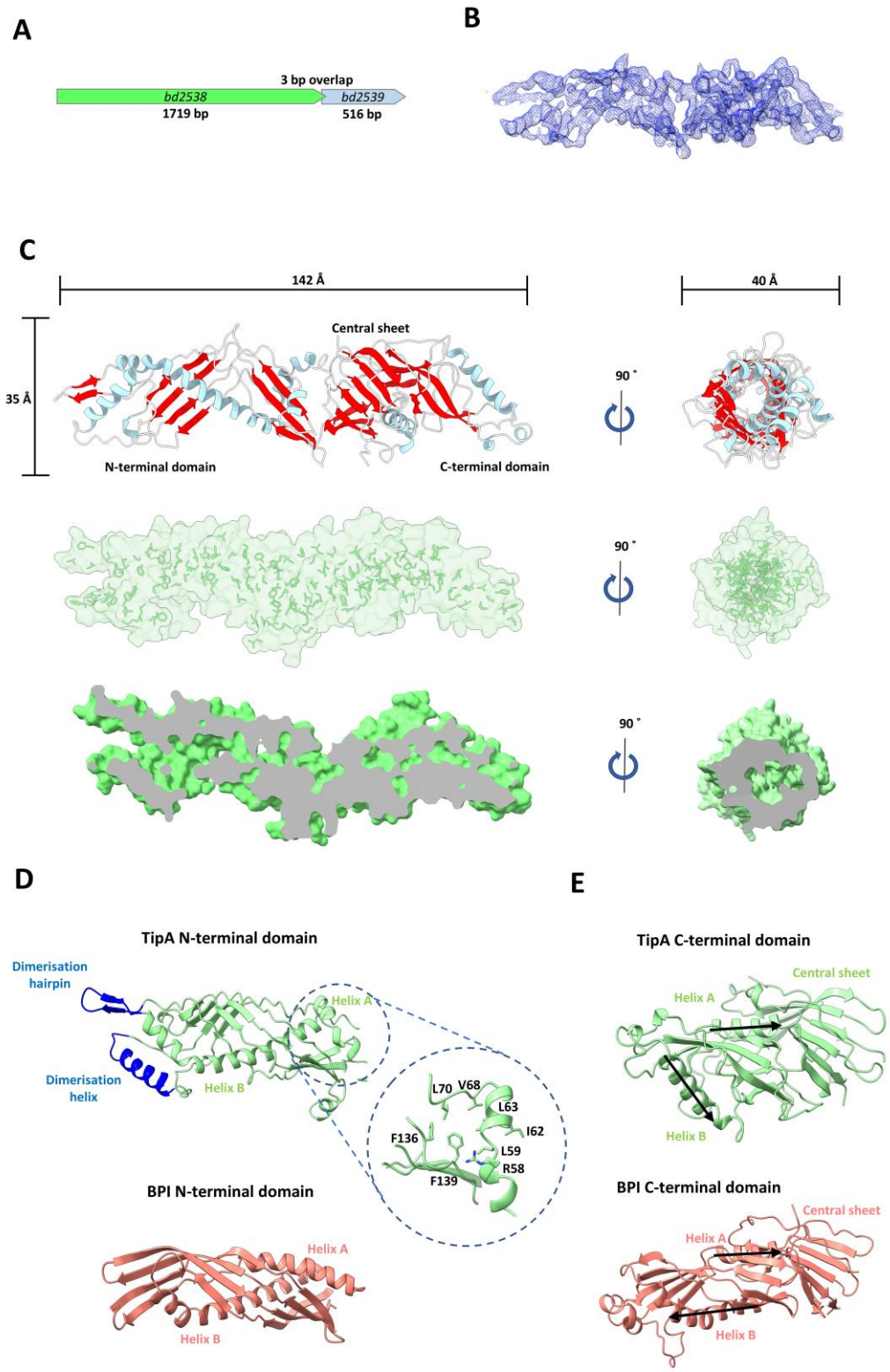
571

572 **QCM-D**

573

574 QCM-D was performed using a Q-sense Analyzer QCM-D System (Biolin Scientific,
575 Gothenburg, Sweden). Silicon QCM-D sensors were fixed into the QCM-D flow cell at a
576 constant temperature of 20 °C and attached to a peristaltic pump. ddH_2O was pumped through
577 the flow cell at a constant rate of 0.1 ml/min, a rate which was subsequently maintained
578 throughout the rest of the experiment. Frequency and dissipation changes (Δf and ΔD) were
579 monitored using multiple harmonics (n = 3, 5, 7, 9, 11, 13) of the resonant frequency. For
580 simplicity we report here only the changes to the 3rd harmonic. Prior to data acquisition flow
581 cells were equilibrated in H_2O for 5 mins to allow for a stable baseline to be acquired. H2O
582 was then exchanged for buffer, which was pumped through the flow cell until the change in
583 frequency of the 3rd harmonic was stabilised. Bilayers consisting of either POPC, *E. coli* polar
584 lipids or POPC:LPS (LPS from *E. coli* O111:B4) were then deposited onto the silicon sensor.
585 This initially involves the formation of small unilamellar vesicles (SUVs), which were prepared
586 by resuspension of a desiccated POPC/E. coli Polar Lipids/POPC:LPS film in buffer in 150
587 mM NaCl, 20 mM HEPES pH 7.5 to a concentration of 0.2 mg/mL and subsequent sonication
588 for 30 min until an optically transparent solution was obtained. POPC:LPS SUVs were
589 prepared at a 10:1 (w/w) ratio. These SUVs were subsequently flowed over the silicon sensor
590 and then ruptured through osmotic shock with H2O, resulting in the characteristic formation
591 of a deposited lipid bilayer. Exchange back into buffer 150 mM NaCl, 20 mM HEPES pH 7.5
592 aids the formation of a stable bilayer, with Δf3 ranging between -35 to -45 Hz. Once bilayers
593 were deposited and baselines stabilized, TipA was introduced into the flow cell at a
594 concentration of 1 mg/ml and flowed over the deposited bilayers for ~45 mins. Excess TipA
595 was then removed by washing sensors in 150 mM NaCl, 20 mM HEPES pH 7.5. QCM-D with
596 TipB was performed as above for TipA with POPC bilayers.
597
598 An *E. coli* Polar Lipid:LPS (10:1 w/w) mixed bilayer was also attempted to be formed and
599 deposited onto silicon surfaces as outlined above. These bilayers were unable to be successfully
600 generated, with SUVs unable to rupture and subsequently washed from the sensor. These
601 surfaces were treated as 'unformed bilayers' and used as control surfaces to monitor TipA
602 interaction with empty silicon sensors.
603
604 **ITC**
605

606 To test whether TipB binds to calcium, 5 mM EDTA and 5 mM EGTA was added to the protein
607 following size exclusion. After 16 hours the protein was passed through a Superdex 75 pg
608 26/60 column (GE Healthcare), which was pre-equilibrated with 20 mM HEPES, 150 mM
609 dialysed against 1 L of 20 mM HEPES, 150 mM NaCl, pH 7.5. $CaCl_2$ was dissolved in the
610 dialysis buffer to a final concentration of 500 µM. ITC was performed by titrating 500 µM
611 $CaCl_2$ into 50 µM TipB in 13 injections (1 x 0.4, 12 x 3 µL injections).
612
613 To test whether TipB binds to TipA-His, purified protein was dialysed against 1 L of 20 mM
614 HEPES, 150 mM NaCl, pH 7.5. ITC was performed by titrating 200 µM TipB into 20 µM TipA
615 in 13 injections (1 x 0.4, 12 x 3 µL injections).
616
617
618 **TLC**
619 TLC was employed to assess the lipid binding ability of TipA. Lipids were extracted from
620 protein samples through chloroform/methanol extraction. Briefly, 2 ml of Bd2538 at 0.5 mg/ml
621 was added to 2 ml methanol and 1 ml chloroform (2:2:1, v/v/v). Samples were vortexed for 5
622 min, followed by a 30 min incubation at 50 °C and then vortexed again for 5 min.
623 Centrifugation at 2,000 x g for 10 min resulted in the formation of two phases, the lower
624 chloroform layer containing lipidic material. This lower phase was extracted and then
625 evaporated. The dried lipids were resuspended in 100 µl chloroform with 5 µl then loaded onto
626 a Silica TLC plate (Sigma) and run in a 6.5:2.5:1 (chloroform:methanol:acetic acid) solvent
627 system. The TLC plate was dried, stained with 10% (w/v) phosphomolybdic acid in ethanol,
628 and finally heated until stained spots could be visualised.
629
630
631



632

633 **Figure 1. The structure of *Bdellovibrio* TULIP protein TipA**

634 **(A)** Schematic of the forward strand ORFs for the gene operon of *bd2538* (green) and *bd2539* (blue). **(B)** 2Fo-Fc map at 2.5 σ surrounding
635 chain C of the crystal structure of TipA after the final refinement. **(C)** Top; Crystal structure of TipA, showing the double-TULIP fold of the
636 Bactericidal permeability-increasing protein, alpha/beta domain superfamily, coloured by secondary structure (beta – red, helix – light blue,
637 coil – white). The lengths of the protein are shown for all three dimensions. Middle; a surface view of TipA with hydrophobic residues shown

638 in stick form, highlighting the hydrophobic nature of the central cavity. Bottom; A clipped surface view showing the inner cavity. (**D)** Top;
639 structural features of the N-terminal TipA TULIP domain, with the dimerisation motif coloured blue. The expanded view shows residues in
640 stick form that cause the N-terminal Helix A to protrude. Bottom; The structure of the N-terminal TULIP domain of bacterial permeability
641 increasing protein (BPI, code 1BP1) for comparison. **(E)** Top; structural features of the C-terminal TipA TULIP domain, with the helix
642 positions highlighted with arrows. The expanded view shows residues in stick form that cause the N-terminal Helix A to protrude. Bottom;
643 The structure of the C-terminal TULIP domain of BPI for comparison.
644
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650

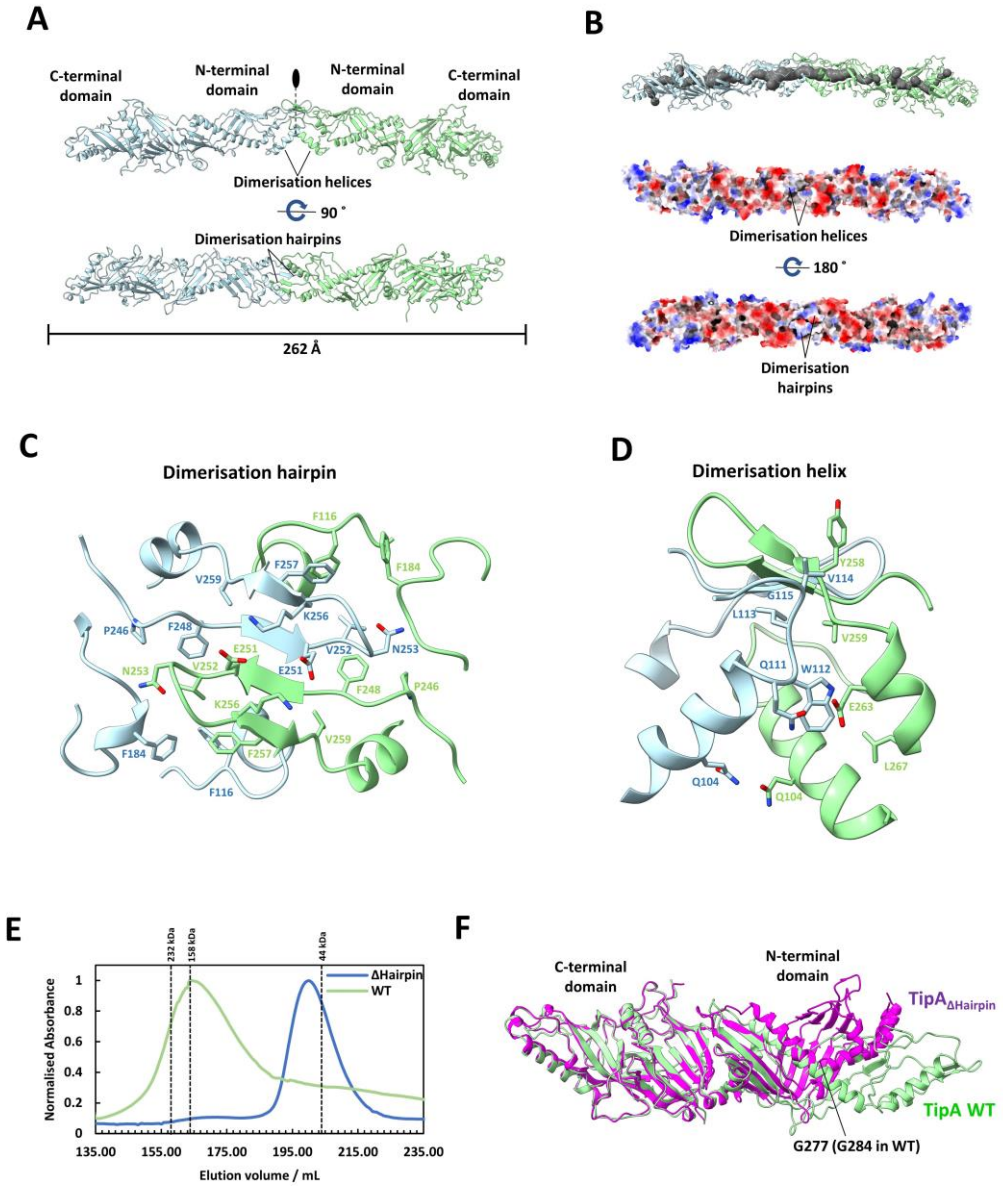


Figure 2. Dimerisation of TipA into a four TULIP-long assembly

(A) Crystal structure of TipA, showing two chains forming an end-on-end homodimer via interaction of their N-terminal TULIP domains. One protomer is shown in light blue and the other is in green with the 2-fold symmetry axis shown as a black oval and dotted line. **(B)** Upper; the dimer forms an elongated cavity within (grey surface, calculated by Mole [52]). Lower; the electrostatic surface view shows that the dimer forms a continuous tube with very few solvent accessible gaps. **(C)** The view down the two-fold axis shows how the two interdigitated dimerisation hairpins (light blue and green) create a polar surface on the outside of the TULIP dimer and a hydrophobic inner face for a continuous hydrophobic channel. **(D)** The dimerisation helix forms polar and hydrophobic interactions with the other protomer's Helix B and dimerisation helix. **(E)** Mutation of the dimerisation hairpin causes a shift in the elution volume of TipA on gel filtration from 165 mL to 200 mL. **(F)** The crystal structure of $TipA_{\Delta Hairpin}$ (magenta) superimposed onto the TipA wild type (WT) structure (green). The C-terminal domain overlays well, but the N-terminal domain is contracted due to twisting of the beta-sheet and bending of the N-terminal Helix B at G277.

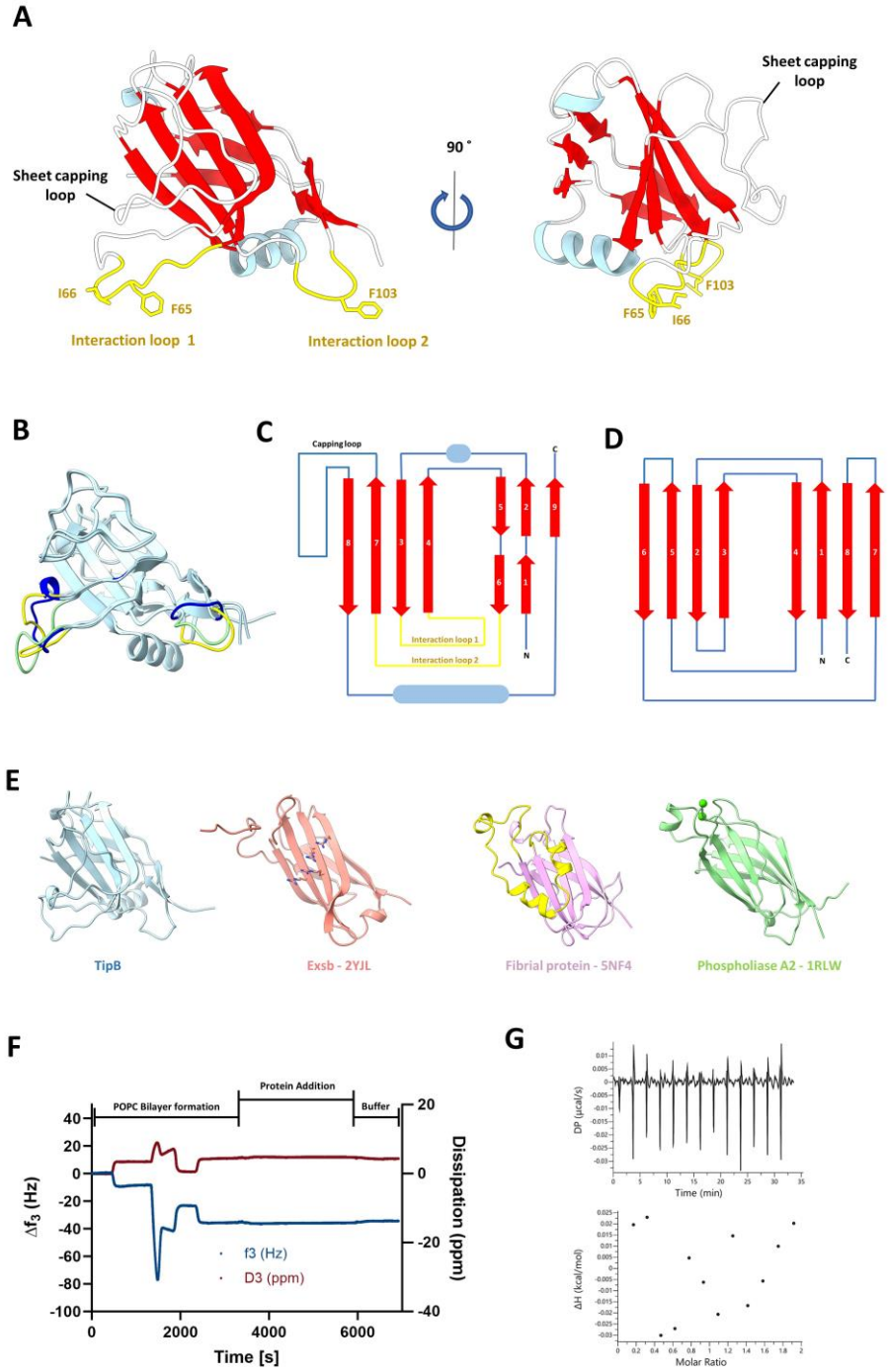


Figure 3. Crystal structure of the TipB adapter protein

(A) Crystal structure of TipB, coloured by secondary structure (beta – red, helix – light blue, coil – white) with the two specialised interaction loops coloured yellow. Key hydrophobic amino acids in the interaction loops are shown in stick form. **(B)** The three chains of TipB in the ASU are superposed to highlight flexibility in the interaction loops (shown blue, green and yellow for each chain). **(C)** The topology of TipB, with strands in red, helices as blue ovals and loops as blue lines. **(D)** The topology of type II C2 domains. **(E)** Crystal structures of proteins identified in structural homology searches with DALI [24], shown in same orientation as TipB (left, light blue). Arginines that form the basic face of the sheet in *Pseudomonas aeruginosa* ExsB [26] (coloured salmon) are shown in stick form; the sheet capping loop of *Porphyromonas gingivalis* [53] (coloured pink) is shown in yellow; and the two calcium ions of human phospholipase A2 [35] (coloured green) are shown as

spheres. **(F)** QCM-D trace showing f3 and d3 signals. Upon protein addition, no significant change in f3 or d3 is observed. **(G)** ITC thermogram of a titration of calcium chloride into TipB showing no binding.

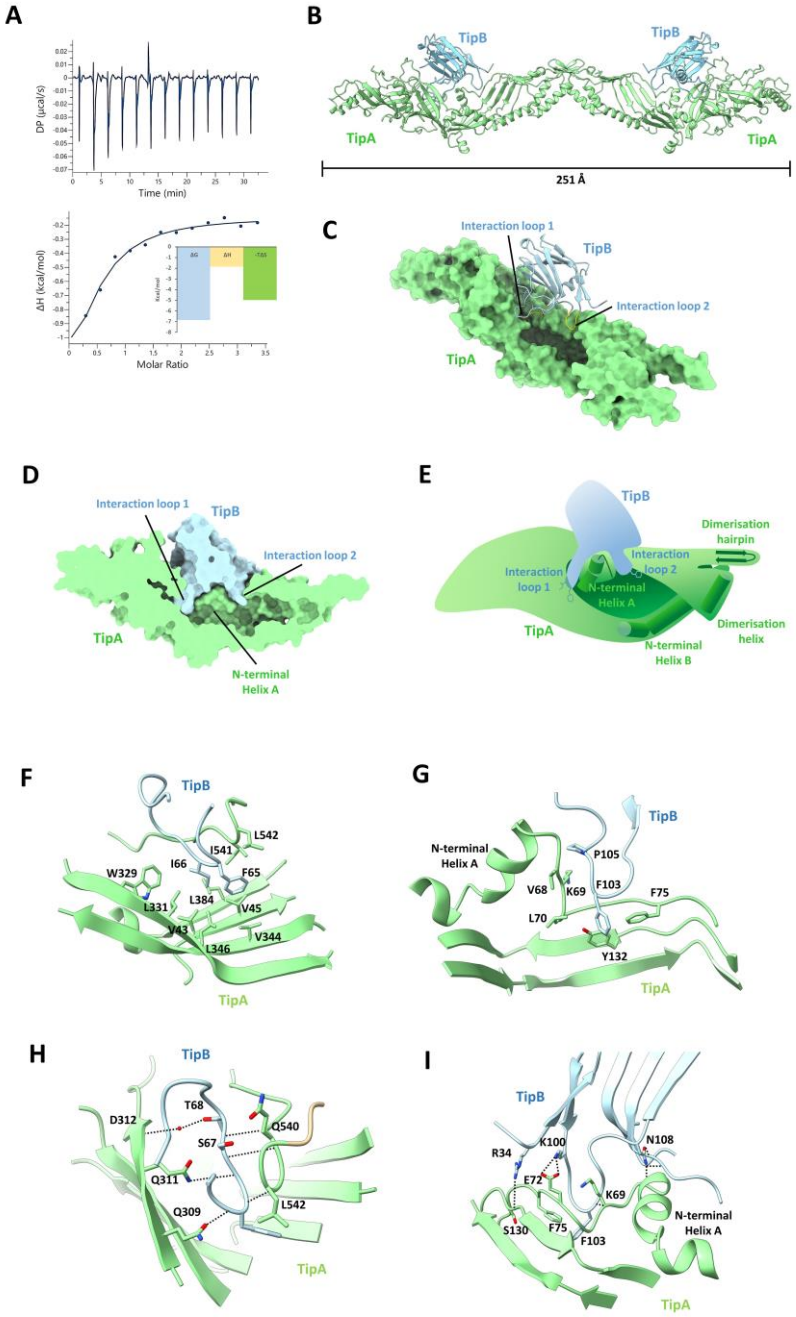


Figure 4. The interaction of the TipA and TipB TULIP:adapter pairing

(A) ITC thermogram of a titration of TipB into TipA, showing binding of the two proteins in solution. The reported energies for binding are shown as an embedded bar graph. **(B)** Crystal structure of the TipAB complex. Tip A is shown in green and forms the same 2-fold dimer as the isolated form through a crystallographic axis. TipB is shown in blue on top of TipA. **(C)** A surface view of TipA is shown, highlighting burial of the two interaction loops of TipB (coloured yellow). **(D)** A clipped surface view of the TipAB complex, showing TipA sat on the TipA N-terminal helix A. **(E)** A cartoon schematic of the interaction with parts of the TipA N-terminal TULIP domain secondary structure visible, and key hydrophobic residues of TipB shown as sticks. **(F)** Hydrophobic interactions of the TipB interaction loop 1. **(G)** Hydrophobic

interactions of the TipB interaction loop 2. **(H)** polar interactions of the TipB interaction loop 1. **(I)** Polar interactions of the TipB interaction loop 2.

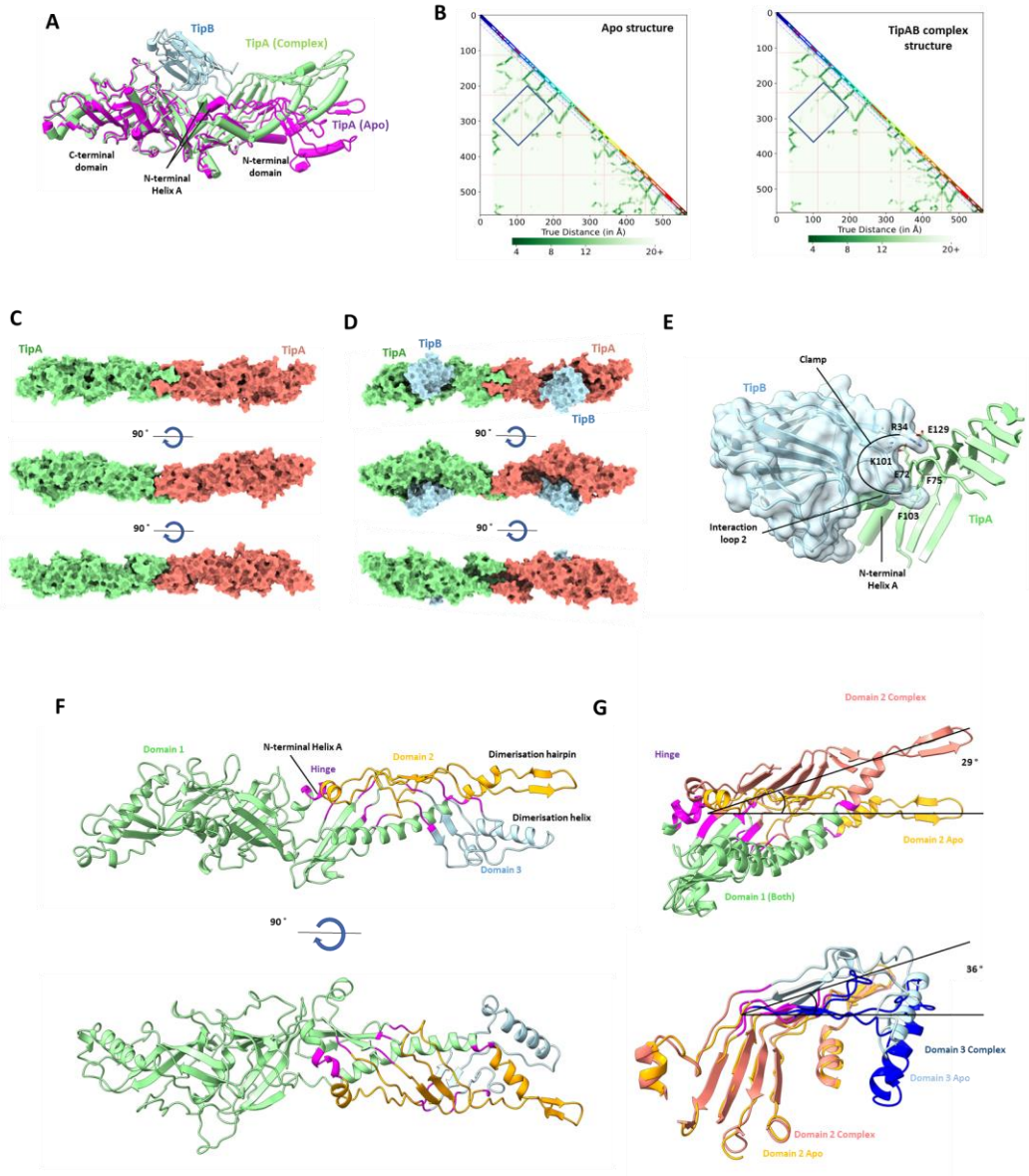


Figure 5. Conformational change of TipA when bound to the TipB adapter

(A) A superimposition of apo TipA (magenta) with the TipAB complex. Large motions in the N-terminal domain of TipA are visible. **(B)** contact plots of TipA for the apo TipA and TipAB structures, generated by DISTEVAL [54]. Both plots have a box surrounding residues that differ significantly between the two structures, which are only found in the N-terminal domain. **(C)** Three orientations of the apo TipA homodimer (one protomer green, one protomer red), showing a mostly-closed tube. **(D)** The same three orientations of the TipA homodimer as **(C)** for the TipAB complex. TipB is shown in blue, and the large opening of the TULIP homodimer stretching between the two TipB proteins is visible. **(E)** The clamp formed by interaction loop 2 and charged residues grasp the bottom and top of the TipA N-terminal beta sheet, respectively. **(F)** Analysis of the apo TipA and complexed TipA structure with DynDom to investigate the motions of the conformational change identifies three domains (coloured green, orange and blue), with motions in the hinge regions (magenta). **(G)** Upper; superpositions show the 29° rotation between Domain 1 and Domain 2 rotate by 29. Lower; superpositions show the 36° movement between domain 2 and domain 3.

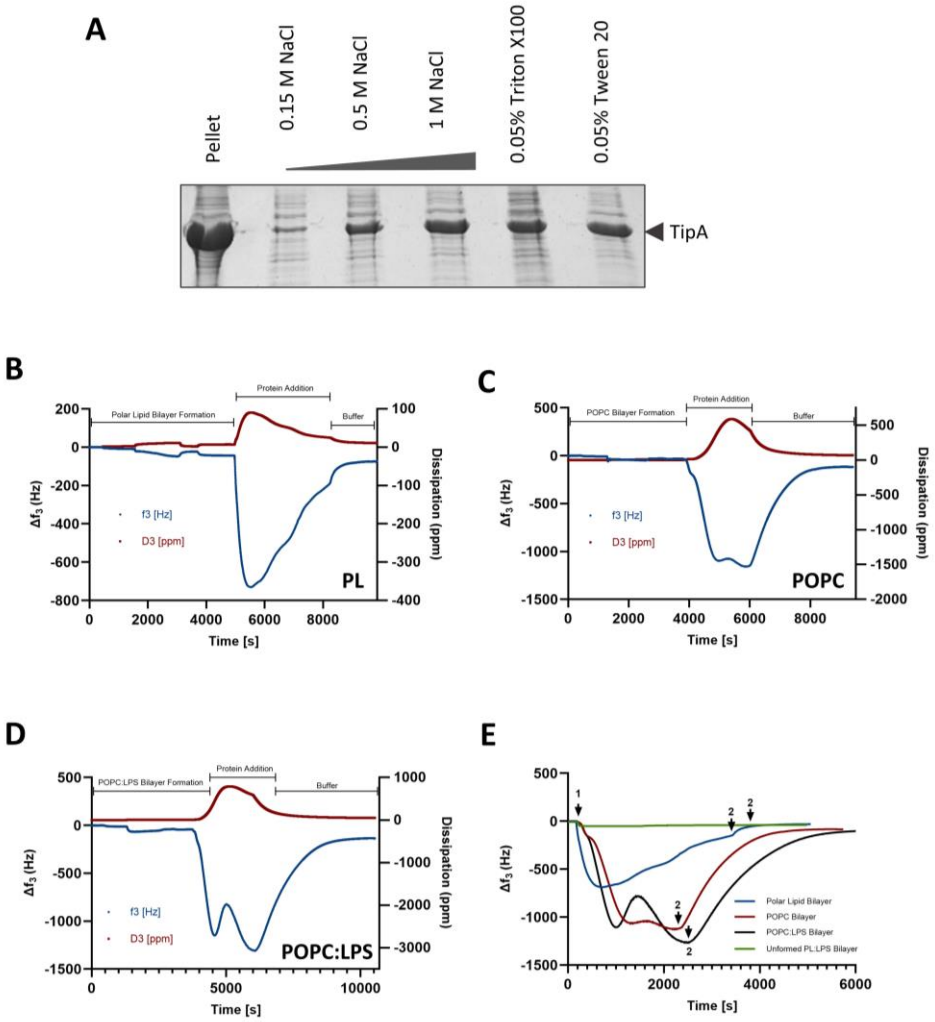


Figure 6. TipA can insert into a range of lipid bilayers

(A) Pellet-binding assays with TipA. SDS-PAGE of pellet and supernatants from various buffer washes. A prominent band for soluble TipA is observed in high salt concentrations or with the presence of detergents. **(B)** QCM-D measurements showing the changes in frequency (blue) and dissipation (red) in response to the mass deposition associated with *E. coli* polar lipid bilayer formation (polar lipid liposomes were deposited onto a silicon surface and ruptured through osmotic shock), followed by the addition of TipA, and subsequent bilayer washing with excess buffer. **(C)** QCM-D measurements as specified in **(A)** showing POPC bilayer formation, TipA addition, and buffer wash. **(D)** QCM-D measurements as specified in **(A)** indicating the formation of a stable POPC:LPS bilayer, TipA addition and subsequent buffer wash. **(E)** A comparison of the changes to frequency (indicative of mass changes), as measured through QCM-D, in response to the addition of TipA to various lipid bilayer compositions (1) and subsequent buffer wash (2). Note we were unable to successfully form a mixed polar lipid, LPS bilayer.

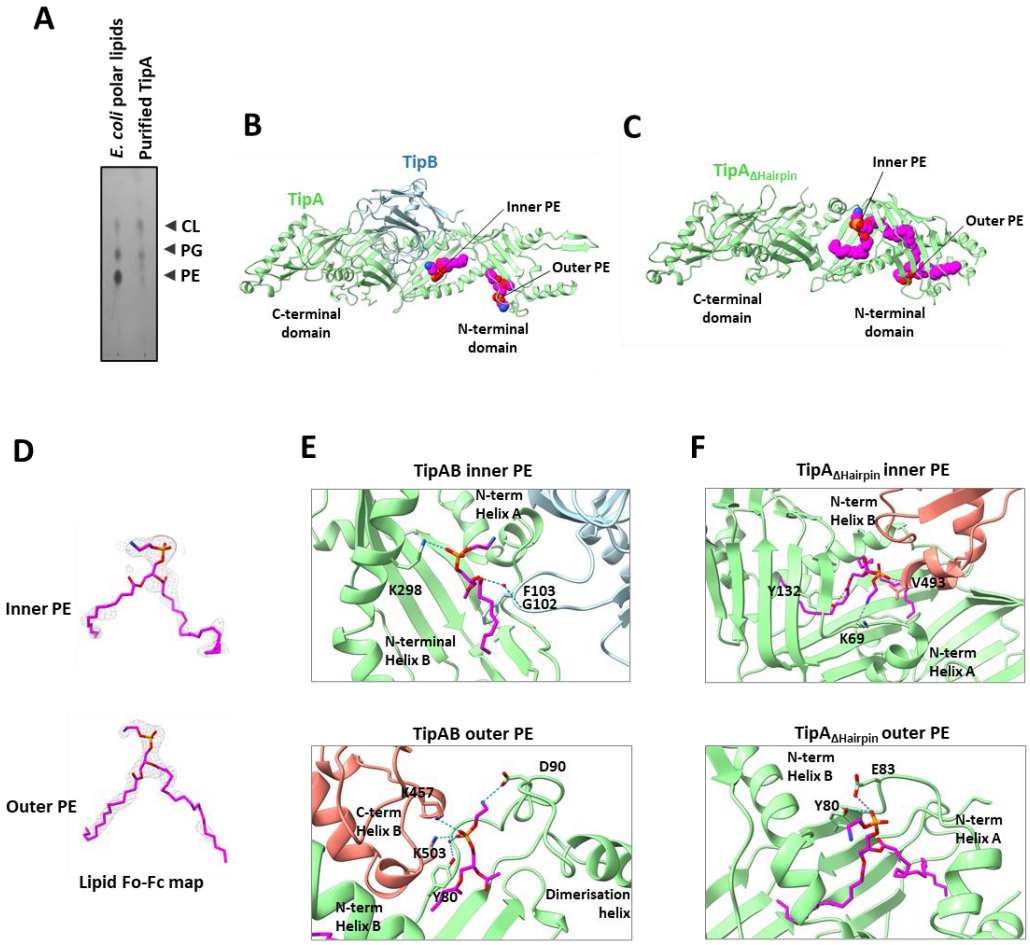


Figure 7. TipA sequesters lipids in the TULIP lumen

(A) TLC of lipids extracted from TipA. All three *E. coli* polar lipids are visible in the TipA lane (cardiolipin (CL), phosphatidylglycerol (PG), phosphatidylethanolamine (PE)), as are some additional species. **(B)** Crystal structure of the TipAB complex, with two lipid head groups shown as spheres (magenta carbons). **(C)** Crystal structure of TipA$_{\Delta Hairpin}$, with two modelled PE molecules shown as spheres (magenta carbons). **(D) 2**Fo-Fc electron density of the two lipids in the TipA$_{\Delta Hairpin}$ structure at 0.8 σ. **(E)** The molecular interactions of TipAB complex and the lipid head groups for the inner PE molecule (top) and outer PE molecule (bottom). TipA is coloured green, TipB is blue, with a symmetry TipA copy coloured salmon. Lipids are shown in stick form and coloured as heteroatoms (carbon – magenta, oxygen – red, nitrogen – blue, phosphorus – orange) **(F)** The molecular interactions of TipA$_{\Delta Hairpin}$ and the lipid head groups for the inner PE molecule (top) and outer PE molecule (bottom). TipA is coloured green with a symmetry TipA copy coloured salmon. Lipids are shown in stick form and coloured as heteroatoms (carbon – magenta, oxygen – red, nitrogen – blue, phosphorus – orange)

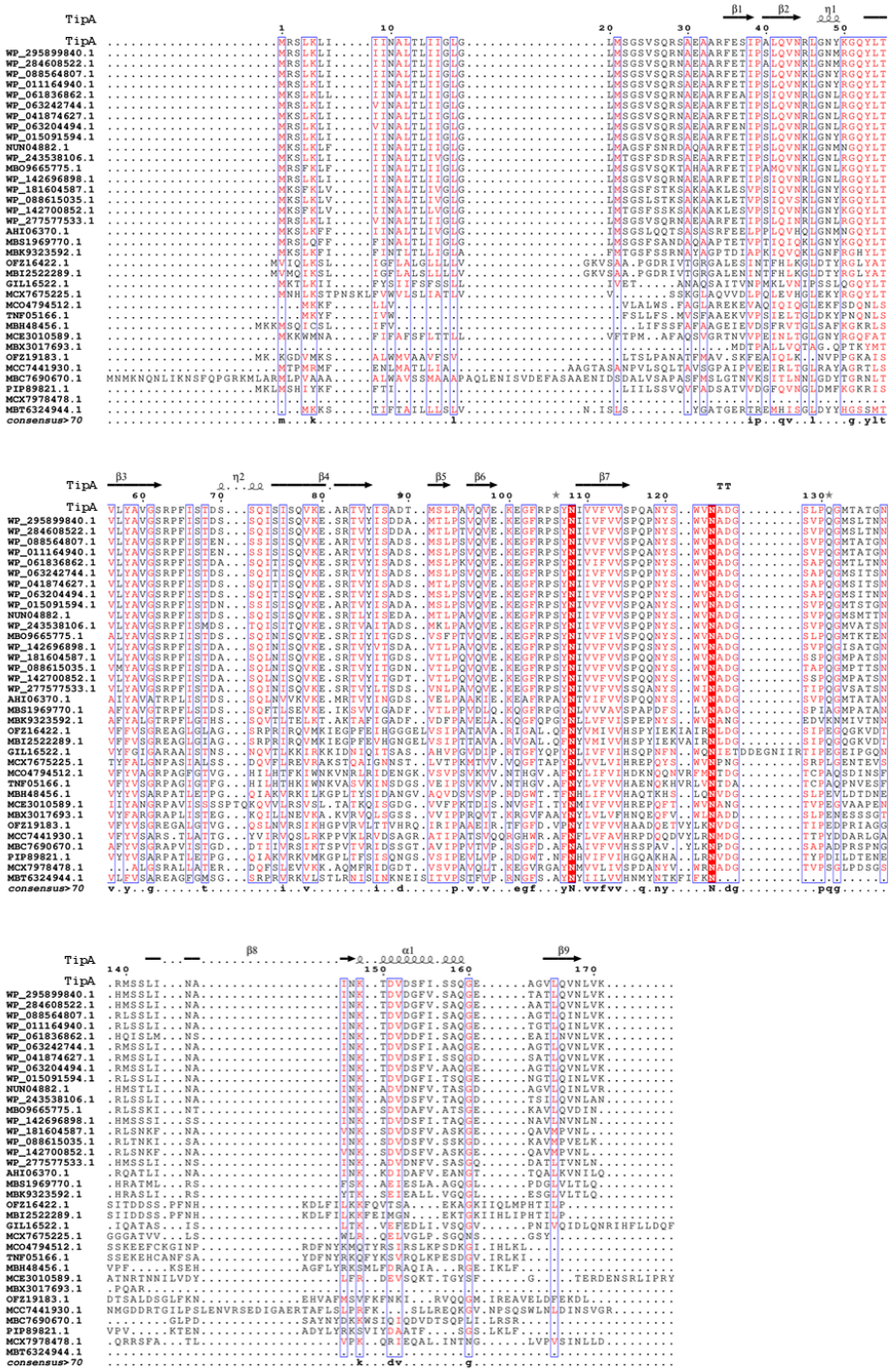


Figure EV1. Sequence alignments of TipA homologues

BlastP [55] was used to identify homologues to the *Bdellovibrio bacteriovorus* HD100 TipA protein. The only absolutely conserved residues are two Asn residiues. Alignments of the sequences obtained from blastp were made using Clustal Omega [56]. ESPRIPT [57] was used to visualise the alignement with secondary structure.

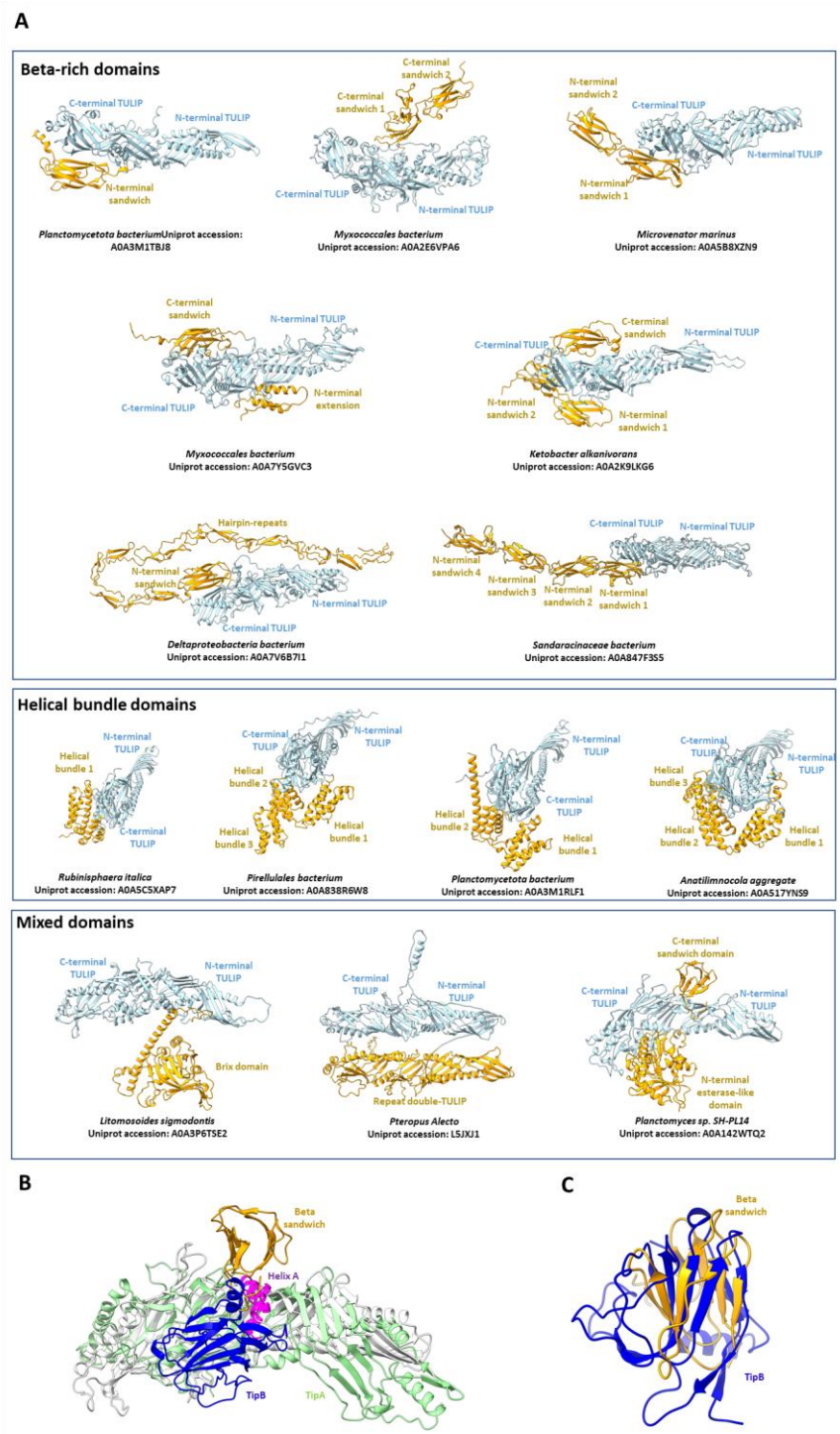
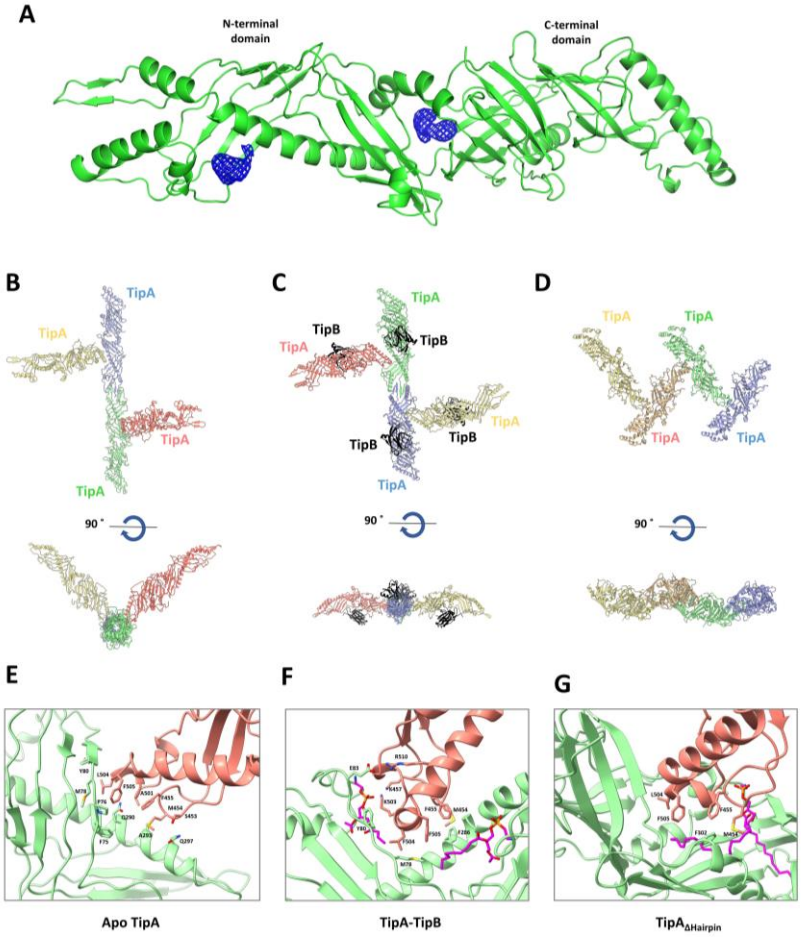


Figure EV2. Additional domains fused to TipA structural homologues

(A) Using Foldseek [38], structural TipA homologues were identified within the AlphaFold [58] AFDB50 database. A number of those homologues had additional domains to the double-TULIP. Representative examples of fusions are shown and comprise beta-rich domains (upper) helical domains (middle) and other domains including second double-TULIPs and enzymes (lower). The double-TULIP domains are coloured light blue, and the accessory domains are coloured orange. Of particular interest is the *Planctomyces sp*. SH-PL14 protein (bottom right) in which there is a similar beta-sandwich domain to TipB fused C-terminally to the TULIP **(B)** Superposition of the TipAB with *Planctomyces sp*. SH-PL14 (Uniprot accession: A0A142WTQ2). TipA is coloured green, TipB is blue, the TULIP domains of A0A142WTQ2 are white and the beta sandwich is orange. The enzymatic domain has been removed for clarity. The beta-sandwiches interact with the N-terminal Helix A in both structures and make interactions with the top of the N-terminal TULIP sheet. **(C)** Superposition of TipB (blue) with the beta sandwich of A0A142WTQ2 (orange), showing similar topology.



759 **Figure EV3. Lipid binding and higher order packing of TipA(A)** Fo-Fc electron density (blue mesh)
760 observed in the apo selenomethionine TipA structure. Large peaks are observed in similar regions to lipid density in the TipAB complex and
761 TipA$_{\Delta Hairpin}$. **(B)** Crystal packing in the apo selenomethionine structure. **(C)** Crystal packing in the TipAB complex structure. **(D)** Crystal
762 packing in the TipA$_{\Delta Hairpin}$ structure. In all structures shown **(B-D)** the C-terminal domain of TipA buries into the N-terminal domain of another
763 TipA protein. **(E)** Molecular interactions of the C-terminal domain and N-terminal of the apo selenomethionine TipA structure. **(F)** Molecular
764 interactions of the C-terminal domain and N-terminal of the TipAB structure. **(G)** Molecular interactions of the C-terminal domain and N-
765 terminal of the TipA$_{\Delta Hairpin}$ structure.

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769 **Table EV1. Data collection and refinement statistics**

	Apo SeMet TipA	TipB	TipAB complex	TipA$_{\Delta Hairpin}$
Wavelength		0.979 Å	0.968 Å	0.968 Å
Resolution range	48.74 - 4.046 (4.191 - 4.046)	29.24 - 2.17 (2.23 - 2.17)	57.66 - 2.6 (2.693 - 2.6)	66.01 - 2.6 (2.72 - 2.6)
Space group	P 31 2 1	C 2 2 21	C 2 2 21	P 21 21 21
Unit cell	252.415 252.415 181.058 90 90 120	106.578 126.162 77.975 90 90 90	89.2741 230.652 131.606 90 90 90	57.9688 97.8339 132.021 90 90 90
Total reflections		376137	596458	307502
Unique reflections	45222 (538)	28055 (2231)	42141 (4178)	23555 (2618)
Multiplicity		13.4 (11.7)	14.1 (13.7)	13.1 (9.0)
Completeness (%)	82.85 (9.99)	99.30 (91.9)	98.36 (85.22)	98.8 (91.5)
Mean I/sigma(I)		16.8 (3.3)	11.6 (0.3)	18.6 (2.7)
Wilson B-factor	188.01	35.30	86.67	60.45
R-merge		0.102 (0.733)	0.117 (3.31)	0.083 (0.681)
R-meas		0.110 (0.798)	0.125 (3.55)	0.089 (0.770)
R-pim		0.041 (0.312)	0.034 (0.946)	0.033 (0.350)
CC1/2		0.999 (0.896)	0.999 (0.333)	0.999 (0.832)
Reflections used in refinement	45184 (538)	28025 (2640)	41504 (3563)	23500 (2098)
Reflections used for R-free	2364 (23)	1360 (115)	2105 (196)	1132 (117)
R-work	0.2766 (0.4538)	0.1774 (0.2220)	0.2463 (0.3946)	0.2283 (0.2910)
R-free	0.3200 (0.5542)	0.2160 (0.2798)	0.2874 (0.4209)	0.2526 (0.3627)
Number of non-hydrogen atoms	12575	3394	5382	4178
macromolecules	12575	3196	5267	3990
ligands	0	0	55	135
solvent	0	198	60	53
Protein residues	1600	415	674	507
RMS(bonds)	0.005	0.009	0.005	0.007
RMS(angles)	1.33	1.01	0.75	1.04
Ramachandran favored (%)	79.99	96.58	94.48	93.01
Ramachandran allowed (%)	18.51	2.93	5.07	6.39
Ramachandran outliers (%)	1.51	0.49	0.45	0.60
Rotamer outliers (%)	0.58	0.55	2.25	3.22
Clashscore	30.35	5.33	12.23	13.31
Average B-factor	239.19	45.22	110.85	83.37
macromolecules	239.19	45.05	111.03	83.25
ligands			115.13	96.48
solvent		47.96	91.11	59.35
Number of TLS groups	1	1	16	1

770 Statistics for the highest-resolution shell are shown in parentheses.

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