bioRxiv preprint doi: https://doi.org/10.1101/2022.06.13.496000; this version posted June 13, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

1 2 2	Dynamic structural adaptations enable the endobiotic predation of bdellovibrio bacteriovorus
3 4	Mohammed Kaplan ^{1,#} , Yi-Wei Chang ^{1,2,#} , Catherine M. Oikonomou ¹ , William J. Nicolas ¹ ,
5	Andrew I. Jewett ³ , Stefan Kreida ^{1,4} , Przemysław Dutka ^{1,5} , Lee A. Rettberg ¹ , Stefano Maggi ¹ and
6	Grant J. Jensen ^{1,6,*}
7	¹ Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA 91125, USA
8	² Current address: Department of Biochemistry and Biophysics, Perelman School of Medicine, University of
9	Pennsylvania, Philadelphia, PA 19104, USA
10	³ Department of Integrative Structural and Computational Biology, The Scripps Research Institute, La Jolla, CA,
11	USA
12	⁴ Department of Microbiology, Tumor and Cell Biology, Karolinska Institute, 17177 Stockholm, Sweden
13	⁵ Division od Chemistry and Chemical Engineering, California Institute of Technology, 1200 California Boulevard,
14	Pasadena, CA 91125, USA
15	⁶ Department of Chemistry and Biochemistry, Brigham Young University, Provo, UT 84604, USA
16	*Corresponding author: <u>grant_jensen@byu.edu</u>
17	[#] equal contribution
18	
19	
20	
21	
22	
23	
24	
25	

bioRxiv preprint doi: https://doi.org/10.1101/2022.06.13.496000; this version posted June 13, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

26 Graphical abstract



32 Abstract

33 Bdellovibrio bacteriovorus is an endobiotic microbial predator that offers promise as a living 34 antibiotic for its ability to kill Gram-negative bacteria, including human pathogens. Even after six 35 decades of study, fundamental details of its predation cycle remain mysterious. Here, we used 36 cryo-electron tomography to comprehensively image the lifecycle of *B. bacteriovorus* at 37 nanometer-scale resolution. In addition to providing the first high-resolution images of predation 38 in a native (hydrated, unstained) state, we also discover several surprising features of the process, 39 including novel macromolecular complexes involved in prev attachment/invasion and a flexible 40 portal structure lining a hole in the prey peptidoglycan that tightly seals the prey outer membrane around the predator during entry. Unexpectedly, we find that B. bacteriovorus does not shed its 41 42 flagellum during invasion, but rather resorbs it into its periplasm for degradation. Finally, 43 following replication and division in the bdelloplast, we observe a transient and extensive 44 ribosomal lattice on the condensed B. bacteriovorus nucleoid.

45 Introduction

46 Predation is a widespread behavior, from the largest eukaryotes to the smallest viruses, that drives 47 evolution and energy flow in biological communities. In bacteria, predatory behavior is common 48 and multiple predation types have been described, including epibiotic strategies in which the 49 predator remains outside the prey and endobiotic strategies in which the predator invades the 50 prey's cytoplasm or periplasm (Pérez et al., 2016). Since its description in 1963 as the first known 51 bacterial parasite of bacteria (Stolp and Starr, 1963), Bdellovibrio bacteriovorus has been a 52 paradigm of periplasmic endobiotic predation, in which a small predator takes up residence in a 53 larger diderm prey's periplasm (Laloux, 2020; Sockett, 2009). The ability of Bdellovibrio-and-54 like-organisms (BALOs) to invade other Gram-negative bacteria, including human pathogens, has 55 rendered them potential candidates for living antibiotics to tackle the crisis of antimicrobial 56 resistance that has emerged in the past few decades (Bratanis et al., 2020; Cavallo et al., 2021; 57 Harini et al., 2013; Iebba et al., 2014; Madhusoodanan, 2019; Negus et al., 2017; Pantanella et al., 58 2018; Raghunathan et al., 2019; Russo et al., 2018; Shatzkes et al., 2017a, 2017b), or even as a 59 possible probiotic agent (Bonfiglio et al., 2020). For example, it has been shown that treating 60 zebrafish infected with *Shigella flexneri* with *B. bacteriovorus* increases the animals' survival rate 61 (Willis et al., 2016). In addition, microbial predation has been suggested to have been involved in 62 pivotal evolutionary events including the genesis of eukaryotic cells, the rise of multicellularity, 63 and pathogenicity (Davidov and Jurkevitch, 2009; Erken et al., 2013; Lyons and Kolter, 2015).

64

65 The predatory lifecycle of *B. bacteriovorus* has been extensively studied by methods including 66 conventional transmission electron microscopy, light/fluorescence microscopy, helium-ion 67 microscopy, atomic force microscopy and biochemical assays (Burnham et al., 1968; Kuru et al.,

68 2017; Makowski et al., 2020; Núñez et al., 2003; Said et al., 2019; Stolp and Starr, 1965), and is 69 the subject of several excellent reviews (see for example: (Cavallo et al., 2021; Laloux, 2020; Negus et al., 2017; Rotem et al., 2014; Sockett, 2009)). In the free-living attack phase, the predator 70 71 is transcriptionally streamlined, with a highly compacted spiral nucleoid (Butan et al., 2011), and 72 a vibrioid cell shape caused by the asymmetric activity of a peptidoglycan hydrolase (Banks et al., 73 2022). At one pole of the cell, a sheathed unipolar flagellum enables high-velocity (up to 160 μ m/s 74 (Lambert et al., 2006; Rendulic, 2004)) motile collisions with prey. The flagellar filament has a 75 distinctive damped waveform due to segments made up of subunits with different helical properties 76 (Thomashow and Rittenberg, 1985a). Initial interaction with, and attachment to, prey is mediated 77 by type IVb (Avidan et al., 2017) and type IVa pili (T4P) (Evans et al., 2007; Mahmoud and Koval, 78 2010; Milner et al., 2014) at the pole opposite the flagellum (the "biting pole"). Following this 79 initial interaction, B. bacteriovorus uses flagellum-independent gliding motility to reach an 80 attachment site on the side of rod-shaped prey cells (Lambert et al., 2011).

81

82 Upon initial attachment, prey quality is assessed in a cyclic-di-GMP-dependent process (Caulton 83 and Lovering, 2020; Hobley et al., 2012; Meek et al., 2019), and if the prey is found to be suitable 84 and not already under attack by another predator (Lerner et al., 2012), the attachment is made 85 permanent and the process of invasion begins. Initially, it was suggested that a "drilling" 86 mechanism caused by flagellar rotation might play a role in the invasion process (Burnham et al., 87 1968; Stolp and Starr, 1965), but later studies found that flagellar motility-compromised mutants 88 can still invade prey, which fits with the roles of pili and gliding motility mentioned above 89 (Lambert et al., 2006). Another early penetration model hypothesized that entry is passive and 90 occurs due to osmotic forces from the flux of solutes and water resulting from structural changes
91 in the prey envelope (Abram et al., 1974).

92

93 Prey invasion involves enzymatic modification of the prey cell wall by B. bacteriovorus at the 94 predator-prey contact point (Kuru et al., 2017; Thomashow and Rittenberg, 1978c, 1978b, 1978a; 95 Tudor et al., 1990) and is associated with the secretion, by type I and II secretion systems, of many 96 enzymes (Pasternak et al., 2014; Rendulic, 2004), including glycanases (Harding et al., 2020; 97 Thomashow and Rittenberg, 1978a), peptidases (Lerner et al., 2012; Tudor et al., 1990) and 98 deacetylases (Lambert et al., 2016). A self-protection protein in B. bacteriovorus inhibits the 99 predator's peptidases, thereby allowing specific modification of the prey cell wall (Lambert et al., 100 2015). These modifications round the prey cell and greatly expand the periplasmic space, 101 providing room for the predator to enter. Entry itself is rapid, occurring in a few minutes or less 102 (Abram et al., 1974; Capeness et al., 2013). With a few exceptions noted (Iida et al., 2009; Lambert 103 et al., 2006), flagella are no longer visible on *B. bacteriovorus* when they enter their prey (Shilo, 104 1969; Thomashow, L. S., 1979).

105

Once *B. bacteriovorus* enters the prey's periplasm, the entry pore is sealed and transpeptidases modify the prey cell wall to render it more robust to osmotic pressure (Kuru et al., 2017; Lerner et al., 2012), forming what is known as the bdelloplast (Starr and Baigent, 1966). If prey-derived cues indicate the availability of sufficient nutrients in the bdelloplast (Rotem et al., 2015), *B. bacteriovorus* enters its growth phase. The predator consumes the cytoplasmic contents of the prey, facilitated by translocation of outer membrane (OM) pore proteins into the prey's cytoplasmic membrane (Tudor and Karp, 1994) and secretion of nucleases to digest the prey's nucleic acids

113 (Bukowska-Faniband et al., 2020). This fuels predator growth, through bidirectional elongation, 114 and replication of its genetic material (Makowski et al., 2019). Once available nutrients are 115 exhausted, the predator septates synchronously into multiple progeny cells by non-binary fission, 116 with the number of progeny cells depending on the size of the prey (Fenton et al., 2010a; Kaljević 117 et al., 2021). Finally, the progeny cells, reset to the attack phase, use an enzyme specific for 118 deacetylated peptidoglycan to lyse the prey cell wall (Harding et al., 2020; Lambert et al., 2016), 119 creating pores through which they exit the bdelloplast and move on in search of new prey (Fenton 120 et al., 2010a). If the first invasion does not yield sufficient nutrients for replication and division, a 121 B. bacteriovorus cell may leave the first bdelloplast and complete its lifecycle in a second prey 122 (Makowski et al., 2019).

123

124 Despite extensive study, the structural details of much of the invasion process remain unclear. 125 Most of the previous work relied on low-resolution imaging methods or conventional electron 126 microscopy preparations, in which dehydration and fixation disrupt cell membranes and obscure 127 macromolecular details. Cryogenic electron tomography (cryo-ET) allows the investigation of 128 cellular processes in a fully-hydrated frozen state with macromolecular resolution (Ghosal et al., 129 2019a; Kaplan et al., 2021a; Oikonomou and Jensen, 2017), but so far it has only been applied to 130 study the ultrastructure of individual *B. bacteriovorus* cells in the attack phase. While these studies 131 revealed important structural features of this stage (Borgnia et al., 2008; Butan et al., 2011; Fenton 132 et al., 2010b), much remains to be learned about the full invasion cycle of *B. bacteriovorus*.

133

Here, we used cryo-ET to image the predation cycle of *B. bacteriovorus* invading three types of
prey: *Vibrio cholerae, Escherichia coli*, and *E. coli* minicells. Our work reveals, for the first time,

- 136 the macromolecular details of each stage of the *B. bacteriovorus* lifecycle and uncovers several
- 137 unexpected features of the process, including absorption of the extracellular flagellum into the
- 138 predator's periplasm during attachment, a flexible portal structure associated with the prey
- 139 peptidoglycan surrounding the predator during entry, and formation of a ribosome lattice around
- 140 the predator's nucleoid after the prey is consumed in the bdelloplast.
- 141
- 142

bioRxiv preprint doi: https://doi.org/10.1101/2022.06.13.496000; this version posted June 13, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

143 **Results**

144 To visualize the predatory lifecycle of *B. bacteriovorus* in a near-native state, we applied cryo-ET 145 imaging to samples of B. bacteriovorus HD100 at various timepoints, from 10 minutes to 16 hours, 146 after addition of prey (Vibrio cholerae, Escherichia coli, or E. coli minicells). Compared to thicker 147 cells, the thinness of E. coli minicells yielded higher-resolution details about the predator-prey 148 interaction. The small size of the minicells also prevented complete entry of the predator, allowing 149 us to capture otherwise fleeting intermediates in the rapid invasion process. Table S1 lists the 150 number of cryo-tomograms we acquired at each stage of the predatory lifecycle of *B. bacteriovorus*, 151 and Table S2 the number of examples we observed of each of the features described below.

152

153

I- Anatomy of the attack-phase *B. bacteriovorus* cell

154 Our cryo-tomograms of attack-phase *B. bacteriovorus* showed features previously described, 155 including a compact spiral nucleoid occupying the center of the cell (Fig. 1A) (Borgnia et al., 2008; 156 Butan et al., 2011). While the nucleoid excluded ribosomes, they were occasionally abundant at 157 its periphery (Fig. S1 and Movie S1), as seen previously by cryo-ET (Borgnia et al., 2008; Butan 158 et al., 2011). Again consistent with previous cryo-ET of attack-phase cells (Borgnia et al., 2008), 159 we saw unidentified tubes (on average, two per cell) in the cytoplasm. The tubes had a uniform 160 diameter of ~8 nm and were typically a few tens of nanometers in length (Fig. S2). Each cell 161 contained a single polar flagellum, sheathed in outer membrane (Fig. 1A), and subtomogram 162 averaging of 79 particles revealed that the structure of the flagellar motor is similar to that recently 163 published from a host-independent strain of *B. bacteriovorus* (Chaban et al., 2018) (Fig. 1B).

164

165 We also saw novel features, including extracellular vesicles with a uniform diameter of ~ 25 nm 166 near cells (Fig. S3A). On the "biting" pole opposite the flagellum, we noted several characteristic 167 features that have not been described before. Rarely, we saw spherical and short filamentous 168 structures in the periplasm (Figs. 1A and S3B). We observed abundant, thin (~3-4 nm wide) 169 fimbriae on the cell surface, with lengths ranging from ~50 to more than 100 nm (Fig. S3A&C). 170 We also observed a novel complex, spanning the periplasm and with a prominent extracellular 171 rosette of density. We call this unidentified structure the "rose-like complex." On average, each 172 cell had 2-3 rose-like complexes at the biting pole (Fig. 1A). Subtomogram averaging of 132 173 particles revealed a molecular complex spanning the entire periplasmic space with associated 174 cytoplasmic densities (a ring ~17 nm in diameter) and extensive extracellular densities. Five 175 distinct extracellular densities could be distinguished in cross-section: two stacked rings and a 176 central cap (Fig. 1C).

177

178 We observed both piliated and non-piliated T4aP basal bodies on the biting pole (Figs. 1A and S4 179 and S5), with empty more abundant than piliated (Table S2). A subtomogram average of 335 non-180 piliated T4aP basal bodies revealed the architecture, including a distinctive extracellular ring 181 present in both non-piliated and piliated basal bodies (Figs. 1D and S4) which was not seen in 182 subtomogram averages of T4P in other species (Chang et al., 2016, 2017; Gold et al., 2015; 183 Treuner-Lange et al., 2020). While all piliated basal bodies had the ring, not all non-piliated basal 184 bodies did (Fig. S5). This could either be because the external ring had disassembled or not yet 185 assembled, or because these structures were not in fact T4aP but rather, e.g. T4bP, which are also 186 involved in adhesion to prey (Avidan et al., 2017). In addition, classifying the non-piliated T4aP 187 using principal component analysis revealed that $\sim 1/2$ of the particles had only the Secretin outer

188 membrane channel and extracellular ring and lacked the lower periplasmic ring (Fig. S6). This 189 might reflect different assembly stages, as it has been shown that T4P in other species utilize an 190 outside-in assembly pathway starting from the Secretin (Friedrich et al., 2014).

191

192

II- Attachment of *B. bacteriovorus* to prey

We observed attachment of *B. bacteriovorus* to *V. cholerae* and *E. coli* minicells (of various sizes).
The earliest event we identified was connection of *B. bacteriovorus* to prey by T4aP, with the tip
of the extended pilus clearly in contact with the prey's outer membrane. At the resolution of our
cryo-tomograms, no distinctive features were visible at the pilus-prey attachment point (Fig. 2A).
Occasionally, we also saw thin fimbriae apparently contacting the outer membrane of the prey cell
(Fig. S7).

199

200 Next, we observed predator and prey in close apposition. In some cases, pilus attachments were 201 still visible; in others the pili had retracted completely. In all cases, we observed non-piliated T4aP 202 basal bodies aligned at the contact site, with rose-like complexes nearby (Figs. 2B and S8). In 203 several attachments to *E. coli* minicells, where finer detail could be resolved, we observed that the 204 non-piliated T4aP basal bodies extended through the prey outer membrane, with their extracellular 205 rings apparently embedded in the prey peptidoglycan (PG) (Figs. 2B and S9). At this stage, we 206 began to observe enlargement of the prey periplasm. In agreement with previous studies (Lambert 207 et al., 2011), with rod-shaped prey the contact site was usually located on the side of the prey cell, 208 and on the pole of the predator (Fig. S10A). In some cases, particularly with smaller, spherical E. 209 *coli* minicells, the attachment site was displaced slightly off the biting pole onto the side of the 210 predator (Fig. S10B-D).

bioRxiv preprint doi: https://doi.org/10.1101/2022.06.13.496000; this version posted June 13, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

211

212 In the next stage, we observed an electron-dense but relatively unstructured plaque of material 213 (henceforth referred to as the "attachment plaque") at the contact point between the predator and 214 the prey. The diameter of this plaque ranged from 15-70 nm and the thickness usually extended 215 from the predator's OM to the prey's PG cell wall (Figs. 2C, S10C and D), suggesting a 216 modification of the prey cell wall at the predator-prey contact point in agreement with previous 217 reports (Kuru et al., 2017). We often observed nonpiliated T4aP basal bodies near or in the plaque 218 (Fig. S10D), as well as rose-like complexes and, very occasionally, prey-attached pili nearby. 219 While we sometimes observed two or even three *B*. *bacteriovorus* attached to the same prey cell 220 with T4aP, only one ever formed an attachment plaque, consistent with the committed attachment 221 observed in previous studies (Fig. S11).

222

223 At around the same time that the attachment plaque formed at the biting pole, an unexpected 224 process began at the other pole. The sheathed flagellum of the *B. bacteriovorus* was resorbed into 225 the periplasmic space, wrapping around the cell. The process seems to initiate with breakage of 226 the flagellar motor at its OM-embedded ring (the L- (lipopolysaccharide) ring, Fig. S12). The L-227 ring remained in the OM. Early in the process, the P- (peptidoglycan) ring was still visible around 228 the flagellar rod but was located more than 20 nm from the OM, compared to 10-11 nm in attack-229 phase cells (Figs. S13 and S14 and Movie S2). Consistent with a decoupling of the P- and L-rings, 230 we observed two examples of B. bacteriovorus cells attached to prey with a plaque and with 231 disrupted OM around the flagellum; in both cases, only the P-ring, and not the L-ring, was visible 232 surrounding the rod of the motor (Fig. S15).

233

234 As flagellar resorption continued, the motor (lacking the L-ring but including the P-ring and rod) 235 was completely internalized to the periplasm and moved off the cell pole, with the hook and basal 236 portion of the filament entering the periplasm (Figs. 3, S16 and Movie S3). The L-ring still 237 encircled the filament at the junction of OM and flagellar sheath (Figs. 3, S16 and Movie S3). 238 Eventually, most or all of the filament was internalized to the periplasm, wrapping around the cell 239 (Figs. S17, S18 and Movies S4-S7). In some cases, we saw the motor further up the side of the 240 cell. In other cases, we could not find the motor, either because it was degraded or because it was 241 located in a part of the cell not visible due to the effect of the missing wedge of information in 242 cryo-ET (Baumeister, 1999). During the absorption process, the exit point of the flagellum 243 sometimes shifted from the pole up the side of the cell (Figs. S18, S19 and Movie S6). In cells 244 with fully internalized filaments, we could no longer identify L-rings in the outer membrane. In 245 some cases, the wrapped filament broke in the periplasm (Movie S4). Figure S20 summarizes this 246 absorption process based on our cryo-ET data.

247

Chemosensory arrays remained visible throughout the attachment phase, although they appeared to be partially degraded (smaller in diameter than in attack-phase cells) in some cases. As in attackphase cells, we observed small, uniformly-sized membrane vesicles in the vicinity of the predator and attached prey (Fig. S21). In some vesicles, densities were visible either inside and/or on the surface (Fig. S21F). We also saw vesicles near the sheath of the flagellum during resorption (Fig. S22).

254

255 III- Invasion of the prey periplasm

256 We captured 18 cryo-tomograms of stalled invasions of *B. bacteriovorus* entering the periplasm 257 of *E. coli* minicells. Note that in these stalled invasions we do not know how long before sample 258 freezing a particular predator entered its prev or whether the non-permissive size of the small prev 259 had incidental effects on the entry process. Still, this paradigm provided an unique opportunity to 260 view fleeting stages of invasion at high resolution. In invasion, the attachment plaque at the contact 261 site was replaced by a portal structure through which the predator entered the prey periplasm (Figs. 262 4, S23, and Movie S8). This portal ring, which bridged the outer membranes of predator and prey, 263 appeared in cross-section as a thin (<5 nm), dark density extending from the prey's PG layer to the 264 outside of the cell, capping the open end of the prey outer membrane (Figs. 4A and S23A). The 265 height of the portal in cross-section, on the order of a few tens of nanometers, varied between cells, 266 and even on opposite sides of the same cell (e.g. compare Figs. 4A and S23A). In eight examples, 267 we observed what appeared to be prev OM blebbing out from the portal (Movie S9). Consistent 268 with a water-tight seal model, the portal appeared to exert considerable force on the B. 269 *bacteriovorus* cell, as previously observed (Abram et al., 1974), reducing the distance between the 270 outer and inner membranes by $\sim 50\%$ at the entry point compared to elsewhere in the cell, and 271 constricting the deformation-resistant cell wall (Figs. 4B-C, S23B-C). Consistent with previous 272 reports of cell flexibility (Borgnia et al., 2008), we observed that B. bacteriovorus could bend 273 considerably to maximally occupy the prey periplasm (Fig. 4A).

274

In all stalled invasions, we observed that the *B. bacteriovorus* cells had fully degraded their absorbed periplasmic flagella and lacked rose-like complexes and fimbriae. Chemosensory arrays were also partially or completely degraded (Fig. S24). Interestingly, while complexes morphologically similar to non-piliated T4aP basal bodies were still present in the predator, they

14

279 lacked the characteristic extracellular ring found at earlier stages of invasion (Fig. S25), and were 280 less abundant than T4aP basal bodies in attack-phase cells. This could be because either the 281 extracellular ring is lost during the invasion process, or these are different complexes, e.g. type II 282 secretion systems.

283

284 In two examples where a non-flagellated *B. bacteriovorus* cell was in the vicinity of a lysed prey, 285 we observed knob-like densities on the predator's biting pole (Fig. S26). Given the lysed prey cell 286 nearby and the lack of attack-phase structures such as flagella or chemosensory arrays in the B. 287 *bacteriovorus*, we think it likely that these cells were pulled out of the prey post-invasion during 288 sample preparation. When present, the knob-like structures could be abundant; we identified 23 289 examples on one cell (Table S2). While leg-like densities could be seen extending from the 290 extracellular domains to the PG layer in individual examples, subtomogram averaging failed to 291 resolve a consistent structure, suggesting flexibility or differing stoichiometry.

292

Even though entry was incomplete, *B. bacteriovorus* in stalled invasions of *E. coli* minicells showed signs of entering growth phase. Some predators had at least partially decondensed their nucleoids, and the prey cytoplasm was considerably reduced in size, presumably consumed by the predator. Perhaps related to this digestion, we observed multiple vesicles with a consistent size of ~25 nm in the prey periplasm (Figs. 4A and S23A). We cannot tell whether they originated from prey or predator membrane, but their size is identical to those we observed in the vicinity of isolated attack-phase and prey-attached *B. bacteriovorus*.

300

301 IV- Growth phase in the bdelloplast

302 We captured 54 cryo-tomograms of the bdelloplast stage from samples of *B. bacteriovorus* 303 invading V. cholerae cells and E. coli minicells large enough to accommodate the entire predator. 304 Once the predator fully entered the prey's periplasm, the entry hole was sealed by a scar consisting 305 of an extracellular bubble of what appeared to be membrane and an amorphous electron density 306 associated with the prev OM and PG beneath it (Figs. 5, S27 and S28 and Movies S10-S13). In 307 invaded V. cholerae, the prey's (non-functional) flagellum remained attached to the bdelloplast 308 (Figs. 5F and S27B, C). The flagellum remained connected to the part of the motor embedded in 309 the OM and PG: the PL-rings and part of the rod. Presumably these parts were separated from the 310 rest of the motor by periplasmic expansion. We did not observe any motor components still 311 associated with the inner membrane. The fact that flagellar relics remained only on V. cholerae 312 bdelloplasts, and not E. coli, could be because the flagellar sheath aids in retention. In V. cholerae 313 bdelloplasts, we sometimes also observed PL-subcomplexes (without associated filaments) 314 resulting from previous flagellar loss events (Ferreira et al., 2019; Kaplan et al., 2020) (Fig. S27B). 315

316 In predators inside bdelloplasts, we saw neither chemosensory arrays nor any relics of flagella, 317 although we occasionally observed filamentous structures in the periplasm that may be remnants 318 of flagellar digestion (Fig. S29). We also sometimes observed these in (flagellated) attack-phase 319 cells (Fig. S3B). Interestingly, they were always located at the pole (the biting pole of attack-phase 320 cells), perhaps reflecting spatial differences in proteolysis. We also could not find any rose-like 321 complexes or T4aP basal bodies with external rings in *B. bacteriovorus* inside bdelloplasts. As in 322 stalled invasions, we did identify putative nonpiliated T4aP basal bodies lacking the external ring. 323 Again, they were less abundant than in attack-phase cells; from 47 cryo-tomograms of stalled 324 invasions or early bdelloplasts, we identified 25 such particles (Table S2). We did occasionally

observe 8-nm-wide cytoplasmic tubes, as seen in other lifecycle stages (Figs. S30). In addition, we
saw variously-sized spherical, nested and horseshoe-shaped vesicles in the predator's cytoplasm,
morphologically similar to those reported in other species (Dobro et al., 2017) (Fig. S31).

328

As in stalled invasions of *E. coli* minicells, we observed many uniformly-sized (~25 nm) vesicles in the bdelloplast periplasm (Figs. 5 and S31 and Movies S11-S13). Consistent with active growth, *B. bacteriovorus* nucleoids were less condensed than in earlier stages and, in concert with the prey cytoplasm shrinking, the predator cell elongated and curled to fill most of the bdelloplast (Fig. S32). Contrary to previous observations by traditional EM (Abram et al., 1974), we could not unambiguously identify a connection between the predator OM and prey inner membrane (Figs. S28, S32, S33 and Movies S10-S11).

336

337 When nearly all of the prey cytoplasm was consumed, the elongated *B. bacteriovorus* cell divided. 338 The number of progeny depends on the size of the bdelloplast (Fenton et al., 2010a), and we 339 observed two or three progeny cells in E. coli and V. cholerae prey (e.g., Fig. 5). In a few cases, 340 division produced an extra, small spherical product, in accordance with previous reports (Burnham 341 et al., 1970) (Fig. S34). In some cases, bdelloplasts contained a very dense sphere of material, 342 presumably containing the remnants of the prey cytoplasm (Figs. 5 and S34). In other cases, not 343 even this remained (a characteristic we use to define an "end-stage bdelloplast"). The characteristic 344 ~25 nm vesicles, however, were still present in end-stage bdelloplasts even after no prey cytoplasm 345 remained.

346

17

347 In some elongated or divided *B. bacteriovorus* in end-stage bdelloplasts of *E. coli*, we observed a 348 remarkable hexagonal lattice of ribosomes coating the nucleoid (Fig. 6 and Movies S13-S17). The 349 lattice spacing was ~ 20 nm, consistent with maximally dense packing of ribosomes (Fig. S35). 350 This arrangement was much more extensive than we and others observed in attack-phase cells 351 (Butan et al., 2011). We measured the distances from ribosomes to the apparent surface of the 352 nucleoid in two tomograms. Of 2,304 ribosomes in one (Fig. 6) and 1,109 in the other (Fig. S36), 353 \sim 80% were located within 10 nm of the nucleoid surface. By comparison, in a simulation of the 354 same number of randomly-packed 20-nm spheres in the same tomographic volumes (see Materials 355 and Methods), only ~20-25% were expected to be located within 10 nm of the nucleoid surface 356 (Figs. 6E and S36), suggesting that the association we observed does not arise simply by chance. 357 To investigate whether the ordered ribosomes shared the same orientation, we produced an ~ 4.7 358 nm-resolution subtomogram average of the ribosomes in a *B. bacteriovorus* cell and mapped it 359 back into the tomographic volume using the positions and orientations determined during 360 averaging. We found that individual particles were apparently randomly oriented on the nucleoid 361 surface (Fig. S37).

362

Movie S18 offers an animated summary of all stages of the *B. bacteriovorus* predatory lifecycle
that we observed in this study.

365 **Discussion**

Here we used cryo-ET imaging to reveal the predation cycle of *B. bacteriovorus in situ* at nanometer-scale resolution (Movie S18). Our results contextualize decades of research on BALO predation and uncover many surprising new details of the process.

369

370 In addition to previously-characterized structures in attack-phase cells such as the flagellum, poly-371 phosphate storage granules, highly-condensed nucleoid, chemosensory array, and type IV pili 372 (Borgnia et al., 2008; Butan et al., 2011; Chaban et al., 2018; Evans et al., 2007; Mahmoud and 373 Koval, 2010), we observed several unidentified structures. At all stages of invasion, B. 374 *bacteriovorus* cells contained 8 nm-wide cytoplasmic tubes, typically two per cell. The identity 375 and function of these tubes, which were also seen previously by cryo-ET in attack-phase cells 376 (Borgnia et al., 2008), remains unknown, but they may serve a cytoskeletal role. In a few attack-377 phase cells, we observed spherical or tubular structures in the periplasm at the biting pole which 378 might be fragments of digested flagella. The biting pole of attack-phase cells also contained 379 abundant fimbriae, shorter (~100 nm or less) and thinner than T4aP and without obvious 380 machinery at their base. Their location suggests a role in prey interaction. Interestingly, mutant 381 strains of *B. bacteriovorus* lacking either T4aP and T4bP genes can still attach to prey (Avidan et 382 al., 2017; Milner et al., 2014); perhaps these fimbriae mediate such adhesion.

383

The most intriguing new structure we observed is what we call the rose-like complex, present in ~2-3 copies on the biting pole of nearly every attack-phase cell we imaged. The periplasmic portion of the rose-like complex is morphologically similar to a recent structure of a tripartite efflux pump (Alav et al., 2021) and since related *B. bacteriovorus* type I secretion systems (T1SS) secrete enzymes that modify the prey during invasion (Rendulic, 2004), it is possible that the rose-like complex is a T1SS. Consistent with a role in early invasion, we observed rose-like complexes on attack-phase cells and at prey contact sites, but not in cells during or after invasion. The function of the elaborate extracellular domains extending nearly 20 nm out from the cell is of particular interest; perhaps they interact with, or breach, the prey envelope.

393

394 Another machine with a notable extracellular domain is the T4aP basal body. The pili observed on 395 the biting pole of *B. bacteriovorus* in early micrographs (Abram and Davis, 1970; Abram et al., 396 1974; Shilo, 1969) were previously identified as T4aP by mutant analysis and immunolocalization 397 (Evans et al., 2007; Mahmoud and Koval, 2010). Our higher-resolution imaging here revealed a 398 novel extracellular ring surrounding the base of the pilus, not seen in previous subtomogram 399 averages of related T4aP in *Thermus thermophilus* and *Myxococcus xanthus* (Chang et al., 2016; 400 Gold et al., 2015). The ring was also present in non-piliated basal bodies, indicating that it is stable 401 in the absence of the pilus. Interestingly, these extracellular densities were observed on attack-402 phase cells in a previous cryo-ET study, but their relation to T4aP was not resolved (Borgnia et al., 403 2008). We observed some non-piliated complexes lacking the extracellular ring, both in attack-404 phase cells and in bdelloplasts, where no complexes with the outer ring were observed. It is 405 possible that these complexes are not T4aP, but rather a related machine containing a Secretin pore 406 in the outer membrane, such as a type II secretion system, which is structurally similar (Ghosal et 407 al., 2019b) and thought to be involved in *B. bacteriovorus* secretion (Dori-Bachash et al., 2008; 408 Rendulic, 2004). In addition, B. bacteriovorus also contains a large repertoire of T4bP genes, 409 which are dispensable for attachment but required for invasion (Avidan et al., 2017; Schwudke et 410 al., 2005). Their products have not been located on the cell and it is possible that some or all of the

411 ring-less basal bodies we saw were T4bP. Alternatively, the extracellular ring may be a transient

412 component of the *B. bacteriovorus* T4aP, perhaps dissociating during the prey entry process.

413

414 The pilin protein PilA is required for invasion and T4aP have been suggested to pull cells into prey, 415 perhaps by attaching to the cell wall (Evans et al., 2007; Mahmoud and Koval, 2010; Milner et al., 416 2014), but mutants lacking the disassembly PilT ATPase are still capable of invasion (Chanyi and 417 Koval, 2014) and the role of T4aP in the process remains a major open question in the field 418 (Sockett, 2009). In our tomograms, multiple T4aP can be seen attached to prey cells and clearly 419 exerting force as they retracted, pulling the prey OM and PG into close contact with the predator. 420 As the membranes were brought into contact, the shortening pili fully disassembled, leaving empty 421 basal bodies. Interestingly, these non-piliated basal bodies continued to mediate attachment and 422 could be seen extending through the prey OM, with their extracellular rings located in the PG layer 423 of the prey, suggesting the function of this novel component. Our results thus suggest that pili 424 themselves do not drive entry, but rather force the initial connection. If the basal bodies without 425 external rings we observed in bdelloplasts were in fact T4aP, it is possible that the rings remained 426 embedded in the prey PG.

427

With a few exceptions noted by (Lambert et al., 2006), *B. bacteriovorus* are known to lose their flagella when entering prey. Our images reveal a surprising mechanism: while attached to a prey cell, the flagellar motor is broken at the L-ring and the filament absorbed into the predator's periplasm, where it is digested. This mechanism differs from all previous observations of flagellar loss due to lifecycle-programmed ejection, response to nutrient deprivation or mechanical breakage, all of which leave a stable subcomplex of the P- and L-rings in the cell wall and outer 434 membrane (Ferreira et al., 2019; Kaplan et al., 2019, 2020, 2021b, 2021b; Zhu and Gao, 2020; 435 Zhu et al., 2019; Zhuang and Lo, 2020; Zhuang et al., 2020). It will be interesting to see whether 436 the *B. bacteriovorus* motor lacks the inter-subunit interactions that likely stabilize the PL-437 subcomplex in other species (Johnson et al., 2021; Tan et al., 2021; Yamaguchi et al., 2020). This 438 process also differs from the breakage of the prey flagellum that occurs as the periplasmic space 439 is expanded into the bdelloplast. In that case, we see that the flagellum remains anchored to the 440 cell by a stub of the motor embedded in the outer membrane. Why the hook/filament is not lost is 441 unclear; perhaps it is locked into the remodeled PG. This process is reminiscent of that recently 442 observed in other species upon cell lysis, where the cytoplasmic flagellar switch complex is lost, 443 while the periplasmic and extracellular components remain (Kaplan et al., 2021c).

444

445 How does *B. bacteriovorus* absorb its flagellum into the periplasm? It is unlikely to be pulled from 446 the motor, which we occasionally saw drift partway up the side of the cell before being fully 447 degraded. Our observation of filaments partially absorbed up to a junction on the side of the cell 448 suggests that the process involves zippering of the flagellar sheath and the outer membrane. 449 Perhaps this is related to the unique lipid composition of the sheath, which is predicted to be even 450 more fluid than the rest of the outer membrane (Thomashow and Rittenberg, 1985b). The vesicles 451 we observed in the vicinity of some absorbed flagella are consistent with previous reports that 452 rotation of sheathed flagella can lead to shedding of outer membrane vesicles (Aschtgen et al., 453 2016; Brennan et al., 2014). However, we do not know whether the vesicles formed because the 454 motor was still rotating during absorption or due to the absorption process itself.

455

22

456 Other bacterial species have been observed to wrap their extracellular (unsheathed) flagella around 457 themselves (Alirezaeizanjani et al., 2020; Cohen et al., 2020; Constantino et al., 2018; Hintsche et 458 al., 2017; Kühn et al., 2017; Tian et al., 2022). This behavior, which Shewanella putrefaciens uses 459 to burrow back out of a tight spot when stuck, is triggered by a mechanical instability in the 460 flagellum that buckles it when the cell can no longer move to alleviate the torque of flagellar 461 rotation (Kühn et al., 2017). Such a situation likely occurs when a *B. bacteriovorus* cell attaches 462 to a prey. Brief continuing flagellar rotation could conceivably wrap the filament around the cell, 463 where fusion of the outer membrane and sheath would bring it into the periplasm. Such increased 464 resistance is consistent with the lateral shift of the biting pole we sometimes observed on attached 465 *B. bacteriovorus* cells. Interestingly, it was recently shown that a discontinuous flagellar filament 466 formed by two different flagellins facilitates screw-like motility in S. putrefaciens (Kühn et al., 467 2018), and is key to wrapping in *Campylobacter jejuni* (Cohen et al., 2020). Multiple flagellins 468 similarly make up distinct segments of the B. bacteriovorus flagellum (Thomashow and Rittenberg, 469 1985a); the reason for this was unknown but now we speculate that it may facilitate flagellar 470 recycling.

471

A major open question is the nature of the pore through which *B. bacteriovorus* enter their prey. Secreted PG-remodeling enzymes, presumably part of the dense plaque we observe at the attachment site that extends to the prey cell wall, are known to create, and subsequently seal, a reinforced circular porthole in the prey PG (see figures 2 and 3 in (Kuru et al., 2017)). How the prey outer membrane is modified remains more of a mystery. Prey cells remain intact and transcriptionally active throughout the initial entry process (Lambert et al., 2010a), so it was proposed that the membranes of prey and predator must fuse (Negus et al., 2017). Instead, we

479 observed what seems to be a proteinaceous collar curving out from the prey PG to seal the hole in 480 the outer membrane and prevent interaction of the two membranes. Presumably this structure is 481 associated with the PG remodeling enzymes, and may even be a modified and reinforced extension 482 of the cell wall, as suggested by (Abram et al., 1974). The portal is dynamic, expanding and 483 contracting to match the cross-section of the cell passing through it, and its height varied between 484 cells (and even on opposite sides of the same cell). As was also seen by traditional thin-section 485 TEM (Abram et al., 1974), the portal exerted considerable force on the *B. bacteriovorus* cell, 486 deforming its PG layer, consistent with a water-tight seal between the cells.

487

488 What provides the force for entry remains enigmatic. Over several decades, various models have 489 been proposed, including flagellar rotation (Stolp and Starr, 1963), retraction of T4aP attached to 490 the prey cell wall (Evans et al., 2007; Mahmoud and Koval, 2010), and attachment to the prey 491 inner membrane as osmotic pressure rapidly expands the periplasm (Abram et al., 1974). Our 492 results rule out all of these models. We see that the *B. bacteriovorus* flagellum is broken, and at 493 least partially absorbed, prior to entry. Similarly, pili are fully disassembled prior to entry. And 494 finally, we see prey periplasmic expansion even before entry, presumably due to secreted enzymes 495 that de-crosslink and sculpt the prey PG during entry (Lerner et al., 2012), with no visible 496 connection between the predator and the now-distant prey inner membrane. It is possible that an 497 expanding periplasmic space provides a suction force. It is also possible that an active mechanism 498 walks the connection point with the portal down the *B. bacteriovorus* cell.

499

Following predator entry, we saw that the portal is sealed by a scar of electron dense material,
likely related to the resealing of the PG sacculus (Kuru et al., 2017; Snellen and Starr, 1974), with

502 a protruding bubble of what appears to be membrane. While previous, traditional EM imaging also 503 observed an electron-dense ring at the sealed penetration pore (Shilo, 1969), no associated bubble 504 was observed, perhaps reflecting the improved preservation and hydration of membrane structures 505 by cryo-EM. Given the blebs around the invasion portal we noticed on some prey cells in stalled 506 invasions, it may be prev OM. Alternatively, it may be outer membrane pinched off from the end 507 of the invading predator. B. bacteriovorus has a highly labile OM, as we saw both with absorption 508 of the sheathed flagellum and in occasional highly-curved cells where the outer membrane fused 509 into a sac, and consistent with previous lipid analysis of its unique membrane (Lambert et al., 510 2008). In either case, the bubble must be somehow tethered to the seal, perhaps through membrane-511 embedded protein complexes.

512

513 While we did not see the connections to the prey cytoplasm during entry observed by (Abram et 514 al., 1974), we did see a few examples of the predator outer and prey inner membranes in close 515 proximity in the bdelloplast. In most cases, however, we only observed vesicles in the bdelloplast 516 periplasm, suggesting that this may be a mechanism for nutrient transfer. Compared to membrane 517 vesicles of other species (Kaplan et al., 2021d; Toyofuku et al., 2019), these vesicles were notable 518 for their relatively small and uniform size (~25 nm in diameter). Similar small periplasmic vesicles 519 may be present in previous traditional EM images, but interpretation is limited by the membrane 520 disruption of that sample preparation (Abram et al., 1974). In our tomograms, we cannot tell which 521 cell(s) are producing the vesicles in bdelloplasts, but we also observed them near isolated attack-522 phase cells as well as budding from *B. bacteriovorus* attached to prey. We also observed them in 523 end-stage bdelloplasts where no remaining prey inner membrane was visible. B. bacteriovorus is 524 known to translocate a pore protein from its outer membrane to the prey inner membrane (Tudor

and Karp, 1994); perhaps similar transferred machinery produces vesicles to deliver prey
cytoplasmic content, and lipids, to the growing predator.

527

528 Following complete consumption of the prey, we observed a striking hexagonal lattice of 529 ribosomes on the surface of the *B. bacteriovorus* condensed nucleoid(s). Similar, though more 530 limited, ribosome associations were previously observed in attack phase, particularly in a B. 531 bacteriovorus mutant with an even more tightly condensed nucleoid than wild-type cells (Borgnia 532 et al., 2008; Butan et al., 2011), and in some attack-phase cells in this study. One possibility for 533 this ribosome lattice is that it is a depletion effect resulting from entropic forces encouraging large 534 objects to aggregate on a surface (Asakura and Oosawa, 1954, 1958; Rocha et al., 2020). However, 535 we did not observe the effect on other cell surfaces such as the inner membrane, nor in other stages 536 of the cell cycle, so we think this unlikely. Eukaryotic ribosomes have been observed to crystallize 537 in hypothermic conditions (Byers, 1967), but we see no relationship between the orientation of 538 neighboring particles consistent with a crystal, or with a polysome (Brandt et al., 2009). We think 539 a more likely possibility is that the ribosomes are independently translating transcripts from the 540 condensing nucleoid. The switch from growth phase to attack phase involves a transcriptional shift 541 activating a few hundred genes, and inactivating many more (Karunker et al., 2013; Lambert et al., 542 2010b). The highly condensed attack-phase nucleoid excludes even small monomeric proteins 543 (Kaljević et al., 2021), so the ribosomes we observe on the surface may be, or recently have been, 544 coupled to transcribing polymerases.

545

546 Together, our results provide the most complete view to date of the unique structures that enable 547 the complex lifecycle of this endobiotic bacterial predator, and raise new questions. We hope our bioRxiv preprint doi: https://doi.org/10.1101/2022.06.13.496000; this version posted June 13, 2022. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

- 548 work spurs further study of this fascinating process and informs potential future applications of
- 549 bacterial predators as living antibiotics (Atterbury and Tyson, 2021).

550 **References**

- Abram, D., and Davis, B.K. (1970). Structural Properties and Features of Parasitic *Bdellovibrio*
- 552 *bacteriovorus*. J Bacteriol *104*, 948–965. https://doi.org/10.1128/jb.104.2.948-965.1970.
- Abram, D., e Melo, J.C., and Chou, D. (1974). Penetration of *Bdellovibrio bacteriovorus* into
- 554 Host Cells. J Bacteriol *118*, 663–680. https://doi.org/10.1128/jb.118.2.663-680.1974.
- Alav, I., Kobylka, J., Kuth, M.S., Pos, K.M., Picard, M., Blair, J.M.A., and Bavro, V.N. (2021).
- 556 Structure, Assembly, and Function of Tripartite Efflux and Type 1 Secretion Systems in Gram-
- 557 Negative Bacteria. Chem. Rev. 121, 5479–5596. https://doi.org/10.1021/acs.chemrev.1c00055.
- Alirezaeizanjani, Z., Großmann, R., Pfeifer, V., Hintsche, M., and Beta, C. (2020). Chemotaxis
- 559 strategies of bacteria with multiple run modes. Sci. Adv. 6, eaaz6153.
- 560 https://doi.org/10.1126/sciadv.aaz6153.
- Asakura, S., and Oosawa, F. (1954). On Interaction between Two Bodies Immersed in a Solution
- of Macromolecules. The Journal of Chemical Physics 22, 1255–1256.
- 563 https://doi.org/10.1063/1.1740347.
- Asakura, S., and Oosawa, F. (1958). Interaction between particles suspended in solutions of macromolecules. J. Polym. Sci. *33*, 183–192. https://doi.org/10.1002/pol.1958.1203312618.
- 566 Aschtgen, M.-S., Lynch, J.B., Koch, E., Schwartzman, J., McFall-Ngai, M., and Ruby, E. (2016).
- 567 Rotation of Vibrio fischeri Flagella Produces Outer Membrane Vesicles That Induce Host
- 568 Development. Journal of Bacteriology 198, 2156–2165. https://doi.org/10.1128/JB.00101-16.
- 569 Atterbury, R.J., and Tyson, J. (2021). Predatory bacteria as living antibiotics where are we 570 now? Microbiology *167*. https://doi.org/10.1099/mic.0.001025.
- 571 Avidan, O., Petrenko, M., Becker, R., Beck, S., Linscheid, M., Pietrokovski, S., and Jurkevitch,
- 572 E. (2017). Identification and Characterization of Differentially-Regulated Type IVb Pilin Genes
- 573 Necessary for Predation in Obligate Bacterial Predators. Sci Rep 7, 1013.
- 574 https://doi.org/10.1038/s41598-017-00951-w.
- 575 Banks, E.J., Valdivia-Delgado, M., Biboy, J., Wilson, A., Cadby, I.T., Vollmer, W., Lambert, C.,
- 576 Lovering, A.L., and Sockett, R.E. (2022). Asymmetric peptidoglycan editing generates cell
- 577 curvature in Bdellovibrio predatory bacteria. Nat Commun 13, 1509.
- 578 https://doi.org/10.1038/s41467-022-29007-y.
- Baumeister, W. (1999). Electron tomography of molecules and cells. Trends in Cell Biology 9,
 81–85. https://doi.org/10.1016/S0962-8924(98)01423-8.
- 581 Bonfiglio, G., Neroni, B., Radocchia, G., Marazzato, M., Pantanella, F., and Schippa, S. (2020).
- Insight into the Possible Use of the Predator Bdellovibrio bacteriovorus as a Probiotic. Nutrients
 12, 2252. https://doi.org/10.3390/nu12082252.

- 584 Borgnia, M.J., Subramaniam, S., and Milne, J.L.S. (2008). Three-Dimensional Imaging of the
- 585 Highly Bent Architecture of Bdellovibrio bacteriovorus by Using Cryo-Electron Tomography.
- 586 JB 190, 2588–2596. https://doi.org/10.1128/JB.01538-07.
- 587 Brandt, F., Etchells, S.A., Ortiz, J.O., Elcock, A.H., Hartl, F.U., and Baumeister, W. (2009). The
- 588 Native 3D Organization of Bacterial Polysomes. Cell *136*, 261–271.
- 589 https://doi.org/10.1016/j.cell.2008.11.016.
- 590 Bratanis, E., Andersson, T., Lood, R., and Bukowska-Faniband, E. (2020). Biotechnological
- 591 Potential of Bdellovibrio and Like Organisms and Their Secreted Enzymes. Front. Microbiol. 11,
- 592 662. https://doi.org/10.3389/fmicb.2020.00662.
- 593 Brennan, C.A., Hunt, J.R., Kremer, N., Krasity, B.C., Apicella, M.A., McFall-Ngai, M.J., and
- Ruby, E.G. (2014). A model symbiosis reveals a role for sheathed-flagellum rotation in the
- release of immunogenic lipopolysaccharide. ELife 3. https://doi.org/10.7554/eLife.01579.
- 596 Bukowska-Faniband, E., Andersson, T., and Lood, R. (2020). Studies on Bd0934 and Bd3507,
- 597 Two Secreted Nucleases from *Bdellovibrio bacteriovorus*, Reveal Sequential Release of
- 598 Nucleases during the Predatory Cycle. J Bacteriol 202, e00150-20, /jb/202/18/JB.00150-20.atom.
- 599 https://doi.org/10.1128/JB.00150-20.
- 600 Burnham, J.C., Hashimoto, T., and Conti, S.F. (1968). Electron microscopic observations on the
- 601 penetration of Bdellovibrio bacteriovorus into gram-negative bacterial hosts. J Bacteriol *96*,
- 602 1366–1381. https://doi.org/10.1128/JB.96.4.1366-1381.1968.
- Burnham, J.C., Hashimoto, T., and Conti, S.F. (1970). Ultrastructure and Cell Division of a
- 604 Facultatively Parasitic Strain of *Bdellovibrio bacteriovorus*. J Bacteriol *101*, 997–1004.
- 605 https://doi.org/10.1128/jb.101.3.997-1004.1970.
- Butan, C., Hartnell, L.M., Fenton, A.K., Bliss, D., Sockett, R.E., Subramaniam, S., and Milne,
 J.L.S. (2011). Spiral Architecture of the Nucleoid in Bdellovibrio bacteriovorus. Journal of
 Bacteriology *193*, 1341–1350. https://doi.org/10.1128/JB.01061-10.
- 609 Byers, B. (1967). Structure and formation of ribosome crystals in hypothermic chick embryo
- cells. Journal of Molecular Biology 26, 155–167. https://doi.org/10.1016/0022-2836(67)90288-4.
- 611 Calakli, F., and Taubin, G. (2012). SSD-C: Smooth Signed Distance Colored Surface
- 612 Reconstruction. In Expanding the Frontiers of Visual Analytics and Visualization, J. Dill, R.
- Earnshaw, D. Kasik, J. Vince, and P.C. Wong, eds. (London: Springer London), pp. 323–338.
- 614 Capeness, M.J., Lambert, C., Lovering, A.L., Till, R., Uchida, K., Chaudhuri, R., Alderwick,
- 615 L.J., Lee, D.J., Swarbreck, D., Liddell, S., et al. (2013). Activity of Bdellovibrio Hit Locus
- 616 Proteins, Bd0108 and Bd0109, Links Type IVa Pilus Extrusion/Retraction Status to Prey-
- 617 Independent Growth Signalling. PLoS ONE 8, e79759.
- 618 https://doi.org/10.1371/journal.pone.0079759.

- 619 Caulton, S.G., and Lovering, A.L. (2020). Bacterial invasion and killing by predatory
- 620 Bdellovibrio primed by predator prey cell recognition and self protection. Current Opinion in
- 621 Microbiology 56, 74–80. https://doi.org/10.1016/j.mib.2020.07.002.
- 622 Cavallo, F.M., Jordana, L., Friedrich, A.W., Glasner, C., and van Dijl, J.M. (2021). Bdellovibrio
- 623 *bacteriovorus* : a potential 'living antibiotic' to control bacterial pathogens. Critical Reviews in
- 624 Microbiology 1–17. https://doi.org/10.1080/1040841X.2021.1908956.
- 625 Chaban, B., Coleman, I., and Beeby, M. (2018). Evolution of higher torque in Campylobacter-
- type bacterial flagellar motors. Scientific Reports 8. https://doi.org/10.1038/s41598-017-181151.
- 628 Chang, Y.-W., Rettberg, L.A., Treuner-Lange, A., Iwasa, J., Søgaard-Andersen, L., and Jensen,
- 629 G.J. (2016). Architecture of the type IVa pilus machine. Science 351, aad2001.
- 630 https://doi.org/10.1126/science.aad2001.
- 631 Chang, Y.-W., Kjær, A., Ortega, D.R., Kovacikova, G., Sutherland, J.A., Rettberg, L.A., Taylor,
- 632 R.K., and Jensen, G.J. (2017). Architecture of the Vibrio cholerae toxin-coregulated pilus
- 633 machine revealed by electron cryotomography. Nature Microbiology 2.
- 634 https://doi.org/10.1038/nmicrobiol.2016.269.
- 635 Chanyi, R.M., and Koval, S.F. (2014). Role of Type IV Pili in Predation by Bdellovibrio
- bacteriovorus. PLoS ONE 9, e113404. https://doi.org/10.1371/journal.pone.0113404.
- 637 Chen, M., Bell, J.M., Shi, X., Sun, S.Y., Wang, Z., and Ludtke, S.J. (2019). A complete data
- 638 processing workflow for cryo-ET and subtomogram averaging. Nat Methods 16, 1161–1168.
- 639 https://doi.org/10.1038/s41592-019-0591-8.
- 640 Chreifi, G., Chen, S., Metskas, L.A., Kaplan, M., and Jensen, G.J. (2019). Rapid tilt-series
- 641 acquisition for electron cryotomography. Journal of Structural Biology *205*, 163–169.
- 642 https://doi.org/10.1016/j.jsb.2018.12.008.
- 643 Cohen, E.J., Nakane, D., Kabata, Y., Hendrixson, D.R., Nishizaka, T., and Beeby, M. (2020).
- 644 Campylobacter jejuni motility integrates specialized cell shape, flagellar filament, and motor, to
- 645 coordinate action of its opposed flagella. PLoS Pathog 16, e1008620.
- 646 https://doi.org/10.1371/journal.ppat.1008620.
- 647 Constantino, M.A., Jabbarzadeh, M., Fu, H.C., Shen, Z., Fox, J.G., Haesebrouck, F., Linden,
- 648 S.K., and Bansil, R. (2018). Bipolar lophotrichous Helicobacter suis combine extended and
- 649 wrapped flagella bundles to exhibit multiple modes of motility. Sci Rep 8, 14415.
- 650 https://doi.org/10.1038/s41598-018-32686-7.
- Davidov, Y., and Jurkevitch, E. (2009). Predation between prokaryotes and the origin of
- 652 eukaryotes. BioEssays *31*, 748–757. https://doi.org/10.1002/bies.200900018.
- Ding, H.J., Oikonomou, C.M., and Jensen, G.J. (2015). The Caltech Tomography Database and
- Automatic Processing Pipeline. Journal of Structural Biology 192, 279–286.
- 655 https://doi.org/10.1016/j.jsb.2015.06.016.

- Dobro, M.J., Oikonomou, C.M., Piper, A., Cohen, J., Guo, K., Jensen, T., Tadayon, J.,
- 657 Donermeyer, J., Park, Y., Solis, B.A., et al. (2017). Uncharacterized Bacterial Structures
- 658 Revealed by Electron Cryotomography. Journal of Bacteriology *199*.
- 659 https://doi.org/10.1128/JB.00100-17.
- 660 Dori-Bachash, M., Dassa, B., Pietrokovski, S., and Jurkevitch, E. (2008). Proteome-Based
- 661 Comparative Analyses of Growth Stages Reveal New Cell Cycle-Dependent Functions in the
- 662 Predatory Bacterium *Bdellovibrio bacteriovorus*. Appl Environ Microbiol 74, 7152–7162.
- 663 https://doi.org/10.1128/AEM.01736-08.
- Eisenstein, F., Danev, R., and Pilhofer, M. (2019). Improved applicability and robustness of fast
- 665 cryo-electron tomography data acquisition. Journal of Structural Biology 208, 107–114.
 666 https://doi.org/10.1016/j.jsb.2019.08.006.
- 667 Erken, M., Lutz, C., and McDougald, D. (2013). The Rise of Pathogens: Predation as a Factor
- 668 Driving the Evolution of Human Pathogens in the Environment. Microb Ecol 65, 860–868.
- 669 https://doi.org/10.1007/s00248-013-0189-0.
- Evans, K.J., Lambert, C., and Sockett, R.E. (2007). Predation by Bdellovibrio bacteriovorus
 HD100 Requires Type IV Pili. JB *189*, 4850–4859. https://doi.org/10.1128/JB.01942-06.
- 672 Fenton, A.K., Kanna, M., Woods, R.D., Aizawa, S.-I., and Sockett, R.E. (2010a). Shadowing the
- 673 Actions of a Predator: Backlit Fluorescent Microscopy Reveals Synchronous Nonbinary
- 674 Septation of Predatory Bdellovibrio inside Prey and Exit through Discrete Bdelloplast Pores. JB
- 675 *192*, 6329–6335. https://doi.org/10.1128/JB.00914-10.
- 676 Fenton, A.K., Hobley, L., Butan, C., Subramaniam, S., and Sockett, R.E. (2010b). A coiled-coil-
- 677 repeat protein 'Ccrp' in Bdellovibrio bacteriovorus prevents cellular indentation, but is not
- 678 essential for vibroid cell morphology: CCRP protein in Bdellovibrio. FEMS Microbiology
- 679 Letters 313, 89–95. https://doi.org/10.1111/j.1574-6968.2010.02125.x.
- 680 Ferreira, J.L., Gao, F.Z., Rossmann, F.M., Nans, A., Brenzinger, S., Hosseini, R., Wilson, A.,
- Briegel, A., Thormann, K.M., Rosenthal, P.B., et al. (2019). γ-proteobacteria eject their polar
- flagella under nutrient depletion, retaining flagellar motor relic structures. PLOS Biology 17,
- 683 e3000165. https://doi.org/10.1371/journal.pbio.3000165.
- 684 Friedrich, C., Bulyha, I., and Søgaard-Andersen, L. (2014). Outside-In Assembly Pathway of the
- Type IV Pilus System in Myxococcus xanthus. J Bacteriol 196, 378–390.
- 686 https://doi.org/10.1128/JB.01094-13.
- 687 Ghosal, D., Kaplan, M., Chang, Y.-W., and Jensen, G.J. (2019a). In Situ Imaging and Structure
- 688 Determination of Bacterial Toxin Delivery Systems Using Electron Cryotomography. In
- 689 Legionella, C. Buchrieser, and H. Hilbi, eds. (New York, NY: Springer New York), pp. 249–
- 690265.
- 691 Ghosal, D., Kim, K.W., Zheng, H., Kaplan, M., Truchan, H.K., Lopez, A.E., McIntire, I.E.,
- 692 Vogel, J.P., Cianciotto, N.P., and Jensen, G.J. (2019b). In vivo structure of the Legionella type II

- 693 secretion system by electron cryotomography. Nature Microbiology
- 694 https://doi.org/10.1038/s41564-019-0603-6.
- 695 Gold, V.A., Salzer, R., Averhoff, B., and Kühlbrandt, W. (2015). Structure of a type IV pilus 696 machinery in the open and closed state. ELife *4*. https://doi.org/10.7554/eLife.07380.
- Hagen, W.J.H., Wan, W., and Briggs, J.A.G. (2017). Implementation of a cryo-electron
- tomography tilt-scheme optimized for high resolution subtomogram averaging. J. Struct. Biol.
- 699 197, 191–198. https://doi.org/10.1016/j.jsb.2016.06.007.
- 700 Harding, C.J., Huwiler, S.G., Somers, H., Lambert, C., Ray, L.J., Till, R., Taylor, G., Moynihan,
- P.J., Sockett, R.E., and Lovering, A.L. (2020). A lysozyme with altered substrate specificity
- facilitates prey cell exit by the periplasmic predator Bdellovibrio bacteriovorus. Nat Commun 11,
- 703 4817. https://doi.org/10.1038/s41467-020-18139-8.
- Harini, K., Ajila, V., and Hegde, S. (2013). Bdellovibrio bacteriovorus : A future antimicrobial
 agent? J Indian Soc Periodontol *17*, 823. https://doi.org/10.4103/0972-124X.124534.
- Heumann, J.M., Hoenger, A., and Mastronarde, D.N. (2011). Clustering and variance maps for
 cryo-electron tomography using wedge-masked differences. Journal of Structural Biology *175*,
 288–299. https://doi.org/10.1016/j.jsb.2011.05.011.
- Hintsche, M., Waljor, V., Großmann, R., Kühn, M.J., Thormann, K.M., Peruani, F., and Beta, C.
- 710 (2017). A polar bundle of flagella can drive bacterial swimming by pushing, pulling, or coiling
- 711 around the cell body. Sci Rep 7, 16771. https://doi.org/10.1038/s41598-017-16428-9.
- 712 Hobley, L., Fung, R.K.Y., Lambert, C., Harris, M.A.T.S., Dabhi, J.M., King, S.S., Basford, S.M.,
- 713 Uchida, K., Till, R., Ahmad, R., et al. (2012). Discrete Cyclic di-GMP-Dependent Control of
- 714 Bacterial Predation versus Axenic Growth in Bdellovibrio bacteriovorus. PLoS Pathog 8,
- 715 e1002493. https://doi.org/10.1371/journal.ppat.1002493.
- 716 Iebba, V., Totino, V., Santangelo, F., Gagliardi, A., Ciotoli, L., Virga, A., Ambrosi, C., Pompili,
- 717 M., De Biase, R.V., Selan, L., et al. (2014). Bdellovibrio bacteriovorus directly attacks
- 718 Pseudomonas aeruginosa and Staphylococcus aureus Cystic fibrosis isolates. Front. Microbiol. 5.
- 719 https://doi.org/10.3389/fmicb.2014.00280.
- 720 Iida, Y., Hobley, L., Lambert, C., Fenton, A.K., Sockett, R.E., and Aizawa, S.-I. (2009). Roles of
- 721 Multiple Flagellins in Flagellar Formation and Flagellar Growth Post Bdelloplast Lysis in
- 722 Bdellovibrio bacteriovorus. Journal of Molecular Biology *394*, 1011–1021.
- 723 https://doi.org/10.1016/j.jmb.2009.10.003.
- Jewett, A. (2021a). Jewett, AI, VISFD Software, <u>https://doi.org/10.5281/zenodo.5559243</u>.
- Jewett, A. (2021b). Jewett, AI., VISFD Tutorials, <u>https://doi.org/10.5281/zenodo.5758648</u>.
- Johnson, S., Furlong, E.J., Deme, J.C., Nord, A.L., Caesar, J.J.E., Chevance, F.F.V., Berry,
- 727 R.M., Hughes, K.T., and Lea, S.M. (2021). Molecular structure of the intact bacterial flagellar
- 728 basal body. Nat Microbiol *6*, 712–721. https://doi.org/10.1038/s41564-021-00895-y.

- 729 Kaljević, J., Saaki, T.N.V., Govers, S.K., Remy, O., van Raaphorst, R., Lamot, T., and Laloux,
- 730 G. (2021). Chromosome choreography during the non-binary cell cycle of a predatory bacterium.
- 731 Current Biology *31*, 3707-3720.e5. https://doi.org/10.1016/j.cub.2021.06.024.
- 732 Kaplan, M., Subramanian, P., Ghosal, D., Oikonomou, C.M., Pirbadian, S., Starwalt-Lee, R.,
- 733 Mageswaran, S.K., Ortega, D.R., Gralnick, J.A., El-Naggar, M.Y., et al. (2019). In situ imaging
- of the bacterial flagellar motor disassembly and assembly processes. The EMBO Journal
- 735 e100957. https://doi.org/10.15252/embj.2018100957.
- 736 Kaplan, M., Sweredoski, M.J., Rodrigues, J.P.G.L.M., Tocheva, E.I., Chang, Y.-W., Ortega,
- 737 D.R., Beeby, M., and Jensen, G.J. (2020). Bacterial flagellar motor PL-ring disassembly
- subcomplexes are widespread and ancient. Proceedings of the National Academy of Sciences
 201916935. https://doi.org/10.1073/pnas.1916935117.
- 740 Kaplan, M., Nicolas, W.J., Zhao, W., Carter, S.D., Metskas, L.A., Chreifi, G., Ghosal, D., and
- 741 Jensen, G.J. (2021a). In Situ Imaging and Structure Determination of Biomolecular Complexes
- 742 Using Electron Cryo-Tomography. In CryoEM, T. Gonen, and B.L. Nannenga, eds. (New York,
- 743 NY: Springer US), pp. 83–111.
- Kaplan, M., Wang, Y., Chreifi, G., Zhang, L., Chang, Y.-W., and Jensen, G.J. (2021b).
- Programmed flagellar ejection in Caulobacter crescentus leaves PL-subcomplexes. Journal of
 Molecular Biology 167004. https://doi.org/10.1016/j.jmb.2021.167004.
- 747 Kaplan, M., Tocheva, E.I., Briegel, A., Dobro, M.J., Chang, Y.-W., Subramanian, P., McDowall,
- A.W., Beeby, M., and Jensen, G.J. (2021c). Loss of the Bacterial Flagellar Motor Switch
- Complex upon Cell Lysis. MBio e0029821. https://doi.org/10.1128/mBio.00298-21.
- 750 Kaplan, M., Chreifi, G., Metskas, L.A., Liedtke, J., Wood, C.R., Oikonomou, C.M., Nicolas,
- 751 W.J., Subramanian, P., Zacharoff, L.A., Wang, Y., et al. (2021d). In situ imaging of bacterial
- outer membrane projections and associated protein complexes using electron cryo-tomography.
- 753 ELife 10, e73099. https://doi.org/10.7554/eLife.73099.
- Karunker, I., Rotem, O., Dori-Bachash, M., Jurkevitch, E., and Sorek, R. (2013). A Global
- 755 Transcriptional Switch between the Attack and Growth Forms of Bdellovibrio bacteriovorus.
- 756 PLoS ONE *8*, e61850. https://doi.org/10.1371/journal.pone.0061850.
- Kazhdan, M., and Hoppe, H. (2013). Screened poisson surface reconstruction. ACM Trans.
 Graph. *32*, 1–13. https://doi.org/10.1145/2487228.2487237.
- 759 Kremer, J.R., Mastronarde, D.N., and McIntosh, J.R. (1996). Computer visualization of three-
- 760 dimensional image data using IMOD. J. Struct. Biol. 116, 71–76.
- 761 https://doi.org/10.1006/jsbi.1996.0013.
- 762 Kühn, M.J., Schmidt, F.K., Eckhardt, B., and Thormann, K.M. (2017). Bacteria exploit a
- polymorphic instability of the flagellar filament to escape from traps. Proceedings of the
- 764 National Academy of Sciences *114*, 6340–6345. https://doi.org/10.1073/pnas.1701644114.

- 765 Kühn, M.J., Schmidt, F.K., Farthing, N.E., Rossmann, F.M., Helm, B., Wilson, L.G., Eckhardt,
- 766 B., and Thormann, K.M. (2018). Spatial arrangement of several flagellins within bacterial
- flagella improves motility in different environments. Nat Commun 9, 5369.
- 768 https://doi.org/10.1038/s41467-018-07802-w.
- 769 Kuru, E., Lambert, C., Rittichier, J., Till, R., Ducret, A., Derouaux, A., Gray, J., Biboy, J.,
- 770 Vollmer, W., VanNieuwenhze, M., et al. (2017). Fluorescent D-amino-acids reveal bi-cellular
- cell wall modifications important for Bdellovibrio bacteriovorus predation. Nat Microbiol 2,
- 772 1648–1657. https://doi.org/10.1038/s41564-017-0029-y.
- Laloux, G. (2020). Shedding Light on the Cell Biology of the Predatory Bacterium Bdellovibrio
 bacteriovorus. Front. Microbiol. *10*, 3136. https://doi.org/10.3389/fmicb.2019.03136.
- 775 Lambert, C., and Sockett, R.E. (2008). Laboratory Maintenance of *Bdellovibrio*. Current
- 776 Protocols in Microbiology 9, 7B.2.1-7B.2.13.
- 777 https://doi.org/10.1002/9780471729259.mc07b02s9.
- T78 Lambert, C., Evans, K.J., Till, R., Hobley, L., Capeness, M., Rendulic, S., Schuster, S.C.,
- Aizawa, S.-I., and Sockett, R.E. (2006). Characterizing the flagellar filament and the role of
- motility in bacterial prey-penetration by Bdellovibrio bacteriovorus. Mol Microbiol *60*, 274–286.
- 781 https://doi.org/10.1111/j.1365-2958.2006.05081.x.
- 782 Lambert, C., Hobley, L., Chang, C.-Y., Fenton, A., Capeness, M., and Sockett, L. (2008). A
- 783 Predatory Patchwork: Membrane and Surface Structures of Bdellovibrio bacteriovorus. In
- Advances in Microbial Physiology, (Elsevier), pp. 313–361.
- Lambert, C., Ivanov, P., and Sockett, R.E. (2010a). A Transcriptional "Scream" Early Response
- of E. coli Prey to Predatory Invasion by Bdellovibrio. Curr Microbiol 60, 419–427.
- 787 https://doi.org/10.1007/s00284-009-9559-8.
- Lambert, C., Chang, C.-Y., Capeness, M.J., and Sockett, R.E. (2010b). The First Bite-Profiling
- the Predatosome in the Bacterial Pathogen Bdellovibrio. PLoS ONE 5, e8599.
- 790 https://doi.org/10.1371/journal.pone.0008599.
- Lambert, C., Fenton, A.K., Hobley, L., and Sockett, R.E. (2011). Predatory Bdellovibrio bacteria
 use gliding motility to scout for prey on surfaces. J Bacteriol *193*, 3139–3141.
- 793 https://doi.org/10.1128/JB.00224-11.
- Lambert, C., Cadby, I.T., Till, R., Bui, N.K., Lerner, T.R., Hughes, W.S., Lee, D.J., Alderwick,
- L.J., Vollmer, W., Sockett, R.E., et al. (2015). Ankyrin-mediated self-protection during cell
- invasion by the bacterial predator Bdellovibrio bacteriovorus. Nat Commun 6, 8884.
- 797 https://doi.org/10.1038/ncomms9884.
- 798 Lambert, C., Lerner, T.R., Bui, N.K., Somers, H., Aizawa, S.-I., Liddell, S., Clark, A., Vollmer,
- 799 W., Lovering, A.L., and Sockett, R.E. (2016). Interrupting peptidoglycan deacetylation during
- 800 Bdellovibrio predator-prey interaction prevents ultimate destruction of prey wall, liberating
- 801 bacterial-ghosts. Sci Rep 6, 26010. https://doi.org/10.1038/srep26010.

- 802 Lerner, T.R., Lovering, A.L., Bui, N.K., Uchida, K., Aizawa, S.-I., Vollmer, W., and Sockett,
- 803 R.E. (2012). Specialized Peptidoglycan Hydrolases Sculpt the Intra-bacterial Niche of Predatory
- 804 Bdellovibrio and Increase Population Fitness. PLoS Pathog 8, e1002524.
- 805 https://doi.org/10.1371/journal.ppat.1002524.
- Lindeberg, T. (1998). Feature Detection with Automatic Scale Selection. International Journal of
 Computer Vision *30*, 79–116. https://doi.org/10.1023/A:1008045108935.
- Liu, J., Chen, C.-Y., Shiomi, D., Niki, H., and Margolin, W. (2011). Visualization of
- 809 bacteriophage P1 infection by cryo-electron tomography of tiny Escherichia coli. Virology 417,
- 810 304–311. https://doi.org/10.1016/j.virol.2011.06.005.
- 811 Lyons, N.A., and Kolter, R. (2015). On the evolution of bacterial multicellularity. Current
- 812 Opinion in Microbiology 24, 21–28. https://doi.org/10.1016/j.mib.2014.12.007.
- 813 Madhusoodanan, J. (2019). Inner Workings: Probing predatory bacteria as an antibacterial
- 814 remedy. Proc Natl Acad Sci USA 116, 22887–22890. https://doi.org/10.1073/pnas.1917513116.
- 815 Mahmoud, K.K., and Koval, S.F. (2010). Characterization of type IV pili in the life cycle of the
- 816 predator bacterium Bdellovibrio. Microbiology *156*, 1040–1051.
- 817 https://doi.org/10.1099/mic.0.036137-0.
- 818 Makowski, Ł., Trojanowski, D., Till, R., Lambert, C., Lowry, R., Sockett, R.E., and Zakrzewska-
- 819 Czerwińska, J. (2019). Dynamics of Chromosome Replication and Its Relationship to Predatory
- 820 Attack Lifestyles in *Bdellovibrio bacteriovorus*. Appl Environ Microbiol 85, e00730-19,
- 821 /aem/85/14/AEM.00730-19.atom. https://doi.org/10.1128/AEM.00730-19.
- 822 Makowski, Ł., Trojanowski, D., and Zakrzewska-Czerwińska, J. (2020). Live-Cell Imaging of
- 823 the Life Cycle of Bacterial Predator Bdellovibrio bacteriovorus using Time-Lapse Fluorescence
- 824 Microscopy. JoVE 61105. https://doi.org/10.3791/61105.
- 825 Martinez-Sanchez, A., Garcia, I., Asano, S., Lucic, V., and Fernandez, J.-J. (2014). Robust
- 826 membrane detection based on tensor voting for electron tomography. Journal of Structural
- 827 Biology 186, 49–61. https://doi.org/10.1016/j.jsb.2014.02.015.
- Mastronarde, D.N. (2005). Automated electron microscope tomography using robust prediction
 of specimen movements. J. Struct. Biol. *152*, 36–51. https://doi.org/10.1016/j.jsb.2005.07.007.
- 830 Meek, R.W., Cadby, I.T., Moynihan, P.J., and Lovering, A.L. (2019). Structural basis for
- 831 activation of a diguanylate cyclase required for bacterial predation in Bdellovibrio. Nat Commun
- 832 10, 4086. https://doi.org/10.1038/s41467-019-12051-6.
- 833 Milner, D.S., Till, R., Cadby, I., Lovering, A.L., Basford, S.M., Saxon, E.B., Liddell, S.,
- 834 Williams, L.E., and Sockett, R.E. (2014). Ras GTPase-Like Protein MglA, a Controller of
- 835 Bacterial Social-Motility in Myxobacteria, Has Evolved to Control Bacterial Predation by
- Bdellovibrio. PLoS Genet 10, e1004253. https://doi.org/10.1371/journal.pgen.1004253.

- 837 Negus, D., Moore, C., Baker, M., Raghunathan, D., Tyson, J., and Sockett, R.E. (2017). Predator
- 838 Versus Pathogen: How Does Predatory *Bdellovibrio bacteriovorus* Interface with the Challenges
- of Killing Gram-Negative Pathogens in a Host Setting? Annu. Rev. Microbiol. 71, 441–457.
- 840 https://doi.org/10.1146/annurev-micro-090816-093618.
- 841 Nicastro, D. (2006). The Molecular Architecture of Axonemes Revealed by Cryoelectron
- 842 Tomography. Science *313*, 944–948. https://doi.org/10.1126/science.1128618.
- 843 Núñez, M.E., Martin, M.O., Duong, L.K., Ly, E., and Spain, E.M. (2003). Investigations into the
- Life Cycle of the Bacterial Predator Bdellovibrio bacteriovorus 109J at an Interface by Atomic
- 845 Force Microscopy. Biophysical Journal *84*, 3379–3388. https://doi.org/10.1016/S0006-846 3405(03)70061.7
- 846 3495(03)70061-7.
- 847 Oikonomou, C.M., and Jensen, G.J. (2017). A new view into prokaryotic cell biology from
- 848 electron cryotomography. Nature Reviews Microbiology 15, 128.
- 849 https://doi.org/10.1038/nrmicro.2016.195.
- 850 Ortega, D.R., Yang, W., Subramanian, P., Mann, P., Kjær, A., Chen, S., Watts, K.J., Pirbadian,
- 851 S., Collins, D.A., Kooger, R., et al. (2020). Repurposing a chemosensory macromolecular
- 852 machine. Nat Commun 11, 2041. https://doi.org/10.1038/s41467-020-15736-5.
- Pantanella, F., Iebba, V., Mura, F., Dini, L., Totino, V., Neroni, B., Bonfiglio, G., Maria, T.,
- 854 Passariello, C., and Schippa, S. (2018). Behaviour of Bdellovibrio bacteriovorus in the presence
- 855 of Gram-positive Staphylococcus aureus. New Microbiol 41, 145–152. .
- 856 Pasternak, Z., Njagi, M., Shani, Y., Chanyi, R., Rotem, O., Lurie-Weinberger, M.N., Koval, S.,
- 857 Pietrokovski, S., Gophna, U., and Jurkevitch, E. (2014). In and out: an analysis of epibiotic vs
- periplasmic bacterial predators. ISME J *8*, 625–635. https://doi.org/10.1038/ismej.2013.164.
- 859 Pérez, J., Moraleda-Muñoz, A., Marcos-Torres, F.J., and Muñoz-Dorado, J. (2016). Bacterial
- 860 predation: 75 years and counting!: Bacterial predation. Environ Microbiol 18, 766–779.
- 861 https://doi.org/10.1111/1462-2920.13171.
- 862 Pettersen, E.F., Goddard, T.D., Huang, C.C., Meng, E.C., Couch, G.S., Croll, T.I., Morris, J.H.,
- 863 and Ferrin, T.E. (2021a). UCSF CHIMERAX : Structure visualization for researchers, educators,
- and developers. Protein Science *30*, 70–82. https://doi.org/10.1002/pro.3943.
- 865 Pettersen, E.F., Goddard, T.D., Huang, C.C., Meng, E.C., Couch, G.S., Croll, T.I., Morris, J.H.,
- and Ferrin, T.E. (2021b). UCSF CHIMERAX : Structure visualization for researchers, educators,
- and developers. Protein Science 30, 70–82. https://doi.org/10.1002/pro.3943.
- 868 Raghunathan, D., Radford, P.M., Gell, C., Negus, D., Moore, C., Till, R., Tighe, P.J., Wheatley,
- 869 S.P., Martinez-Pomares, L., Sockett, R.E., et al. (2019). Engulfment, persistence and fate of
- 870 Bdellovibrio bacteriovorus predators inside human phagocytic cells informs their future
- therapeutic potential. Sci Rep 9, 4293. https://doi.org/10.1038/s41598-019-40223-3.
- 872 Rendulic, S. (2004). A Predator Unmasked: Life Cycle of Bdellovibrio bacteriovorus from a
- 873 Genomic Perspective. Science *303*, 689–692. https://doi.org/10.1126/science.1093027.

- 874 Rocha, B., Paul, S., and Vashisth, H. (2020). Role of Entropy in Colloidal Self-Assembly.
- 875 Entropy 22, 877. https://doi.org/10.3390/e22080877.

876 Rotem, O., Pasternak, Z., and Jurkevitch, E. (2014). Bdellovibrio and Like Organisms. In The

877 Prokaryotes, E. Rosenberg, E.F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson, eds.

- 878 (Berlin, Heidelberg: Springer Berlin Heidelberg), pp. 3–17.
- 879 Rotem, O., Pasternak, Z., Shimoni, E., Belausov, E., Porat, Z., Pietrokovski, S., and Jurkevitch,
- 880 E. (2015). Cell-cycle progress in obligate predatory bacteria is dependent upon sequential
- sensing of prey recognition and prey quality cues. Proc Natl Acad Sci USA 112, E6028–E6037.
- 882 https://doi.org/10.1073/pnas.1515749112.
- 883 Russo, R., Kolesnikova, I., Kim, T., Gupta, S., Pericleous, A., Kadouri, D., and Connell, N.
- 884 (2018). Susceptibility of Virulent Yersinia pestis Bacteria to Predator Bacteria in the Lungs of
- 885 Mice. Microorganisms 7, 2. https://doi.org/10.3390/microorganisms7010002.
- 886 Said, N., Chatzinotas, A., and Schmidt, M. (2019). Have an Ion on It: The Life-Cycle of
- 887 Bdellovibrio bacteriovorus Viewed by Helium-Ion Microscopy. Adv. Biosys. 3, 1800250.
- 888 https://doi.org/10.1002/adbi.201800250.
- 889 Schwudke, D., Bernhardt, A., Beck, S., Madela, K., Linscheid, M.W., Appel, B., and Strauch, E.
- 890 (2005). Transcriptional Activity of the Host-Interaction Locus and a Putative Pilin Gene of
- 891 Bdellovibrio bacteriovorus in the Predatory Life Cycle. Curr Microbiol 51, 310–316.
- 892 https://doi.org/10.1007/s00284-005-0030-1.
- 893 Shatzkes, K., Tang, C., Singleton, E., Shukla, S., Zuena, M., Gupta, S., Dharani, S., Rinaggio, J.,
- 894 Connell, N.D., and Kadouri, D.E. (2017a). Effect of predatory bacteria on the gut bacterial
- microbiota in rats. Sci Rep 7, 43483. https://doi.org/10.1038/srep43483.
- Shatzkes, K., Singleton, E., Tang, C., Zuena, M., Shukla, S., Gupta, S., Dharani, S., Rinaggio, J.,
 Kadouri, D.E., and Connell, N.D. (2017b). Examining the efficacy of intravenous administration
 of predatory bacteria in rats. Sci Rep 7, 1864. https://doi.org/10.1038/s41598-017-02041-3.
- 899 Shilo, M. (1969). Morphological and Physiological Aspects of the Interaction of Bdellovibrio
- 900 with Host Bacteria. In Current Topics in Microbiology and Immunology, W. Arber, W. Braun, F.
- 901 Cramer, R. Haas, W. Henle, P.H. Hofschneider, N.K. Jerne, P. Koldovský, H. Koprowski, O.
- 902 Maaløe, et al., eds. (Berlin, Heidelberg: Springer Berlin Heidelberg), pp. 174–204.
- 903 Snellen, J.E., and Starr, M.P. (1974). Ultrastructural aspects of localized membrane damage in
- 904 Spirillum serpens VHL early in its association with Bdellovibrio bacteriovorus 109D. Arch
- 905 Microbiol 100, 179–195. https://doi.org/10.1007/BF00446316.
- Sockett, R.E. (2009). Predatory Lifestyle of *Bdellovibrio bacteriovorus*. Annu. Rev. Microbiol.
 63, 523–539. https://doi.org/10.1146/annurev.micro.091208.073346.
- Starr, M.P., and Baigent, N.L. (1966). Parasitic interaction of Bdellovibrio bacteriovorus with other bacteria. J Bacteriol *91*, 2006–2017. https://doi.org/10.1128/JB.91.5.2006-2017.1966.

- 910 Stolp, H., and Starr, M.P. (1963). Bdellovibrio bacteriovorus gen. et sp. n., a predatory,
- 911 ectoparasitic, and bacteriolytic microorganism. Antonie van Leeuwenhoek 29, 217–248.
- 912 https://doi.org/10.1007/BF02046064.

Stolp, H., and Starr, M.P. (1965). Bacteriolysis. Annu. Rev. Microbiol. 19, 79–104.

- 914 https://doi.org/10.1146/annurev.mi.19.100165.000455.
- 915 Tan, J., Zhang, X., Wang, X., Xu, C., Chang, S., Wu, H., Wang, T., Liang, H., Gao, H., Zhou,
- 916 Y., et al. (2021). Structural basis of assembly and torque transmission of the bacterial flagellar
- 917 motor. Cell S009286742100430X. https://doi.org/10.1016/j.cell.2021.03.057.
- 918 Tang, G., Peng, L., Baldwin, P.R., Mann, D.S., Jiang, W., Rees, I., and Ludtke, S.J. (2007).
- 919 EMAN2: An extensible image processing suite for electron microscopy. Journal of Structural 920 Dick and 157, 28, 46 https://doi.org/10.1016/j.jch.2006.05.000
- 920 Biology 157, 38–46. https://doi.org/10.1016/j.jsb.2006.05.009.
- 921 Thomashow, L.S., and Rittenberg, S.C. (1985a). Waveform analysis and structure of flagella and
- basal complexes from Bdellovibrio bacteriovorus 109J. J Bacteriol *163*, 1038–1046.
- 923 https://doi.org/10.1128/jb.163.3.1038-1046.1985.
- 924 Thomashow, L.S., and Rittenberg, S.C. (1985b). Isolation and composition of sheathed flagella
- from Bdellovibrio bacteriovorus 109J. J Bacteriol *163*, 1047–1054.
- 926 https://doi.org/10.1128/jb.163.3.1047-1054.1985.
- 927 Thomashow, M.F., and Rittenberg, S.C. (1978a). Intraperiplasmic growth of Bdellovibrio
- bacteriovorus 109J: solubilization of Escherichia coli peptidoglycan. J Bacteriol 135, 998–1007.
 https://doi.org/10.1128/JB.135.3.998-1007.1978.
- 930 Thomashow, M.F., and Rittenberg, S.C. (1978b). Intraperiplasmic growth of Bdellovibrio
- 931 bacteriovorus 109J: N-deacetylation of Escherichia coli peptidoglycan amino sugars. J Bacteriol
- 932 135, 1008–1014. https://doi.org/10.1128/jb.135.3.1008-1014.1978.
- 933 Thomashow, M.F., and Rittenberg, S.C. (1978c). Intraperiplasmic growth of Bdellovibrio
- 934 bacteriovorus 109J: attachment of long-chain fatty acids to escherichia coli peptidoglycan. J
- 935 Bacteriol 135, 1015–1023. https://doi.org/10.1128/jb.135.3.1015-1023.1978.
- 936 Thomashow, L. S., R., S.C. (1979). Descriptive biology of the bdellovibrios. In
- 937 Developmental Biology of Prokaryotes. Parish, J.H. (ed.). Berkeley, CA: University of
- 938 California Press. 115–138. .
- Tian, M., Wu, Z., Zhang, R., and Yuan, J. (2022). A new mode of swimming in singly
- 940 flagellated *Pseudomonas aeruginosa*. Proc. Natl. Acad. Sci. U.S.A. *119*, e2120508119.
 941 https://doi.org/10.1073/pnas.2120508119.
- Toyofuku, M., Nomura, N., and Eberl, L. (2019). Types and origins of bacterial membrane
- 943 vesicles. Nature Reviews Microbiology 17, 13–24. https://doi.org/10.1038/s41579-018-0112-2.
- 744 Treuner-Lange, A., Chang, Y.-W., Glatter, T., Herfurth, M., Lindow, S., Chreifi, G., Jensen,
- G.J., and Søgaard-Andersen, L. (2020). PilY1 and minor pilins form a complex priming the type

- 946 IVa pilus in Myxococcus xanthus. Nat Commun *11*, 5054. https://doi.org/10.1038/s41467-020947 18803-z.
- 948 Tudor, J.J., and Karp, M.A. (1994). Translocation of an outer membrane protein into prey
- 949 cytoplasmic membranes by bdellovibrios. J Bacteriol 176, 948–952.
- 950 https://doi.org/10.1128/jb.176.4.948-952.1994.
- 951 Tudor, J.J., McCann, M.P., and Acrich, I.A. (1990). A new model for the penetration of prey
- 952 cells by bdellovibrios. Journal of Bacteriology *172*, 2421–2426.
- 953 https://doi.org/10.1128/JB.172.5.2421-2426.1990.
- 954 Willis, A.R., Moore, C., Mazon-Moya, M., Krokowski, S., Lambert, C., Till, R., Mostowy, S.,
- and Sockett, R.E. (2016). Injections of Predatory Bacteria Work Alongside Host Immune Cells
- to Treat Shigella Infection in Zebrafish Larvae. Current Biology 26, 3343–3351.
- 957 https://doi.org/10.1016/j.cub.2016.09.067.
- 958 Yamaguchi, T., Makino, F., Miyata, T., Minamino, T., Kato, T., and Namba, K. (2020).
- 959 Structure of the molecular bushing of the bacterial flagellar motor (Molecular Biology).
- 260 Zheng, S.Q., Keszthelyi, B., Branlund, E., Lyle, J.M., Braunfeld, M.B., Sedat, J.W., and Agard,
- 961 D.A. (2007). UCSF tomography: an integrated software suite for real-time electron microscopic
- tomographic data collection, alignment, and reconstruction. J. Struct. Biol. 157, 138–147.
- 963 https://doi.org/10.1016/j.jsb.2006.06.005.
- Zhu, S., and Gao, B. (2020). Bacterial Flagella Loss under Starvation. Trends in Microbiology
 https://doi.org/10.1016/j.tim.2020.05.002.
- Zhu, S., Schniederberend, M., Zhitnitsky, D., Jain, R., Galán, J.E., Kazmierczak, B.I., and Liu, J.
 (2019). *In Situ* Structures of Polar and Lateral Flagella Revealed by Cryo-Electron Tomography.
 Journal of Bacteriology *201*. https://doi.org/10.1128/JB.00117-19.
- Zhuang, X.-Y., and Lo, C.-J. (2020). Construction and Loss of Bacterial Flagellar Filaments.
 Biomolecules *10*, 1528. https://doi.org/10.3390/biom10111528.
- 271 Zhuang, X., Guo, S., Li, Z., Zhao, Z., Kojima, S., Homma, M., Wang, P., Lo, C., and Bai, F.
- 972 (2020). Live-cell fluorescence imaging reveals dynamic production and loss of bacterial flagella.
- 973 Molecular Microbiology 114, 279–291. https://doi.org/10.1111/mmi.14511.
- 274 Zivanov, J., Nakane, T., Forsberg, B.O., Kimanius, D., Hagen, W.J., Lindahl, E., and Scheres,
- 975 S.H. (2018). New tools for automated high-resolution cryo-EM structure determination in
- 976 RELION-3. ELife 7, e42166. https://doi.org/10.7554/eLife.42166.
- 977

978 Figure legends

979 Figure 1: Anatomy of attack-phase B. bacteriovorus. A) A slice through an electron cryo-980 tomogram of an attack phase *B. bacteriovorus* cell, with enlarged views of the flagellated (red) 981 and biting (blue) poles. White rectangles highlight the flagellar motor (upper panel) and non-982 piliated T4aP basal body (lower panel), and the white ellipse highlights a rose-like complex. 983 Question mark points to a cross-section through a periplasmic tubular structure, and white arrow 984 to a type IVa pilus. Scale bars are 50 nm. B-D) Central slices through subtomogram averages of 985 the *B. bacteriovorus* flagellar motor (B), rose-like complex (C), and non-piliated T4aP basal body 986 (D). White arrows in D point to the extracellular ring of the T4aP basal body. Scale bars are 20 987 nm. Dashed black lines indicate a composite of slices through the tomogram at different z-heights 988 in (A), and a composite of subtomogram averages aligned on the outer and inner membrane, 989 respectively, in (C). OM = outer membrane, IM = inner membrane.

990

Figure 2: Attachment of *B. bacteriovorus* to prey. A) A slice through an electron cryo-tomogram showing a *B. bacteriovorus* cell attached to a prey (*E. coli* minicell) vi a T4aP. OM = outer membrane, IM = inner membrane. B) A Slice through an electron cryo-tomogram of *B. bacteriovorus* attached to prey (*E. coli* minicell) showing non-piliated T4aP basal bodies (white ellipses) penetrating to the prey's PG layer. C) A slice through an electron cryo-tomogram of *B. bacteriovorus* attached to prey with a polar attachment plaque. PG = peptidoglycan. Scale bars 100 nm.

998

Figure 3: *B. bacteriovorus* flagellar absorption. A) A slice through an electron cryo-tomogram
showing a *B. bacteriovorus* cell attached to a prey via an attachment plaque with its flagellum

resorbing into the periplasm. Enlargements in the red-boxed areas highlight different parts of the absorbed flagellum. Scale bar is 50 nm in the main panel, 20 nm in the enlargements. **B)** A 3D segmentation of panel (A) and an enlarged view illustrating different parts of the absorbed flagellum.

1005

1006 Figure 4: *B. bacteriovorus* prev invasion. A) A slice through an electron cryo-tomogram (left) 1007 and enlarged view (right) showing a stalled invasion by a *B. bacteriovorus* of an *E. coli* minicell. 1008 Blue arrows and inset schematic highlight the portal. B) Right (top): Cross-section through the yz1009 plane of the tomogram shown in (A) along the black dotted line indicated on the left. Note that this 1010 slice does not include the portal. Right (bottom): Average density profile taken along the white 1011 dashed line (inside the white rectangle) in the top panel. The distance between the predator's inner 1012 (IM) and outer membranes (OM) is indicated (20 nm). C) Similar to (B) but for a vz slice where 1013 the portal is visible. The distance between the predator's inner and outer membranes is indicated 1014 (10 nm). The schematics in the right panels of (A-C) represent the white-boxed areas in the 1015 corresponding slices, with the portal shown in blue, the prey outer membrane in grey, and the 1016 predator inner and outer membranes in black. Scale bars 100 nm in the left panel of (A), and 50 1017 nm in other panels.

1018

Figure 5: Anatomy of the bdelloplast. A-C) Slices (at different z-levels) through an electron cryo-tomogram of a *V. cholerae* bdelloplast containing two *B. bacteriovorus* after predator division. D) A 3D segmentation of the bdelloplast shown in (A-C). E) Enlargement (left) and 3D segmentation (right) of the white-boxed area in (A), highlighting the features of the seal. F) Enlargement (left) and 3D segmentation (right) of the white-boxed area in (C), rotated 180°,

- highlighting the prey flagellar relic. OM= outer membrane. Scale bars 100 nm in (A-C) and 50 nmin (E, F).

Figure 6: Ribosomal nucleoid lattice in the end-stage bdelloplast. A, B) Slices (at different z-levels) through an electron cryo-tomogram of an end-stage E. coli bdelloplast containing two B. *bacteriovorus* cells after predator division, highlighting the hexagonal arrangement of ribosomes around the nucleoids. C, D) Rotated views of a 3D segmentation of the nucleoids and ribosomes of the cryo-tomogram shown in (A, B). Scale bars 50 nm. E) Distances of individual ribosomes from the nucleoid surface measured in the 3D segmentations of the cryo-tomogram shown in (A&B) ("experiment," solid line), compared to a simulation of randomly distributed 20 nm-wide spheres packed in the same segmented volume ("random," dashed line). See also Movie S16.

1046 Acknowledgements

- 1047 This project was funded by the National Institutes of Health (grant R01 AI127401 to G.J.J) and a
- 1048 Baxter postdoctoral fellowship from Caltech to M.K. S.K. is supported by the Swedish Research
- 1049 Council (2019-06293). Cryo-ET work was performed in the Beckman Institute Resource Center
- 1050 for Transmission Electron Microscopy at the California Institute of Technology and the Howard
- 1051 Hughes Medical Institute Janelia Farm CryoEM Facility. We thank Daniel Villanueva Avalos for
- 1052 making the summary animation. We are deeply grateful to Prof. Liz Sockett (University of
- 1053 Nottingham) for the gift of the *B. bacteriovorus* strain and helpful advice and comments.

















