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Axon tension regulates fasciculation/defasciculation through the control of axon shaft zippering

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ABSTRACT

1 While axon fasciculation plays a key role in the development of neural networks, very little is
2 known about its dynamics and the underlying biophysical mechanisms. In a model system composed
3 of neurons grown *ex vivo* from explants of embryonic mouse olfactory epithelia, we observed that
4 axons dynamically interact with each other through their shafts, leading to zippering and unzippering
5 behaviour that regulates their fasciculation. Taking advantage of this new preparation suitable for
6 studying such interactions, we carried out a detailed biophysical analysis of zippering, occurring
7 either spontaneously or induced by micromanipulations and pharmacological treatments. We show
8 that zippering arises from the competition of axon-axon adhesion and mechanical tension in the
9 axons, and provide the first quantification of the force of axon-axon adhesion. Furthermore, we
10 introduce a biophysical model of the zippering dynamics, and we quantitatively relate the individual
11 zipper properties to global characteristics of the developing axon network. Our study uncovers a new
12 role of mechanical tension in neural development: the regulation of axon fasciculation.
13

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14 INTRODUCTION

15 In the developing nervous system, complex neural networks are built through the growth of axons from
16 their neuronal cell body of origin towards their target(s), according to specific pathfinding patterns [1].
17 These patterns are genetically controlled by molecular cues mediating interactions between axons and
18 their environment, including other axons, cells and the extracellular matrix [2]. The establishment of
19 axonal projections from a given neural tissue to its final target is in many cases a multistep process,
20 in which individual axons are sequentially guided from one area to another by a series of cues inducing
21 specific decisions at the level of axonal growth cones [3]. In many organisms and especially in vertebrates,
22 given the generally high number of neurons generated in the various areas, and the need for their massive
23 projections from one area to another, a fundamental principle governing axon pathfinding resides in the
24 control of the fasciculation and defasciculation of their axons. This control is believed to be exerted
25 at the level of axonal growth cones, which may choose to grow along other axons (fasciculation) or to
26 detach from other axons (defasciculation) (reviewed in [4]). On the one hand, fasciculation ensures robust
27 coordinated growth of a number of axons along the paths initially established by pioneering axons [5].
28 On the other hand, axon defasciculation, often associated to branching, is in many cases required for
29 individual axons to reach with precision their specific target(s), which can be distributed in large areas
30 [6, 7]. For example, motor axons emerging from spinal somatic motoneurons fasciculate in tight bundles,
31 they migrate in fascicles within spinal nerves, and they thereafter defasciculate to allow each individual
32 axon subpopulation to innervate a specific muscle cell group [8, 9].

33 While the interaction of growth cones with other axons has been the focus of numerous studies [4, 6,
34 10, 11, 12], other aspects of the process of axon fasciculation have received much less attention. In par-
35 ticular, while it seems obvious that tight fasciculation of axons is aided by adhesion between their shafts,
36 very little is known about the dynamics of shaft-shaft interactions, the underlying biophysical mecha-
37 nisms, and their potential role in the regulation of axon fasciculation. The aim of the present paper was to
38 address these issues, by analyzing axon-axon interactions and the resulting fasciculation/defasciculation
39 processes in a convenient setting. We chose the mouse olfactory epithelium as a model system. This
40 system has the advantage of comprising a single population of neurons, the olfactory sensory neurons
41 (OSNs). During their normal development from the olfactory epithelium (OE) towards their target in the
42 olfactory bulb (OB), OSN axons undergo a massive fasciculation step to form branches of the olfactory
43 nerve, followed by their defasciculation and rearrangement in the OB to project to their specific target
44 cells distributed throughout the OB glomerular layer [13, 14, 15, 16, 17].

45 Since it is currently technically challenging to image mouse OSN axon fasciculation and defasciculation
46 dynamics *in vivo*, and impossible to manipulate *in vivo* the individual axons in order to assess their
47 biophysical properties, we chose to perform our study on embryonic OE cultured explants, grown on
48 a permissive planar substrate. Using time lapse imaging, we recorded OSN axons as they grow from

49 the explants, and characterized their dynamic interactions. Surprisingly, we observed that OSN axons
50 interact extensively with each other through their axon shafts, leading to zippering and unzipping
51 behaviours that trigger their fasciculation or defasciculation, respectively. In the present paper, we
52 characterize the dynamics of these axon-axon shaft interactions, assess quantitatively the biophysical
53 parameters of these processes, and develop a biophysical model of this dynamics. Micromanipulations of
54 individual zippers, as well as pharmacologically induced perturbations of the fasciculated network, are
55 used to demonstrate unzipping by forces of functionally relevant magnitude. Our analysis supports a
56 framework in which axonal mechanical tension regulates fasciculation through the control of axon shaft
57 zippering.

58 RESULTS

59 Progressive fasciculation in cultures of olfactory epithelium explants is due to axon shaft 60 zippering

61 OSN axons grow in cultures as unbranched axons. In our experimental conditions (see MATERIALS AND
62 METHODS), the growth of these axons from OE explants was characterized by a sequence of three main
63 stages: 1) advance of the growth cones and initiation of an axon network (first 24–48 h), 2) mainte-
64 nance of the growth cones at distance from the explants, but with little or no further growth (48–72 h),
65 and 3) retraction of the growth cones and collapse of the network (3–5 d). We analysed in detail the
66 intermediate stage, during which axon shafts interact and form bundles.

67 During this stage, the initial axon network, composed of individual axons or bundles of few axons,
68 progressively evolves into a less dense network with thicker bundles, indicating that individual axons
69 and/or small bundles fasciculate together to form larger bundles (Fig. 1 and Video 1). To characterize this
70 process quantitatively, we selected a typical area of the network and manually segmented (see MATERIALS
71 AND METHODS) the images into vertices (junction points and crossings of axon segments) and edges (lines
72 connecting the vertices). Fig. 1D-G show the results of such a segmentation over a 178 min time interval,
73 the red dashed lines representing the segmented edges, and star symbols representing the vertices of
74 the network. Based on this image analysis, we determined the total length of the network and the
75 total number of vertices (junction points), and found that both these quantities decreased approximately
76 linearly with time (Fig. 1H). This trend was observed in 5 out of 6 quantitatively analysed experiments
77 from different cultures, with an average reduction of $(20 \pm 16)\%$ in length of the network over the
78 duration of the recordings (178 to 295 min). Topologically, such dynamics is reminiscent of the well-
79 known coarsening of two-dimensional foams [18, 19]; the underlying structures and forces, however, are
80 substantially different (see DISCUSSION).

81 To understand the processes that lead to this coarsening of the axon network, we examined its

82 dynamics on finer time scales. On time intervals of the order of minutes, we observed elementary
83 zipper processes, as shown in Fig. 2A, B. In an advancing zipper process, two axons or axon fascicles
84 progressively adhere to each other in a longer segment of contact and form a larger fascicle. Receding
85 zippers leading to defasciculation of axons were also observed. The zipper vertex at the meeting point of
86 the axons moves with a velocity of the order of $1 \frac{\mu\text{m}}{\text{min}}$ until it reaches a position of equilibrium (Fig. 2A).
87 In the example of Fig. 2B, two adjacent advancing zippers lead to a clear decrease in the total length of
88 the network.

89 Numerous and frequent zippers were observed throughout the network, as demonstrated in Fig. 3,
90 showing a selected time interval from the network dynamics of Fig. 1. Blue arrows in Fig. 3A,B,C point
91 to vertices that will zipper in the following frames. Red dashed lines with arrows show the resulting
92 zippered segment. The zipper processes in the upper left corner (the area marked by rectangle in
93 Fig. 3E and enlarged in Fig. 3G-J) lead to a reduction in the number of vertices, from 3 to 1 (star
94 symbols in Fig. 3G and J).

95 Axon shafts adhere to form two types of zippers: simple or entangled

96 To assess the axonal structure of the zippers, we performed scanning electron microscopic analyses of
97 our cultures (Fig. 4A). We observed at high magnification that numerous network vertices displayed
98 structures as shown in Fig. 4B,C, in which individual or small bundles of axons are adherent to each
99 other along a defined segment, while remaining parallel to each other. A zipper with such structure is
100 free to increase or decrease the length of the zippered segment, depending on the balance of the forces
101 acting at the zipper vertex; we call such zippers "simple zippers".

102 More rarely, we observed zippers with entangled structure (Fig. 4E,F). Such structure may prevent
103 the zipper from unzipping past the entanglement point; further zippering, however, remains possible.
104 In some instances, axons would cross on top of each other, without forming a zippered segment (Fig. 4D).
105 Such crossings (identified at the light microscopy level by a lack of visible adherent segment and by no
106 change in the axon direction), are marked by green symbols in Fig. 1D-G and were not counted in the
107 totals of Fig. 1H. While the entangled zippers cannot be distinguished from the simple zippers with light
108 microscopy, we examined high-magnification ($1000\times$) SEM pictures (Fig. 4 – figure supplement 1) to
109 find the following abundances of the three types of network vertices: 54 % (134 out of 247 vertices) were
110 simple zippers, 28 % (69 out of 247) entangled zippers, and 18 % (44 out of 247) crossings.

111 Besides axon shafts and their bundles, thin lateral protrusions emerging from the shafts are observed
112 in the network (Fig. 4G). These protrusions appear highly dynamic and occasionally mediate interactions
113 at a distance when they extend and touch another axon shaft.

114 The observations reported above indicate that the progressive coarsening of the axon network results
115 from zippering events driven by adhesion between the axon shafts.

116 Manipulation of axon tension alters the relative abundance of zippering and unzippering

117 In recorded time lapse sequences of network evolution in 13 explants, we typically observed that the
118 axon network coarsened in a manner similar to Fig. 1, or in some cases remained stable when the
119 recording was performed over shorter time intervals. This indicates that in general, zippering events
120 dominated over unzippering events. In a few cases, however, a de-coarsening dynamics was seen in a
121 limited area of the network. Upon examining these cases, we noticed that they were associated with an
122 apparent contraction of the explant, thus generating a pulling force on the axonal network (Fig. 1 – figure
123 supplement 1). Stimulated by this observation, we sought a pharmacological manipulation by which a
124 similar effect could be induced in a controlled manner.

125 First, we envisaged treatments aiming at rapidly enhancing growth cone motility, in view of increasing
126 the pulling force exerted by the GC on axon shafts. Since the molecular cues having such effects on OSN
127 explant cultures remain unknown, we tested in a first approach Foetal Bovine Serum (FBS), assuming
128 that some of growth factors it contains may stimulate axon outgrowth. Interestingly, while no obvious
129 effect on the growth cones was observed upon FBS addition to the culture, we found that the application
130 of 5% FBS reliably induced the explant pull. This is likely due to a cell-rounding effect of FBS on
131 cultured neurons, previously demonstrated in [20]. In Fig. 5A-D, an example is shown, with a resulting
132 de-coarsening in the axon network. Often, however, the FBS-induced pull resulted instead in a rapid
133 collapse of the entire axon network onto the explant, due presumably to a disturbance of the attachments
134 of the axons to the substrate.

135 As an alternative means to influence axon tension, we tested blebbistatin, a well known inhibitor of
136 neuronal Myosin II (NMII), previously shown to decrease cell cortex / membrane tension in a variety of
137 non-neuronal cells [21, 22]. Somewhat surprisingly, in our culture system 10 μ M blebbistatin (dissolved
138 in dimethyl sulfoxide (DMSO)) did not show visible effect on axon tension, but rather had a stabilizing
139 effect on the network: the spontaneous coarsening was inhibited while the individual zippers remained
140 mobile. No such effect was observed in control experiments in which only DMSO was added.

141 We took advantage of the stabilizing effect of blebbistatin to facilitate the analysis of the coarsening or
142 de-coarsening effects generated by subsequent treatments. In Fig. 5E-H and Fig. 5I-L, two examples are
143 shown in which FBS was applied to the pre-stabilized network and rapidly induced clear de-coarsening
144 in parts of the network, with unzippering /defasciculation dominating over zippering events.

145 In the example of Fig. 5E-H, the edge of the explant (visible near the left border of each frame)
146 retreated by approximately 20 μ m to the left, thus stretching the axon network in the horizontal direction.
147 Fig. 5 – figure supplement 1 evaluates how three candidate axon paths were deformed during the first
148 20 min after FBS was added (i.e., in between the frames F and G of Fig. 5). The lengths of the paths
149 increased by (8 to 23) μ m, i.e by 4 to 10% (panel E in Fig. 5 – figure supplement 1), while at the same
150 time, the paths tended to become more straight (panel F). The axon segments thus became significantly

151 stretched and also aligned in the direction of the pull, as expected for an object under increased tension.
152 The stretching of the axons by $\sim 15\mu\text{m}$ is expected to result in a significant tension increase of over 1 nN
153 (see DISCUSSION). This tension increase is achieved within 20 min of FBS addition and precedes the
154 changes in network configuration seen in Fig. 5G-H.

155 As a complement to the observed de-coarsening induced by a pulling force, we sought to perturb
156 the network dynamics by decreasing the tension in the axons. In previous literature, cytochalasin, an
157 inhibitor of actin polymerization, was shown to significantly lower the tension of PC-12 neurites [23].
158 Indeed we found that in our system, $2\mu\text{M}$ cytochalasin B (dissolved in DMSO) induces a change in
159 network dynamics consistent with a drop of average axon tension. In the example in Fig. 6A-C, the
160 application of cytochalasin B induced coarsening (panel C) in a network that was previously stable
161 (panels A-B). As the networks generally have a tendency to coarsen, we sought to better isolate the
162 effect of cytochalasin by applying it to networks that were pre-stabilized by blebbistatin. As shown in
163 the example frames in Fig. 6D-F and in the graphs in Fig. 6G-I, cytochalasin B induces strong network
164 coarsening within 30 min of application. No such effect was observed in control experiments in which
165 only DMSO was applied.

166 Force balance at the level of a zipper: competition of axon tension and axon-axon adhesion

167 To understand the conditions leading to zippering or unzippering, we analyze the force balance in a
168 zipper of two axons (Fig. 7). The contributing forces originate from the mechanical tension in each axon,
169 the adhesion between each axon and the substrate, and the adhesion between axons in the zippered
170 segment. In the following, we combine the mechanical tension T_0 (i.e., tensile energy per unit length of
171 the axon) and the axon-substrate adhesion (i.e., energy of adhesion per unit length of the axon) into an
172 effective tension parameter T . The zipper will be in static equilibrium when the effective tensile forces
173 are in balance with the force of adhesion between the axons. Consider for simplicity a symmetric zipper,
174 in which the tensions in both axons are equal to each other, $T=T_1=T_2$. At the vertex of the zipper
175 (Fig. 7A), the force balance condition in the direction parallel to the zippered segment is given by

$$176 \quad S = 2T \left(1 - \cos \frac{\beta}{2} \right) \quad (1)$$

177 where β is the zipper angle (Fig. 7A) and S is the force arising due to the adhesion between the axon
178 shafts. The force S may also be understood as the axon-axon adhesion energy per unit length of
179 the zippered segment, and Eq. 1 derived from the minimization of total energy (see MATERIALS AND
180 METHODS). If the tension T changes so that the equilibrium condition (Eq. 1) is no longer satisfied,
181 zippering (in the case of tension decrease) or unzippering (in the case of tension increase) will result
182 until a new equilibrium value of the zipper angle β is reached. Such changes in axon tension may occur

183 due to a rearrangement of the network configuration in the vicinity of the zipper (and hence a change in
184 forces pulling on the segments of the zipper), or directly from changes in the basal tension generated by
185 the pull of the growth cones.

186 The balance of forces at a junction of three axon segments was previously considered in [24, 25,
187 26, 27], and used to analyze the distribution of tensions in a branched axon network. Provided that
188 the junction is not strongly attached to the substrate [26], at a static branch point the tension force
189 vectors in the three segments must add to zero (and there is no axon-axon adhesion force). Breaking this
190 balance results in a fast shift in the position of the branch point and adjustment of the branch angles
191 [25], however the material composition of the branches does not immediately change. In contrast, when
192 the force balance of Eq. 1 is broken, new portions of the two unbranched zippering axons are brought
193 into contact, increasing the length of the zippered segment at the expense of the unzipped segments.

194 Measurement of axon tension allows to estimate the axon-axon adhesion energy

195 To support the explanatory framework presented above, we carried out micro-manipulation experiments
196 designed to measure the magnitude of the inter-axon adhesion force S and to investigate the dynamics
197 of individual zippers. To determine S , we used observations of zippers in static equilibrium combined
198 with measurements of the axon tension T . As seen from Eq. 1, the knowledge of T and of the zipper
199 angle β permits to calculate the magnitude of the adhesion force S .

200 It is known from previous literature that the typical value of mechanical tension in an isolated axon
201 grown in culture is of the order of 1 nN ([23] reports a wide range of rest tension values, with the
202 most common tension around 0.5 nN). Approaches using calibrated needles or Microelectromechanical
203 systems (MEMS) had been successfully used to measure the tension of axons of dorsal root ganglia
204 (DRG) neuron and PC12 neurites [23, 28], as well as motor neuron axons in *Drosophila* embryo [29]. In
205 our case, the small diameter of OSN axons (about 200 nm) makes the use of such approaches difficult,
206 because of the likely physical contact of the manipulator with the substrate resulting in an incorrect force
207 reading, as well as in the detachment of the axon from the substrate. Optical tweezers technique would
208 in principle allow the manipulation using microbeads attached to axons without touching the substrate,
209 but does not permit to achieve manipulation forces comparable to 1 nN. Therefore, we decided to use
210 the Biomembrane Force Probe (BFP) technique, in which a red blood cell is used as a force transducer
211 (Fig. 8). In this technique, streptavidin-coated glass microbeads (3 μm diameter) attached to biotinylated
212 axons are manipulated by a biotinylated red blood cell aspirated in a micropipette [30, 31]. By measuring
213 the deformation of the red blood cell, one can calculate the force with which the bead is manipulated.

214 Using the BFP technique, we determined the tension in a collection of thin network segments, pre-
215 sumably individual axons, even though we cannot exclude that some of them might have been fascicles
216 of several axons. The basis of the measurement is force equilibrium between the calibrated force of the

217 probe acting on and deforming the axon, and a restoring force, which arises from the tension in the
218 axon shaft. The measurement is described in Fig. 8. The initially straight axon (Fig. 8A) is deformed
219 by displacing the micropipette and holding it in a fixed position (Fig. 8B). The force equilibrium is
220 reached: the pulling force F_{BFP} is balanced by the projection of the axon tension in the transverse
221 direction $2T \sin \delta$, where δ is the angle of deflection of the axon (i.e. $180^\circ - 2\delta$ is the angle at the apex
222 of axon deformation). This operation is repeated for larger displacements (Fig. 8C), until the red blood
223 cell detaches from the bead, which generally occurs at a deformation angle of about $\delta \approx 5^\circ$. Fig. 8D
224 shows the time course of the pulling force measured on the probe during this experiment, as well as the
225 measured deformation angle. The force plateaux (labelled 1 to 5 in Fig. 8D and marked by black boxes)
226 correspond to the time intervals during which the micropipette position was held fixed. To extract the
227 value of the tension in the axon, a linear fit is performed on the transverse projection of pulling force vs.
228 $\sin \delta$ (Fig. 8E). The slope of the fit line gives the tension $T=906$ pN in the case of the experiment shown
229 in Fig. 8. The non zero intercept of the fit arises from calibration effects described in MATERIALS AND
230 METHODS. Out of a several dozen measurements performed, we obtained a collection of 8 measurements
231 from 7 axons that included at least 3 plateaux in each.

232 For one of the axons, two distinct values of tension were measured early and late in the experiment:
233 (432 ± 157) pN and (1665 ± 219) pN. This increase in tension was likely caused by the strong stretching
234 of the axon that occurred during this particular experiment—see Fig. 8 – figure supplement 1. Such
235 stretching is unlikely to occur during spontaneous dynamics of the axon network (without added drugs),
236 and we excluded the post-stretching data from the analysis. This experiment indicates, however, that
237 the FBS-induced pulling (Fig. 5) may have lead to very significant increases in axon tension.

238 Using the slope values and their errors calculated from the 7 remaining linear fits, we estimated the
239 distribution of the tensions in the axon population, shown in Fig. 8F. The distribution is sharply peaked
240 near 678 pN, with the mean value of 679 pN and the interquartile range (529–833) pN.

241 Technical limitations were encountered in these experiments, including the uncontrollable bead lo-
242 calization along the axons and with respect to zippers, as well as early detachment of beads from the
243 red blood cell upon pulling. The 7 measurements included in Fig. 8F correspond to the most robust
244 ones, and were obtained with beads that were not necessarily in the vicinity of a zipper vertex. It was
245 therefore not feasible to correlate the measured tension values with measured zipper angles on the level
246 of individual axons. Rather, we chose to obtain a separate set of measurements of equilibrium zipper
247 angles.

248 As zippers that are entangled (as in Fig. 4E,F) may remain static without satisfying the equilibrium
249 Eq. 1, we restricted our measurements to zippers that were observed to be mobile before reaching a
250 static configuration. In the videorecordings of the developing network, we selected 17 such zippers that
251 were approximately symmetric and appeared to consist of single axons (or possibly thin fascicles). We

252 measured the zipper angles of the equilibrated configurations (requiring stability over at least 5 min), and
253 based on these values estimated the distribution of equilibrium zipper angles in the zipper population
254 (Fig. 7C). The distribution is sharply peaked around 42° , with mean of 51.2° and interquartile range
255 $(34-60)^\circ$.

256 Based on the measured distributions of axon tensions and of equilibrium zipper angles, we then esti-
257 mated the axon-axon adhesion force S . First, we assumed that the two distributions are related to each
258 other through Eq. 1 and determined the value of S resulting in their best mutual match (see MATERIALS
259 AND METHODS), obtaining $S=88$ pN. In an alternative procedure, we estimated a joint distribution of the
260 axon tensions and equilibrium zipper angles (treating the two variables as independent), and used Eq. 1
261 to compute the corresponding distribution of adhesion parameters S (see MATERIALS AND METHODS).
262 This procedure allows for the expected variability of the values of S among zippers (e.g. due to different
263 areas of contact), and gives a maximum interquartile range of $S=(52-186)$ pN, with a median of 102 pN.

264 Induced or spontaneous dynamics of individual zippers

265 To determine the axon adhesion force more directly, not relying on the measurement of axon tension,
266 we attempted to unzipper selected zippers using a calibrated pulling force. These attempts were not
267 successful, due to insufficient strength of the bond between the bead and the red blood cell. This
268 resulted in the detachment of the red blood cell before any significant effect on the zipper. To overcome
269 this limitation, we bypassed the red blood cell and bead and used the pipette to drag the axon directly.
270 This allowed us to use forces sufficiently large to induce unzipping at the price of losing the knowledge
271 of the force magnitude. Fig. 9 and the corresponding Video 2 show an example. By dragging one of the
272 axons of a zipper, we increased the zipper angle beyond its equilibrium value, leading to unzipping
273 accompanied by a decrease of angle (Fig. 9A-D). Then, the axon was released by lifting the pipette. The
274 axons snapped back to a smaller zipper angle which initiated a re-zipping process accompanied by an
275 increase of the angle (Fig. 9E-F), leading finally to the recovery of the initial configuration. Additional
276 similar manipulations performed on other zippers gave either the same results (Video 3), or in some
277 instances, no unzipping (Video 4). However, this latter case is likely to be due to the structural
278 organization of these particular zippers involving entangled axons (Fig. 4E,F).

279 Similarly to these cases of induced unzipping/rezipping, we view the numerous individual zip-
280 pering processes observed in the developing network (Fig. 2 and 3) as arising from force perturbations
281 that act on a zipper and move it to a new equilibrium configuration. These perturbations may consist
282 in changes in the network geometry in the vicinity of the zipper, or in changes in mechanical tension
283 within the axons that constitute the zipper. To characterize such spontaneous zipping dynamics, we
284 tracked 17 individual zipping processes within the developing network and measured how the zipper
285 configuration evolved. All 17 zippers selected for this analysis started from approximately stationary

286 initial configurations, and reached a final configuration that remained stationary for at least 5 min. Se-
 287 lected typical examples are shown in Fig. 10. The distance of the zipper vertex from the final equilibrium
 288 position is plotted as a function of time in Figs. 10A, B (the time point when equilibrium is reached is
 289 defined as $t=0$ min). It can be seen that in both advancing (Fig. 10A) and receding (Fig. 10B) zippers,
 290 the zipper vertex moves with a velocity in the range $(0.3-2) \frac{\mu\text{m}}{\text{min}}$. Fig. 10C shows, that while some zippers
 291 (R3 and A6) converge with an approximately constant velocity, others (R4 and A5) have a weakly expo-
 292 nential velocity profile, with the velocity gradually decreasing as equilibrium is approached. The former
 293 case, in which the zipper stops rather abruptly near the equilibrium position, is observed in roughly $2/3$
 294 of the evaluated examples. In Fig. 10D, the smoothed zipper angle is plotted as a function of time for 3
 295 advancing and 2 receding zippers. In these examples, the angle increases with time for advancing zippers
 296 (A1, A4, A6) and decreases with time for receding zippers (R4, R5). In some other cases (typically those
 297 in which the zipper configuration was complex, e.g. influenced by side processes) the time dependence
 298 of the zipper angle was more irregular. The full dynamics of the zippers R4 and R5 is shown in Video 5
 299 and Video 6.

300 Dynamical biophysical model of zippering driven by imbalance of tension and adhesion forces

301 Our analysis of equilibrium zipper configurations (cf. Eq. 1) was based on viewing the zippers as arising
 302 from the interplay of mechanical tension and inter-axon adhesion forces. To assess if the observed
 303 zipper dynamics is consistent with this framework, we developed a basic biophysical dynamical model,
 304 formulated as an effective equation of motion for the zipper vertex (see MATERIALS AND METHODS for
 305 the underlying assumptions and a full derivation). Consider the instantaneous configuration shown in
 306 Fig. 11A; here the axons are fixed at the points A,B,C (these may correspond to entangled connections
 307 with the rest of the network, to immobile adhesion points with the substrate, or to the soma or the growth
 308 cone), while the zipper vertex $V(x, y)$ is mobile. The condition for static equilibrium of the vertex (given
 309 by Eq. 1 in the case of a symmetric zipper and by Eqs. 9,10 in the general asymmetric case) takes into
 310 account the mechanical tension in the axons and the force arising from axon-axon adhesion. When the
 311 vertex is moving, however, additional forces arise from energy dissipation. As shown below, including
 312 these frictional forces in the force balance condition permits to obtain an equation of motion, specifying
 313 the velocity of the vertex.

314 We first describe the frictional force arising from the stretching or shortening of axons (which neces-
 315 sarily occurs during zippering or unzippering). Within the linear viscoelasticity framework, the viscous
 316 stress in each axon is proportional to the local strain rate. Assuming a uniform elongation strain in
 317 between the axon fixed points, the strain rate is simply expressed as $\frac{\dot{L}}{L}$, where L is the total length of
 318 the segments of the axon. During axon elongation or shortening, the total force acting in a cross-section

319 of the axon is therefore

$$320 \quad \tau = T + \eta^{\updownarrow} \frac{\dot{L}}{L} \quad (2)$$

321 where T denotes, as before, the axon tension, and η^{\updownarrow} is the elongation viscosity constant.

322 In addition to axon elongation/shortening, another possible source of energy dissipation consists in
 323 changes in the axon configuration in the immediate vicinity of the vertex. When the vertex advances
 324 during zippering, new regions of the axons undergo bending/unbending (internal structural changes),
 325 with corresponding viscoelastic losses. Possible non-equilibrium binding effects at the newly adhering
 326 membrane region may also result in dissipation. These energy losses are expected to result in a localized
 327 frictional force that acts at the vertex and is anti-parallel to the vertex velocity component along the
 328 axis of the zipper; i.e. this frictional force is collinear with the adhesion force $-S\widehat{VC}$. The magnitude
 329 of the combined zipper adhesion/friction force is

$$330 \quad \chi = S - \eta^Z \vec{u} \cdot (-\widehat{VC}) = S - \eta^Z u^Z \quad (3)$$

331 where η^Z is a friction constant and u^Z is the "zippering velocity", given by the projection of vertex
 332 velocity in the direction of advancing zipper (see Fig. 11A). Thus the friction force acts in the direction
 333 of the adhesion force during unzippering and in the opposite direction during zippering.

334 The balance of forces at a moving vertex may now be readily expressed. Consider for simplic-
 335 ity the case of a symmetric zipper (the asymmetric case is treated in MATERIALS AND METHODS
 336 and the APPENDIX). The dynamics preserves the symmetry, i.e., an initially symmetric configuration
 337 ($T_1=T_2=T$, $\alpha_1=\alpha_2=\beta/2$, $L_1=L_2=L$) will remain symmetric during the course of zippering. Aligning the
 338 zippering direction (i.e., the direction of the zippered segment) with the y axis, we have $u^Z = \dot{y}$ in Eq. 3
 339 and $\dot{L} = (1 - \cos \beta/2)\dot{y}$ in Eq. 2. Replacing now, in the equilibrium equation Eq. 1, T by τ (Eq. 2) and S
 340 by χ (Eq. 3), we obtain the condition expressing the total force balance in a moving vertex. Rearranging
 341 to express the zippering velocity \dot{y} , we get the equation of motion for a symmetric zipper

$$342 \quad \dot{y} = \frac{S - 2T \left(1 - \cos \frac{\beta}{2}\right)}{2\eta^{\updownarrow} \frac{(\cos \beta/2 - 1)^2}{L} + \eta^Z} \quad (4)$$

343 The terms $\cos \beta/2$ and L on the right hand side are nonlinear functions of y , and are straightforwardly
 344 expressed in terms of the coordinates of the fixed points A, B, C. The resulting differential equation
 345 (Eq. 4) cannot be solved in closed analytical form, but the predicted vertex trajectory $y(t)$ can be
 346 obtained by numerical integration.

347 We tested the equation of motion Eq. 4 by comparing it with the experimental recordings of induced
 348 zippering/unzippering dynamics in our system. We measured the zippering velocity \dot{y} and the zipper
 349 angle β during the experiment shown in Fig. 9A-F; these quantities were evaluated at 1 s intervals and

350 smoothed using a Gaussian kernel of half-width 10s. Fig. 9G demonstrates that the zipper velocity is
 351 linearly related to $1 - \cos \beta/2$. In this plot, the fast transients resulting from the axon manipulation are
 352 shown as pale blue dashed curves, while the zippering/unzippering dynamics induced by the manipulation
 353 (once the axons relaxed into an approximately symmetric configuration) is shown as solid curves. The
 354 straight red line indicates the best linear fit (from which the fast transient manipulation segments were
 355 excluded). Comparing now to Eq. 4, we see that such linear dependence is predicted when the friction
 356 in the vertex (i.e. term proportional to η^Z) dominates over the elongation friction ($\eta^Z u^Z \gg \eta^\dagger \frac{\dot{L}}{L}$). The
 357 slope of the linear fit is predicted to equal $-2T/\eta^Z$, while the predicted intercept is S/η^Z . From the ratio
 358 of the intercept ($0.0825 \frac{\mu\text{m}}{\text{s}}$) and the slope ($-4.1692 \frac{\mu\text{m}}{\text{s}}$), we therefore obtain an estimate of the ratio of
 359 the axon adhesion force S to the axon tension T . This dynamical estimate gives $S/T = 0.04$, as compared
 360 with the typical value $S/T = \frac{162 \text{ pN}}{679 \text{ pN}} = 0.24$ that we obtained from the analysis of static configurations. The
 361 induced zippering experiment of Fig. 9 was performed with axon bundles located close to the explant
 362 boundary; these have larger total tension T than the single axons forming the zippers used in our static
 363 analysis, while the adhesion parameter S is expected to scale sub-linearly with the number of axons in
 364 the bundle; this may explain the lower dynamic S/T ratio. Assuming a tension of order 2 nN, the slope
 365 of the fit indicates a value for the vertex friction constant of order $\eta^Z \sim 10^{-3} \frac{\text{Ns}}{\text{m}}$.

366 We now discuss the zippering dynamics in the case of an asymmetric zipper. The general equation
 367 of motion for the vertex is presented in the APPENDIX, and includes (in addition to the elongation
 368 and zippering friction introduced above) the friction of the axons with the substrate. Fig. 12 shows
 369 representative trajectories of the vertex obtained by numerical integration of the general equation of
 370 motion. The panel 12A displays a contour plot of the energy landscape $E(x, y)$, defined as the total
 371 tensile and adhesive energy of the zipper configuration with vertex located at (x, y) ; this energy is given by
 372 Eq. 7 (in MATERIALS AND METHODS). The energy landscape plotted in Fig. 12A corresponds to a zipper
 373 constituted by axons with tensions $T_1=1 \text{ nN}$ and $T_2=1.5 \text{ nN}$ and mutual adhesion strength $S=0.2 \text{ nN}$.
 374 The marked "final point" denotes the static equilibrium point of the landscape. The "initial point" of the
 375 trajectories in Fig. 12A corresponds to the equilibrium zipper configuration for $T_1=T_2=1 \text{ nN}$. Following
 376 a rapid increase (between time $t=0 \text{ s}$ and $t=5 \text{ s}$) of the tension in the right axon by 0.5 nN , the zipper
 377 undergoes relaxation to the new equilibrium, driven by the force given by the gradient of the energy
 378 landscape displayed (dashed) in Fig. 12A. It is seen that different forms of dominating friction (black
 379 for viscous elongation, red for substrate friction, blue for vertex-localized friction) lead to distinct paths
 380 (Fig. 12A) as well as time courses (Fig. 12B,D) of the trajectory. For comparison, the red dashed curve
 381 in Fig. 12A shows the gradient path, which would correspond to an isotropic and geometry-independent
 382 vertex friction tensor \vec{H} (see MATERIALS AND METHODS).

383 Our experimental observations (as in Fig. 9) show that a typical response of a zipper to a fast
 384 asymmetric perturbation consists of a fast lateral equilibration, followed by a slower dynamics during

385 which the vertex moves parallelly to the zippered segment. Such trajectory arises from our model in case
386 of dominant zipper friction (blue line), while it cannot be achieved through the other friction mechanisms
387 alone. We conclude that the velocity of zippering is primarily limited by the internal friction localized
388 at the zipper vertex.

389 Our observations of spontaneous zippering processes in the developing network showed that the
390 zippering velocity typically remained approximately constant (for $\sim 2/3$ of the events), with abrupt stop
391 near the equilibrium point (see Fig. 10). The velocity profiles obtained from the model in case of zippering
392 resulting from abrupt perturbation, in contrast, are exponential or double-exponential (Fig. 12D). An
393 approximately constant velocity of zippering is obtained in the model, however, when the tension is
394 assumed to increase gradually over an extended interval of minutes, rather than abruptly (Fig. 12E).
395 The corresponding trajectories are shown in Fig. 12C. In this case the paths obtained for different
396 dominating forms of friction are similar to each other. This is a consequence of the gradual increase in
397 tension: for all of the friction types considered, the relaxation dynamics is then sufficiently fast to allow
398 the zipper vertex to closely track the equilibrium point of the energy landscape, which evolves on the time
399 scale of minutes. These results suggest that in the developing network, the zippering is driven by gradual,
400 rather than abrupt, changes in the forces that act at the zipper vertex. The resulting reconfiguration of
401 the zipper may then act as a gradual perturbation acting on the zippers in the immediate vicinity.

402 To summarize, the comparison of predictions of the dynamical model with experimental observations
403 supports a framework in which the zippering arises from an imbalance of tension and adhesion forces at
404 the zipper vertex, and in which the zippering velocity is limited predominantly by friction arising from
405 internal energy dissipation in the immediate vicinity of the moving vertex.

406 Topological changes and loop stability in the evolving axon network

407 Following the analysis of the statics and dynamics of individual zippers, we establish a connection to the
408 global dynamics of developing axon network.

409 The gradual decrease of the total network length with time (Fig. 1H) indicates that in our experi-
410 mental setting, zippering is overall more frequent than unzippering. The observed decrease of the total
411 number of vertices (Fig. 1H) is likewise a natural consequence of zippering. An advancing zipper vertex
412 may eventually encounter another vertex and combine with it, resulting in a zipper consisting of thicker
413 fascicles.

414 A process of this type repeatedly observed in the developing network (Fig. 3G-J) consisted of a
415 gradual collapse of triangular loops, with the three vertices eventually converging into a single-vertex
416 quasi-stable configuration (as illustrated in Fig. 13A). During this process, the loops typically retained
417 their shape, i.e. the three zipper angles remained approximately constant during the collapse. Such
418 dynamics is expected to result from a decrease in the tension of the axons that constitute the loop,

419 such that the equilibrium zipper angle becomes larger than the current zipper angles. In such case,
420 no stable redistribution of angles is possible and the vertices advance synchronously, keeping the loop
421 shape invariant. This combined dynamics is therefore distinct from the elementary zippering process we
422 considered in Fig. 11, where it was assumed that the fixed points A, B, C were immobile, and consequently
423 the zipper angle gradually increased as the zipper approached equilibrium. A strong support for this
424 interpretation of the mechanism of loop collapse is provided by the experiments in which we used FBS to
425 generate a pull on the network, hence increasing axon tension. As seen in Fig. 5F-H, this manipulation
426 leads to the rapid opening and expansion of triangular loops in the de-coarsening areas of the network.

427 Our induced zippering experiments and model analysis showed that the zippering transients resulting
428 from sudden perturbations last for minutes, while the coarsening of the network develops over hours.
429 Such separation of time scales indicates that the network is locally near the quasi-equilibrium state cor-
430 responding to the momentaneous values of the axon tensions. The network statistics reported in Fig. 1H
431 exhibit robustly monotonous time course and low volatility, which is consistent with this assumption
432 and shows that large abrupt perturbations do not dominate the network dynamics. At a given time, the
433 majority of vertices in the network are seen to be approximately static or fluctuating around an equilib-
434 rium position, while the proportion of steadily advancing or receding zippers is minor (see Video 1). In
435 the following analysis, we will assume that the majority of the zippers have a zipper angle that is close
436 to the equilibrium value given by Eq. 1 (see also DISCUSSION).

437 Progressive fasciculation is reflected in the network distribution of zipper angles

438 The observed decrease in total network length implies that larger fascicles are gradually formed. The
439 limits of optical microscopy resolution did not allow us to reliably determine the size of fascicles forming
440 individual zippers. However, the structure of the fascicles determines their tension, and is therefore
441 expected to be reflected in the equilibrium zipper angles (cf. Eq. 1). To examine this relation, we
442 extracted the distribution of the zipper angles in the network. At each analyzed time point, the network
443 was manually segmented as in Fig. 1D-G and the angles between the graph edges were measured. At
444 each zipper vertex, the zippering angle was selected as the sharpest of the three angles between the edges,
445 unless the observed configuration indicated otherwise. Crossings (marked by green stars in Fig. 1D-G)
446 were excluded from the statistics.

447 The analysis included a total of 5 experiments in which the network coarsened (each lasting for 178 to
448 295 min), with 7 to 10 time points per experiment at which the zipper angle distribution was extracted.
449 The typical shape of the distribution is shown in Fig. 14A. Note the marked under-representation of
450 sharp zippering angles (below 20°). In the example of Fig. 14A (which corresponds to Fig. 1H), the
451 median angle of the distribution shifted to lower values during the 3 h interval (from 60° to 49°).

452 Evaluating the relation between the zipper angle distribution and the network coarsening, we found

453 a consistent trend in the 5 analysed experiments. The median zipper angle β_M overall showed a positive
 454 correlation with the total network length L (with the 5 correlation coefficients in the range (0.26–0.69)).
 455 Two examples are shown in the scatter plots of Fig. 14B, where *experiment 1* corresponds to the time
 456 interval in Fig. 1H.

457 To propose an explanation of this observation, we return to the distribution of single-axon tensions
 458 obtained using the BFP technique (Fig. 8F). We assume that the distribution of tensions of zipper-forming
 459 axons (either single or fasciculated) matches the distribution from the BFP experiments. Treating the
 460 tensions of individual axons in a fascicle of size n as independent random variables, it follows that the
 461 mean of the fascicle tension distribution scales as $\bar{T} \sim n$ and its standard deviation as $\sigma(T) \sim \sqrt{n}$.

462 To evaluate how this is reflected in the distribution of zippering angles, we use Eq. 1 with an appro-
 463 priately rescaled adhesion strength S . The adhesion force between two fascicles scales with their contact
 464 area and therefore with the fascicle surface. For a fascicle composed of n axons, the surface is expected to
 465 scale as $\sim \sqrt{n}$ (assuming that the cross-section of the fascicle remains approximately circular, rather than
 466 flattened by strong adhesion to the substrate, which is supported by the SEM micrographs presented in
 467 Fig. 4). Using these scaling rules and Eq. 6 (which follows from Eq. 1, see MATERIALS AND METHODS),
 468 we can transform the distribution of tensions $p(T)$ into the distribution of zippering angles $q(\beta)$.

469 To qualitatively assess the changes of $q(\beta)$ with fascicle growth, we made two simplifications: (i) we re-
 470 placed the experimental distribution of tensions with a lognormal distribution¹, $p(T) = \text{PDF}_{\log}(\bar{T}, \sigma(T))$,
 471 of the same mean $\bar{T} = \bar{T}_{\text{BFP}} = 0.68 \text{ nN}$ and std $\sigma(T) = \sigma_{\text{BFP}} = 0.25 \text{ nN}$, and (ii) we used a single value of
 472 S (appropriately scaled with n), ignoring its possible variance. The distribution of zipper angles is then
 473 given by Eq. 6, using the lognormal distribution of tensions with the two parameters related through scal-
 474 ing with n as $\bar{T} = n\bar{T}_{\text{BFP}}$, $\sigma(T) = \sqrt{n}\sigma_{\text{BFP}}$, and using the scaled value of adhesive strength $S = \sqrt{n} \cdot 0.17 \text{ nN}$.
 475 As shown in Fig. 14C, this analysis predicts that a coarsening-induced increase in mean fascicle size n
 476 leads to a lower median zipper angle, in agreement with the trend seen in the experimental data. The
 477 orange curve in Fig. 14C is the angle distribution with parameters set to the BFP-derived values, while
 478 the green curve is the predicted distribution after rescaling of fascicle size n by factor 1.50. This factor
 479 was obtained from the data in Fig. 1H and from the expected scaling $n \sim 1/D$, where D is the total
 480 network length per unit area.

481 **The structure of a sensory neurite plexus in *Xenopus* embryo is consistent with the dynamical**
 482 **zippering framework**

483 Strong connection points can be established between our dynamical observations and the *in vivo* ob-
 484 servations of Roberts and Taylor [32], who studied the formation of the sensory neurite plexus on the

¹We verified numerically that the lognormal approximation of tension distribution for fascicles of size $n \geq 2$ closely corresponds to the tension distribution obtained by n -fold convolution of the single-axon distribution.

485 basal lamina of trunk skin in *Xenopus* embryos. In [32], the neurite network on the trunk and the
486 inside skin surface was examined using electron microscopy at magnification 1000–2500, and the angles
487 between neurites that fasciculated or crossed (“incidence angles”) were determined. As shown in the
488 inset of Fig. 15, the distribution of the fasciculation incidence angles in [32] is similar to the distribution
489 of zipper angles measured in our system. Small angles (between 0° – 30°) are notably absent from the
490 recorded angle distributions (see also Fig. 14A), while these angles would be a priori expected to be
491 equally represented in an isotropically growing network (and overrepresented in a network with a pre-
492 ferred direction of growth). Roberts and Taylor proposed that this was a result of zippering processes
493 analogous to the ones that we directly observed in our study. Thus, if a growing axon encounters another
494 axon at an initially small incidence angle and starts following it (Fig. 13B1,B2), the segment behind the
495 growth cone subsequently zippers and the incidence angle increases until the equilibrium zipper angle
496 is reached (Fig. 13B3). Our observations of zippering dynamics are consistent with this proposal. The
497 under-representation of small angles in the zipper angle distributions (Figs. 14A,B and 15) thus further
498 supports our inference that most zippers are close to local equilibrium during the development of the
499 network.

500 In addition to extracting the distribution of incidence angles for fasciculated neurites, Roberts et al.
501 determined the probability for two neurites to cross (rather than fasciculate). This crossing probability
502 $\Pi(\beta_{\text{inc}})$ was found to depend strongly on the incidence angle β_{inc} (Fig. 15). Using our analysis framework,
503 we can quantitatively explain this observed dependence. A given pair of axons will not fasciculate (zipper)
504 if their equilibrium zippering angle β_{eq} is smaller than their initial incidence angle β_{inc} . Any zippered
505 segment formed in this situation would be unstable towards unzipping; the axons are therefore expected
506 to cross (Fig. 13B4). Given that there is a distribution of equilibrium zippering angles in the network
507 (see the previous section), the probability that two randomly chosen axons with initial incidence angle
508 β_{inc} will cross (rather than zipper) equals the probability of their equilibrium angle β_{eq} being smaller
509 than the initial incidence angle β_{inc} . This probability—the cumulative distribution function evaluated
510 at β_{inc} —is computed in Fig. 15, starting from the angle distribution taken from [32]. A good agreement
511 with the crossing probabilities reported in [32] is seen. We thus successfully applied our framework to
512 explain the network properties observed in the *in vivo* study of [32], indicating that this framework is
513 not limited to dynamics in culture.

514 DISCUSSION

515 Axon fasciculation is generally thought to be controlled during development at the level of growth cones,
516 which may adhere to other axons in their environment (hence driving fasciculation), or may separate
517 from other axons (hence driving defasciculation). Here we provide strong evidence for an additional

518 mechanism playing a critical role in regulating axon fasciculation, which does not involve growth cones,
519 but takes place at the level of axon shafts, through zippering of individual axons or small bundles of
520 axons. In our culture of embryonic olfactory epithelium explants, this mechanism resulted in a substantial
521 reorganization of the structure of a grown axon network, on the time scale of 10 hours. The process of
522 axon zippering has been rarely reported or discussed in previous literature. Axon zippering *in vivo* was
523 inferred in [32], and was noticed for axons growing in culture in [33] and [34]. The process of zippering
524 and the underlying biophysical mechanisms, however, were never studied. We thus decided to take
525 advantage of our novel culture system, which presents the right balance of axon tension and axon-axon
526 adhesion forces, to gain insight into this phenomenon. We undertook a detailed characterization of axon
527 zippering and un-zippering, in view of understanding its dynamics and possible biological significance.

528 Axon zippering as the result of competition between mechanical tension and axon-axon ad- 529 hesion

530 Using a combination of experimental observations and biophysical modelling, we showed that axon
531 zippering arises from the competition of two principal forces: axon-axon adhesion and mechanical tension.
532 The adhesion force favours an increase in the length of the zippered segment. The mechanical tension
533 tends to minimize the total length of the axons, thus favouring un-zippering. The relative strength of
534 these two forces determines the vertex angle between axons in a zipper that reached static equilibrium.
535 We used the BFP technique to measure the mechanical tensions of the OSN axons grown *ex vivo*,
536 and obtained values (interquartile range (529–833) pN) comparable to tensions reported in the previous
537 literature for PC-12 neurites grown in culture, on average around 650 pN [23]. Combining this information
538 with measurements of the geometry of zippers in static equilibrium, we extracted the magnitude of the
539 axon-axon adhesion force, obtaining approximately $S \approx 100$ pN (with an upper bound on its spread, the
540 interquartile range (52–186) pN). To our knowledge, this is the first measurement of the force of adhesion
541 between axon shafts, in any system. From the EM images in Fig. 4, we estimate that the fraction of
542 circumference participating in contact between two axon shafts is in the range (15–35)%. Assuming that
543 25% of the circumference adhered and converting the adhesion force S to the adhesion energy per unit
544 membrane area, one obtains $6 \times 10^{-16} \frac{\text{J}}{\mu\text{m}^2}$. This is comparable to the energy density for E-cadherin
545 mediated cell-cell adhesion, which we estimate from the separation force measurements of [35] to be
546 $(2 \times 10^{-16} \text{ to } 4 \times 10^{-15}) \frac{\text{J}}{\mu\text{m}^2}$ (obtained as $\frac{F}{3\pi R}$, where F is the separation force and R is the cell radius).

547 We were able to unzip selected zippers by manipulating them with micropipettes, with consequent re-
548 zippering after the manipulation was stopped. By fitting such induced zippering/un-zippering dynamics
549 to a basic biophysical model, we obtained an independent estimate of the axon-axon adhesion force,
550 consistent with the estimate based on static observations. Comparing the shape of the observed zipper
551 trajectories with the trajectories predicted by the dynamical model, we inferred that the zippering

552 dynamics is limited by energy dissipation arising at the zipper vertex, and estimated the corresponding
553 friction coefficient η^Z . Taking into account the typical zipper vertex velocity of $(0.3-2.0) \frac{\mu\text{m}}{\text{min}}$, the rate
554 of energy dissipation during zippering or unzippering is of order $\sim 10^{-17} \frac{\text{J}}{\text{min}}$. Our results provide a
555 first systematic characterization of the statics and dynamics of individual axon zippers. The dynamical
556 biophysical model that we developed (see MATERIALS AND METHODS) makes it possible to include axon
557 zippering in mathematical models of axon guidance and bundling. Such studies previously focused on
558 the dynamics of the growth cone, and modelled growth cone guidance by diffusible guidance cues [36],
559 the influence of tension forces and anchor points/focal adhesions on growth cone trajectory [37, 38], as
560 well as contact interactions of growth cones with other axons [39, 40]. The previous modelling studies
561 did not, however, consider the dynamics of axon shafts.

562 In the previous literature, the tendency to fasciculate was often interpreted as arising from differential
563 adhesion, i.e., the growth cone having stronger adhesion to another axon than to the substrate [32, 41].
564 The structure of the small fascicles observed in our system, with axons travelling on top of each other
565 (Fig. 4), does indicate that adhesion between axon shafts is stronger than axon-substrate adhesion. We
566 note, however, that for the zippering of axon shafts to occur, such differential advantage is in principle
567 not necessary. Two axon shafts (or axon fascicles) adhered to the substrate can gain mutual adhesion
568 energy by initiating zippering, while remaining adhered to the substrate (as in the configurations shown
569 in Fig. 4B-C). In our biophysical analysis, we assumed that adhesion to the substrate was preserved
570 during zippering, and we did not model the possible subsequent slower rearrangements (involving loss of
571 substrate contact for some axons) in the internal structure of the zippered fascicle.

572 Structure and dynamics of the axon network in light of the zippering framework

573 A striking observation, in our culture system, is that the axon network, initially established as a complex
574 network of individual axons or small bundles of axons, progressively coarsens in time, leading to the
575 formation of large fascicles of axons. The coarsening persists over time scales of about 10 hours, which
576 is much longer than the typical time scale for an individual zippering process (10 min). It is therefore
577 unlikely that the coarsening is a result of protracted equilibration of zipper configurations under sta-
578 tionary force conditions. A possible explanation of the slow coarsening dynamics may lie in a gradual
579 decrease of the average axon tension as the culture matures, which would lead to increasing domination
580 of axon-axon adhesion forces over tension, hence favouring zippering. This proposal is supported by the
581 observed sequence of stages of the culture maturation: axon elongation in the early stage, insignificant
582 elongation in the intermediate stage and axon retraction in the final stage. According to [28], axonal
583 elongation is possible only when the axon tension exceeds a certain threshold (estimated as 1 nN for
584 PC-12 axons); in our system, the arrest of growth in the intermediate stage may thus have resulted from
585 a decrease of tension below such threshold. Further indications of decreased tension are observed near

586 the end of the intermediate stage, when some growth cones visibly loose their grip to the substrate (see
587 Video 7), and hence can no longer generate axon tension by pulling [42].

588 To directly test if a decrease in average axon tension leads to coarsening, we used cytochalasin, a
589 drug that was previously shown to significantly decrease the tension of PC-12 neurites [23]. Indeed we
590 found that when the drug was applied to a slowly coarsening or stabilized network, a marked increase
591 in coarsening rate resulted within 30 min of the application (Fig. 6). Apart from the more pronounced
592 coarsening, cytochalasin did not change the structure of the network, and the axons did not become
593 visibly slack; this is consistent with the expected reduction of tension in all directions in the evolving
594 network, with enough tension remaining to keep the axons taut. While we did not measure the axon ten-
595 sion in cytochalasin-treated cultures, we did observed morphological changes (the growth cone acquiring
596 a stub-like shape with suppressed filopodia, and a reduction in number of axonal side processes) consis-
597 tent with previous studies [23, 43] in which cytochalasin-induced reduction of axon tension was assessed.
598 Similarly to [43], we also observed that in cytochalasin-treated cultures, the axons took a longer time
599 (16 min in two experiments, compared to 6 and 10 min for untreated network) to detach from the sub-
600 strate and retract when exposed to trypsin, presumably because longer proteolysis of adhesion molecules
601 is needed before the reduced tension can detach the axons [43].

602 As a second strategy aiming to perturb axon tension, we used blebbistatin, an inhibitor of NMII,
603 previously shown to decrease cell cortex/membrane tension in non-neuronal cells [21, 22] and to decrease
604 tension generated in smooth muscle [44]. In growth cones of isolated DRG neurons, Sayyad et al. [45]
605 showed that blebbistatin increased the force exerted by lamellipodia, but surprisingly reduced the force
606 exerted by filopodia. Other studies found that the blebbistatin-dependent NMII inhibition may have
607 opposite effects on axon extension, depending on the substrate. For example, Ketschek et al. showed
608 in [46] that while inhibition of NMII promotes peripheral dorsal root ganglia (DRG) axon extension on
609 polylysine, it decreases axon extension on laminin. Hur et al. [47], who observed a positive effect of
610 blebbistatin on DRG axon extension on a laminin substrate (thus the opposite result), discussed how
611 these puzzling differences may be due to differences in laminin concentrations, or by the different adhesive
612 properties of polylysine (used in [47] and in our study) and polyornithine (used by [46]). These studies
613 suggest that the while blebbistatin decreases cell cortex contractility, its effect on axon shaft tension may
614 depend on additional factors. In our model system, we did not observe an obvious effect of blebbistatin
615 on axon tension or on axon extension. Further analyses would be required to explain why blebbistatin
616 has a stabilizing effect on OSN axons grown in our experimental conditions.

617 Since the biological agents having the ability to specifically stimulate axon growth or motility in
618 our cultured explants are currently unknown, we tested FBS for this purpose, because of its established
619 content of a variety of bioactive molecules and growth factors. It turned out that FBS did not have
620 any significant boosting effect on OSN growth cones but, very interestingly, it induced the apparent

621 contraction of the whole explant itself, leading to the generation of pulling forces on axons from the
622 explant core. This apparent contraction of the explant is likely to be the result of changes in the shape
623 of the individual cells constituting the explant, if such changes lead to an overall rounding of previously
624 flattened or elongated cells. In line with this hypothesis, Jalink and Moolenaar observed in [20] that the
625 serum induced a rapid rounding of cultured differentiating neural cells, an effect which may likely be due
626 to lysophosphatidic acid, which by itself induces both cell rounding and neurite retraction [48].

627 A quantitative estimate of the increase in axon tension due to the FBS-induced pull may be obtained
628 from the measured stretch of the axons and the expected axon elongation stiffness. The measurements of
629 [23, 28] showed the spring constant of PC12 and DRG neurites (which have baseline tension and length
630 comparable to the axons in our system) to be of order $100 \frac{\text{pN}}{\mu\text{m}}$. Assuming a similar axon stiffness for our
631 system, the axon stretch of $\sim 15 \mu\text{m}$ in the FBS experiments (Fig. 5 – figure supplement 1) is expected to
632 generate a tension increase of over 1 nN. This is further supported by our tension measurements in the
633 experiment of Fig. 8 – figure supplement 1, where a pull of $6 \mu\text{m}$ was correlated with increase of tension
634 by about 1.2 nN. The estimated FBS-induced tension increase of at least 1 nN (possibly several nN) is
635 very significant compared to the typical axon tension (under 1 nN) that we recorded in the untreated
636 networks. This tension increase, which was achieved within 20 min of the application of FBS, preceded
637 a marked and rapid de-coarsening in parts of the network (Fig. 5).

638 We have thus shown on the network level that the extent of axon fasciculation can be regulated by
639 changes in axon tension: an overall decrease in tension leads to zippering-driven coarsening, while an
640 overall increase in tension leads to unzipping and de-coarsening. The estimated tension increase (of
641 order 1 nN) generated endogenously by the FBS-induced explant pull is comparable in magnitude to
642 active changes in axon tension demonstrated in previous literature, such as the tension recovery within
643 30 min after axon unloading *in vitro* [28] and *in vivo* [29]. Our results therefore demonstrate the control
644 of axon zippering by tension changes of functionally relevant magnitude.

645 As the axon network coarsens, the zippers become predominantly formed by axon fascicles, rather
646 than by individual axons. We derived the expected distribution of tensions among the fascicles and
647 combined it with the expected scaling of the fascicle-fascicle adhesion force (proportional to the surface
648 area of the fascicle) to predict how the distribution of equilibrium zipper angles in the network depends
649 on the mean number of axons per fascicle. This theoretical prediction was consistent with the observed
650 relation between the median zipper angle and network coarsening, thus supporting the framework in
651 which zippering is controlled by the competition between tension and adhesion not only for individual
652 axons, but also on the fascicle level.

653 A direct test our theoretical models, be it on the single-zipper level or on the network level, would
654 require a measurement of tension changes in the evolving network and following pharmacological manip-
655 ulations. The tension generated by the pull of the growth cone may be efficiently assessed using traction

656 force microscopy [49]. In this technique, the net traction force is determined from the deformation of a
657 suitable hydrogel substrate with embedded tracer beads, and corresponds to the axon tension directly
658 behind the growth cone [50]. One may expect, however, that far behind the growth cone, where the
659 zipper vertices are located, the axon tension can differ, due to force dissipation at substrate attachments
660 along the axon and due to the tension generated directly within the axon shaft. A recently developed
661 contact-less technique that may allow the monitoring of axon tension near the zipper vertex is thermal
662 fluctuation spectroscopy (TFS), adapted to transverse fluctuations of long protrusions. In [51], this
663 technique was used to obtain time-resolved estimates of axial tension in PC12 neurites. Each tension
664 measurement by TFS requires, however, to expose the axon to hundreds of short laser pulses, and relies
665 on a fitting of the measurements to a phenomenological biomechanical model of the axon shaft, in order
666 to extract combinations of viscoelastic parameters. A TFS procedure validated in our system would
667 potentially allow the monitoring of increases or decreases in axon tension, with sufficient temporal reso-
668 lution (~ 10 s) to correlate these tension changes with the dynamics of individual zippering events in the
669 developing network.

670 Topological analogies between progressive axon fasciculation and the coarsening of liquid 671 foams

672 Topologically, the structure of the axon network observed in our study is similar to the structure of foams
673 (froths) with a low liquid fraction. A froth consists of gas bubbles that are separated by liquid film walls,
674 with surface tension in the walls [19]. The typical structure of a "two-dimensional froth" (obtained
675 when the foam is restricted between two closely spaced glass plates) is shown in Fig. 13C. Similarly
676 to the axon network (Fig. 1), the structure is defined by vertices (junctions) at which three segments
677 under mechanical tension meet. In froths [18, 19], the tensions in the three walls are equal, resulting
678 in approximately 120° angles at the junction. In the axon network, the triple junctions are formed
679 predominantly by axon zippers. At each junction, the mechanical tension in the zippered segment is
680 necessarily the largest (being the sum of tensions in the unzipped segments), but is effectively lowered
681 by the axon-axon adhesion force (see Eq. 1); the angles between the segments are generally unequal.
682 Closed loops that are formed by axon or fascicle segments are the topological equivalent of bubbles in
683 the froth.

684 In froths, the walls of a bubble with fewer than 6 sides are on average curved, making the bubble
685 slightly rounded rather than polygonal; this is associated with a surplus of air pressure [19]. Gas diffusion
686 out (into the bubbles with lower pressure) leads to the shrinking and disappearance of the rounded
687 bubbles. This $T2$ process is illustrated for a three-sided bubble in Fig. 13D1, D2. A second type
688 of topological rearrangement (denoted $T1$ in the literature) observed in coarsening froths is shown in
689 Fig. 13D3, D4. In this process, two bubbles (at top and bottom) come into contact, pushing out the

690 bubbles at left and right; the bubble walls are reconnected. Through a combination of $T2$ and $T1$
691 rearrangements, the froth coarsens [18, 19]; typically the coarsening will proceed indefinitely (until the
692 size of the container is reached).

693 No comparable coarsening mechanism, associated with pressure differences, exists in the axon net-
694 work. However, we have repeatedly observed the shrinking and disappearance of loops formed by three
695 axon segments (see RESULTS and Fig. 13A); this is a topological analogue of the $T2$ process known
696 from foam dynamics. The reconnection of bubble walls (the $T1$ process) has no topological analogue
697 in the axon network. Rather, an attempt to implement such a process leads to a complete unzipping
698 (Fig. 13E1,E2), which is topologically equivalent to the third elementary process of foam dynamics—a
699 wall rupture [18]. Such a process was observed in the axon network only infrequently, consistent with
700 the overall predominance of zippering over unzipping.

701 As explained in RESULTS, the shrinking or expansion of a loop of axon segments can result from the
702 zipper angles at the loop vertices being smaller than or larger than the equilibrium zipper angle, respec-
703 tively. This is directly supported by our observations of expanding triangular loops in the experiments in
704 which a pull was generated on the network (Fig. 5). Basic geometry implies that on average, the zipper
705 angle is 60° at vertices that form a triangular loop (as in Fig. 13A), and 90° at vertices that form an
706 analogous rectangular loop. The equilibrium angles recorded from static zippers formed by single axons
707 or small fascicles (Fig. 7C) are predominantly below 90° , with the mean value of 51° . This suggests that
708 at the early stages of network coarsening, rectangular loops (with sides formed by single axons) are un-
709 stable towards expansion, while triangular loops are closer to equilibrium and may either slowly retract
710 or slowly expand. Such tendency may change in the later stages of network development, however, as
711 axon fascicles can form loops with complex structure and modified equilibrium angles. A more detailed
712 study would be required to characterize the stability of loops in the evolving axon network and its role
713 in the coarsening process.

714 Analogies between froths and biological tissues consisting of closely packed cells have been investigated
715 in previous literature (e.g., [52, 53]). Such analogies are complicated by two aspects: (i) the active cortical
716 mechanics of the cells (which make the interfacial tension dependent on the cell configuration [52, 54]),
717 (ii) and restrictions on the volume of animal cells (which prevent pronounced coarsening). However,
718 in [53], a growing plant tissue (meristem of *Arabidopsis thaliana*) was converted into a "living froth"
719 when oryzalin was used to depolymerize microtubules attached to the cell walls. In the resulting tissue,
720 the topology and geometry of the cell interfaces was consistent with a typical froth, and pronounced
721 coarsening of the structure was observed during the plant growth. The system we investigated—the
722 zippering axon network—presents a remarkable example of an *ex vivo* system exhibiting both topological
723 and dynamical analogies to froths. In our system, the coarsening is not limited by any cell volume
724 restrictions, and can proceed rapidly, on the time scale of hours. However, structural features such as

725 complex loop configurations and entangled zippers can limit the final extent of coarsening.

726 Axon zippering *in vivo*: its regulation and functional significance

727 We report in the present paper that OSN axons grown on a planar glass substrate covered homogeneously
728 with polylysine and laminin display extensive zippering behaviour, raising the possibility that these
729 axons, and more generally other types of axons with adhesive interactions, may zipper *in vivo*.

730 While a direct imaging of zippering dynamics *in vivo* is difficult to achieve in mice with current
731 methods, strong indirect evidence for axon zippering has been obtained in some other model organisms.
732 In *Xenopus* embryos, the geometry of the sensory neurite plexus on the basal lamina of trunk skins
733 shows features that are likely to be the result of axon zippering, as proposed by Roberts and Taylor [32]
734 and discussed in the last subsection of RESULTS. This *in vivo* configuration has strong similarities to
735 the *ex vivo* axonal network we studied—a result of the shared planar character and absence of obstacles
736 to zippering in these two systems. In *C. elegans*, axon fascicles from the left and right ventral nerve
737 cords fuse into a single fascicle if a specific medial interneuron is ablated at a late stage, when the axons
738 have already reached their targets [55]. This "axon flip-over" phenotype appears very likely to be due to
739 axon shaft zippering, as evidenced by the abnormal fasciculation profiles observed between shafts which
740 never fasciculate in non-manipulated animals. It was further shown that this phenotype was absent in
741 immobilized animals, indicating that axon zippering was facilitated by mechanical forces exerted during
742 the wriggling locomotion of the worm [55]. From a mechanistic point of view, the inhibition of zippering
743 in wild type animals is due to secretion by the medial interneuron of a 2-immunoglobulin-domain protein,
744 which was proposed to bind and inhibit the activity of homophilic molecules expressed by the left/right
745 contralaterally analogous axons [55]. This study nicely illustrates the ability of axon shafts to zipper
746 *in vivo*, in this case with detrimental developmental consequences. It further provides a framework in
747 which inhibition of axon-axon adhesion negatively regulates zippering.

748 We next discuss the zippering potential of axons in the mammalian nervous system. In the de-
749 veloping neural systems, the high volume of extracellular space [56] is highly compatible with zipper-
750 ing/unzippering of axons. Despite its reduction as the development proceeds, this extracellular space
751 (still representing 15% of the volume of the adult cortical tissue [57]) still provides a suitable environ-
752 ment for axon shaft dynamic interactions, as far as individual axons are not separated from each other
753 by glial or parenchyme boundaries. Central and peripheral myelinated axons will obviously lose their
754 zippering/unzippering abilities as soon as the myelination process begins. Similarly, unmyelinated axons
755 within peripheral nerves, which become separated from each other by Schwann cell cytoplasmic processes
756 as the nerves mature [58], will not be able to zipper thereafter. However, no such isolation by glial cells of
757 unmyelinated axons occurs in the central nervous system, where shaft-shaft contacts persist in numerous
758 areas.

759 Our work with OE explants was motivated by the projection pattern of the *in vivo* olfactory system,
760 where the growth of OSN axons from the OE towards the OB leads to the formation of fascicles. Inter-
761 estingly, the type of glial cell associated to these axon bundles, the olfactory ensheathing cells (OECs),
762 never myelinate, cover nor even separate individual axons from each other: they instead wrap large
763 bundles containing up to thousands of naked axons [59]. Within these bundles, olfactory sensory axons
764 are free to interact directly with each other, from early development onwards, because of the continuous
765 production of OSNs throughout life [60]. One can thus speculate that the zippering we observed *in vitro*
766 may occur and serve some functions *in vivo*.

767 An appealing hypothesis is that the zippering of olfactory axons may participate in the sorting of
768 olfactory axons. This sorting is indeed critical given the facts that each OSN expresses one odorant
769 receptor (OR) gene, picked out of a large repertoire of roughly 1000 OR genes, and that the axons of all
770 OSNs expressing the same OR (these OSNs are distributed across a large zone of the OE) converge into
771 a few glomeruli of the OB (reviewed in [16, 17]). This projection pattern results from a multistep process
772 involving the regulated expression of adhesion and guidance cues, some of which under the control of
773 ORs [13, 14, 15, 16, 61, 62, 63]. In addition, an OR-independent pre-sorting of OSN axons, leading to
774 the segregation of Class I vs. Class II OSN axon types within the nerve branches, has been reported
775 [64]. An OR-dependent sorting ultimately ensures that the axons of OSNs expressing the same OR are
776 segregated from each other in purely innervated glomeruli. We did not carry out labelling to distinguish
777 axon subtypes expressing for example specific adhesion molecules in our cultures, and therefore we do
778 not know if the dynamic axon-axon interactions we observed *in vitro* are related to any sorting process.
779 Since it takes about 4 days for newborn OSNs to express their OR [65], the OSN growing in our cultures
780 probably do not express their OR at the time point of our analyses, precluding that any OR-dependent
781 sorting would occur in these cultures. It remains to be investigated if zippering of olfactory axons, in
782 conjunction with OR-specific adhesion between axon shafts or OR-specific tension differences, may play
783 a role in the axon sorting process *in vivo*.

784 More generally, what are the expected functional consequences of regulated axon shaft zippering *in*
785 *vivo*? First, early on as a growth cone navigates towards its target, the ability to zipper can regulate the
786 probability with which it would cross a fascicle or not, as we illustrated in RESULTS with the Roberts
787 and Taylor data [32]. Second, once growth cones are already at distance on the way towards their
788 target area, the extent to which their shafts are fasciculated may be regulated through zippering or
789 unzippering. During both the development and maturation of neural networks, ephaptic interactions
790 between axons may be favoured in tightly fasciculated segments, thus influencing the synchrony of
791 transmitted action potentials, or generating ectopic spikes [66]. One could speculate that controlling
792 the degree of fasciculation of axons through a regulation of zippering may be used to modulate such
793 ephaptic interactions. Third, the resulting structure of the fascicles may have important consequences

794 for subsequent steps of development and maturation of the networks. Indeed, while tightly fasciculated
795 small bundles of pioneer axons constitute a robust path for follower axons, loosening the axons within
796 fascicles might be beneficial for their myelination. Finally, in pathological contexts of axon regeneration
797 following injury, or of axon demyelination, the unmyelinated axons or axon segments may zipper up in
798 tracts. In tightly bundled tracts of partially demyelinated axons, ephaptic interactions are predicted to
799 permit recovery of robust conduction [67].

800 In light of our analysis, there are two principal ways by which zippering and hence the extent of
801 fasciculation may be regulated *in vivo*. In a developing network, the growth cone activity (or shaft
802 cytoskeleton activity [68]) can change the axon tension [29] and hence influence fascicle structure on
803 fast time scales (dozen minutes). On a slower time scale, fasciculation can be regulated by changes in
804 CAM expression or their post-translational modifications. For example, it has long been established
805 that axonal N-CAM is involved in axon-axon adhesion and regulated post-translationally by addition or
806 removal of polysialic acid (PSA); high levels of PSA on N-CAM decrease cell-cell adhesion [69, 70, 71].
807 Axon fasciculation is also regulated by external guidance cues, through a variety of signalling pathways.
808 For example, matrix metalloproteases promote motor axon fasciculation in *Drosophila* [72], secreted Slit2
809 promotes motor axon fasciculation via an autocrine and/or juxtacrine mechanism in the mouse embryo
810 [73], EphA4 expressed by otic mesenchyme cells regulates in a non-cell autonomous manner the spiral
811 ganglion axon fasciculation in the mouse auditory system [74], and Neuropilin1 mediates inter-axonal
812 communications before and within the plexus region of the limb, thus regulating the fasciculation of
813 sensory and motor projections [75]. It remains to be established if and how these signals affect axon-
814 axon adhesion or possibly axon tension.

815 The tension of an axon shaft is influenced by the traction force generated by its growth cone [76],
816 which in turn depends on the mechanical properties of its environment (reviewed in [77]), likely through
817 micro-scale elastic deformation of adhesion complexes between the axon actin network and the substrate
818 [78, 79]. In [50, 80], the growth cone traction force was found to increase linearly with the substrate
819 stiffness (before saturation); a similar relation was found in non-neuronal cells [81, 82] (reviewed in [83]).
820 Spatial changes in substrate stiffness may therefore regulate the distal axon shaft tension and hence the
821 extent of zippering, potentially triggering fasciculation/defasciculation of a population of axons during
822 development, when their growth cones arrive to a specific target area. In [50], a ~ 1 nN gradual in-
823 crease in growth cone net traction force was recorded for DRG neurons plated onto a stiff substrate.
824 In our experiments with FBS-induced explant pull, we observed marked defasciculation following an
825 estimated tension increase of comparable magnitude (see previous subsection). This suggests that sig-
826 nificant changes in fasciculation may result from growth cone transitions between tissues with distinct
827 elastic properties. Similarly, the general increase in stiffness of brain tissue during development [84] may
828 gradually increase the GC traction force and as a consequence facilitate unzipping and defasciculation

829 as the growing tracts differentiate. In comparison, the substrate stiffness in our *ex vivo* experiments is
830 more homogeneous and static, simplifying the zippering-driven dynamics.

831 In conclusion, our work shows that adhesion-driven zippering of axon shafts can induce the formation
832 of axon fascicles without a direct involvement of the growth cones. However, active changes in the pulling
833 force at the growth cone, and hence in axon tension, may be used as a mechanism to control the extent
834 of zippering and to regulate fasciculation/defasciculation. Mechanical tension has been shown to play
835 important roles in neural development (reviewed in [84, 85]), and recent studies have demonstrated that
836 changes in axon tension can affect the formation of neural circuits by regulating neurite differentiation
837 [86], axon branch survival [27], as well as synaptic structure [87]. Our work introduces a novel role
838 of axon tension in neural circuit assembly: the regulation of fasciculation/defasciculation through the
839 control of axon shaft zippering.

840 MATERIALS AND METHODS

841 Olfactory epithelium explant cultures

842 All animal procedures were approved by the Île de France Ethics Committee. Pregnant female Swiss mice
843 were sacrificed by cervical elongation at embryonic day 13.5 (E13.5), embryo were extracted from the
844 uterus, and olfactory epithelium explants were prepared from the postero-dorsal quarter of the septum
845 and turbinates as follows. First, these posterior and dorsal parts of septa and turbinates were cut into
846 pieces in L15 medium (Gibco 21083) maintained on ice at 4 °C, before being subsequently incubated for
847 30 min at 25 °C in a solution of 1:1 of Trypsin 0.25 % (Gibco 25050) and Pancreatin 4X USP (Gibco
848 02-0036DG) to allow the OE to separate from the lamina propria. Enzymatic reactions were stopped by
849 adding 10 % Fetal Bovine Serum (FBS, Gibco 10270), and the biological material was rinsed in ice-cold
850 L15 containing 5 % FBS. Pieces of tissue were transferred into a glass Petri dish in which the OE sheets
851 were cut, using a micro-scalpel, into small pieces of about (100–200) μm diameter each. Explants were
852 then carefully transferred into 50 mm diameter IBIDI video dishes (Biovalley) that included a 35 mm
853 glass coverslip (for Biomembrane Force Probe experiments), or into IBIDI μ -slide 8 well #1.5 polymer
854 coverslip (Biovalley 80826) (for time lapse acquisition), previously coated with poly-L-lysine (0.2 $\frac{\text{mg}}{\text{ml}}$,
855 Sigma P1524) and Laminin (0.02 $\frac{\text{mg}}{\text{ml}}$, Sigma L2020), and maintained in culture (37 °C, 5 % CO_2) until
856 the day of experiment in a culture medium of DMEM/F12 (Gibco 31331) containing 1 % N_2 (Gibco
857 17502), 0.1 $\frac{\text{mg}}{\text{ml}}$ Gentamycin (Sigma G1272), 1.5 % D-Glucose (Sigma G8769), 1 % BSA (Sigma A4161)
858 and 7 $\frac{\mu\text{g}}{\text{ml}}$ Ascorbic acid (Sigma A4403). We typically prepared and put in culture 40 to 60 explants per
859 set of experiments coming from 10 to 12 embryos.

860 Videomicroscopy

861 10mM Hepes was added to the explant cultures 1 h before starting time lapse acquisitions. In some
862 experiments, the cultures were treated with FBS (Gibco, 5% final concentration), blebbistatin (Sigma
863 B0560, 10 μ M in culture medium containing a final concentration of 0.1% DMSO), cytochalasin B (Sigma
864 C6762, 2 μ M in culture medium containing a final concentration of 0.1% DMSO), or trypsin (Gibco 25050,
865 0.25% in culture medium). Videomicroscopy was performed on a Leica DMI 6000B inverted microscope
866 in a thermostated chamber (37 °C, 7% CO₂ at the rate 10¹/h, (87–95)% relative humidity) using a
867 DIC 63 \times NA 1.40 IMM, or a dry phase contrast objective 40 \times NA 0.75 Leica HCX PL APO, and a
868 CCDcoolSNAP HQ2 camera driven by Metamorph 7.1, in a multiple acquisition mode. Typically, 9 Z
869 steps with an interval of 1 μ m were acquired each minute for each of the 8 to 10 positions chosen around
870 explants. Recording of each experiment lasted (2–19) h.

871 Analysis of the videomicroscopy recordings

872 The pool of recordings of network evolution contained 13 explants where no drug was added, 15 explants
873 where cytochalasin was added (pre-treated with blebbistatin in 11 cases), and 10 explant where FBS was
874 added (pre-treated with blebbistatin in 5 cases). Major criteria for selection for quantitative analysis
875 were good contrast, culture survival, and sufficient area and density of the network. The reported data
876 are based on analyses performed for (i) $N = 6$ recorded experiments with no added drug (originating
877 from 3 individual animals), of which network coarsening did not occur in one experiment, which was
878 therefore excluded from statistical analyses, and (ii) $N = 12$ recorded experiments in which cytochalasin
879 was applied (originating from 6 individual animals), 8 of which were pretreated with blebbistatin.

880 The quantitative analysis was performed on video recordings in which the axonal network showed
881 clear evolution lasting over 1 h. Initial preprocessing and manual segmentation were performed using the
882 distribution Fiji [88] of the project ImageJ [89]. The field was cropped to restrict the region of interest
883 and several images (frames) were chosen from the course of the recording. The network of axons was
884 then manually segmented by drawing individual selection lines over the image. In some cases, successive
885 frames were consulted to decide whether a line is an axon to include or a transient side-process. The
886 list of segmentation selections was exported to Matlab [90], where a set of custom-made functions was
887 used to: (i) convert the list of selection lines into a graph data structure, (ii) detect cordless loops in
888 the graph, (iii) semi-automatically measure zipper angles or determine crossing points, (iv) calculate
889 the network statistics (notably the zipper angle distribution, total network length, number of vertices,
890 average area of cordless loops), and (v) determine correlations between these statistics.

891 The segmentation selections underlying the analysis shown in Fig. 6 can be found in the
892 Figure 6—source data 1 source file. They can be displayed in ImageJ by opening the TIFF file and
893 the corresponding ZIP file with the segmentation; the segmentation can be laid over the image by check-

ing the box 'Show All' on the 'ROI Manager' window. The Matlab script and input data which can be used to perform the fully automatic post-segmentation steps are provided in Figure 6—source data 2. Segmentation coordinates for Fig. 1 are provided in Figure 1—source data 1.

Regarding the data of dynamics of individual zippers, 17 events were measured by manually tracking the coordinates of the zipper vertex in consecutive frames. The measurement was accepted only if the vertex remained in static equilibrium 5 min before and after the transition. Zipper measurements were obtained from 14 explants originating from 4 mother animals. They were chosen from the networks of low density to minimize disturbance from the areas adjacent to the zipper.

Scanning Electron Microscopy

Explants were cultured on a 14 mm diameter coverslip (as described above), fixed 1 h at 4 °C in 2% glutaraldehyde prepared in 0.1 M sodium cacodylate buffer, rinsed in cacodylate buffer, dehydrated in a series of graded ethanol baths, and dried using a critical point dryer (Quorum Technologies CPD7501). They were finally mounted on a carbon stub and sputter-coated. Observations were made using a Cambridge Instruments Stereoscan 260 scanning electron microscope equipped with a digital camera.

Force measurements with a Biomembrane Force Probe (BFP)

The implementation of the BFP method [30] was adapted from [31]. Basically, this method uses a force transducer composed of a biotinylated red blood cell (RBC) held by a glass micropipette (treated with BSA), and a streptavidin-coated glass microbead (3 μm diameter), linked to the RBC by a streptavidin-biotin bond. In our experimental design, the bead was attached to axons of the culture previously treated for surface biotinylation. Within the range of forces measured in our experiments, the RBC force-deformation relation is linear, and the RBC behaves as a spring of stiffness k determined by the geometry of the probe and by the aspiration pressure ΔP within the pipette:

$$k = R_p \Delta P \frac{\pi}{\left(1 - \hat{R}_p\right) \log\left(\frac{4}{\hat{R}_c \hat{R}_p}\right) - \left(1 - \frac{1}{4} \hat{R}_p - \frac{3}{8} \hat{R}_p^2 + \hat{R}_c^2\right)} \quad (5)$$

where R_p (0.6–1.0 μm) and R_c (0.75–1.2 μm) are the internal pipette radius and the radius of contact between the RBC and the bead respectively. The hat designates the corresponding radius divided by the radius of the aspirated unstrained RBC (2–3 μm). An adjustment of the pressure allows to set up the desired stiffness, $k=100\text{--}400 \frac{\text{pN}}{\mu\text{m}}$. By measuring the extension of the RBC, we could calculate the force exerted by the probe on an attached axon.

For these experiments, 2 days *in vitro* (DIV) OE explants, cultured in 50 mm IBIDI dishes, were biotinylated using EZ-Link™ Sulfo-NHS-SS-Biotin (Thermoscientific 21328) according to the manufacturer instructions. Dishes were then transferred into the thermostated chamber (37 °C) of the Le-

925 ica DMIRB inverted microscope equipped with micropipette manipulators and a CCD digital camera
926 (purchased from JAI, Yokohama, Japan). Streptavidin beads were added to the culture, a micropipette
927 (1.5–2 μm inner diameter) was filled with the culture medium and fixed onto the mechanical micropipette
928 manipulator [31]. The diameter of the pipette was measured using the 40 \times objective and the CCD cam-
929 era. Biotinylated RBCs were added to the culture medium [31].

930 Then, a RBC was aspirated into the micropipette ($\Delta P=200\text{--}250\text{ Pa}$) and put in contact for at least
931 2 min with a bead attached to an axon or a small axon bundle. After an adhesive contact had been
932 formed between the bead and the RBC, the pipette was slowly moved (see Video 8) in order to pull or to
933 push the axon(s), being recorded by the CCD camera. In favourable cases, when the bead-axon contact
934 adhesion was sufficiently strong, the pulling or pushing of the pipette lead to a deformation (elongation
935 or compression respectively) of the RBC, often resulting in lateral deflection of the axon (see Video 8).
936 After the pipette movement, we paused to let system relax; an equilibrium would be formed between the
937 force induced by the probe and the transverse projection of the reaction force of the axon axial tension.
938 Several steps of pulling or pushing had been performed for each bead, gradually increasing applied force
939 and axon deflection, until the bead detached from the RBC. The whole process was recorded on the
940 CCD camera and the recording analysed.

941 Analysis of BFP data

942 The analysis was based on the captured recordings, recorded at rate of 65 fps. The BFPTool software
943 package [91] was used to subdivide each recorded video into intervals suitable for automated analysis,
944 and then to track the pipette and bead position with sub-pixel precision. For the algorithms used, please
945 refer to [91]. The distance of the centre of the bead and a fixed point on the pipette tip were used to
946 represent the length of RBC. The length of unloaded RBC was determined from a frame where the bead
947 first touches the approaching RBC. Having established the RBC stiffness (from geometry and pressure,
948 using Eq. 5), the applied force was calculated for every frame. The force was corrected by a projection
949 to the direction normal to manipulated axon.

950 Then, the stable plateaux would be identified in the force time course, and for each, the average force
951 F_i applied by the probe (over the duration of the plateau i) and the angle of axon deflection δ_i would be
952 determined. Such pairs of values, $(2 \sin \delta_i, F_i \sin \phi_i)$, constituted our data points for each experiment (ϕ_i
953 represents angle between pipette axis and axon axis). Finally, linear interpolation of the acquired data
954 points was performed to obtain the tensile force within the axon as the slope of the interpolating line.
955 Non-zero intercept of the interpolation line was often present; this happens when the selected reference
956 distance does not truly correspond to the unstrained size of the RBC. With the slope determined from
957 several plateaux (we chose experiments having at least three), this offset does not influence the resulting
958 calculated axonal tension.

959 The uncertainty of the tension measurement has three sources. The uncertainty of stiffness of the
 960 BFP, $k \approx 14\%$, given by the limited precision of measurement of probe radii and aspiration pressure
 961 (see Eq. 5). The uncertainty of RBC deformation measurement; while pipette pattern matching is
 962 generally very robust and precise, the tracking of the bead centre is more sensitive to perturbations
 963 and can introduce an error of (10–50) nm—see [91]. Lastly, the most important source of measurement
 964 uncertainty is the deflection angle δ_i ; the value of the deflection angle is small ($<5^\circ$) while the precision
 965 of measurement is limited by diffuse edges of axons at $\Delta\delta_i \approx 0.5^\circ$. The change of angle is small between
 966 consecutive frames (at 65 fps), so the precision can be improved by averaging several measurements,
 967 giving an upper limit of $\delta(\delta_i) \leq 25\%$ for the smallest angles.

968 The axon tension is obtained by a linear regression of time-averaged quantities; the variability of
 969 applied force and deformation angle over the duration of each plateau is shown by error bars in Fig. 8E).
 970 This error was used to assign weights to data points during the linear interpolation. The final reported
 971 error of axon tension measurement is the standard deviation of the slope.

972 Calculation of distributions of biophysical parameters in the axonal population

973 Each BFP experiment resulted in a value of tension and its experimental uncertainty for the given axon.
 974 This pair of parameters was used to construct the corresponding normal distribution, representing the
 975 tension of each axon. These Gaussian distributions were added and their sum normalized, to approximate
 976 the distribution of tensions within the whole axonal population.

977 Similarly, the set of the measured equilibrium zipper angles (described in RESULTS) was transformed
 978 into a distribution, by convolving the dataset with a Gaussian kernel (using Matlab’s kernel distribution
 979 functions).

980 Two complementary approaches were used to estimate the value of axon-axon adhesion strength
 981 S : in the first, the measured distribution of tensions and the distribution of zipper angles are fully
 982 determined by each other, while in the second approach, the tensions and angles are treated as statistically
 983 independent variables. In the first approach, a fixed value of S was assumed, and Eq. 1 was used to
 984 transform the measured distribution of tensions $p(T)$ into a distribution of angles $q(\beta)$:

$$985 \quad q(\beta) = \frac{p(T(\beta))}{\left| \frac{dT}{d\beta} \right|} = p\left(\frac{S}{2\Phi}\right) \frac{S \sqrt{2-\Phi}}{4 \Phi^{3/2}}, \text{ where } \Phi = 1 - \cos \frac{\beta}{2} \quad (6)$$

986 where the relation between T and β is specified by Eq. 1. The correspondence (evaluated as correla-
 987 tion) between the distribution $q(\beta)$ and the experimentally obtained angle distribution was maximal for
 988 $S=88$ pN (correlation coefficient 0.813). As shown in Fig. 8 – figure supplement 2A, the experimentally
 989 determined distribution was wider than the transformed distribution, suggesting that in reality the zip-
 990 pers do not all have the same value of S . In the second approach, we estimated an upper bound on the

991 spread of S values. We constructed the joint distribution, Fig. 8 – figure supplement 2B, of tensions and
 992 angles as the product of the measured tension and angle distributions (thus treating the tension and the
 993 angle as mutually independent), and computed the distribution of adhesion strengths S defined by this
 994 joint distribution and Eq. 1. To do so, the values of S were discretized in 1 pN bins and the probabilities
 995 of (tension, angle) pairs that gave S in the given bin were integrated. The resulting distribution of S is
 996 shown in Fig. 8 – figure supplement 2C. As the tensions and the angles are in reality expected to be par-
 997 tially dependent, the obtained interquartile range $S=(52-186)$ pN should be viewed as the upper bound
 998 on the spread of S values. The obtained median (102 pN) is consistent with the value of S obtained from
 999 the first approach. The Matlab scripts for performing the calculations described in this subsection are
 1000 provided as source files associated with Fig. 8 – figure supplement 2.

1001 Dynamical model of axon zippering

1002 For the general asymmetric axon zipper (as in Fig. 11A, with mobile vertex V and fixed points A, B,
 1003 C), the static equilibrium condition and the equation of motion were derived as follows.

1004 We assumed that the vertex motion is sufficiently slow to allow the tension forces to keep the axon
 1005 segments straight during zippering or unzippering; this is consistent with the experimental observations
 1006 (Fig. 3). Mechanical stresses were assumed to be uniform along each axon. We neglected elastic forces
 1007 arising from axon bending in the immediate vicinity of the zipper; in zippers formed by single axons or
 1008 small fascicles, the axons form a sharp bend (Fig. 3), indicating that the bending rigidity is low.

1009 This assumption is further supported by the following quantitative arguments. Considering the
 1010 flexural rigidity of a microtubule $(EI)_{MT} \lesssim 1 \times 10^{-1} \text{ nN}\mu\text{m}^2$ [92], at most 10 microtubules in each axon
 1011 [93], and a radius of curvature of the axons $R \approx 1 \mu\text{m}$ at the vertex, the density of energy of flexure can
 1012 be estimated as $\frac{10 \cdot (EI)_{MT}}{2R^2} \lesssim 1 \times 10^{-1} \text{ nN}$ [94, p. 127], i.e. an order of magnitude lower than the axial
 1013 tensile energy density (the axon tension, see RESULTS). For a bundle of axons, the bending energy is
 1014 expected to scale quadratically with the number of axons, while the tension scales linearly. The two
 1015 energy densities are therefore expected to become comparable in the vicinity of the zipper vertex only
 1016 for bundles of $\gtrsim 10$ axons. We note that while the energy stored in the elastic flexure is neglected in
 1017 our model, the energy dissipation resulting from the disruption of microtubule-associated cross-linking
 1018 proteins and other bending-related structural changes is included in the empirical vertex-localized friction
 1019 force introduced in RESULTS.

1020 When formulating the dynamical model of zippering, we further assume that the tension in the
 1021 constituent axons is constant in time. As the time scale for a simple zippering or unzippering process
 1022 is of order 10–20 min (see RESULTS), one cannot in principle exclude active adjustments of axon tension
 1023 accompanying the zippering or unzippering, or a coupling to active intracellular transport processes.
 1024 In previous literature, a recovery of tension within 15–60 min was shown for axons that were made

1025 slack following a large rapid distension [29]. Compared to such distension experiments, however, the
 1026 unzipping dynamics is gradual, and we assume no active tension regulation.

1027 Under these assumptions, the instantaneous zipper configuration is fully specified by the Cartesian
 1028 coordinates (x, y) of the vertex. The total tension/adhesion energy of the configuration is given by

$$1029 \quad E(x, y) = T_1(|VA| + |VC|) + T_2(|VB| + |VC|) - S|VC| \quad (7)$$

1030 where $|VX|$ denotes the length of the given axon segment, T_1 and T_2 the values of (effective) tension in
 1031 the two axons (equivalent to the tensile energy per unit length), and S the energy of inter-axon adhesion
 1032 per unit length of the adhered segment VC . (Treating the tensions T_1 and T_2 as constants independent
 1033 of the axon length, we neglect possible Hookean elasticity contributions.) The spatial gradient of the
 1034 potential energy E defines the mechanical conservative force \vec{F}_v that effectively acts at the vertex and
 1035 drives the dynamics. The vector \vec{F}_v thus points in the direction along which the energy decreases fastest
 1036 upon a displacement of the zipper vertex. One straightforwardly obtains

$$1037 \quad \vec{F}_v = -\nabla E(x, y) = T_1 \widehat{VA} + T_2 \widehat{VB} + (T_1 + T_2 - S) \widehat{VC} \quad (8)$$

1038 where \widehat{VA} indicates the unit vector in the VA direction (and similarly for VB, VC). The right-hand
 1039 side of Eq. 8 can be interpreted as the vector sum of the forces with which the axon segments VA, VB
 1040 and VC pull at the vertex. The last term, $-S \widehat{VC}$, in Eq. 8 is the force of inter-axon adhesion, which
 1041 has magnitude S and is always oriented anti-parallelly to the zippered axon segment VC .

1042 A zipper is in a static equilibrium when $\vec{F}_v = \vec{0}$. Spatial components of the force \vec{F}_v can be con-
 1043 veniently expressed in terms of the zipper angles α_1 and α_2 (see Fig. 11A). In the direction along the
 1044 zippered segment, the force equilibrium condition then becomes

$$1045 \quad -(T_1 + T_2 - S) + T_1 \cos \alpha_1 + T_2 \cos \alpha_2 = 0 \quad (9)$$

1046 while in the perpendicular direction

$$1047 \quad T_1 \sin \alpha_1 - T_2 \sin \alpha_2 = 0. \quad (10)$$

1048 Given the parameters T_1, T_2 and S , the Eqs. 9,10 specify the angles α_1 and α_2 , and hence the equilibrium
 1049 vertex position (x, y) . It is readily shown that the equilibrium defined by Eqs. 9,10 is stable (i.e., $E(x, y)$
 1050 has a local minimum at the equilibrium point). In the special case of a symmetric zipper (i.e. $T_1=T_2$),
 1051 Eq. 10 implies $\alpha_1=\alpha_2$ and Eq. 10 becomes equivalent to Eq. 1 used in our static data analysis.

1052 A nonzero driving force \vec{F}_v will result in motion of the vertex, with a velocity $\vec{u} = (\dot{x}, \dot{y})$ such that \vec{F}_v

1053 is balanced by an effective frictional force acting at the zipper vertex. (Expressed in terms of energy, the
 1054 rate of change of $E(x, y)$ when the vertex moves must equal the rate of energy dissipation in the entire
 1055 zipper configuration—see APPENDIX). Assuming a frictional force proportional to the vertex velocity,
 1056 the resulting equation of motion is

$$1057 \quad \vec{F}_v = \vec{\vec{H}}(x, y)\vec{u} \quad (11)$$

1058 where the friction tensor $\vec{\vec{H}}$ is independent of \vec{u} but may in general depend on the zipper configuration ge-
 1059 ometry, specified by the vertex position (x, y) . In the simplest case of isotropic and geometry-independent
 1060 friction, $\vec{\vec{H}} = c \cdot \mathbb{1}$ is a constant multiple of unit tensor and the integration of Eq. 11 results in a trajectory
 1061 that follows the gradient of $E(x, y)$. In case of anisotropic and/or geometry-dependent friction, however,
 1062 the vertex trajectory deviates from this path.

1063 The form of the vertex friction tensor depends on the dominant mechanism of energy dissipation.
 1064 In the main text, we introduced two forms of internal energy dissipation in the axons—the viscosity of
 1065 elongation/shortening, and the vertex-localized dissipation. As the corresponding frictional forces are
 1066 collinear with the axon tension and with the axon-axon adhesion force, respectively, one may substitute
 1067 the "dynamically corrected" tension and adhesion magnitudes (Eqs. 2,3) into the static equilibrium
 1068 condition (Eqs. 9,10), and obtain two coupled equations for the zipper velocity components \dot{x} , \dot{y} . It is
 1069 straightforward to see that in general, the vertex friction tensor resulting from either of these frictional
 1070 forces is anisotropic. $\vec{\vec{H}}$ depends on the geometry (i.e., the positions of the fixed points A, B, C) in the
 1071 case of elongation viscosity, but is geometry-independent in the case of vertex-localized friction.

1072 A third form of energy dissipation—friction between the axons and the substrate—is evaluated in
 1073 the APPENDIX. In this case, no simple prescription for generalizing the static equilibrium equation at
 1074 the zipper vertex is available. The corresponding equation of motion is derived by integrating the energy
 1075 dissipated along the axons, and equating the total rate of dissipative energy loss with the rate of gain
 1076 from conservative energy. The Rayleigh dissipation function formalism is used for the unified treatment
 1077 of all three forms of friction we consider.

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1086 ADDITIONAL FILES

1087 **Video 1.** Development and coarsening of axon network over 12 h, corresponding to Fig. 1.

1088 **Video 2.** Induced unzipping experiment corresponding to Fig. 9.

1089 **Video 3.** Induced unzipping of a zipper segment delimited by two vertices on either side. The two
1090 constitutive bundles separate, similarly to the scheme shown in Fig. 13C1,C2.

1091 **Video 4.** Induced unzipping experiment. The vertex does not recede, despite the large increase in
1092 zipper angle resulting from the manipulation by micropipette.

1093 **Video 5.** Receding zipper R4 from Fig. 10.

1094 **Video 6.** Receding zipper R5 from Fig. 10.

1095 **Video 7.** Growth cone losing grip on the substrate.

1096 **Video 8.** Illustration of a BFP experiment with overlays that mark the results of pipette and bead
1097 tracking.

1098 **Video 9.** Development of axon network over 135 min, with decoarsening visible after $t=65$ min, corre-
1099 sponding to Fig. 1 – figure supplement 1.

1100 **Video 10.** Development of axon network over 240 min, treated with FBS at $t=90$ min, corresponding
1101 to Fig. 5A-D.

1102 **Video 11.** Development of axon network over 142 min, pretreated with blebbistatin, and treated with
1103 FBS at $t=79$ min, corresponding to Fig. 5E-H.

1104 **Video 12.** Development of axon network over 142 min, pretreated with blebbistatin, and treated with
1105 FBS at $t=79$ min, corresponding to Fig. 5I-L.

1106 **Video 13.** Development of axon network over 165 min, treated with cytochalasin at $t=65$ min, corre-
1107 sponding to Fig. 6A-C (red in graphs G-I).

1108 **Video 14.** Development of axon network over 159 min, pretreated with blebbistatin, and treated with
1109 cytochalasin at $t=65$ min, corresponding to Fig. 6D-F (blue in graphs G-I).

1110 **Video 15.** Development of axon network over 159 min, pretreated with blebbistatin, and treated with
1111 cytochalasin at $t=65$ min, orange data in Fig. 6G-I.

1112 **Video 16.** Development of axon network over 129 min, pretreated with blebbistatin, and treated with
1113 cytochalasin at $t=67$ min, purple data in Fig. 6G-I.

1114 **Video 17.** Development of axon network over 166 min, pretreated with blebbistatin, and treated with
1115 cytochalasin at $t=75$ min, cyan data in Fig. 6G-I.

1116 **Figure 1—source data 1.** Segmentation coordinates (D-G), plot data (H).

1117 **Figure 4 – figure supplement 1—source data 1.** Coordinates of indicated points.

1118 **Figure 5 – figure supplement 1—source data 1.** Coordinates of paths (A-D), plot data (E,F).

1119 **Figure 6—source data 1.** ZIP archive; contains 5 ZIP archives, one per each analysed video shown
1120 in the Fig. 6G,H,I. Each video archive contains analysed frames (TIFF format) and corresponding seg-

1121 mentation selection data (ImageJ-generated ZIP archives). Data can be displayed using ImageJ, please
1122 refer to MATERIALS AND METHODS.

1123 **Figure 6—source data 2.** ZIP archive; contains source code (figure6_source_code.m) and 5 ZIP archives
1124 with selection input data. Running the code (with the 5 input archives in the same directory) performs
1125 data processing and statistical analysis, and outputs the data shown in plots G, H and I (see MATERIALS
1126 AND METHODS).

1127 **Figure 6—source data 3.** Plot data (G, H and I).

1128 **Figure 7—source data 1.** Estimated angle distribution (C) and underlying experimental angle data.

1129 **Figure 8—source data 1.** Time course of force and angle (D), plateaux averages and fit parameters
1130 (E), estimated tension distribution (F) and underlying experimental tension data.

1131 **Figure 8 – figure supplement 2—source data 1** Estimated angle distribution (Fig. 7C), angle dis-
1132 tribution as transformation of estimated tension distribution (Fig. 8F) and adhesion parameter S estimate
1133 (A), estimated angle and tension distributions (B), estimated distribution of adhesion parameter S (C).

1134 **Figure 8 – figure supplement 2—source data 2.** Source code to process input data from Figure 8
1135 – figure supplement 2—source data 1.

1136 **Figure 9—source data 1.** Velocity and angle data, fit parameters (G).

1137 **Figure 10—source data 1.** Plot data (A, B and D).

1138 **Figure 12—source data 1.** Source code of zipper model to generate plot data (A-E).

1139 **Figure 14—source data 1.** Data of histograms (A), correlations (B) and distributions (C).

1140 **Figure 14—source data 2.** Source code to generate angle distributions (C) using Eq. 6, see MATERI-
1141 ALS AND METHODS.

1142 **Figure 15—source data 2.** Crossing probabilities and angles—data [32] and distributions estimates.

1143 APPENDIX

1144 This appendix formulates the effective equation of motion for the zipper vertex, taking into account
1145 three forms of energy dissipation (i.e., three distinct frictional forces). It complements the section
1146 Dynamical model of axon zippering in MATERIALS AND METHODS, where only two forms of friction were
1147 considered and a symmetric zipper was assumed. We use the Euler-Lagrange formalism with Rayleigh
1148 function, calculated as the total energy dissipation rate in the whole zipper configuration.

1149 Assumptions

1150 The following assumptions are used:

1151 **Assumption 1.** The axons are modelled as one-dimensional viscoelastic filaments with negligible bend-
1152 ing energy.

1153 **Assumption 2.** The axon segments remain straight between the vertex and the fixation point. (The
1154 straightening dynamics is assumed to be faster than zippering dynamics.)

1155 **Assumption 3.** Longitudinal strain is assumed to be uniform along each axon. (The strain redistribu-
1156 tion along axons is assumed to be faster than the zippering dynamics.)

1157 **Assumption 4.** The dissipative forces are linear functions of local velocities and are mutually indepen-
1158 dent.

1159 Euler-Lagrange formalism with Rayleigh dissipation function

1160 Please refer to Fig. 11A for the geometry of the zipper configuration and for basic notation. The potential
1161 energy U of the configuration is given by the sum of tensile and adhesive energies $E(x, y)$, as expressed
1162 in Eq. 7 of main text. The kinetic energy E_K is negligible. The Lagrange function $L = E_K - U$ therefore
1163 becomes

$$1164 \quad L(x, y) = -(T_1 + T_2 - S) \underbrace{\sqrt{(y - y_C)^2 + (x - x_C)^2}}_{=L_C} - T_1 \underbrace{\sqrt{(y - y_A)^2 + (x - x_A)^2}}_{=L_A} - T_2 \underbrace{\sqrt{(y - y_B)^2 + (x_B - x)^2}}_{=L_B}.$$

1165 where $L_A = |VA|$, $L_B = |VB|$, $L_C = |VC|$ (see Fig. 11A). The equation of motion for the zipper vertex
1166 is given by the Euler-Lagrange equations

$$1167 \quad \frac{\partial L}{\partial q_i} - \frac{d}{dt} \frac{\partial L}{\partial \dot{q}_i} = Q_i \quad (\text{A1})$$

1168 where q_i are the spatial coordinates of the vertex, \dot{q}_i are the corresponding velocities, and Q_i are frictional
1169 forces to be derived from the Rayleigh dissipation function. On the left-hand side, the second term van-
1170 ishes for the velocity-independent Lagrangian, while the calculation of the first term is straightforward.

1171 Assumption 4 allows us to use the formalism of Rayleigh dissipation function D to express the non-
1172 conservative forces in Eq. A1. This function is defined as $D = \sum_{i,j} \frac{1}{2} K_{ij} \dot{q}_i \dot{q}_j$ and the generalized forces
1173 are given as $Q_i = -\frac{\partial D}{\partial \dot{q}_i}$. Here K_{ij} is a symmetric, positive definite matrix of generalized coefficients of
1174 friction. The coefficients are independent on velocity but may depend on the coordinates q_i . Evaluating
1175 the left- and right-hand side of Eq. A1, the equation of motion becomes, in Cartesian coordinates,

$$1176 \quad -(T_1 + T_2 - S) \frac{x - x_C}{L_C(x, y)} - T_1 \frac{x - x_A}{L_A(x, y)} - T_2 \frac{x - x_B}{L_B(x, y)} = -K_{12}(x, y) \dot{y} - K_{11}(x, y) \dot{x} \quad (\text{A2})$$

$$1177 \quad -(T_1 + T_2 - S) \frac{y - y_C}{L_C(x, y)} - T_1 \frac{y - y_A}{L_A(x, y)} - T_2 \frac{y - y_B}{L_B(x, y)} = -K_{21}(x, y) \dot{x} - K_{22}(x, y) \dot{y}. \quad (\text{A3})$$

1178

1179 To complete these equations, the coefficients $K_{ij}(q_i)$ must be specified. In the next section, three
1180 distinct forms of friction are introduced. The general form of the corresponding matrix K_{ij} is given in
1181 section Rayleigh function.

1182 **Energy dissipation rates**

1183 In our model, we consider friction forces of three distinct origins. The elongational viscosity η^\uparrow and
 1184 the vertex-localized zippering friction η^Z were already discussed in the main text. In addition, here we
 1185 introduce a frictional interaction with the substrate, while allowing for anisotropy with respect to the
 1186 axon shaft orientation.

1187 **Substrate friction**

1188 This type of friction arises due to the motion of the axons with respect to the substrate, and depends
 1189 on the entire geometry of the zipper configuration. Consider an axon segment j connecting the vertex
 1190 with one of the fixed points A,B,C (see Fig. 11A). The frictional force acting on an element dl of this
 1191 segment is assumed to be a linear function of the element velocity $\vec{v}(l)$, where l denotes the distance from
 1192 the fixed point (see Fig. 11B). We allow for anisotropic friction, with the friction coefficient eigenvalue
 1193 η^\parallel for motion parallel to the axon segment (axial friction) and η^\perp for motion normal to the axon
 1194 segment (transverse friction). The rate of energy dissipation in the axon element dl is then given by
 1195 $dR(l) = dR^\parallel(l) + dR^\perp(l) = \frac{1}{2}\eta^\parallel(\vec{v}(l) \cdot \vec{t}_j)^2 dl + \frac{1}{2}\eta^\perp(\vec{v}(l) \cdot \vec{n}_j)^2 dl$, where \vec{t}_j and \vec{n}_j denote the unit vectors
 1196 tangent and normal to the axon segment j .

1197 The integration of the dissipation rate in the whole axon segment is simple in the case of transverse
 1198 friction. Here $\vec{v}(l) \cdot \vec{n}_j = \frac{l}{L_j} \vec{u} \cdot \vec{n}_j$, where L_j is the length of the axon segment and \vec{u} is the vertex velocity.
 1199 The dissipation rate in the whole segment j is therefore

$$1200 \quad R_j^\perp = \frac{1}{2}\eta^\perp(\vec{u} \cdot \vec{n}_j)^2 \int_0^{L_j} \left(\frac{l}{L_j}\right)^2 dl = \frac{1}{6}\eta^\perp L_j(\vec{u} \cdot \vec{n}_j)^2.$$

1201 The total transverse dissipation rate in each axon is given by the sum of these contributions from the
 1202 two segments that constitute the axon.

1203 The axial friction case is slightly more complicated, as the elongations within the two axon segments
 1204 cannot be treated independently. However, as we assume uniform strain all along the axon, the rate of
 1205 change in length of a given segment may be obtained as the rate of change in length of the whole axon,
 1206 multiplied by the proportion of this segment in the total length of the axon. I.e., for the two segments
 1207 constituting the left axon in Fig. 11A, we have $\dot{L}_A = \frac{L_A}{L}\dot{L}$ and $\dot{L}_C = \frac{L_C}{L}\dot{L}$, where $L = L_A + L_C$. The
 1208 rate of change in the total length of the axon is simply expressed in terms of the vertex velocity as
 1209 $\dot{L} = -\vec{u} \cdot (\vec{t}_A + \vec{t}_C)$. (Note that the segment elongation rates cannot be obtained directly by a projection
 1210 of the vertex velocity. Consider the counter-example in which the two axon segments have identical
 1211 direction (the axon is straight), and the zipper vertex moves along this direction. Then the total length
 1212 of the axon is unchanged and there is no elongation within either segment, while $\vec{u} \cdot \vec{t}_A$ and $\vec{u} \cdot \vec{t}_C$ are
 1213 nonzero.)

1214 The local elongation velocity within segment j can now be obtained as $\frac{l}{L_j} \dot{L}_j$, where l is the distance
 1215 from the fixed point. By integration similar to the case of transverse friction, we obtain the total energy
 1216 dissipation rate due to axial friction in the segment VA ,

$$1217 \quad R_A^{\parallel} = \frac{1}{2} \eta^{\parallel} \left(\frac{L_A}{L} \dot{L} \right)^2 \int_0^{L_A} \left(\frac{l}{L_A} \right)^2 dl = \frac{1}{6} \eta^{\parallel} \frac{L_A^3}{(L_A + L_C)^2} (\vec{u} \cdot (\vec{t}_A + \vec{t}_C))^2$$

1218 and similarly for the segment VC .

1219 Elongational viscosity

1220 As discussed in the main text section RESULTS, this friction is due to the viscosity of axon elongation.
 1221 Consider again the left axon in Fig. 11A, composed of the segments VA and VC . The strain rate $\dot{\epsilon} = \frac{\dot{L}}{L}$
 1222 can be expressed as $\frac{\vec{u} \cdot (\vec{t}_A + \vec{t}_C)}{L_A + L_C}$ and, according to Assumption 3, is uniform in the whole axon. The rate of
 1223 energy dissipation in an element dl of the axon is $dR^{\updownarrow} = \frac{1}{2} \eta^{\updownarrow} \dot{\epsilon}^2 dl$, which trivially integrates to the total
 1224 dissipation rate in the whole axon, $R^{\updownarrow} = \frac{1}{2} \eta^{\updownarrow} \frac{1}{L_A + L_C} (\vec{u} \cdot (\vec{t}_A + \vec{t}_C))^2$.

1225 Zippering friction

1226 As discussed in the main text section RESULTS, this friction is a phenomenological description of the
 1227 dissipation processes occurring in the immediate vicinity of the zipper vertex. We assume that the
 1228 corresponding dissipation rate depends only on the velocity of zippering u^Z , given by the projec-
 1229 tion of the vertex velocity \vec{u} on the zipper axis \vec{t}_C (see Fig. 11A). Therefore, the dissipation rate is
 1230 $R^Z = \frac{1}{2} \eta^Z (u^Z)^2 = \frac{1}{2} \eta^Z (\vec{u} \cdot \vec{t}_C)^2$.

1231 Rayleigh function

1232 Combining the dissipation mechanisms introduced in the previous sections, the total dissipation rate for
 1233 the left axon (consisting of segments VA and VC) is

$$1234 \quad D = \frac{1}{2} \eta^{\parallel} \left[\frac{1}{3} \frac{L_A^3 + L_C^3}{(L_A + L_C)^2} \right] (\vec{u} \cdot (\vec{t}_A + \vec{t}_C))^2 + \frac{1}{2} \eta^{\updownarrow} \frac{1}{L_A + L_C} (\vec{u} \cdot (\vec{t}_A + \vec{t}_C))^2 + \quad (A4)$$

$$\frac{1}{2} \eta^{\perp} (\vec{u} \cdot \vec{n}_A)^2 \frac{1}{3} L_A + \frac{1}{2} \eta^{\perp} (\vec{u} \cdot \vec{n}_C)^2 \frac{1}{3} L_C + \frac{1}{4} \eta^Z (\vec{u} \cdot \vec{t}_C)^2$$

1235 (where for the convenience of notation, we assigned half of the vertex-localized zippering friction to the
 1236 left axon and half to the right axon).

1237 Note that each term in Eq. A4 consists of three distinct parts, e.g.

$$1238 \quad R_j^{\parallel} = \frac{1}{2} \underbrace{\eta^{\parallel}}_{\text{friction constant}} \underbrace{\frac{1}{3} L_j \left(\frac{L_j}{L_A + L_C} \right)^2}_{\text{geometric factor}} \underbrace{(\vec{u} \cdot (\vec{t}_A + \vec{t}_C))^2}_{\text{velocity projection}}$$

1239 The geometric factor depends on the dimensions of the zipper configuration, while the velocity projection
 1240 selects the component of vertex velocity \vec{u} in the appropriate direction.

1241 The whole Rayleigh function can be conveniently written in matrix notation, where the friction
 1242 constants and the geometric factors are combined into a diagonal matrix $\vec{\vec{A}}$ and the velocity projection
 1243 is achieved using another matrix $\vec{\vec{P}}$ that acts on the vertex velocity \vec{u} :

$$1244 \quad \vec{\vec{A}} = \frac{1}{2} \begin{bmatrix} \eta^{\parallel} \left[\frac{1}{3} \frac{L_A^3 + L_C^3}{(L_A + L_C)^2} \right] + \eta^{\updownarrow} \frac{1}{L_A + L_C} & 0 & 0 & 0 \\ 0 & \eta^{\perp} \frac{1}{3} L_A & 0 & 0 \\ 0 & 0 & \eta^{\perp} \frac{1}{3} L_C & 0 \\ 0 & 0 & 0 & \frac{1}{2} \eta^Z \end{bmatrix}$$

1245 and

$$1246 \quad \vec{\vec{P}} = \begin{bmatrix} \vec{t}_A + \vec{t}_C \\ \vec{n}_A \\ \vec{n}_C \\ \vec{t}_C \end{bmatrix} = \begin{bmatrix} t_{A,x} + t_{C,x} & t_{A,y} + t_{C,y} \\ n_{A,x} & n_{A,y} \\ n_{C,x} & n_{C,y} \\ t_{C,x} & t_{C,y} \end{bmatrix}.$$

1247 In this notation, the matrix of Rayleigh coefficients K_{ij} for the left axon can be obtained as $\vec{\vec{K}} = \vec{\vec{P}}^T \vec{\vec{A}} \vec{\vec{P}}$.
 1248 (The Rayleigh function in Eq. A4 is then reproduced as $D = 1/2 K_{ij} \dot{q}_i \dot{q}_j = (\vec{\vec{P}} \vec{u})^T \vec{\vec{A}} \vec{\vec{P}} \vec{u}$.) Note that as the
 1249 elements of the matrices $\vec{\vec{A}}$ and $\vec{\vec{P}}$ depend on the coordinates (x, y) of the vertex, the Rayleigh coefficients
 1250 are, in general, functions of x and y . The matrix of Rayleigh coefficients for the whole configuration is
 1251 given as the sum of the matrices for the left and right axon.

1252 With the matrix of Rayleigh coefficients determined, the equation of motion for the zipper vertex
 1253 (Eqs. A2,A3) becomes

$$1254 \quad \vec{\vec{K}}^{-1}(x, y) \begin{pmatrix} x \left(\frac{T_1}{L_A(x, y)} + \frac{T_2}{L_B(x, y)} + \frac{T_1 + T_2 - S}{L_C(x, y)} \right) - \frac{T_1 x_A}{L_A(x, y)} - \frac{T_2 x_B}{L_B(x, y)} - \frac{(T_1 + T_2 - S) x_C}{L_C(x, y)} \\ y \left(\frac{T_1}{L_A(x, y)} + \frac{T_2}{L_B(x, y)} + \frac{T_1 + T_2 - S}{L_C(x, y)} \right) - \frac{T_1 y_A}{L_A(x, y)} - \frac{T_2 y_B}{L_B(x, y)} - \frac{(T_1 + T_2 - S) y_C}{L_C(x, y)} \end{pmatrix} = \begin{pmatrix} \dot{x} \\ \dot{y} \end{pmatrix}.$$

1256 This system of two coupled nonlinear differential equations is readily solved numerically.

1257 REFERENCES

- 1258 [1] Alain Chédotal and Linda J. Richards. “Wiring the brain: the biology of neuronal guidance”.
 1259 *Cold Spring Harbor Perspectives in Biology* 2.6 (2010), a001917. ISSN: 1943-0264. DOI: 10.1101/
 1260 cshperspect.a001917.
- 1261 [2] Alex L. Kolodkin and Marc Tessier-Lavigne. “Mechanisms and molecules of neuronal wiring: a
 1262 primer”. *Cold Spring Harbor Perspectives in Biology* 3.6 (2011). ISSN: 1943-0264. DOI: 10.1101/
 1263 cshperspect.a001727.

- 1264 [3] Fanny Mann, William A. Harris, and Christine E. Holt. “New views on retinal axon development:
1265 a navigation guide”. *The International Journal of Developmental Biology* 48.8 (2004), pp. 957–964.
1266 ISSN: 0214-6282. DOI: 10.1387/ijdb.041899fm.
- 1267 [4] M. G. Honig, G. G. Petersen, U. S. Rutishauser, and S. J. Camilli. “In vitro studies of growth
1268 cone behavior support a role for fasciculation mediated by cell adhesion molecules in sensory axon
1269 guidance during development”. *Developmental Biology* 204.2 (1998), pp. 317–326. ISSN: 0012-1606.
1270 DOI: 10.1006/dbio.1998.9093.
- 1271 [5] Jonathan Raper and Carol Mason. “Cellular Strategies of Axonal Pathfinding”. *Cold Spring Harbor*
1272 *Perspectives in Biology* 2.9 (2010). ISSN: 1943-0264. DOI: 10.1101/cshperspect.a001933.
- 1273 [6] Jicheng Tang, Urs Rutishauser, and Lynn Landmesser. “Polysialic acid regulates growth cone
1274 behavior during sorting of motor axons in the plexus region”. *Neuron* 13.2 (1994), pp. 405–414.
1275 ISSN: 0896-6273. DOI: 10.1016/0896-6273(94)90356-5.
- 1276 [7] Valerie A. Schneider and Michael Granato. “Motor axon migration: a long way to go”. *Develop-*
1277 *mental Biology* 263.1 (2003), pp. 1–11. ISSN: 0012-1606.
- 1278 [8] D. Bonanomi and S. L. Pfaff. “Motor Axon Pathfinding”. *Cold Spring Harbor Perspectives in*
1279 *Biology* 2.3 (2010), a001735–a001735. ISSN: 1943-0264. DOI: 10.1101/cshperspect.a001735.
- 1280 [9] Rosa-Eva Huettl, Teresa Haehl, and Andrea B. Huber. “Fasciculation and guidance of spinal motor
1281 axons in the absence of FGFR2 signaling”. *PloS One* 7.7 (2012), e41095. ISSN: 1932-6203. DOI:
1282 10.1371/journal.pone.0041095.
- 1283 [10] C. H. Lin and P. Forscher. “Cytoskeletal remodeling during growth cone-target interactions.” *The*
1284 *Journal of Cell Biology* 121.6 (1993), pp. 1369–1383. ISSN: 0021-9525, 1540-8140. DOI: 10.1083/
1285 jcb.121.6.1369.
- 1286 [11] David Van Vactor. “Adhesion and signaling in axonal fasciculation”. *Current Opinion in Neurobi-*
1287 *ology* 8.1 (1998), pp. 80–86. ISSN: 0959-4388. DOI: 10.1016/S0959-4388(98)80011-1.
- 1288 [12] Katherine Kalil. “Chapter 3 Growth cone behaviors during axon guidance in the developing cerebral
1289 cortex”. In: *Progress in Brain Research*. Ed. by R. Ranney Mize and Reha S. Erzurumlu. Vol. 108.
1290 Elsevier, 1996, pp. 31–40.
- 1291 [13] Brian Key and James St John. “Axon Navigation in the Mammalian Primary Olfactory Pathway
1292 Where to Next?” *Chemical Senses* 27.3 (2002), pp. 245–260. ISSN: 0379-864X, 1464-3553. DOI:
1293 10.1093/chemse/27.3.245.

- 1294 [14] Stéphane Nedelec, Caroline Dubacq, and Alain Trembleau. “Morphological and molecular features
1295 of the mammalian olfactory sensory neuron axons: What makes these axons so special?” *Journal*
1296 *of Neurocytology* 34.1 (2005), pp. 49–64. ISSN: 0300-4864, 1573-7381. DOI: 10.1007/s11068-005-
1297 5047-7.
- 1298 [15] Jörg Strotmann and Heinz Breer. “Formation of glomerular maps in the olfactory system”. *Semi-*
1299 *nars in Cell & Developmental Biology*. OlfactionAnimal Stem Cell Types 17.4 (2006), pp. 402–410.
1300 ISSN: 1084-9521. DOI: 10.1016/j.semcdb.2006.04.010.
- 1301 [16] Peter Mombaerts. “Axonal Wiring in the Mouse Olfactory System”. *Annual Review of Cell and De-*
1302 *velopmental Biology* 22.1 (2006), pp. 713–737. ISSN: 1081-0706. DOI: 10.1146/annurev.cellbio.
1303 21.012804.093915.
- 1304 [17] Kensaku Mori and Hitoshi Sakano. “How Is the Olfactory Map Formed and Interpreted in the
1305 Mammalian Brain?” *Annual Review of Neuroscience* 34.1 (2011), pp. 467–499. DOI: 10.1146/
1306 annurev-neuro-112210-112917.
- 1307 [18] J. A. Glazier and D. Weaire. “The kinetics of cellular patterns”. *Journal of Physics: Condensed*
1308 *Matter* 4.8 (1992), p. 1867. ISSN: 0953-8984. DOI: 10.1088/0953-8984/4/8/004.
- 1309 [19] D. L. Weaire and Stefan Hutzler. *The Physics of Foams*. Clarendon Press, 2001. 268 pp. ISBN:
1310 978-0-19-851097-0.
- 1311 [20] K. Jalink and W. H. Moolenaar. “Thrombin receptor activation causes rapid neural cell rounding
1312 and neurite retraction independent of classic second messengers.” *The Journal of Cell Biology* 118.2
1313 (1992), pp. 411–419. ISSN: 0021-9525, 1540-8140. DOI: 10.1083/jcb.118.2.411.
- 1314 [21] Elisabeth Fischer-Friedrich, Anthony A. Hyman, Frank Jülicher, Daniel J. Müller, and Jonne He-
1315 lenius. “Quantification of surface tension and internal pressure generated by single mitotic cells”.
1316 *Scientific Reports* 4 (2014), p. 6213. ISSN: 2045-2322. DOI: 10.1038/srep06213.
- 1317 [22] Yareni A. Ayala, Bruno Pontes, Barbara Hissa, Ana Carolina M. Monteiro, Marcos Farina, Vivaldo
1318 Moura-Neto, Nathan B. Viana, and H. Moysés Nussenzveig. “Effects of cytoskeletal drugs on actin
1319 cortex elasticity”. *Experimental Cell Research* 351.2 (2017), pp. 173–181. ISSN: 0014-4827. DOI:
1320 10.1016/j.yexcr.2016.12.016.
- 1321 [23] T. J. Dennerll, H. C. Joshi, V. L. Steel, R. E. Buxbaum, and S. R. Heidemann. “Tension and
1322 compression in the cytoskeleton of PC-12 neurites. II: Quantitative measurements.” *The Journal of*
1323 *Cell Biology* 107.2 (1988), pp. 665–674. ISSN: 0021-9525, 1540-8140. DOI: 10.1083/jcb.107.2.665.
- 1324 [24] D. Bray. “Mechanical tension produced by nerve cells in tissue culture”. *Journal of Cell Science*
1325 37.1 (1979), pp. 391–410. ISSN: 0021-9533, 1477-9137.

- 1326 [25] Barry G Condron and Kai Zinn. “Regulated neurite tension as a mechanism for determination of
1327 neuronal arbor geometries in vivo”. *Current Biology* 7.10 (1997), pp. 813–816. ISSN: 0960-9822.
1328 DOI: 10.1016/S0960-9822(06)00343-5.
- 1329 [26] Orit Shefi, Amir Harel, Dmitri B. Chklovskii, Eshel Ben-Jacob, and Amir Ayali. “Biophysical con-
1330 straints on neuronal branching”. *Neurocomputing*. Computational Neuroscience: Trends in Research
1331 2004 58–60 (2004), pp. 487–495. ISSN: 0925-2312. DOI: 10.1016/j.neucom.2004.01.085.
- 1332 [27] Sarit Anava, Alon Greenbaum, Eshel Ben Jacob, Yael Hanein, and Amir Ayali. “The Regulative
1333 Role of Neurite Mechanical Tension in Network Development”. *Biophysical Journal* 96.4 (2009),
1334 pp. 1661–1670. ISSN: 0006-3495. DOI: 10.1016/j.bpj.2008.10.058.
- 1335 [28] T. J. Dennerll, P. Lamoureux, R. E. Buxbaum, and S. R. Heidemann. “The cytomechanics of
1336 axonal elongation and retraction.” *The Journal of Cell Biology* 109.6 (1989), pp. 3073–3083. ISSN:
1337 0021-9525, 1540-8140. DOI: 10.1083/jcb.109.6.3073.
- 1338 [29] Jagannathan Rajagopalan, Alireza Tofangchi, and M. Taher A. Saif. “Drosophila Neurons Actively
1339 Regulate Axonal Tension In Vivo”. *Biophysical Journal* 99.10 (2010), pp. 3208–3215. ISSN: 0006-
1340 3495. DOI: 10.1016/j.bpj.2010.09.029.
- 1341 [30] E. Evans, K. Ritchie, and R. Merkel. “Sensitive force technique to probe molecular adhesion and
1342 structural linkages at biological interfaces”. *Biophysical Journal* 68.6 (1995), pp. 2580–2587. ISSN:
1343 00063495. DOI: 10.1016/S0006-3495(95)80441-8.
- 1344 [31] Christine Gourier, Antoine Jegou, Julien Husson, and Frédéric Pincet. “A Nanospring Named
1345 Erythrocyte. The Biomembrane Force Probe”. *Cellular and Molecular Bioengineering* 1.4 (2008),
1346 pp. 263–275. ISSN: 1865-5025, 1865-5033. DOI: 10.1007/s12195-008-0030-x.
- 1347 [32] Alan Roberts and J. S. H. Taylor. “A scanning electron microscope study of the development of
1348 a peripheral sensory neurite network”. *Development* 69.1 (1982), pp. 237–250. ISSN: 0950-1991,
1349 1477-9129.
- 1350 [33] A. G. Voyiadjis, M. Doumi, E. Curcio, and T. Shinbrot. “Fasciculation and defasciculation of
1351 neurite bundles on micropatterned substrates”. *Annals of Biomedical Engineering* 39.1 (2011),
1352 pp. 559–569. ISSN: 1573-9686. DOI: 10.1007/s10439-010-0168-2.
- 1353 [34] Joshua Barry, Yuanzheng Gu, and Chen Gu. “Polarized Targeting of L1-CAM Regulates Axonal
1354 and Dendritic Bundling in vitro”. *The European journal of neuroscience* 32.10 (2010), pp. 1618–
1355 1631. ISSN: 0953-816X. DOI: 10.1111/j.1460-9568.2010.07447.x.
- 1356 [35] Yeh-Shiu Chu, William A. Thomas, Olivier Eder, Frederic Pincet, Eric Perez, Jean Paul Thiery, and
1357 Sylvie Dufour. “Force measurements in E-cadherin-mediated cell doublets reveal rapid adhesion
1358 strengthened by actin cytoskeleton remodeling through Rac and Cdc42”. *The Journal of Cell*
1359 *Biology* 167.6 (2004), pp. 1183–1194. ISSN: 0021-9525. DOI: 10.1083/jcb.200403043.

- 1360 [36] Geoffrey J. Goodhill and Jeffrey S. Urbach. “Theoretical analysis of gradient detection by growth
1361 cones”. *Journal of neurobiology* 41.2 (1999), pp. 230–241.
- 1362 [37] Guo-Hua Li, Cheng-de Qin, and Li-Wen Wang. “Computer model of growth cone behavior and
1363 neuronal morphogenesis”. *Journal of Theoretical Biology* 174.4 (1995), pp. 381–389. ISSN: 0022-
1364 5193. DOI: 10.1006/jtbi.1995.0106.
- 1365 [38] Huyen Nguyen, Peter Dayan, Zac Pujic, Justin Cooper-White, and Geoffrey J. Goodhill. “A mathe-
1366 matical model explains saturating axon guidance responses to molecular gradients”. *eLife* 5 (2016),
1367 e12248. ISSN: 2050-084X. DOI: 10.7554/eLife.12248.
- 1368 [39] H. G. E. Hentschel and A. van Ooyen. “Models of axon guidance and bundling during development”.
1369 *Proceedings of the Royal Society of London B: Biological Sciences* 266.1434 (1999), pp. 2231–2238.
1370 ISSN: 0962-8452, 1471-2954. DOI: 10.1098/rspb.1999.0913.
- 1371 [40] Debasish Chaudhuri, Peter Borowski, and Martin Zapotocky. “Model of fasciculation and sorting in
1372 mixed populations of axons”. *Physical Review E* 84.2 (2011), p. 021908. DOI: 10.1103/PhysRevE.
1373 84.021908.
- 1374 [41] A. Acheson, J. L. Sunshine, and U. Rutishauser. “NCAM polysialic acid can regulate both cell-
1375 cell and cell-substrate interactions.” *The Journal of Cell Biology* 114.1 (1991), pp. 143–153. ISSN:
1376 0021-9525, 1540-8140. DOI: 10.1083/jcb.114.1.143.
- 1377 [42] Philip Lamoureux, Robert E. Buxbaum, and Steven R. Heidemann. “Direct evidence that growth
1378 cones pull”. *Nature* 340.6229 (1989), pp. 159–162. DOI: 10.1038/340159a0.
- 1379 [43] Paul C. Letourneau, Terri A. Shattuck, and Alice H. Ressler. ““Pull” and “push” in neurite elonga-
1380 tion: Observations on the effects of different concentrations of cytochalasin B and taxol”. *Cell Motil-
1381 ity and the Cytoskeleton* 8.3 (1987), pp. 193–209. ISSN: 1097-0169. DOI: 10.1002/cm.970080302.
- 1382 [44] Paul H. Ratz and John E. Speich. “Evidence that actomyosin cross bridges contribute to “passive”
1383 tension in detrusor smooth muscle”. *American Journal of Physiology - Renal Physiology* 298.6
1384 (2010), F1424–F1435. ISSN: 1931-857X, 1522-1466. DOI: 10.1152/ajprenal.00635.2009.
- 1385 [45] Wasim A. Sayyad, Ladan Amin, Paolo Fabris, Erika Ercolini, and Vincent Torre. “The role of
1386 myosin-II in force generation of DRG filopodia and lamellipodia”. *Scientific Reports* 5 (2015),
1387 p. 7842. ISSN: 2045-2322. DOI: 10.1038/srep07842.
- 1388 [46] Andrea R. Ketschek, Steven L. Jones, and Gianluca Gallo. “Axon extension in the fast and slow
1389 lanes: Substratum-dependent engagement of myosin II functions”. *Developmental Neurobiology*
1390 67.10 (2007), pp. 1305–1320. ISSN: 1932-846X. DOI: 10.1002/dneu.20455.

- 1391 [47] Eun-Mi Hur, In Hong Yang, Deok-Ho Kim, Justin Byun, Saijilafu, Wen-Lin Xu, Philip R. Nicovich,
1392 Raymond Cheong, Andre Levchenko, Nitish Thakor, and Feng-Quan Zhou. “Engineering neuronal
1393 growth cones to promote axon regeneration over inhibitory molecules”. *Proceedings of the National
1394 Academy of Sciences* 108.12 (2011), pp. 5057–5062. ISSN: 0027-8424, 1091-6490. DOI: 10.1073/
1395 pnas.1011258108.
- 1396 [48] K. Jalink, T. Eichholtz, F. R. Postma, E. J. van Corven, and W. H. Moolenaar. “Lysophosphatidic
1397 acid induces neuronal shape changes via a novel, receptor-mediated signaling pathway: similarity
1398 to thrombin action”. *Cell Growth & Differentiation* 4.4 (1993), p. 247.
- 1399 [49] Robert W. Style, Rostislav Boltanskiy, Guy K. German, Callen Hyland, Christopher W. MacMinn,
1400 Aaron F. Mertz, Larry A. Wilen, Ye Xu, and Eric R. Dufresne. “Traction force microscopy in physics
1401 and biology”. 10.23 (2014), pp. 4047–4055. ISSN: 1744-6848. DOI: 10.1039/C4SM00264D.
- 1402 [50] Daniel Koch, William J. Rosoff, Jiji Jiang, Herbert M. Geller, and Jeffrey S. Urbach. “Strength
1403 in the Periphery: Growth Cone Biomechanics and Substrate Rigidity Response in Peripheral and
1404 Central Nervous System Neurons”. *Biophysical Journal* 102.3 (2012), pp. 452–460. ISSN: 0006-3495.
1405 DOI: 10.1016/j.bpj.2011.12.025.
- 1406 [51] Fernanda Gárate, Timo Betz, María Pertusa, and Roberto Bernal. “Time-resolved neurite mechan-
1407 ics by thermal fluctuation assessments”. *Physical Biology* 12.6 (2015), p. 066020. ISSN: 1478-3975.
1408 DOI: 10.1088/1478-3975/12/6/066020.
- 1409 [52] Jos Käfer, Takashi Hayashi, Athanasius F. M. Marée, Richard W. Carthew, and F. Graner. “Cell
1410 adhesion and cortex contractility determine cell patterning in the Drosophilaretina”. *Proceedings
1411 of the National Academy of Sciences* 104.47 (2007), pp. 18549–18554. ISSN: 0027-8424, 1091-6490.
1412 DOI: 10.1073/pnas.0704235104.
- 1413 [53] Francis Corson, Olivier Hamant, Steffen Bohn, Jan Traas, Arezki Boudaoud, and Yves Couder.
1414 “Turning a plant tissue into a living cell froth through isotropic growth”. *Proceedings of the National
1415 Academy of Sciences* 106.21 (2009), pp. 8453–8458. ISSN: 0027-8424, 1091-6490. DOI: 10.1073/
1416 pnas.0812493106.
- 1417 [54] M. Lisa Manning, Ramsey A. Foty, Malcolm S. Steinberg, and Eva-Maria Schoetz. “Coaction
1418 of intercellular adhesion and cortical tension specifies tissue surface tension”. *Proceedings of the
1419 National Academy of Sciences* 107.28 (2010), pp. 12517–12522. ISSN: 0027-8424, 1091-6490. DOI:
1420 10.1073/pnas.1003743107.
- 1421 [55] Oscar Aurelio, David H. Hall, and Oliver Hobert. “Immunoglobulin-Domain Proteins Required for
1422 Maintenance of Ventral Nerve Cord Organization”. *Science* 295.5555 (2002), pp. 686–690. ISSN:
1423 0036-8075, 1095-9203. DOI: 10.1126/science.1066642.

- 1424 [56] A. Lehmenkühler, E. Syková, J. Svoboda, K. Zilles, and C. Nicholson. “Extracellular space parameters in the rat neocortex and subcortical white matter during postnatal development determined by diffusion analysis”. *Neuroscience* 55.2 (1993), pp. 339–351. ISSN: 0306-4522. DOI: 10.1016/0306-
1425 4522(93)90503-8.
1426
1427
- 1428 [57] Natalya Korogod, Carl CH Petersen, and Graham W. Knott. “Ultrastructural analysis of adult mouse neocortex comparing aldehyde perfusion with cryo fixation”. *eLife* 4 (2015), e05793. ISSN: 2050-084X. DOI: 10.7554/eLife.05793.
1429
1430
- 1431 [58] L. G. Elfvin. “The ultrastructure of unmyelinated fibers in the splenic nerve of the cat”. *Journal of Ultrastructure Research* 1.4 (1958), pp. 428–454. ISSN: 0022-5320.
1432
- 1433 [59] Ying Li, Pauline M. Field, and Geoffrey Raisman. “Olfactory ensheathing cells and olfactory nerve fibroblasts maintain continuous open channels for regrowth of olfactory nerve fibres”. *Glia* 52.3 (2005), pp. 245–251. ISSN: 0894-1491. DOI: 10.1002/glia.20241.
1434
1435
- 1436 [60] A. I. Farbman. “Developmental biology of olfactory sensory neurons”. *Seminars in Cell Biology* 5.1 (1994), pp. 3–10. ISSN: 1043-4682.
1437
- 1438 [61] Hirofumi Nishizumi and Hitoshi Sakano. “Developmental regulation of neural map formation in the mouse olfactory system”. *Developmental Neurobiology* 75.6 (2015), pp. 594–607. ISSN: 1932-846X. DOI: 10.1002/dneu.22268.
1439
1440
- 1441 [62] Bolek Zapiec, Olaf Christian Bressel, Mona Khan, Andreas Walz, and Peter Mombaerts. “Neuropilin-1 and the Positions of Glomeruli in the Mouse Olfactory Bulb”. *eneuro* 3.5 (2016), ENEURO.0123-16.2016. ISSN: 2373-2822. DOI: 10.1523/ENEURO.0123-16.2016.
1442
1443
- 1444 [63] Alexis Assens, Julien A. Dal Col, Anthony Njoku, Quentin Dietschi, Chenda Kan, Paul Feinstein, Alan Carleton, and Ivan Rodriguez. “Alteration of Nrp1 signaling at different stages of olfactory neuron maturation promotes glomerular shifts along distinct axes in the olfactory bulb”. *Development* 143.20 (2016), pp. 3817–3825. ISSN: 0950-1991, 1477-9129. DOI: 10.1242/dev.138941.
1445
1446
1447
- 1448 [64] Thomas Bozza, Anne Vassalli, Stefan Fuss, Jing-Ji Zhang, Brian Weiland, Rodrigo Pacifico, Paul Feinstein, and Peter Mombaerts. “Mapping of Class I and Class II Odorant Receptors to Glomerular Domains by Two Distinct Types of Olfactory Sensory Neurons in the Mouse”. *Neuron* 61.2 (2009), pp. 220–233. ISSN: 0896-6273. DOI: 10.1016/j.neuron.2008.11.010.
1449
1450
1451
- 1452 [65] Diego J. Rodriguez-Gil, Dianna L. Bartel, Austin W. Jaspers, Arie S. Mobley, Fumiaki Imamura, and Charles A. Greer. “Odorant receptors regulate the final glomerular coalescence of olfactory sensory neuron axons”. *Proceedings of the National Academy of Sciences* 112.18 (2015), pp. 5821–5826. ISSN: 0027-8424, 1091-6490. DOI: 10.1073/pnas.1417955112.
1453
1454
1455
- 1456 [66] Hemant Bokil, Nora Laaris, Karen Blinder, Mathew Ennis, and Asaf Keller. “Ephaptic interactions in the mammalian olfactory system”. *J Neurosci* 21 (2001), pp. 1–5.
1457

- 1458 [67] S. Reutskiy, E. Rossoni, and B. Tirozzi. “Conduction in bundles of demyelinated nerve fibers:
1459 computer simulation”. *Biological Cybernetics* 89.6 (2003), pp. 439–448. ISSN: 0340-1200, 1432-0770.
1460 DOI: 10.1007/s00422-003-0430-x.
- 1461 [68] Matthew O’Toole, Phillip Lamoureux, and Kyle E. Miller. “Measurement of Subcellular Force
1462 Generation in Neurons”. *Biophysical Journal* 108.5 (2015), pp. 1027–1037. ISSN: 0006-3495. DOI:
1463 10.1016/j.bpj.2015.01.021.
- 1464 [69] S. Hoffman and G. M. Edelman. “Kinetics of homophilic binding by embryonic and adult forms of
1465 the neural cell adhesion molecule.” *Proceedings of the National Academy of Sciences of the United
1466 States of America* 80.18 (1983), p. 5762.
- 1467 [70] R. Sadoul, M. Hirn, H. Deagostini-Bazin, G. Rougon, and C. Goridis. “Adult and embryonic mouse
1468 neural cell adhesion molecules have different binding properties”. *Nature* 304.5924 (1983), pp. 347–
1469 349. ISSN: 0028-0836.
- 1470 [71] U. Rutishauser, M. Grumet, and G. M. Edelman. “Neural cell adhesion molecule mediates initial
1471 interactions between spinal cord neurons and muscle cells in culture”. *The Journal of Cell Biology*
1472 97.1 (1983), pp. 145–152. ISSN: 0021-9525.
- 1473 [72] Crystal M. Miller, Andrea Page-McCaw, and Heather T. Broihier. “Matrix metalloproteinases
1474 promote motor axon fasciculation in the *Drosophila* embryo”. *Development* 135.1 (2008), pp. 95–
1475 109. ISSN: 0950-1991, 1477-9129. DOI: 10.1242/dev.011072.
- 1476 [73] Alexander Jaworski and Marc Tessier-Lavigne. “Autocrine/juxtacrine regulation of axon fas-
1477 ciculation by Slit-Robo signaling”. *Nature Neuroscience* 15.3 (2012), pp. 367–369. ISSN: 1097-6256,
1478 1546-1726. DOI: 10.1038/nn.3037.
- 1479 [74] Thomas M. Coate, Steven Raft, Xiumei Zhao, Aimee K. Ryan, E. Bryan Crenshaw, and Matthew
1480 W. Kelley. “Otic Mesenchyme Cells Regulate Spiral Ganglion Axon Fasciculation through a Pou3f4/EphA4
1481 Signaling Pathway”. *Neuron* 73.1 (2012), pp. 49–63. ISSN: 08966273. DOI: 10.1016/j.neuron.
1482 2011.10.029.
- 1483 [75] Rosa-Eva Huettl, Heidi Soellner, Elisa Bianchi, Bennett G. Novitch, and Andrea B. Huber. “Npn-1
1484 Contributes to Axon-Axon Interactions That Differentially Control Sensory and Motor Innervation
1485 of the Limb”. *PLoS Biol* 9.2 (2011), e1001020. DOI: 10.1371/journal.pbio.1001020.
- 1486 [76] Matthew O’Toole, Phillip Lamoureux, and Kyle E. Miller. “A Physical Model of Axonal Elongation:
1487 Force, Viscosity, and Adhesions Govern the Mode of Outgrowth”. *Biophysical Journal* 94.7 (2008),
1488 pp. 2610–2620. ISSN: 0006-3495. DOI: 10.1529/biophysj.107.117424.
- 1489 [77] Ahmad Ibrahim Mahmoud Athamneh and Daniel Marcel Suter. “Quantifying mechanical force in
1490 axonal growth and guidance”. *Frontiers in Cellular Neuroscience* (2015), p. 359. DOI: 10.3389/
1491 fncel.2015.00359.

- 1492 [78] Ahmad I. M. Athamneh, Alexander X. Cartagena-Rivera, Arvind Raman, and Daniel M. Suter.
1493 “Substrate Deformation Predicts Neuronal Growth Cone Advance”. *Biophysical Journal* 109.7
1494 (2015), pp. 1358–1371. ISSN: 0006-3495. DOI: 10.1016/j.bpj.2015.08.013.
- 1495 [79] Cecile O. Mejean, Andrew W. Schaefer, Kenneth B. Buck, Holger Kress, Alla Shundrovsky, Jason
1496 W. Merrill, Eric R. Dufresne, and Paul Forscher. “Elastic Coupling of Nascent apCAM Adhesions
1497 to Flowing Actin Networks”. *PLOS ONE* 8.9 (2013), e73389. ISSN: 1932-6203. DOI: 10.1371/
1498 journal.pone.0073389.
- 1499 [80] Kristian Franze, Jens Gerdemann, Michael Weick, Timo Betz, Steve Pawlizak, Melike Lakadamyali,
1500 Johannes Bayer, Katja Rillich, Michael Gögler, Yun-Bi Lu, Andreas Reichenbach, Paul Janmey, and
1501 Josef Käs. “Neurite Branch Retraction Is Caused by a Threshold-Dependent Mechanical Impact”.
1502 *Biophysical Journal* 97.7 (2009), pp. 1883–1890. ISSN: 00063495. DOI: 10.1016/j.bpj.2009.07.
1503 033.
- 1504 [81] Marion Ghibaudo, Alexandre Saez, Léa Trichet, Alain Xayaphoummine, Julien Browaeys, Pascal
1505 Silberzan, Axel Buguin, and Benoît Ladoux. “Traction forces and rigidity sensing regulate cell
1506 functions”. 4.9 (2008), pp. 1836–1843. ISSN: 1744-6848. DOI: 10.1039/B804103B.
- 1507 [82] Ai Kia Yip, Katsuhiko Iwasaki, Chaitanya Ursekar, Hiroaki Machiyama, Mayur Saxena, Huiling
1508 Chen, Ichiro Harada, Keng-Hwee Chiam, and Yasuhiro Sawada. “Cellular Response to Substrate
1509 Rigidity Is Governed by Either Stress or Strain”. *Biophysical Journal* 104.1 (2013), pp. 19–29.
1510 ISSN: 0006-3495. DOI: 10.1016/j.bpj.2012.11.3805.
- 1511 [83] Patrick C. Kerstein, Robert H. Nichol IV, and Timothy M. Gomez. “Mechanochemical regulation
1512 of growth cone motility”. *Frontiers in Cellular Neuroscience* (2015), p. 244. DOI: 10.3389/fncel.
1513 2015.00244.
- 1514 [84] Kristian Franze. “The mechanical control of nervous system development”. *Development* 140.15
1515 (2013), pp. 3069–3077. ISSN: 0950-1991, 1477-9129. DOI: 10.1242/dev.079145.
- 1516 [85] Kristian Franze, Paul A. Janmey, and Jochen Guck. “Mechanics in neuronal development and
1517 repair”. *Annual Review of Biomedical Engineering* 15 (2013), pp. 227–251. ISSN: 1545-4274. DOI:
1518 10.1146/annurev-bioeng-071811-150045.
- 1519 [86] Phillip Lamoureux, Gordon Ruthel, Robert E. Buxbaum, and Steven R. Heidemann. “Mechanical
1520 tension can specify axonal fate in hippocampal neurons”. *The Journal of Cell Biology* 159.3 (2002),
1521 pp. 499–508. ISSN: 0021-9525, 1540-8140. DOI: 10.1083/jcb.200207174.
- 1522 [87] Scott Siechen, Shengyuan Yang, Akira Chiba, and Taher Saif. “Mechanical tension contributes
1523 to clustering of neurotransmitter vesicles at presynaptic terminals”. *Proceedings of the National
1524 Academy of Sciences* 106.31 (2009), pp. 12611–12616. ISSN: 0027-8424, 1091-6490. DOI: 10.1073/
1525 pnas.0901867106.

- 1526 [88] Johannes Schindelin, Ignacio Arganda-Carreras, Erwin Frise, Verena Kaynig, Mark Longair, To-
1527 bias Pietzsch, Stephan Preibisch, Curtis Rueden, Stephan Saalfeld, Benjamin Schmid, Jean-Yves
1528 Tinevez, Daniel James White, Volker Hartenstein, Kevin Eliceiri, Pavel Tomancak, and Albert Car-
1529 dona. “Fiji - an Open Source platform for biological image analysis”. *Nature methods* 9.7 (2012).
1530 ISSN: 1548-7091. DOI: 10.1038/nmeth.2019.
- 1531 [89] Caroline A. Schneider, Wayne S. Rasband, and Kevin W. Eliceiri. “NIH Image to ImageJ: 25 years
1532 of image analysis”. *Nature Methods* 9.7 (2012), pp. 671–675. ISSN: 1548-7091. DOI: 10.1038/nmeth.
1533 2089.
- 1534 [90] The Mathworks, Inc. *Matlab release 2015b*. Version 8.6.0.267246. Nattick, MA, USA, 2015.
- 1535 [91] Daniel Šmít, Coralie Fouquet, Mohamed Doulazmi, Frédéric Pincet, Alain Trembleau, and Martin
1536 Zapotocky. “BFPTool: a software tool for analysis of Biomembrane Force Probe experiments”.
1537 *BMC Biophysics* 10.1 (2017), p. 2. ISSN: 2046-1682. DOI: 10.1186/s13628-016-0033-2.
- 1538 [92] Francesco Pampaloni, Gianluca Lattanzi, Alexandr Jonáš, Thomas Surrey, Erwin Frey, and Ernst-
1539 Ludwig Florin. “Thermal fluctuations of grafted microtubules provide evidence of a length-dependent
1540 persistence length”. *Proceedings of the National Academy of Sciences* 103.27 (2006), pp. 10248–
1541 10253. ISSN: 0027-8424, 1091-6490. DOI: 10.1073/pnas.0603931103.
- 1542 [93] R. Fadić, J. Vergara, and J. Alvarez. “Microtubules and caliber of central and peripheral processes
1543 of sensory axons”. *The Journal of Comparative Neurology* 236.2 (1985), pp. 258–264. ISSN: 1096-
1544 9861. DOI: 10.1002/cne.902360209.
- 1545 [94] Raymond J. Roark, Warren C. Young, and Richard G. Budynas. *Roark’s formulas for stress and*
1546 *strain*. 7th ed. New York: McGraw-Hill, 2002. 852 pp. ISBN: 978-0-07-072542-3.

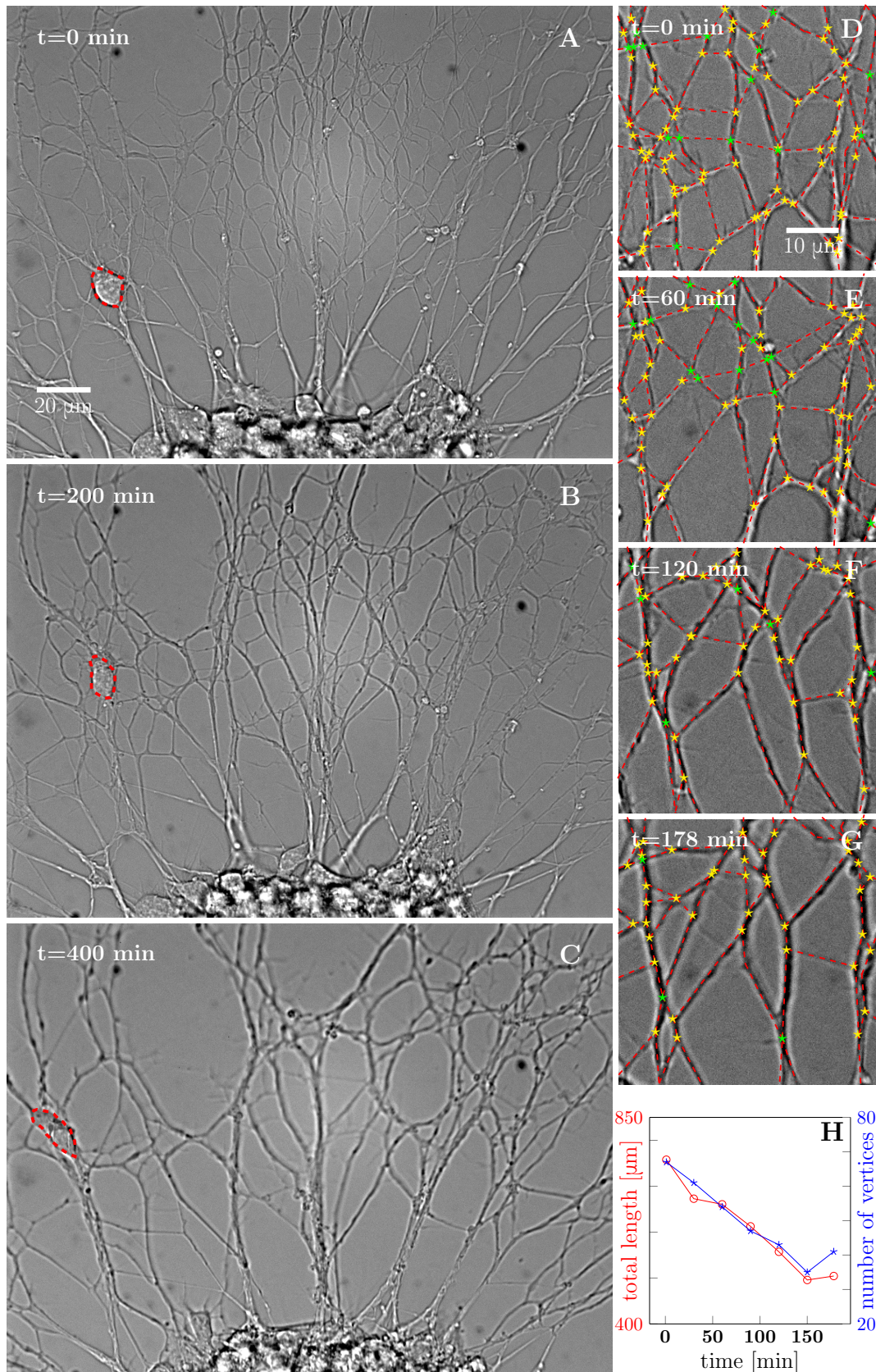


Figure 1: A-C: Evolution of the axonal network growing from an explant during 400 min time lapse recording, after 2 days of incubation; the red dashed outline delineates a travelling ensheating cell. Progressive coarsening of the network and decrease of total length and density can be seen. D-G: Red dashed lines outline the edges of the network while yellow stars indicate junctions between axons or axon bundles, and green stars indicate crossings. H: Quantification of total length and number of vertices of the network section, depicted in panels D-G, as a function of time (based on 7 manually segmented video frames). Segmentation coordinates for panels D-G and data from panel H data are available in Figure 1—source data 1.

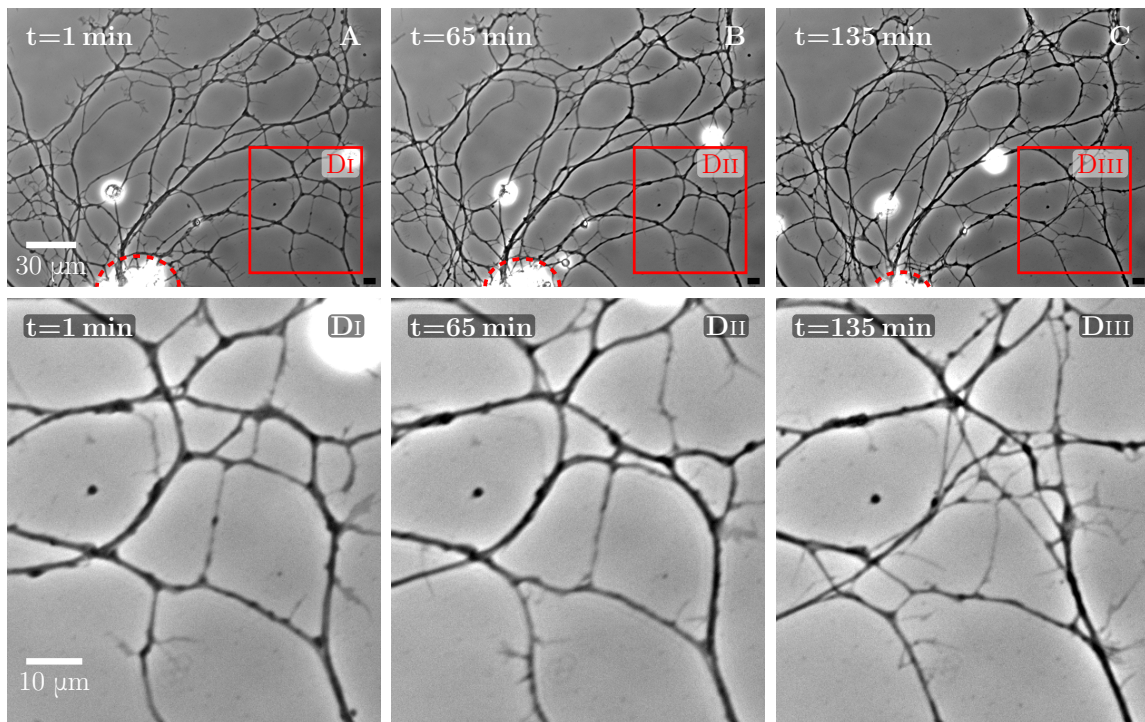


Figure 1 – figure supplement 1: An example of spontaneous defasciculation correlated with explant contraction. A-C: Full field view of network evolution, from an experiment with no added drugs. The edge of the explant is marked by the red dashed line. After $t=65$ min, the explant edge starts to move out of the field and pulls on the outgrown axon network. This causes defasciculation and an increase of network length in the area marked by the red square, labelled DI-DIII. The panels DI-DIII show magnified views of the marked area. An increase in network density is apparent in the panel DIII. The time-lapse recording spanning $t=1$ min through $t=135$ min is provided as supplementary Video 9.

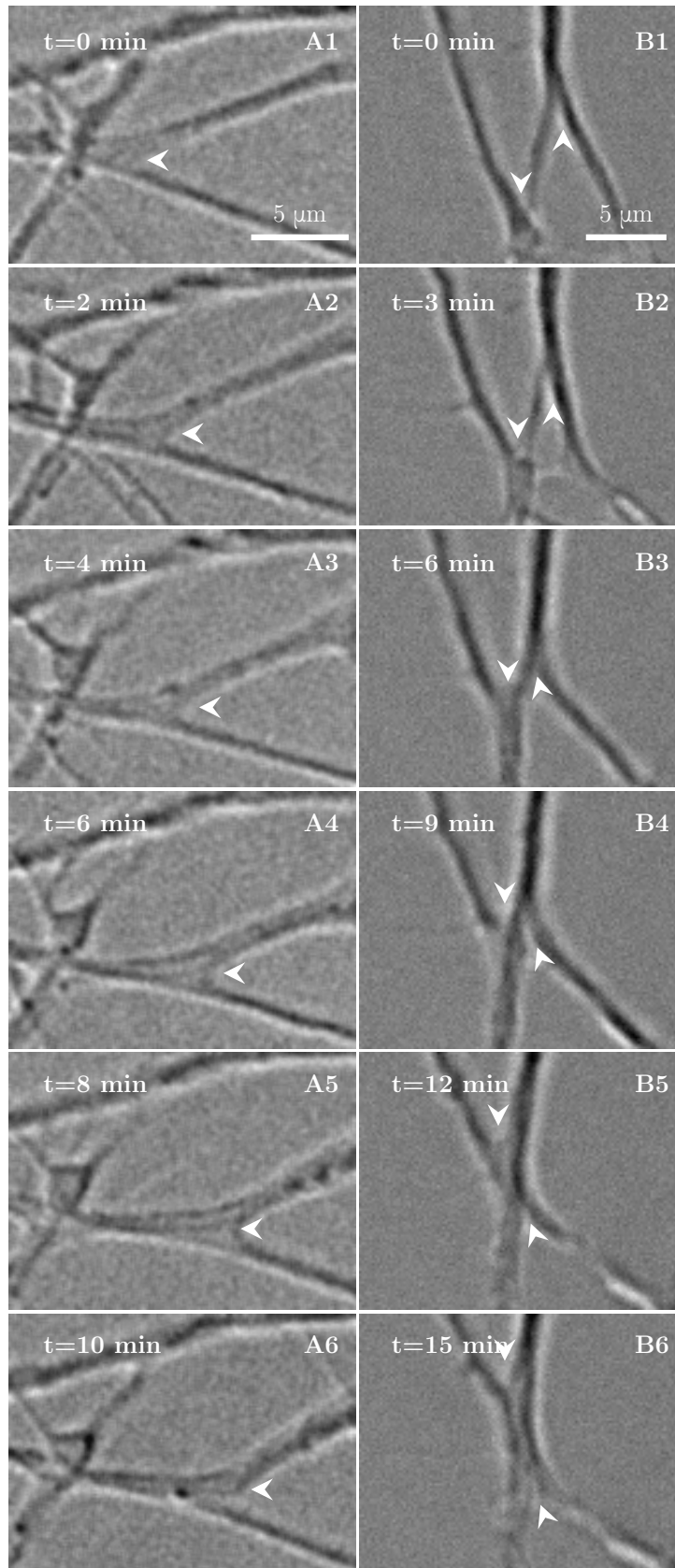


Figure 2: High magnification images of individual axon zippers and their evolution in time. Zipper vertices are marked by arrowheads. A: advancing zipper, B: two associated advancing zippers. Total length of the network segments in B decreases during the zippering process.

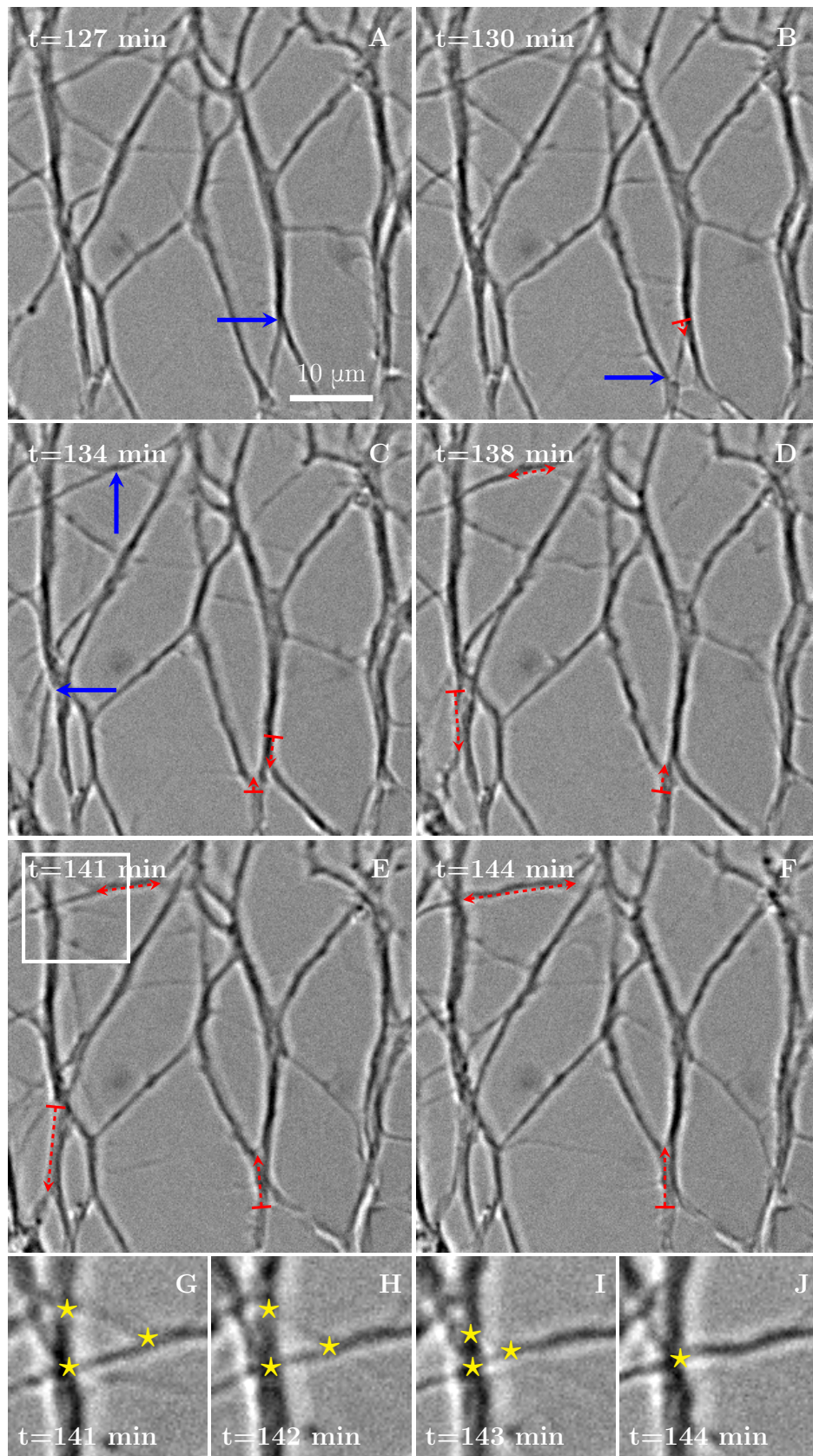


Figure 3: A-F: 6 time-frames extracted from the video shown in Fig. 1D-G. The blue arrows indicate vertices that will start to zipper in the following frame, the red dashed arrows illustrate the direction and the increase in length of the advancing zipper. If two arrowheads are present, there are two vertices extending a single segment. Frames G to J are enlargements of the inset in panel E in the period between the frames E and F, illustrating three vertices (marked by stars in frames G to I) merging into a single vertex (in frame J).

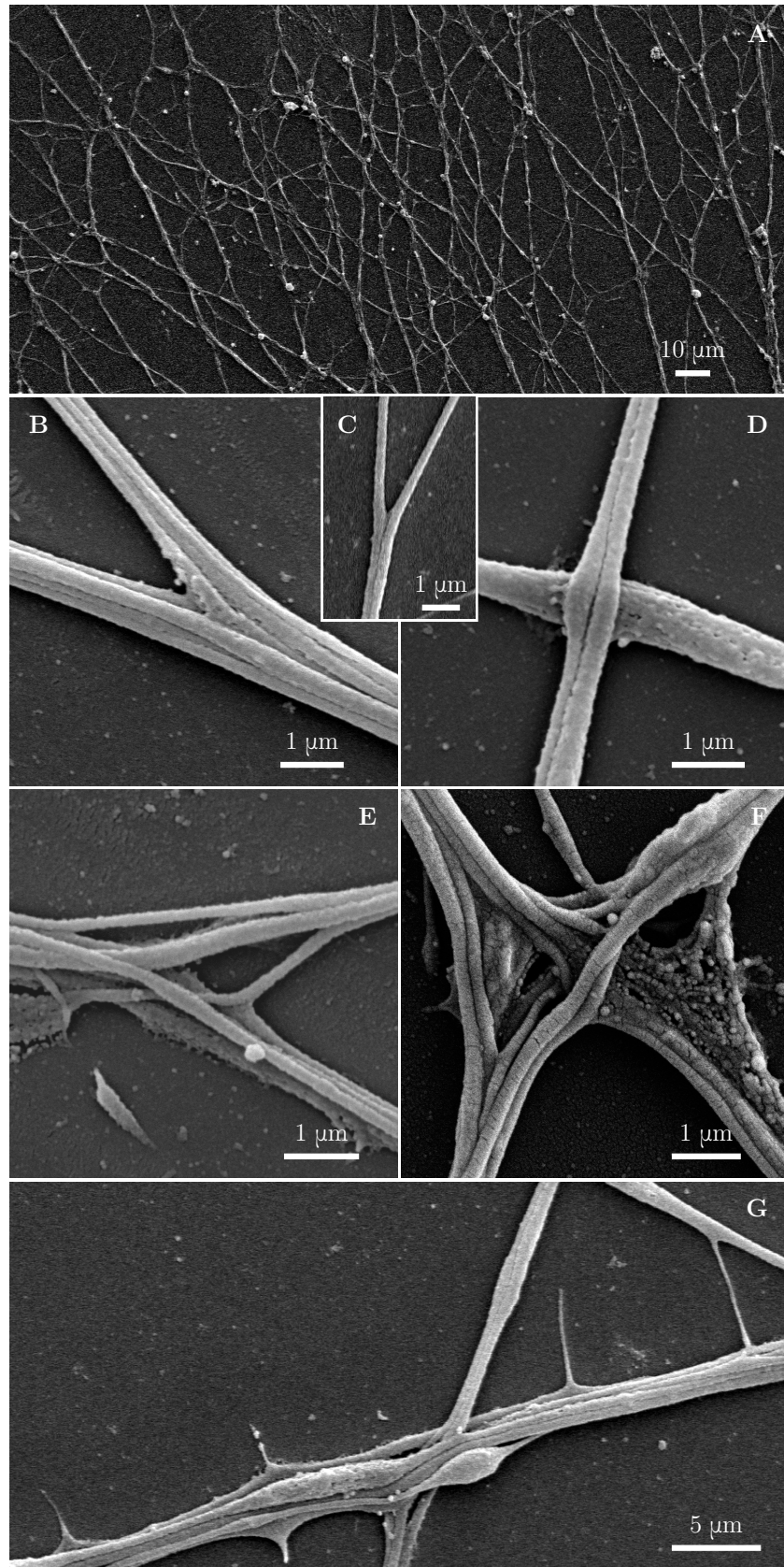
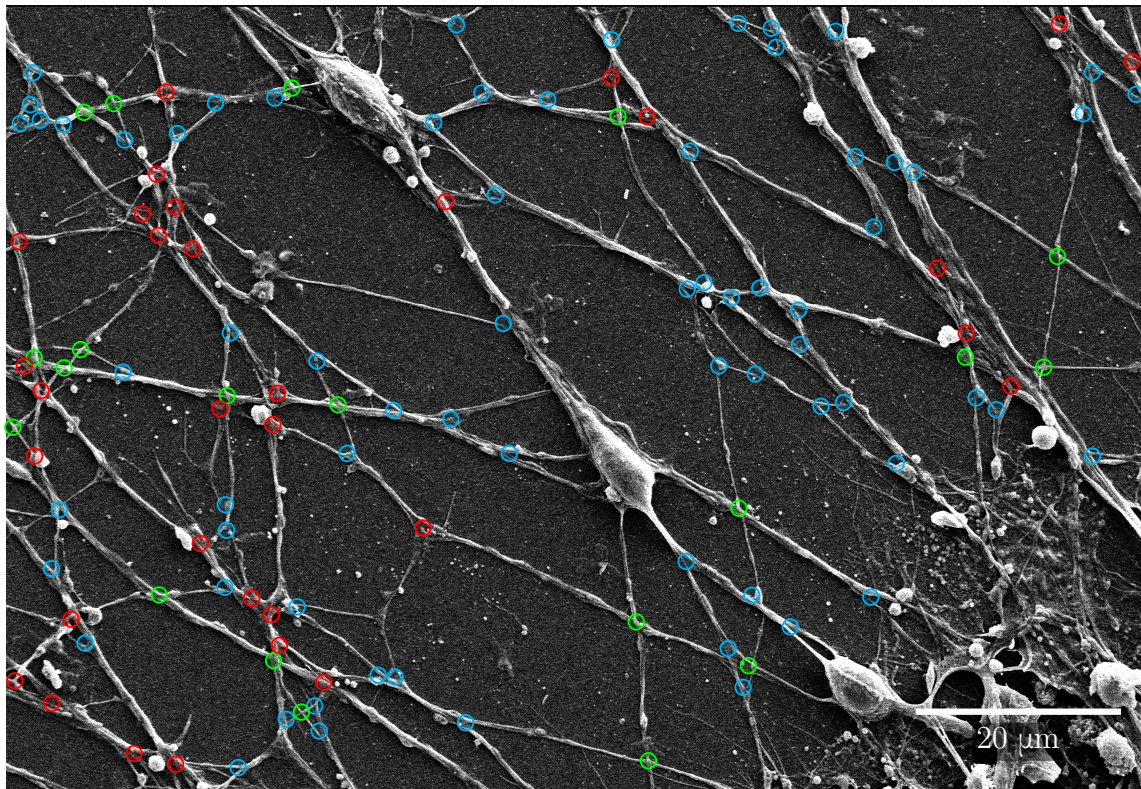


Figure 4: Fine morphological characterization of zippers with scanning electron microscopy. Panel A illustrates a large area of the culture observed at low magnification. B-C illustrate a laminar vertex structure formed between small axon bundles (B) or between individual axons (C). D illustrates crossing of axons. E and F illustrate more complex, entangled vertices. Such configurations are unlikely to easily unzip. G shows thin lateral protrusions, often seen along axon shafts. These protrusions can attach to nearby axons and pull on them.



○ simple zippers (69) ○ crossings (20) ○ entangled zippers (32)

Figure 4 – figure supplement 1: Quantification of abundance of axon crossings, simple zippers and entangled zippers. The SEM image was examined to assign simple zippers (mobile; marked in blue), entangled zippers (unable to recede; marked in red) and crossings of the axons (marked in green). The corresponding counts are indicated in the legend. The same type of analysis of a second SEM image provided the following results: 65 simple zippers, 37 entangled zippers and 24 crossings. Coordinates of selected points are available in Figure 4 – supplement 1—source data 1.

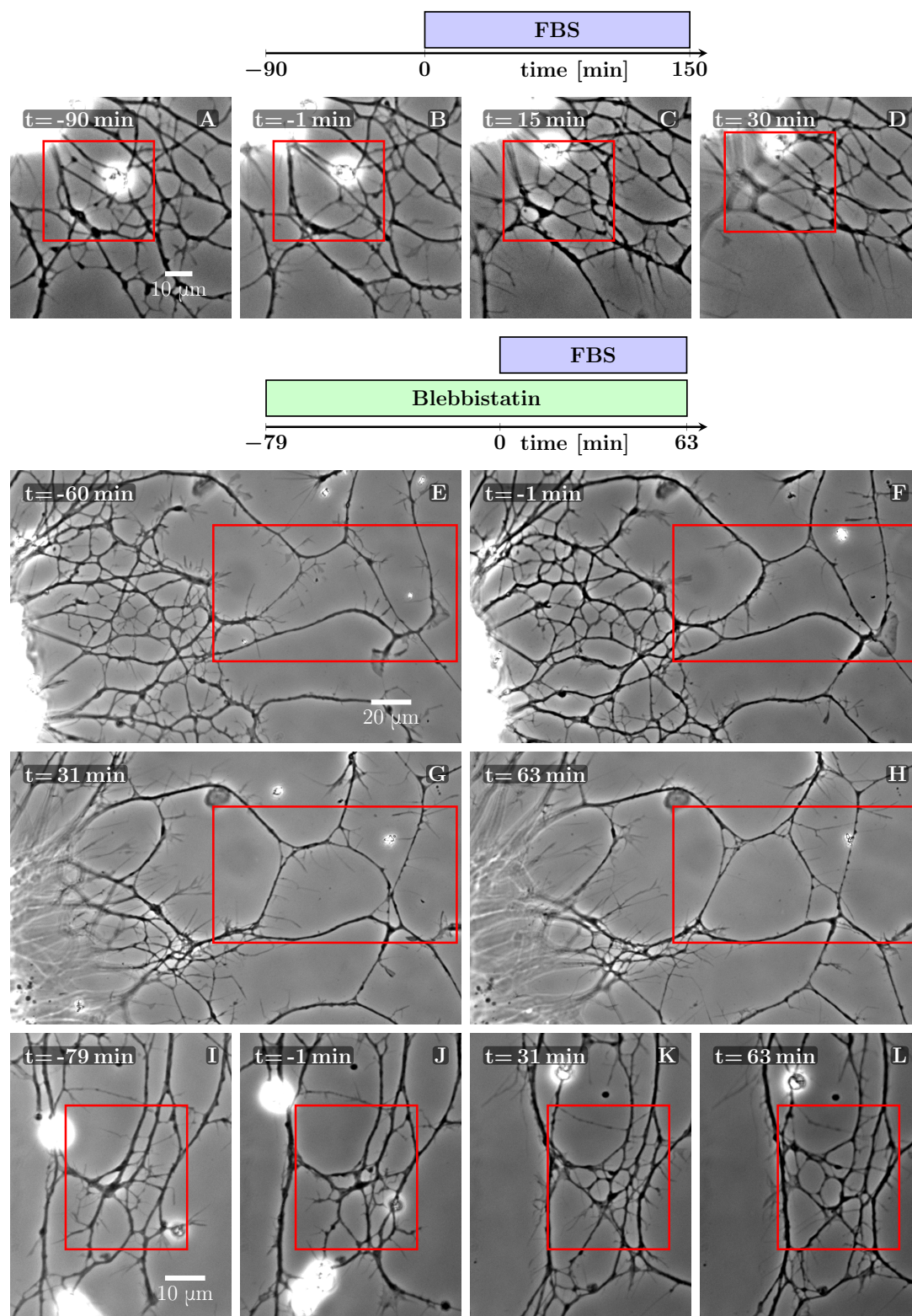


Figure 5: Defasciculation resulting from FBS-induced pull on the network. The schemes indicate the protocol of drug addition for the experiments that are shown on the frames below the schemes. A-D: FBS was added to the culture at $t=0$ min. Decoarsening of part of the network (marked by the red rectangle) is visible. At a later stage, the network collapses. The full recording is provided as supplementary Video 10. E-H: The culture was pretreated by blebbistatin added before $t=-60$ min. Little change is visible between -60 and -1 min. At $t=0$ min FBS is added, after which progressive movement of the explant border to the left can be observed, exerting a pulling force on the axons. As a result, unzipping occurs, the network defasciculates and several new loops appear in the area marked by the red rectangle. The full recording is provided as supplementary Video 11. I-L: The culture was pretreated with blebbistatin ($t=-79$ min) and the network remained mostly unchanged until FBS was added ($t=0$ min). Defasciculation is visible in the frames K and L, where the area of interest is marked by the red rectangle. The full recording is provided as supplementary Video 12.

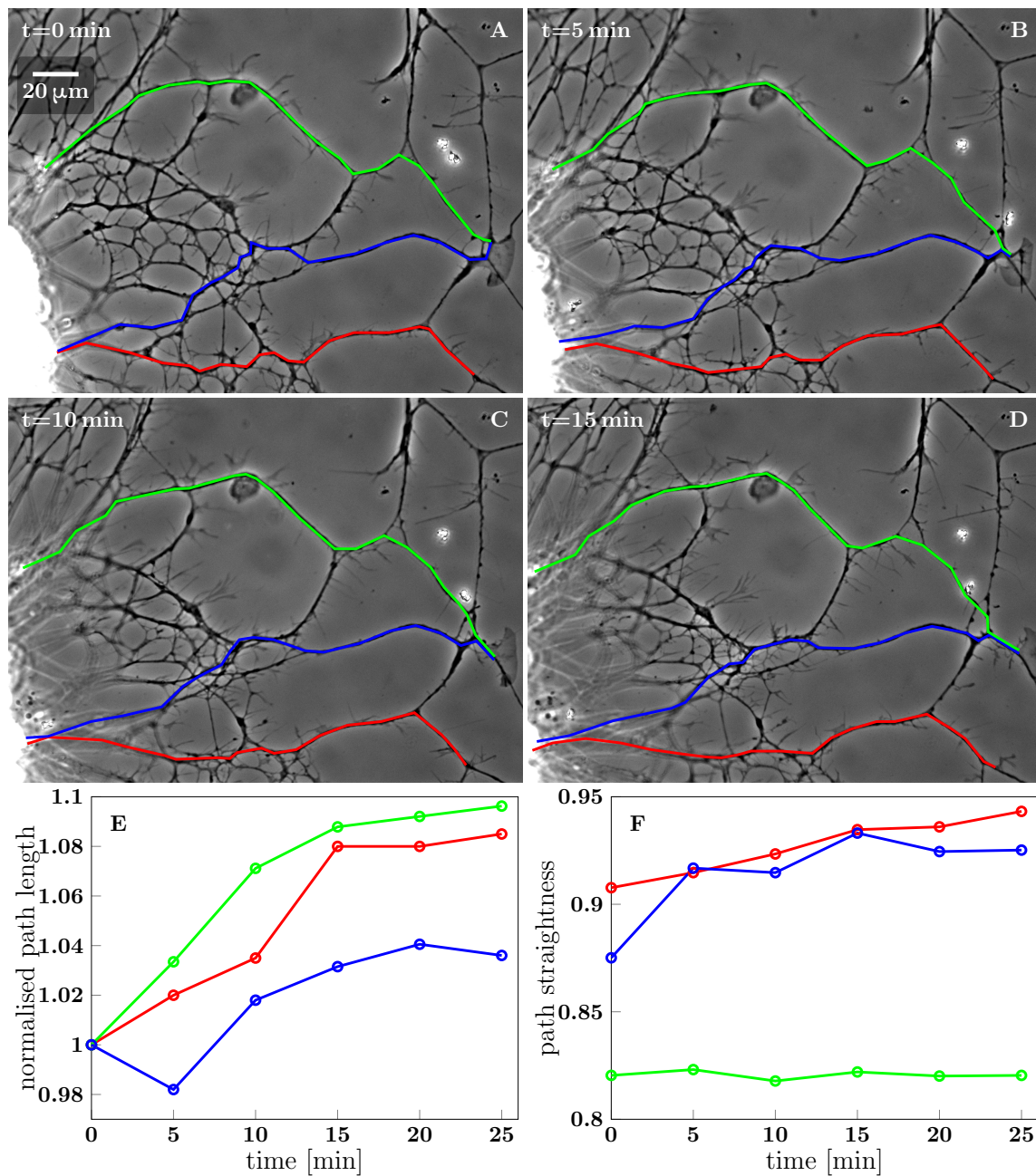


Figure 5 – figure supplement 1: Stretching of the axons due to FBS-induced pull on the network. A-D: Network configurations in the first 15 min after FBS was added to the culture. Three candidate paths of axons growing from the right edge of the explant are marked in green, blue and red, with the green and blue paths terminating in a growth cone. E: Time course of the total length of the three segmented paths, each normalized to the initial path length. F: Time course of the path straightness, defined as the ratio of the direct-line distance between the initial and final points of the path to the total path length (i.e. straightness of 1 corresponds to a straight line). The colours of the datapoints in E and F correspond to the colours of the paths in A-D. The straightness of the blue and red paths approaches 1.0, while the green path is prevented from such marked straightening by the immovable obstacle in the middle of the path. The coordinates of the path segments in panels A-D are available in Figure 5 – supplement 1—source data 1.

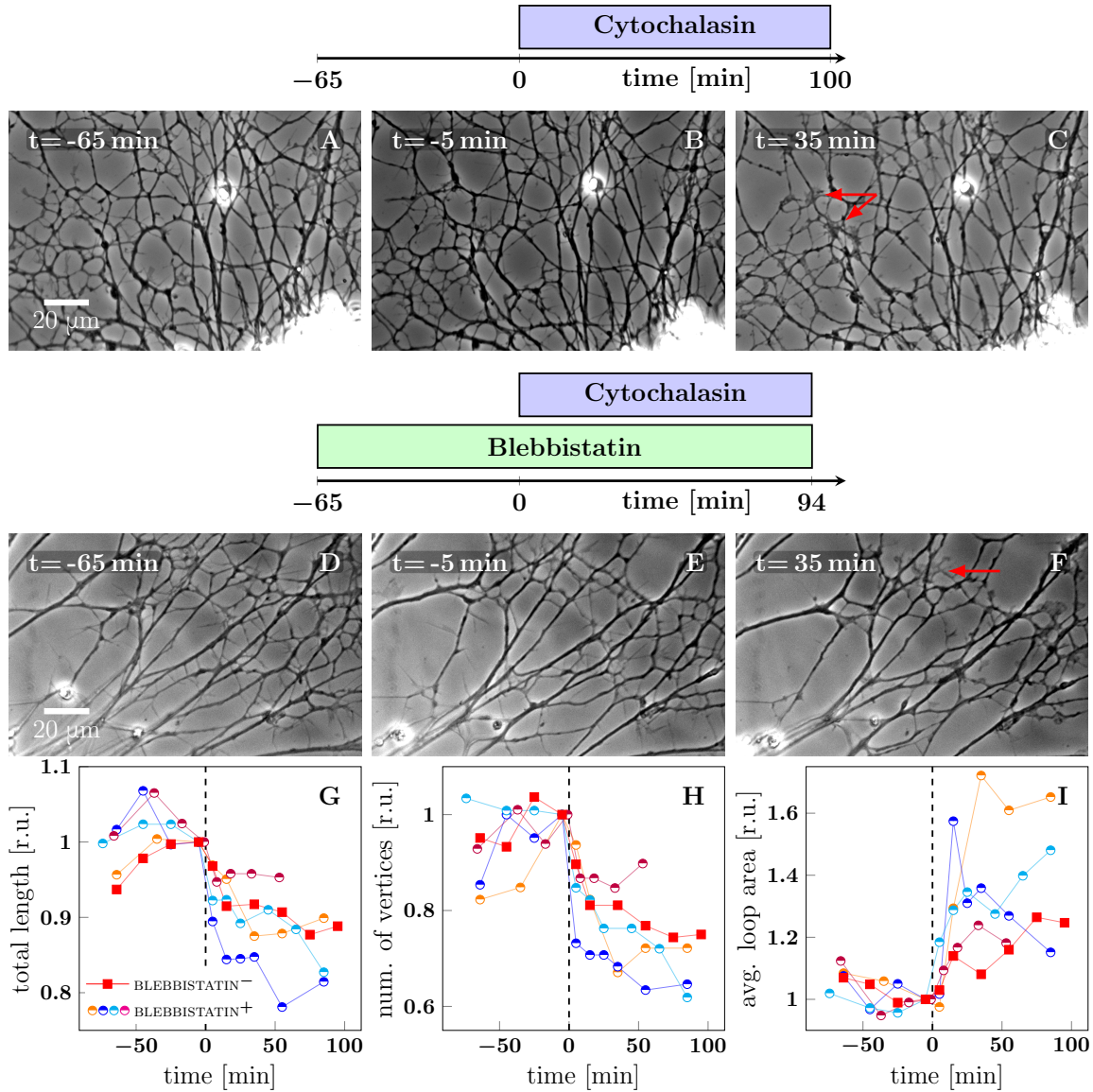


Figure 6: Cytochalasin-induced fasciculation of axon shafts. The schemes indicate the protocol of drug addition for the experiments that are shown on the frames below the schemes. A-C: Cytochalasin was added to the culture at $t=0$ min. While there is little visible change between -65 and -1 min, the network exhibits coarsening between 0 and 35 min. The full recording is provided as supplementary Video 13. D-F: The culture was pretreated with blebbistatin before $t=-65$ min. Little change is visible between -65 and -1 min. After cytochalasin addition at $t=0$ min, the culture exhibits coarsening. The full recording is provided as supplementary Video 14. The red arrows in frames C and F indicate prominent lamellipodia, which appear after the addition of cytochalasin. G-I: The network statistics for the experiment of panel A-C (red squares), panel D-F (blue half-circles), and 3 other experiments with protocol equivalent to D-F, shown as supplementary Video 15 (orange half-circles), Video 16 (purple half-circles) and Video 17 (cyan half-circles). G: Total length of the axon network in the field. H: Total number of vertices of the axon network in the field. I: Average area of cordless closed loop of the axonal network. The networks were manually segmented and analysed as indicated in MATERIALS AND METHODS. In G-I, the data was aligned by the time of cytochalasin addition marked $t=0$ min and normalized by the value of the last measured timepoint before the drug was added. A sharp decrease of total length and of the number of vertices, as well as increase of average loop area, is seen within 30 min after $t=0$ min, indicating coarsening of the network triggered by cytochalasin addition. Segmentation data and frames are available in Figure 6—source data 1 (please consult MATERIALS AND METHODS), source code used to generate the network statistics and input data is in Figure 6—source data 2, the data points plotted in panels G-I are in Figure 6—source data 3.

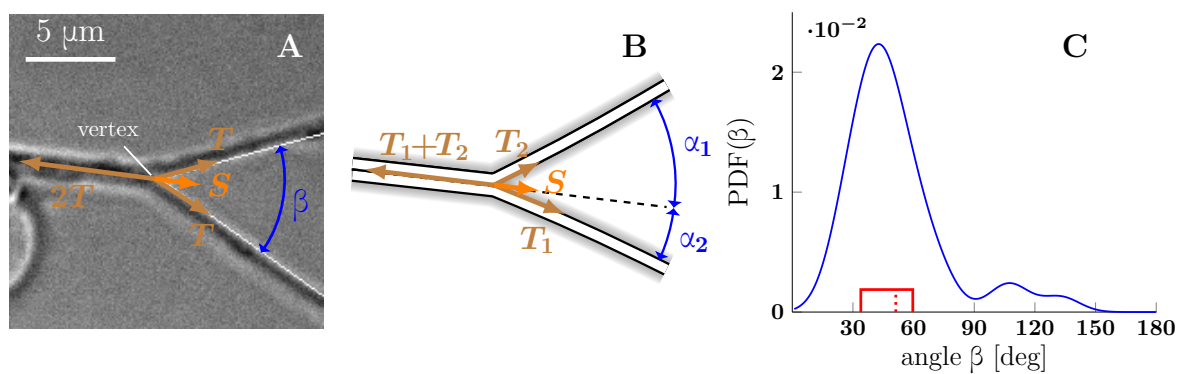


Figure 7: A: Illustration of a symmetric zipper. The zipper angle β is marked in blue. The arrows denote the vectors of tension T and axon-axon adhesive force S . B: illustration of an asymmetric zipper, the markings are the same as in A, but the tensions within the axons differ ($T_1 \neq T_2$). C: distribution of initial and final equilibrium angles of measured zippers (17 zippers, 34 measurements) originating from 4 distinct cultures (each obtained from a different mother animal), transformed into a probability distribution using convolution with Normal kernel. The red dashed line marks the average angle value (51.2°) and the solid red box delimits the interquartile range ($34\text{--}60^\circ$). The values correspond to the full zipper angle β , which equals $\beta = \alpha_1 + \alpha_2$ in asymmetric case. Individual distributions of the angles α_1 , α_2 were not recorded, because of prevailing symmetry of measured zippers. The distribution includes only those zippers, which were stable at least 5 min before and after the dynamics. The measured angles and the distribution of panel C are available in Figure 7—source data 1.

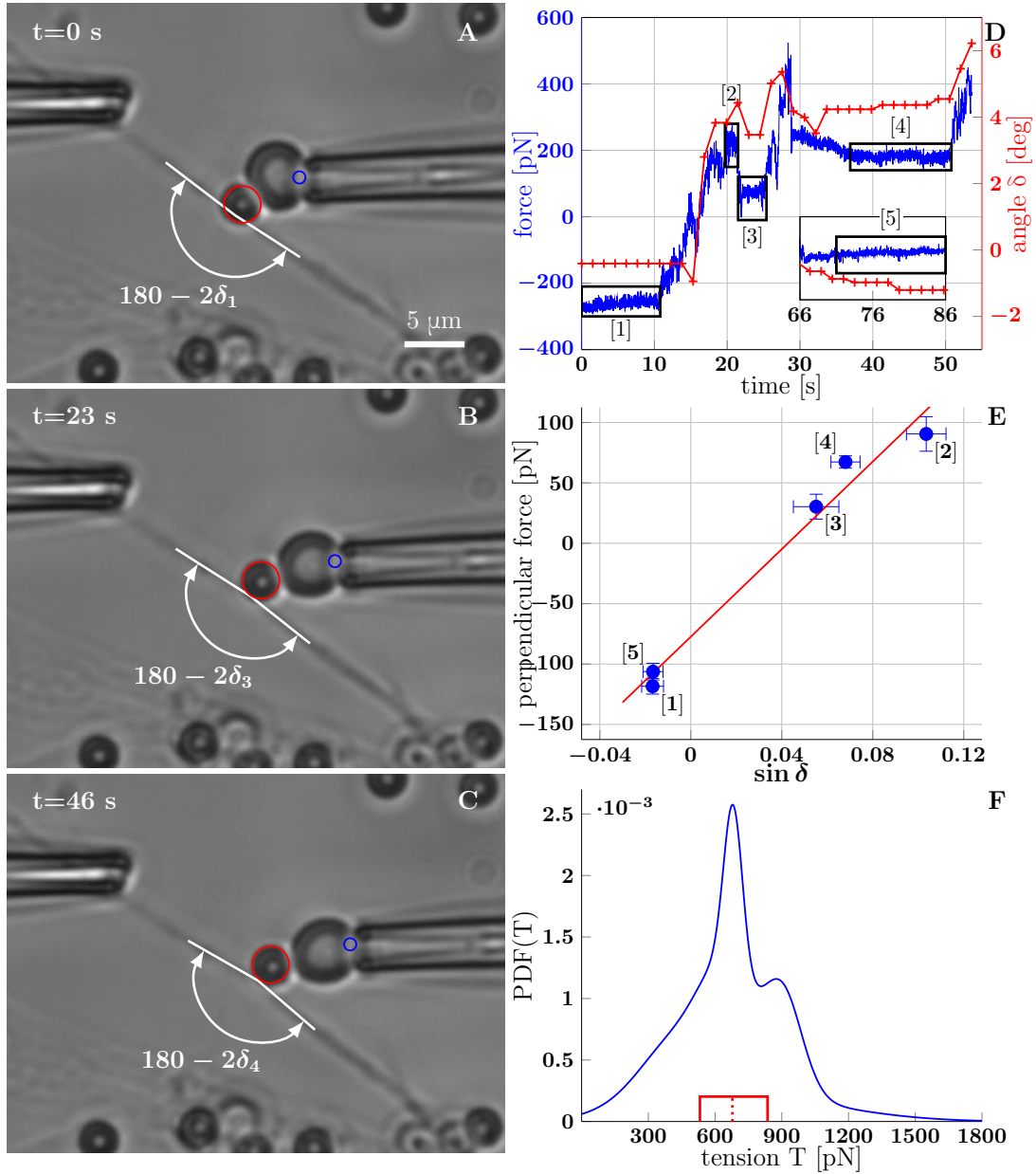


Figure 8: A-C illustrate a BFP experiment. A: The bead is slightly pushed against the axon (with deflection angle $\delta_i \gtrsim -1^\circ$), negative deformation (compression) of the RBC is recorded. B,C: Different stages of the probe exerting a pulling force on the axon; the RBC undergoes positive deformation (extension), the axon deflection angle $\delta > 0^\circ$. Index i of δ_i corresponds to the numbering of plateaux in panel D and data points in panel E. The tracked point on pipette and the tracked bead are marked by blue and red circles. D: Time dependence of the force measured on the probe (each frame at 65 fps), and the angle (measured each second). The deflection angle $\delta < 0^\circ$ corresponds to deflection by pushing, $\delta > 0^\circ$ means deflection by pulling. The deflection angle determines the lateral projection of axial tension acting at the apex, i.e. lateral tensile force $2T \sin \delta$. E: Blue data points represent time-averaged qualities of individual plateaux (labelled by appropriate numbers), abscissa corresponds to average deformation $\sin \delta$ and ordinate to average perpendicular probe force F_{BFP}^\perp . The error bars represent a standard deviation of the quantities during each plateau. The red line is a linear fit of BFP data points, i.e. F_{BFP}^\perp vs. $\sin \delta$, the slope corresponds to axon tension $2T$. Goodness of the fit is $R^2=0.97$. F: Distribution of axon tensions, calculated as a normalized sum of linear fit results from all BFP experiments—each fit j was represented by a Normal distribution, with mean at \bar{T}_j given by the fit slope, and standard deviation $\sigma(T_j)$, given by the standard deviation of data points from the fitted line. The tension mode value is 678 pN, mean 679 pN (designated by the dashed red line), interquartile range (529–833) pN (delimited by the red solid box). The distribution of tension is based on $N=7$ measurements, containing at least 3 force plateaux each, originating from 4 distinct cultures (each obtained from a different mother animal). The time course of force and angle (D), plateaux points and the fit (E), mean values of tension of all experiments and the distribution values (F) are available in the Figure 8—source data 1.

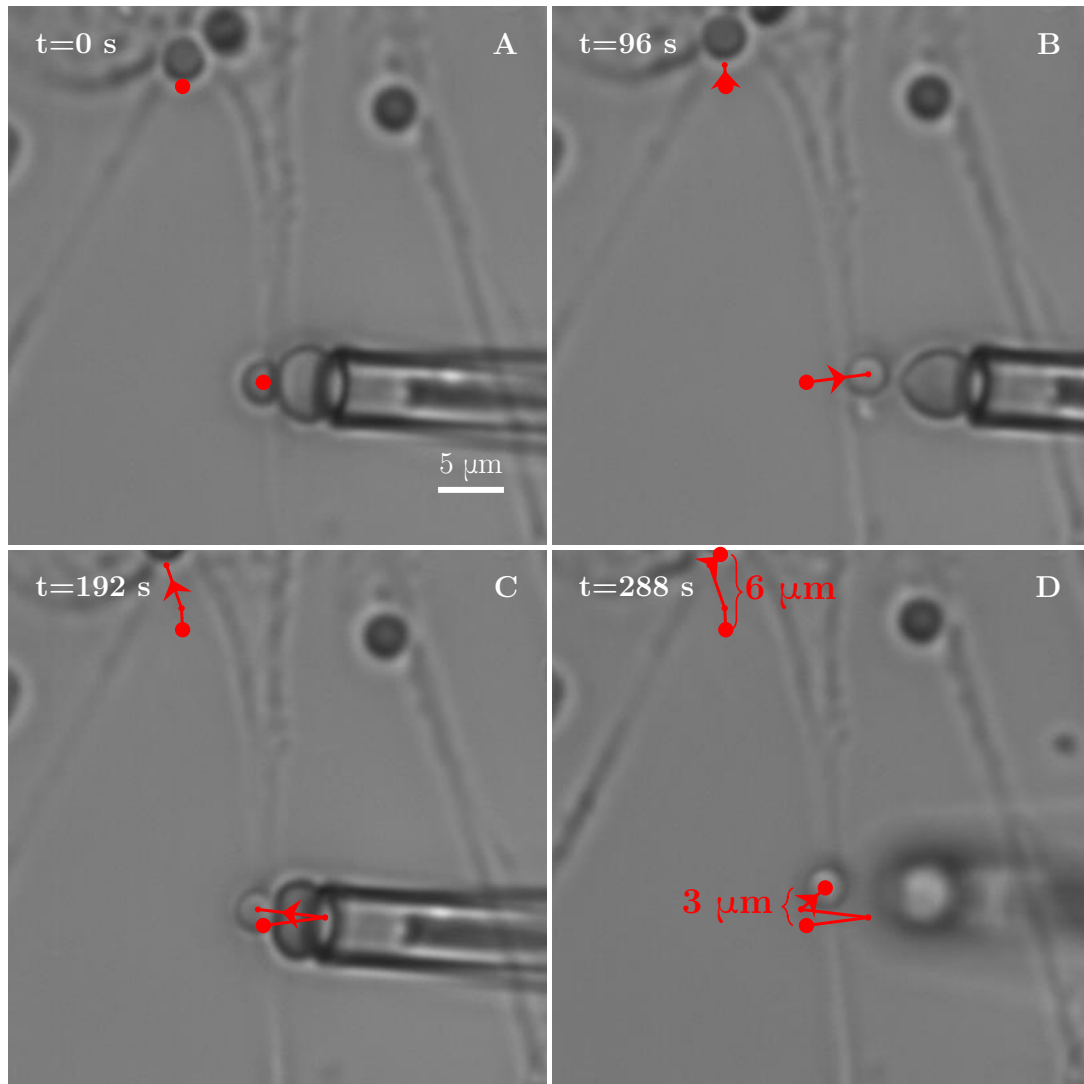


Figure 8 – figure supplement 1: A: Axon stretching observed during a BFP manipulation experiment. The two red dots serve as tracer points. The explant in the upper part of the field gradually moves upwards, pulling the proximal section of the measured bundle along, while no immediate retraction of the rest of the bundle is observed. B-D: Distance between the two points increases by 3 μm, demonstrating the stretching of the axon. The stretching is likely responsible for observed increase in tension within the axon between the frames A-D. Comparing the tension measurements at the beginning of the video and at the later stages, we observe an increase from (432 ± 157) pN to (1665 ± 219) pN.

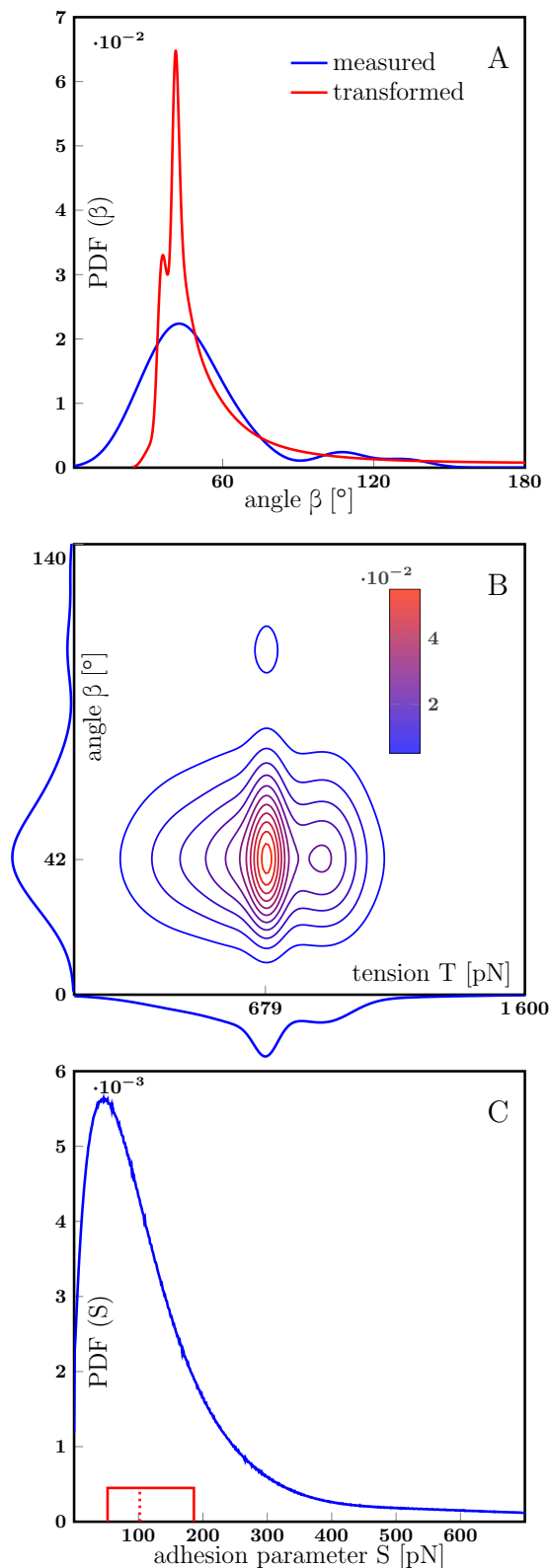


Figure 8 – figure supplement 2: A: Distribution of static equilibrium angles measured from initial and final equilibria of zippers *in vitro* (blue line), and expected distribution of equilibrium angles based on transformation of the tension probability distribution (Eq. 6 in MATERIALS AND METHODS) (red line). The adhesion parameter S of the $\text{PDF}(T) \rightarrow \text{PDF}(\beta)$ transformation was optimized to achieve maximal correlation between the distributions with the result $S=88$ pN, and correlation coefficient 0.813. B: Contour plot of the joint probability density of axon tension T and static equilibrium vertex angle β . The two distributions were considered independent, the joint probability is product of marginal PDFs, which are shown along the corresponding coordinate lines. C: Distribution of adhesion parameter S , calculated by screening the values obtained from Eq. 1 and the joint distribution in panel B. Interquartile range of S is (52–186) pN (delimited by the solid red box), while the median of the distribution is 102 pN, designated by the dashed line. The data of experimental and transformed distributions of angles (A), experimental distributions of angles and tension (B) and probability distribution of adhesion coefficient (C) are available in Figure 8 – supplement 2—source data 1, source code used to process the data is in Figure 8 – supplement 2—source data 2.

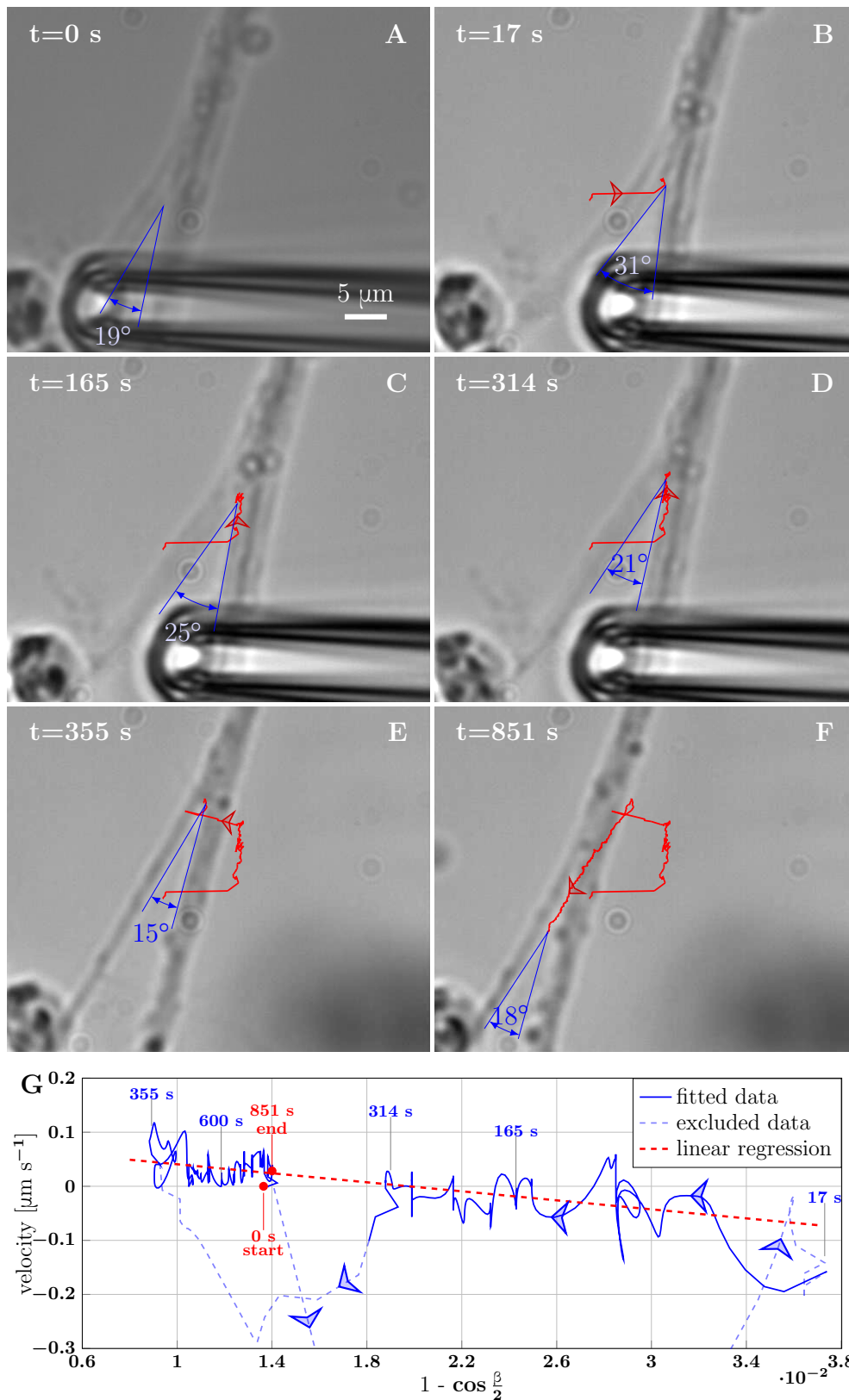


Figure 9: A: Initial state of the zipper before the manipulation. B: Angle increases as vertex shifts to the side with the initial pipette displacement. C-D: The axons unzipper, slowly. E: Pipette is removed and axon released, the vertex shifts strongly to the left to equilibrate the lateral force imbalance. F: Axons zipper back toward the initial configuration. G: Blue line (with time stamps) represents velocity and (a function of) angle of the vertex at each time point of the manipulation. Data points belonging to the full line were fitted using linear regression (dashed red line)—the ratio of slope and intercept of the line provide estimate of the ratio of tensile and adhesive forces. The goodness of the fit is $R^2=0.48$. The pale-blue dashed line corresponds to transients arising during manipulation (excluded from the regression). The values of angle were smoothed by a 20 frame Gaussian filter (for 1 fps recording), the velocity was calculated using convolution of positional data with derivative of the same Gaussian filter. The blue arrows show the direction of increasing time. Velocity, angle data and fit of panel G are available in Figure 9—source data 1.

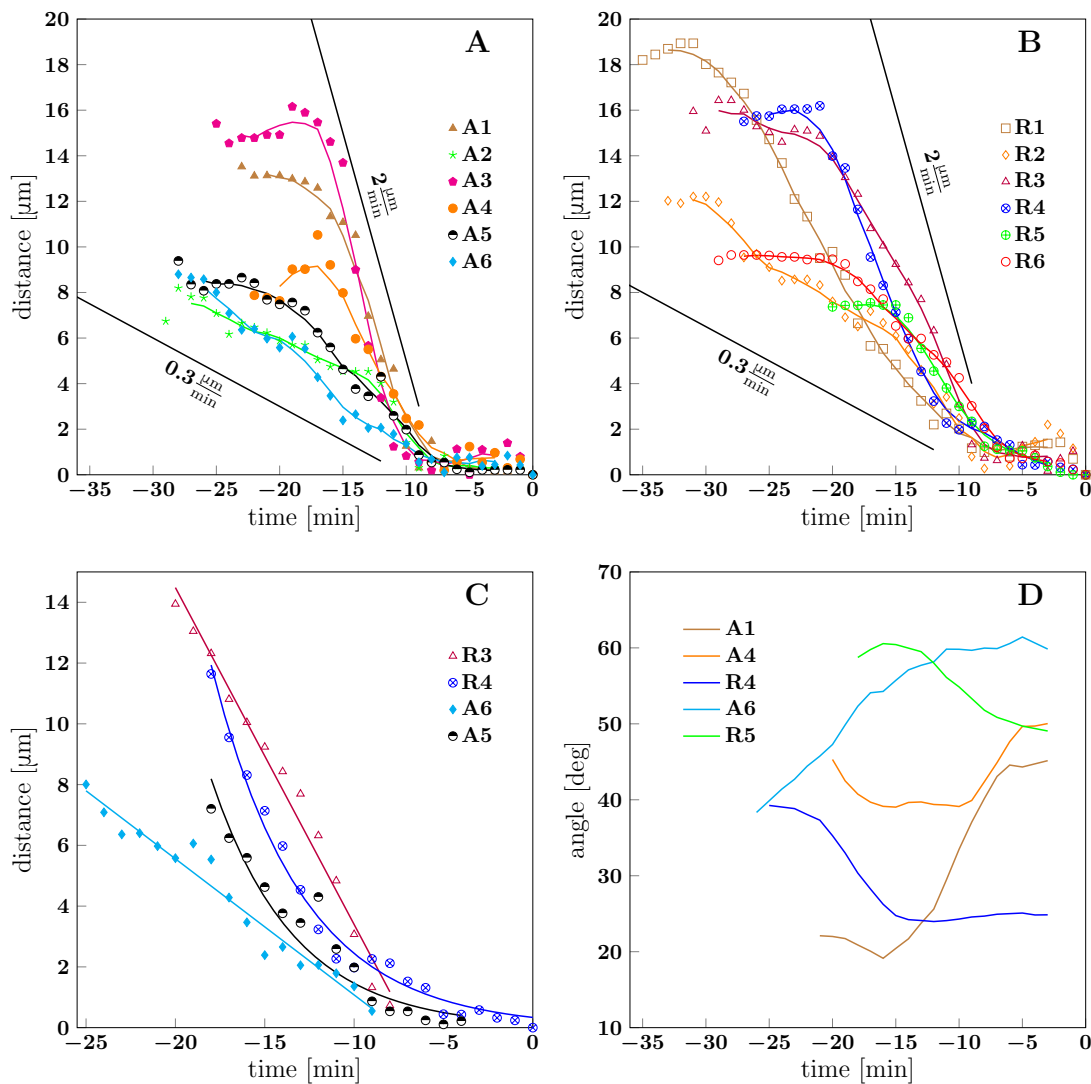


Figure 10: A and B show the convergence to equilibrium for advancing and receding zippers, respectively. The distance between the zipper vertex location at the given time and the final equilibrium position is given. The lines with slopes $(0.3-2.0) \frac{\mu\text{m}}{\text{min}}$ delimit the typical zippering and unzipping velocities. C: Fits illustrating linear or exponential convergence in time. Linear fit equations: $d_{R3}(t) = -1.11(t + 6.94)$ and $d_{A6}(t) = -0.45(t + 7.59)$; exponential fit equations: $d_{R4}(t) = 14.95 \exp\{-0.20(t + 19.14)\}$ and $d_{A5}(t) = 10.42 \exp\{-0.22(t + 19.12)\}$. D: Time course of zipper angles, smoothed by 5 frame window. Note that angle increases for advancing zippers and decreases for receding zippers. Data plotted in panels A, B and D are available in Figure 10—source data 1.

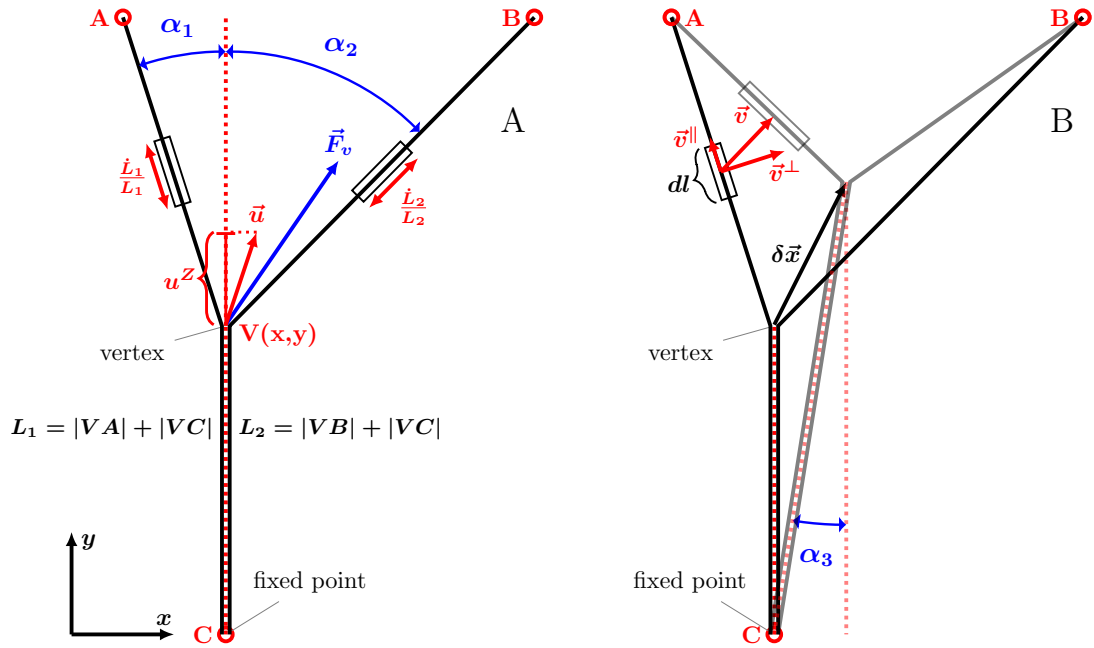


Figure 11: A: illustration of the zippering dynamics model. L_1 and L_2 denote the lengths of the two axons. The red dotted line represents the adhered zipper segment and its extension beyond the vertex (i.e. zipper axis), aligned with the y -axis in the figure. The blue vector \vec{F}_v represents the conservative forces (i.e. tension and adhesion) and the red vector \vec{u} the resulting vertex velocity limited by friction. Projection of the velocity to the zipper axis, u^Z , determines the zipper internal dissipative force, as $f^Z = -\eta^Z u^Z$. The strain rate, \dot{L}/L , determines the elongational viscous dissipative force, i.e. $f^\Downarrow = -\eta^\Downarrow \dot{L}/L$. B: illustration for the APPENDIX. $\delta\vec{x}$ represents a small displacement of the vertex. Vector \vec{v} is the velocity of the element dl , in contrast to the velocity of the vertex, \vec{u} , in panel A. Axial and perpendicular substrate friction forces f^{\parallel} and f^{\perp} are proportional to the element velocity components \vec{v}^{\parallel} and \vec{v}^{\perp} .

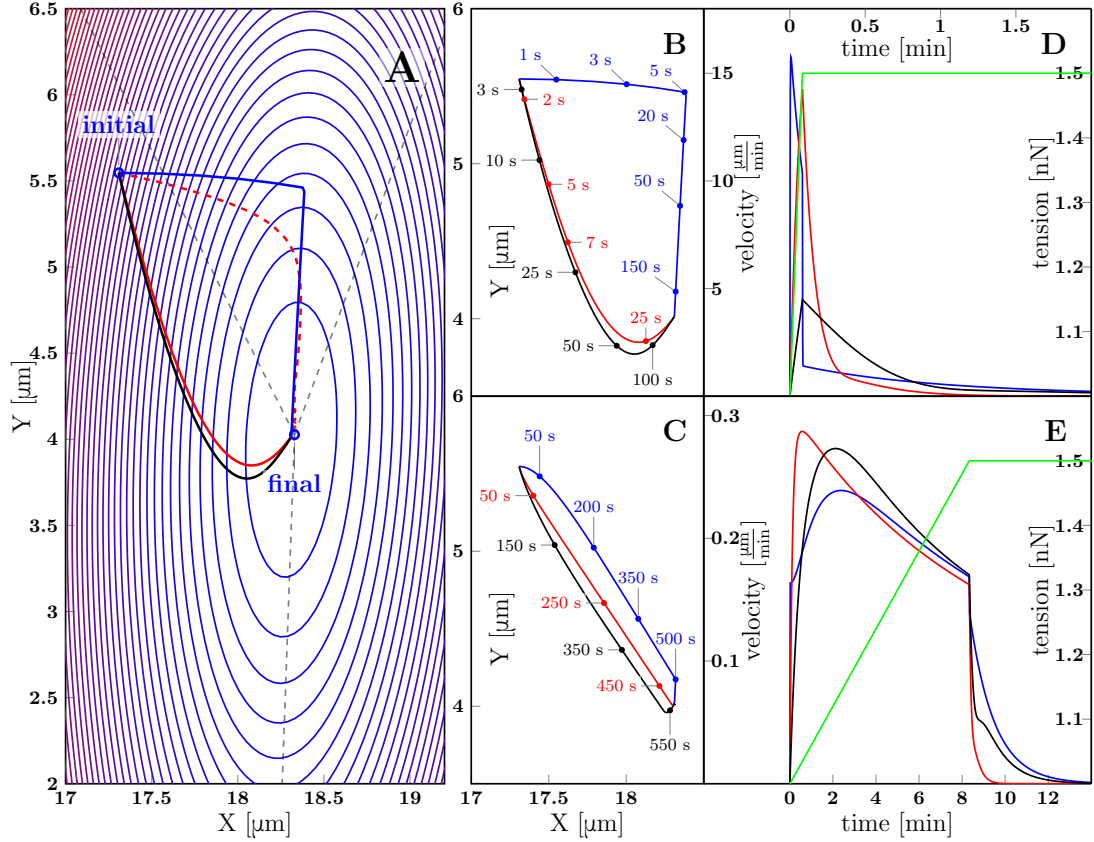


Figure 12: Zippering dynamics resulting from applying a perturbation to a zipper initially in equilibrium, converging to a new equilibrium. A: The landscape of tensile and adhesive energy (Eq. 7) for the new equilibrium condition (specified by the parameter values: left tension $T_1=1 \frac{\text{nJ}}{\text{m}}$, right tension $T_2=1.5 \frac{\text{nJ}}{\text{m}}$ (up from $T_2=1 \frac{\text{nJ}}{\text{m}}$ in the initial equilibrium), axon-axon adhesion $S=0.2 \frac{\text{nJ}}{\text{m}}$). Blue contours indicate locations of equal energy. Gray dashed lines shows axons in the final equilibrium, dashed red line is the gradient trajectory between equilibria,. The full lines indicate zipper vertex trajectories following a rapid increase of the right tension. Red: trajectory with dominant substrate friction ($f^{\parallel} + f^{\perp}$), blue: trajectory with dominant zippering friction (f^Z), black: trajectory with dominant elongation friction (f^{\Downarrow}). The trajectories with dominant friction types are represented by the same colour code across all panels. B: The same trajectories as in A, with time stamps. The tension in the right axon increased rapidly over 5 s and then was kept constant (see green line in panel D). C: Trajectories during gradual perturbation, with dominant zippering friction (blue), substrate friction (red) and elongation friction (black) over 1000 s. The tension was gradually growing in the right axon over 500 s and then was kept constant (see green line in panel E). D, E: velocity of vertex during transition, colour code corresponds to panels B and C, green line represents the prescribed tensile force in the right axon during the transition. For each experiment, one friction constant was set to a particular value to probe its effect on the trajectory, others were set to zero. The following values were used: axial substrate friction $\eta^{\parallel}=200 \text{ Pa s}$, transverse substrate friction $\eta^{\perp}=200 \text{ Pa s}$, elongation friction $\eta^{\Downarrow}=3000 \text{ nN s}$, zippering friction $\eta^Z=3 \frac{\text{nN s}}{\mu\text{m}}$ (in this case a small substrate friction value $\eta^{\parallel}=\eta^{\perp}=1 \text{ Pa s}$ was introduced to avoid a singularity when the motion direction was perpendicular to the zippering axis). Source code of the implemented zipper model used to generate the data is available in Figure 12—source data 1.

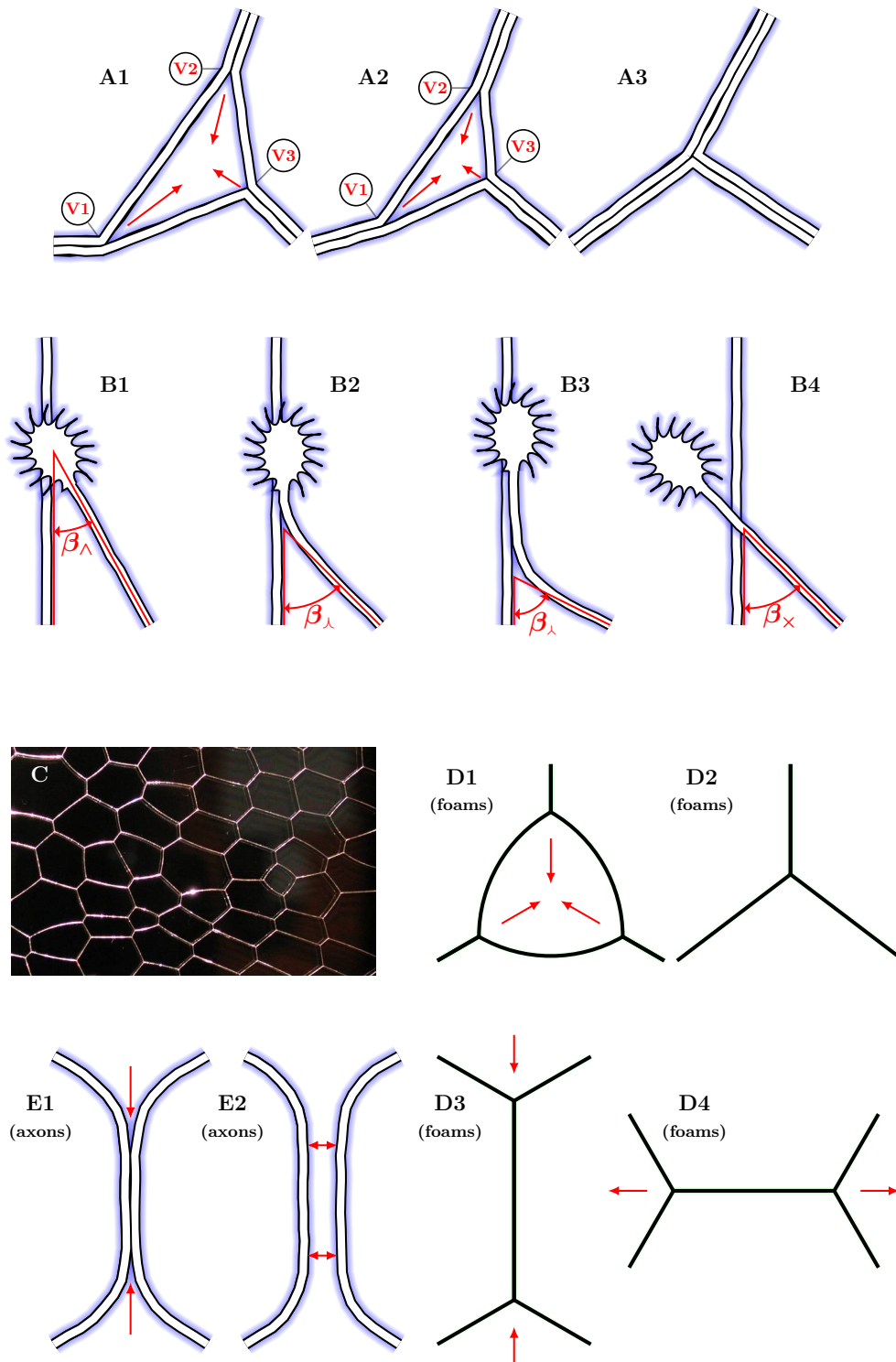


Figure 13: A1-A3: Two vertices are lost during the process of closing of a loop formed by three axon segments. The initial configuration starts to zipper at one or more vertices, gradually decreasing the total network length. A single junction formed by 3 pairs of fully zippered axons remains. B1-B4: Possible outcomes of initial contact of two axons. B1: growth cone (GC) interacts with the shaft of another axon, B2: small incidence angle allows incoming GC to adhere and follow the shaft, B3: the two shafts zipper, increasing the contact angle, B4: when the incidence angle exceeds the equilibrium zipper angle, no stable zippered segment can be formed and the growth cone crosses over. C: Photograph of structure formed by a liquid foam restricted between two glass plates. The gas bubbles are separated by liquid walls that meet at triple junctions. D1-D4: Schemes illustrating the elementary topological processes in liquid foams. D1: A three-sided bubble with curved walls, containing gas under excess pressure. D2: The gas diffuses to neighbouring cells and the three-sided bubble gradually collapses. This process is called *T2*. D3-D4: In foams, the *T1* process leads to a reconnection of bubble walls, preserving the number of vertices of the network. E1-E2: In the axon network, separation rather than reconnection results from unzipping. E1: Two vertices delimiting a zippered segment start to recede, E2: Once the adhered segment length decreases to zero, the two axons detach and separate (see experimental example in Video 3). Panel C is adapted from https://commons.wikimedia.org/wiki/Category:Foam#/media/File:2-dimensional_foam.jpg by Klaus-Dieter Keller, released into the public domain by the author.

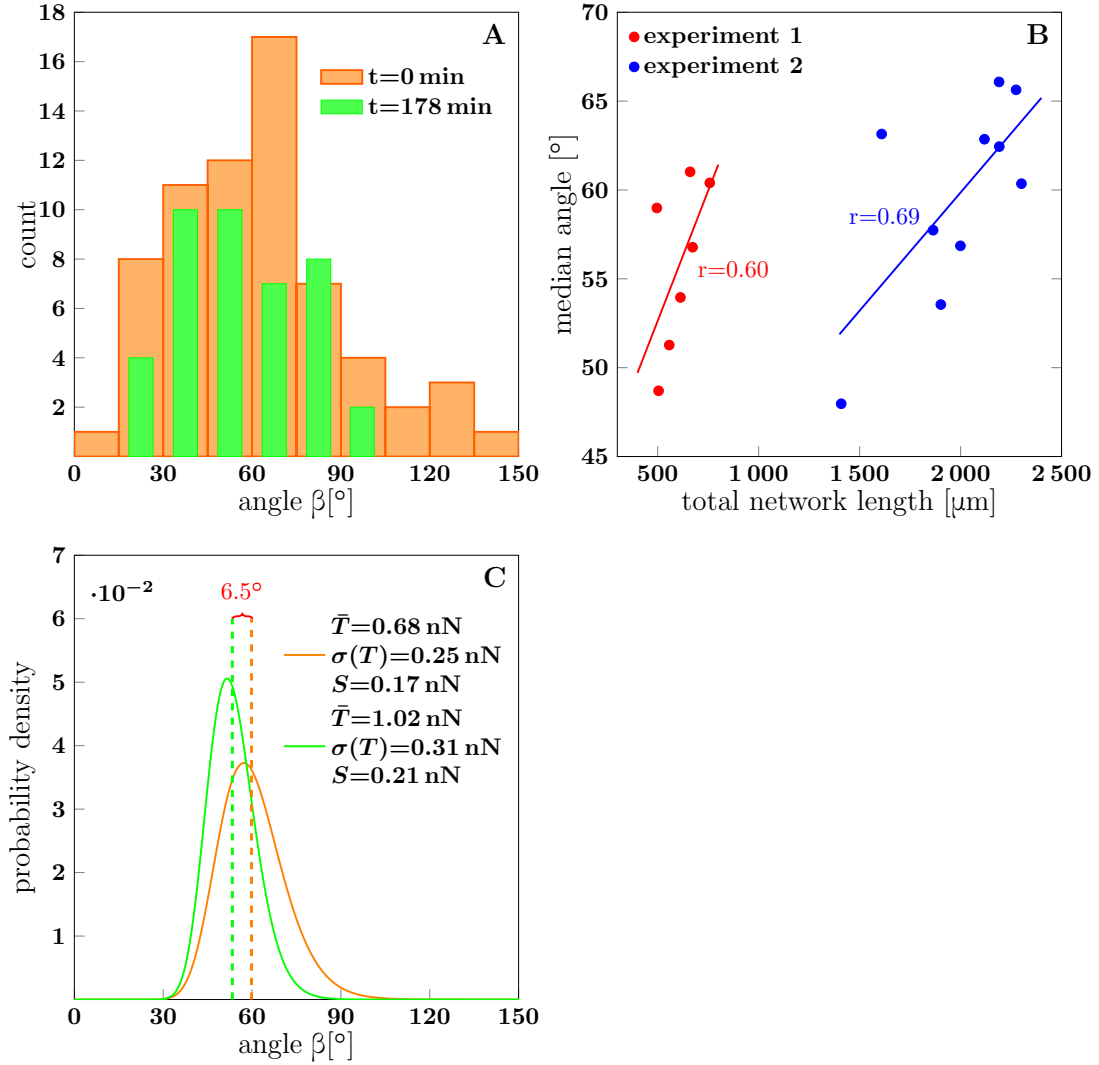


Figure 14: A: The distribution of zipper angles in the network configurations of Fig. 1D ($t=0$ min, total 66 vertices) and Fig. 1G ($t=178$ min, total 44 vertices). B: Correlation between median angle β_M and the total network length L in two experiments; r denotes the Pearson correlation coefficient. C: Predicted equilibrium zipper angle distribution $\text{PDF}(\beta)$ obtained as a transformation of distribution of fascicle tension $\text{PDF}(T)$ using Eq. 6 (see main text and MATERIALS AND METHODS). The distribution of tensions was approximated by a lognormal distribution $\text{PDF}_{\log}(\bar{T}, \sigma(T))$. The distribution plotted in orange corresponds to the values of tension from BFP experiments, $\bar{T}_{\text{BFP}}=0.68$ nN, $\sigma_{\text{BFP}}(T)=0.25$ nN, and adhesion parameter $S_0=0.17$ nN adjusted to match the initial median angle of *experiment 1*. The distribution plotted in green corresponds to parameters rescaled with mean fascicle size n as $\bar{T} \sim n$, $\sigma(T) \sim \sqrt{n}$, $S \sim \sqrt{n}$, with increase in mean fascicle size ($1.50\times$) corresponding to Fig. 1D-G (see main text). The change in median angle in panel C is 6.5° , as compared to 7.5° given by the trendline in panel B (*experiment 1*). The data of histograms (A), correlations (B) and distributions (C) are available in Figure 14—source data 1. The source code used to generate distributions in panel C is available in Figure 14—source data 2.

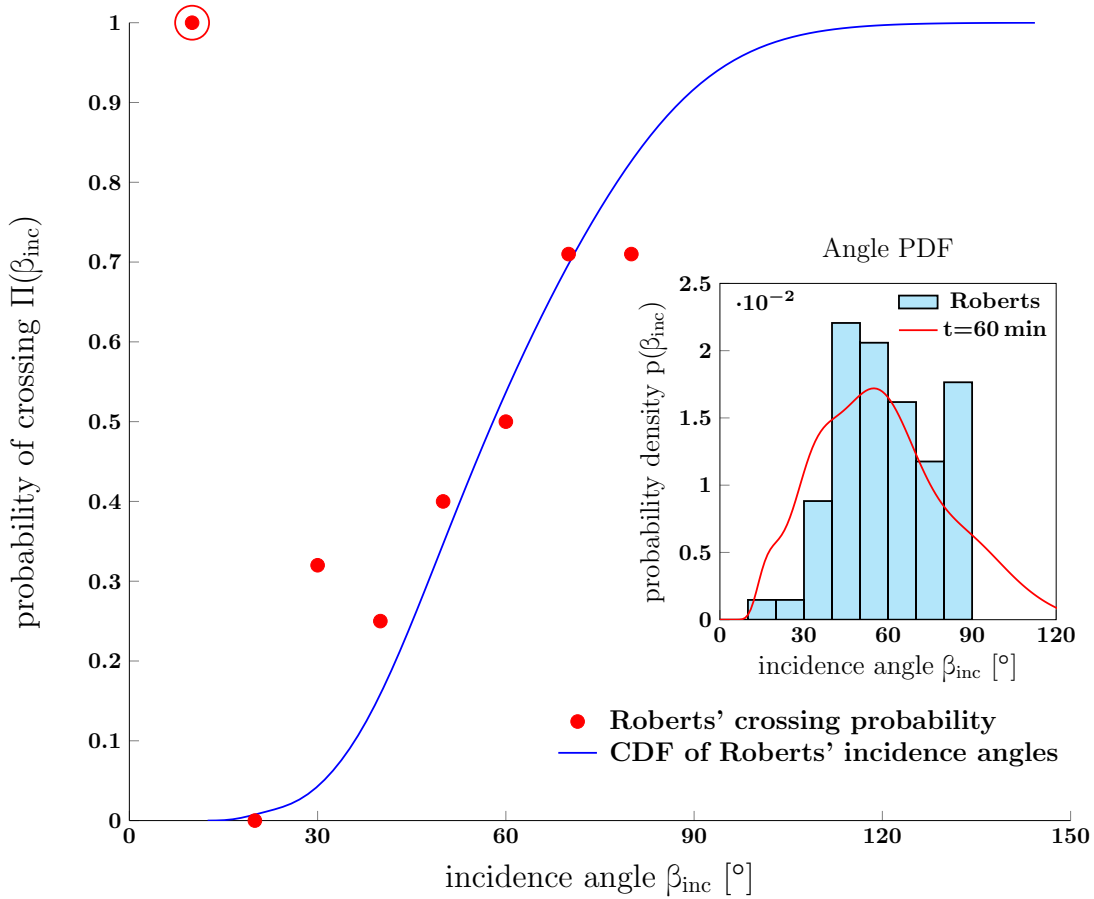


Figure 15: The red dots show data from Ref. [32] (referred to as Roberts in the figure): the observed probability of crossing of two neurites as function of incidence angle β_{inc} . The blue line is the cumulative distribution function of angles of incidence observed by Roberts ($p(\beta_{\text{inc}})$), calculated as $\Pi(\beta_{\text{inc}}) = \Pi(\beta_{\text{eq}} < \beta_{\text{inc}}) = \int_0^{\beta_{\text{inc}}} p(\beta) d\beta$. The PDF was constructed using kernel method based on the Roberts' histogram. The crossing probability of interval (0–10) $^\circ$ is an outlier (marked by the red ring) based on a single observed case. Inset: Histogram of angles of incidence as observed by Roberts. The red line is the PDF of vertex angles measured in our *in vitro* system (Fig. 1 at 60 min). The crossing probabilities and angles, experimental data and the distributions, are available in Figure 15—source data 1.