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1 **DNA-loop extruding condensin complexes can traverse one another**

2

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4

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10

11 **Condensin, a key member of the Structure Maintenance of Chromosome (SMC) protein**  
12 **complexes, has recently been shown to be a motor that extrudes loops of DNA<sup>1</sup>. It remains**  
13 **unclear, however, how condensin complexes work together to collectively package DNA into**  
14 **chromosomes. Here, we use time-lapse single-molecule visualization to study mutual interactions**  
15 **between two DNA-loop-extruding yeast condensins. We find that these one-side-pulling motor**  
16 **proteins are able to dynamically change each other's DNA loop sizes, even when located large**  
17 **distances apart. When coming into close proximity, condensin complexes are, surprisingly, able**  
18 **to traverse each other and form a new type of loop structure, which we term Z-loop – three**  
19 **double-stranded DNA helices aligned in parallel with one condensin at each edge. Z-loops can**  
20 **fill gaps left by single loops and can form symmetric dimer motors that reel in DNA from both**  
21 **sides. These new findings indicate that condensin may achieve chromosomal compaction using a**  
22 **variety of looping structures.**

23

24 The spatial organization of chromosomes is critical to life at the cellular level. Structural Maintenance  
25 of Chromosomes (SMC) complexes including condensin, cohesin, and the Smc5/6 complex are key  
26 players for DNA organization in all organisms<sup>2-5</sup>. An increasing amount of evidence suggests that the  
27 underlying principle of DNA organization by SMC complexes is to actively create and enlarge loops  
28 of DNA, a process named loop extrusion<sup>6</sup>. Polymer simulations<sup>7,8</sup> and chromosome conformation  
29 capture (Hi-C) data on topologically associating domains<sup>9-12</sup> suggested the formation of such DNA  
30 loops, while recent *in vitro* single-molecule studies provided clear experimental evidence of  
31 condensin's DNA translocase activity<sup>13</sup> and its ability to extrude loops of DNA<sup>1</sup>.

32 It remains to be seen how DNA loop extrusion by individual condensins relates to the condensation of  
33 DNA into mitotic chromosomes. Current modelling has so far assumed that translocating SMC  
34 complexes block when they collide, resulting in a string of loops clamped together at their stems by  
35 adjacent condensins<sup>9,12</sup>. Recent polymer simulations<sup>14</sup>, however, showed that this assumption fails to  
36 explain the high degree of compaction observed in mitotic chromosomes<sup>15</sup> if considering asymmetric  
37 extrusion of loops by condensin, the property found in *in vitro* experiments<sup>1</sup>. Experimental evidences  
38 for both condensin<sup>16</sup> and cohesin<sup>17-20</sup>, suggested mutual interactions and a close spacing of SMC  
39 proteins<sup>21</sup>. Here, we study the cooperative action of condensin complexes by time-lapse single-  
40 molecule visualization. The data reveal a set of distinct interactions between DNA loop-extruding  
41 condensins, including the re-shuffling of individual loop sizes and the striking ability of condensins to  
42 traverse one another to form a dimeric motor that reels in DNA from both sides and creates a novel  
43 type of condensed DNA.

44 To study the interaction between multiple condensin-mediated DNA loops, we imaged the extrusion of  
45 DNA loops by budding yeast condensin on 48.5-kilobasepair (kbp)  $\lambda$ -DNA substrates that were  
46 tethered at both ends to a passivated surface and stained with Sytox orange<sup>1</sup> (SxO) (Fig. 1a). Upon  
47 addition of condensin and ATP, we observed DNA loops as bright fluorescent spots (Fig. 1b), which  
48 could be stretched into loops by applying an inplane buffer flow perpendicular to the attached DNA.  
49 While our previous study<sup>1</sup> focused on the properties of single loops at a protein concentration of 1 nM,  
50 we here explored slightly higher concentrations (2–10 nM). Notably, such concentrations, at which we  
51 observe a few condensins binding per DNA molecule (on average 1 condensin per 12±4 kbp,  
52 measured at 4 nM;  $n=10$ ; Methods), approach the *in vivo* situation in the yeast nucleus, where a rough  
53 estimate (Supplementary Information) indicates 1 condensin per ~10 kbp of DNA<sup>22,23</sup>.

54 We first consider the case where two condensins bound at different positions along the same DNA  
55 molecule and subsequently extruded individual loops. In this case, we observed two locally compacted  
56 DNA regions, which could be stretched into loops under buffer flow (Fig. 1c). Since yeast condensin  
57 extrudes DNA loops asymmetrically<sup>1</sup>, where the side from which DNA is reeled into the loop is  
58 presumably set by the orientation of the Ycg1/Brn1 DNA-anchor site<sup>1,24</sup>, two individual DNA loops

59 either maintain a finite gap between them (Fig. 1d, Extended Data Fig. 1a,b) or converge towards each  
60 other (Fig. 1e). We observed a 25:75% distribution of mutually non-converging or converging loops  
61 (Fig. 1f). This ratio perfectly agrees with the expected distribution for a random orientation of two  
62 condensins, given that only one out of four possible orientations of two condensins (anchor sites  
63 facing towards each other) should produce non-converging loops.

64 Unexpectedly, we found that loops can influence each other, even if separated far apart. Upon  
65 initiation of a second loop, the pre-existing loop often began to shrink (70% of cases;  $n = 40$ ) (Fig. 1g,  
66 Supplementary Video 1, Extended Data Fig. 1c,d). The changes in DNA length of the two loops  
67 exhibited a clear anticorrelation (Fig. 1h, 1i, and Extended Data Fig. 1e), showing that the new DNA  
68 loop extruded by the second condensin grew at the expense of the original one. Loop shrinkage was  
69 more pronounced at higher DNA tension (Extended Data Fig. 1f) and could also be solely induced by  
70 increasing the tension by applying a larger buffer flow (Extended Data Fig. 2a-c, Supplementary  
71 Video 2). These results show that DNA in a loop can slip back through the condensin, caused by an  
72 increase in DNA tension that occurs as a second condensin starts reeling in DNA. Notably, loop  
73 slippage occurred mostly from the non-anchor site of condensin (Extended Data Fig. 2a-c,  
74 Supplementary Video 2), while at higher ionic strength conditions (e.g. 125 mM NaCl, 5 mM MgCl<sub>2</sub>),  
75 where the strength of condensin's DNA anchor is reduced,<sup>1</sup> it occurred from both sides of condensin  
76 (Extended Data Fig. 2d-g). The finding that loop extrusion of a remotely located condensin on the  
77 same DNA substrate can induce shrinkage of an already extruded loop implicates that it is possible to  
78 redistribute individual DNA loop sizes (Fig. 1j).

79 Surprisingly, separate individual loops were *not* the majority class of DNA structures in the  
80 experiments with higher condensin concentrations. Instead of individual parallel loops, we  
81 predominantly observed a higher-order DNA structure that appeared as an elongated line of high  
82 fluorescence intensity with a single condensin located at both edges (Fig. 2a, b, Extended Data 3 for  
83 quantification). Imaging under a sideways flow revealed that the observed structure consisted of three  
84 dsDNA stretches connected in parallel (Fig. 2b, Extended Data Fig. 4 and Supplementary Video 3).  
85 We name this structure a *Z-loop*, since its shape resembles the letter Z. The probability to observe Z-  
86 loops increased with the condensin concentration and became the majority pattern for concentrations  
87 higher than 6 nM (Fig. 2c). Similar data were obtained at physiological salt concentrations (125 mM  
88 NaCl, 5 mM MgCl<sub>2</sub>, 10 nM condensin, Extended Data Fig. 5a).

89 Real-time imaging of the flow-stretched DNA revealed the characteristic formation of a Z-loop (Fig.  
90 2d, Supplementary Video 4, Extended Data Fig. 6): After a single loop had been extruded, a locally  
91 compacted region – presumably a small loop formed by an additional condensin – appeared within the  
92 initial loop (453 s) and approached to the stem of the single loop (459 s). This ‘nested loop’ of two  
93 smaller parallel loops did not stop at this point, but instead began to extend towards the DNA outside

94 of the initial loop (540 s) and continued to stretch until it either hit the tethered end of DNA (629 s) or  
95 until the motion stalled, presumably due to the tension in the DNA. To trace the position of the two  
96 condensins during Z-loop formation, we co-imaged DNA and condensin labelled with a single  
97 fluorophore (ATTO647N) (Fig. 2f and Supplementary Video 5, Extended Data Fig. 7). This revealed  
98 that after some time  $\Delta t_1$  after the initiation of the first loop, an additional condensin bound to a  
99 position within the initial DNA loop (59 s) and subsequently approached the stem of the loop (63 s),  
100 where the first condensin was located. After a brief waiting time  $\Delta t_2$ , one of these condensins then  
101 moved away from the stem of the loop and translocated along the DNA outside of the loop, resulting  
102 in a Z-loop (92 s). To identify which of the two condensins co-localized at the ‘leading edge’ of the Z-  
103 loop, we examined events where the first condensin had photobleached before the binding of the  
104 second condensin (Fig. 2f, Extended Data Fig. 8, Supplementary Video 6). These experiments  
105 unambiguously show that it was the second condensin that, strikingly, traversed the first condensin at  
106 the base of the first DNA loop.

107 We then quantified the data. The initial lag time  $\Delta t_1$ , the interval between the start of the initial loop  
108 extrusion and the start of the loop-within-a-loop formation, decreased with protein concentration (Fig.  
109 2g), as expected, since it should correlate with the time lag between binding events of the first and  
110 second condensin. The second lag time  $\Delta t_2$ , the interval between the end of the formation process of a  
111 loop within a loop (i.e. when the second condensin reached the first one) and the start of Z-loop  
112 formation was short ( $7\pm 6$  s) and independent of protein concentration. This quantifies the time that  
113 two condensins spent in close proximity to each other before the second condensin traversed the first  
114 one. The DNA-loop expansion rate (Fig. 2h, Methods) was similar for single loops and loops within  
115 loops ( $0.7\pm 0.4$  kbp/s and  $0.9\pm 0.3$  kbp/s, respectively), consistent with the notion that the observed  
116 compaction of the single loop is induced by a second condensin reeling in, at the same speed, a loop  
117 within the initial loop. The observed rate of Z-loop formation was lower ( $0.1\pm 0.1$  kbp/s), likely due to  
118 the high tension in the DNA tether after the full extrusion of a single loop ( $\sim 0.4$  pN). Indeed, at high  
119 tensions, the average rates of single loop and Z-loop formation were similarly low (Extended Data Fig.  
120 9g). To compare single- and Z-loop formation at low tension, we measured their respective rates in a  
121 single-tethered assay where only one end of the DNA was attached to the surface and DNA was flow-  
122 stretched (Extended Data Fig. 10a,b). This yielded speeds of  $0.8\pm 0.4$  kbp/s and  $1.3\pm 0.6$  kbp/s,  
123 respectively, i.e., a speed of Z-loop formation that was in fact higher than that for single loops. Once  
124 formed, Z-loops were even more stable than single loops (Extended Data Fig. 5b,c).

125 Z-loops also formed when two separate loops formed individually on the DNA tether and mutually  
126 collided (Fig. 2i, Extended Data Fig. 6d, Supplementary Video 7). However, in our double-tethered  
127 DNA assay, these events were rare ( $\sim 7\%$ ), largely because the increase in DNA tension during loop  
128 formation often stalled the two condensin motors before they would merge, whereas these events

129 occurred more frequently in our single-tethered DNA assay, where the DNA continually exhibits a  
130 low tension. Thus, the double-tethered assay favors Z-loop formation by binding of the second  
131 condensin within the loop formed by the first one, since the DNA within that loop is not under tension.

132 Our data reveal the characteristic pathway of two condensins that traverse each other and, as a result,  
133 form a Z-loop (Fig. 3a). Upon forming a loop within a loop, the second condensin approaches the first  
134 condensin, shortly pauses ( $\Delta t_2 \sim 7$  s), and then reaches out to the *trans* DNA outside of the first loop.  
135 Here, regardless of the two possible relative orientations of two condensins (zoomed images in Fig.  
136 3a), the second condensin can in principle reach out to the DNA next to the anchor site or to the site  
137 opposite to the anchor of the first condensin (route I or II, respectively). After the direction is chosen,  
138 the second condensin traverses the first condensin and translocates along the DNA, forming a three-  
139 stranded Z-loop. Interestingly, these two routes I and II lead to qualitatively different loops, viz., a Z-  
140 loop that reels in DNA from both sides (top) or only from one side (bottom). By comparing the  
141 relative direction of single-loop growth before stalling and the direction of subsequent Z-loop  
142 extension (Fig. 3b), we, surprisingly, did not observe a 50:50% distribution of both types, as could be  
143 expected for random relative orientations of the two condensins, but rather a 75:25% distribution of  
144 two-side-pulling versus one-side-pulling Z-loops (Fig. 3c). The observed strong bias to form two-side-  
145 pulling Z-loops likely originates from a preference for the second condensin to traverse to DNA  
146 beyond the anchor site of the first condensin (route I). In the (rare) cases that a Z-loop initiated very  
147 early (i.e. for small  $\Delta t_1$ ) and thus the second condensin bound within the initial loop before the first  
148 condensin was stalled, we observed that the Z-loop expanded symmetrically to both directions,  
149 directly confirming two-side pulling (Fig. 3d, Supplementary Video 9). Two-side pulling was  
150 observed more frequently in single-tethered DNA where the DNA substrate was under low tension  
151 (Fig. 3e, Extended Data Fig. 10b). For double-tethered DNA, once Z-loops were fully extended, they  
152 occasionally (~30%) slipped DNA from one or both of the edges, leading to random diffusion along  
153 the DNA tether over time (Extended Data Fig. 10h). Remarkably, the data show that condensin, which  
154 individually is a one-side pulling motor, can cooperatively reel in DNA from both sides in the form of  
155 a two-side pulling Z-loop driven by a condensin dimer.

156 These discoveries of interactions between DNA-loop extruding condensin complexes have important  
157 implications for understanding the fundamental mechanisms of chromosome organization. The finding  
158 that multiple DNA loops can change their sizes via slippage adds valuable information to the current  
159 picture of loop-extrusion dynamics, which so far only considered the formation, growth, and  
160 dissociation of loops. Whereas our previous discovery of an asymmetry of DNA-loop extrusion posed  
161 a problem, since this mechanism would leave gaps in-between loops<sup>14</sup>, the newly discovered Z-loops  
162 may extend along the unextruded parts of DNA, thereby filling such gaps (e.g. Fig. 3f, top, and  
163 Supplementary Video 10). Notably, in this case, a Z-loop does not reduce the DNA end-to-end length

164 more than two individual loops (Extended Data Fig. 10c-g), but it rather changes its topology into a  
165 more condensed Z structure. If Z-loops initiate rapidly after the nucleation of single loops, they result  
166 in two condensins anchored close to each other that frequently yield a two-side-pulling condensin  
167 dimer that reels in DNA symmetrically (Fig. 3g, bottom). Notably, in this case, the resulting extruded  
168 loop is split into two loops, contrary to the common view of a single loop being extruded. Rather than  
169 individual parallel loops, Z-loops might be the norm, given their frequent occurrence. Most  
170 importantly, the unanticipated ability of condensin complexes to traverse one another has direct  
171 consequences for modeling of chromosomes<sup>8,11</sup>. This transit of the condensin barrier may constitute a  
172 special case of a more general phenomenon of obstacle bypassing by SMCs<sup>25,26</sup>. Models of  
173 chromosome compaction will need to consider these findings that SMC proteins exhibit the ability to  
174 form a rich variety of looping structures.

175

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184 performed the imaging experiments, J. K. contributed in image analyses, I. A. S. developed condensin  
185 fluorescence labeling strategies and purified protein complexes, C. D. and C. H. H. supervised the  
186 work, all authors wrote the manuscript.

187 **Competing interests:** All authors declare that they have no competing interests.

188 **Data and materials availability:** Original imaging data and protein expression constructs are  
189 available upon request. The Matlab source code used for quantification of the number of condensins  
190 and the amount of DNA sizes is available at  
191 [https://github.com/jacobkers/BN\\_CD18\\_EK\\_CondensinTrack](https://github.com/jacobkers/BN_CD18_EK_CondensinTrack).

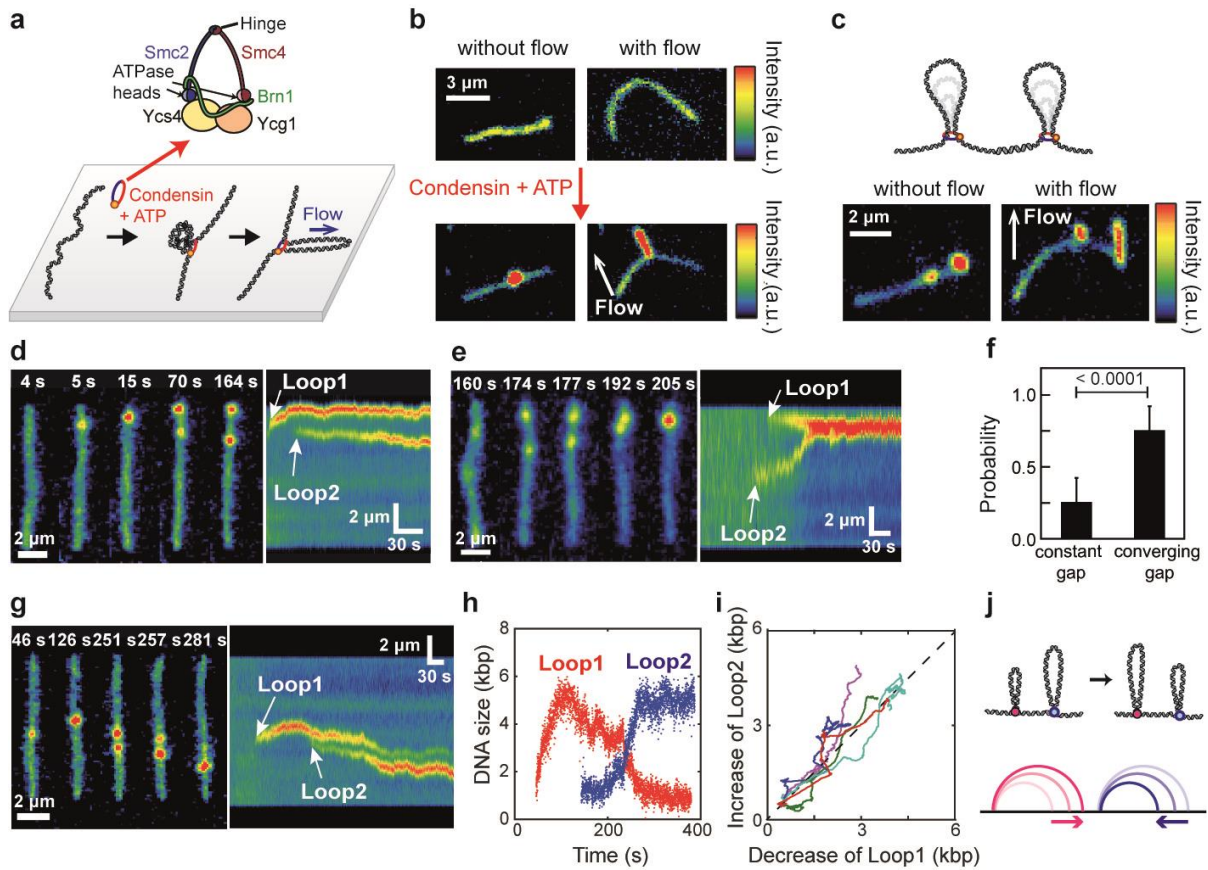
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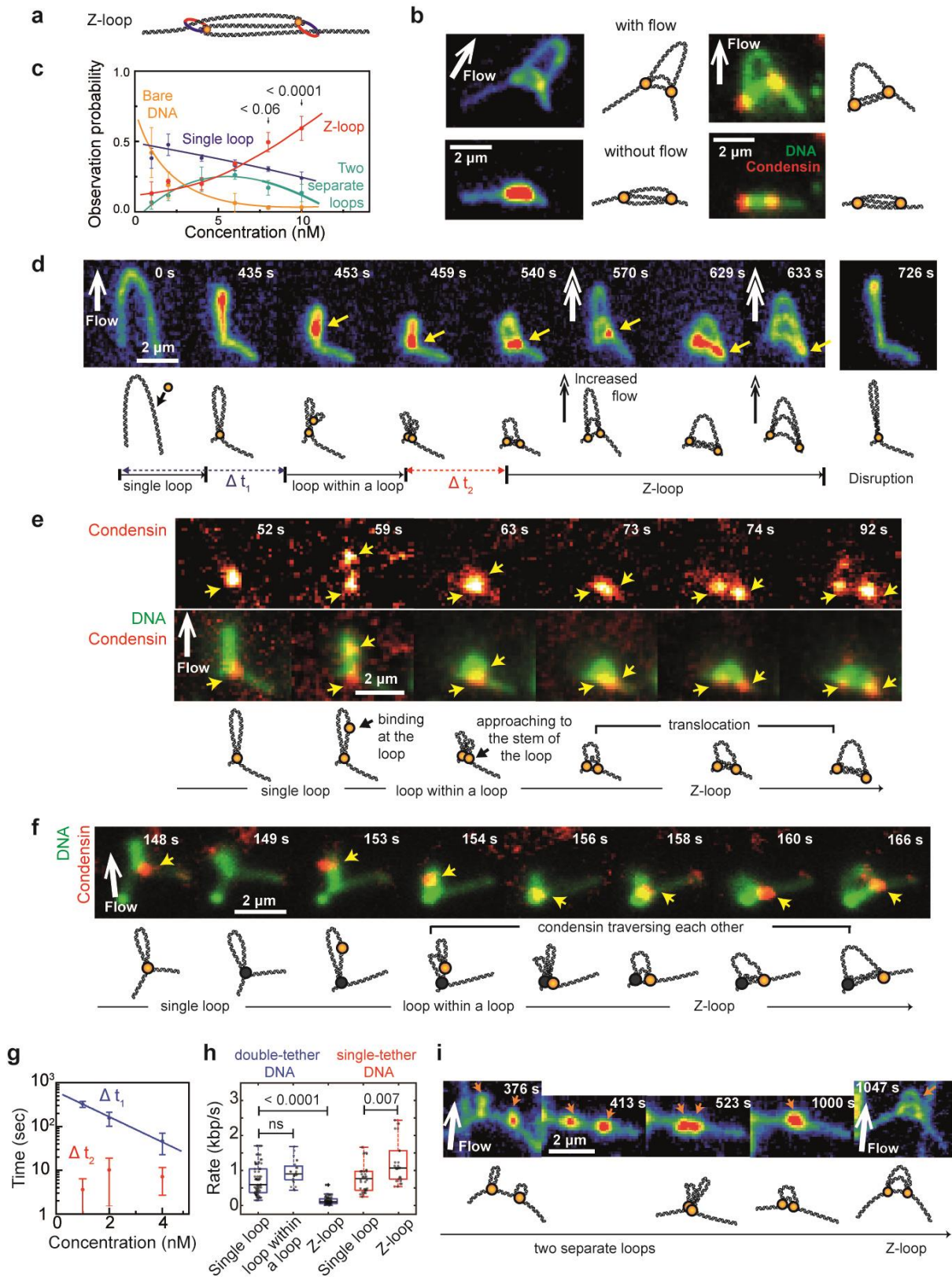
254 **Figure legends**



255

256 **Figure 1 | Interactions between multiple condensin-mediated DNA loops.**

257 **a**, Cartoon of the *S. cerevisiae* condensin featuring a large (~50 nm) ring structure (top), and schematic  
 258 (bottom) and snapshots **b**, showing single-molecule visualizations of DNA loop extrusion on double-  
 259 tethered SxO-stained DNA. Snapshots in (b) represent 21 independent experiments from 2  
 260 independently purified batches of condensin. **c**, Schematic and snapshots of two separate loops along a  
 261 DNA molecule, representative of 14 independent experiments. Arrows in (b,c) indicate direction of  
 262 buffer flow. **d**, **e**, Snapshots (left) and fluorescence-intensity kymographs (right) of two DNA loops  
 263 that diverge (d) or converge (e). Representative of 16 independent experiments. **f**, Probability that two  
 264 loops maintain a constant gap or mutually converge. Data shows the mean  $\pm$  95% confidence interval.  
 265 *P*-value is determined by two-tailed student's t-test ( $n=32$  molecules, 11 independent experiments). **g**,  
 266 Snapshots and kymograph showing the initial formation and shrinkage of a first loop (Loop 1) upon  
 267 initiation of a second loop (Loop 2). **h**, Corresponding DNA size changes of the two loops in panel (g)  
 268 versus time. (g-h) are representative of 16 independent experiments. **i**, Simultaneous change of DNA  
 269 loop size for Loop2 versus for Loop1 ( $n=5$  molecules, 3 independent experiments, Extended data 1e  
 270 for more examples). Dashed line has slope 1, indicating that Loop2 grows at the expense of a  
 271 shrinkage of Loop1. **j**, Schematic diagram depicting DNA size exchange between two loops in real  
 272 space (left) and in one-dimensional genomic space (right)<sup>14,27</sup>.

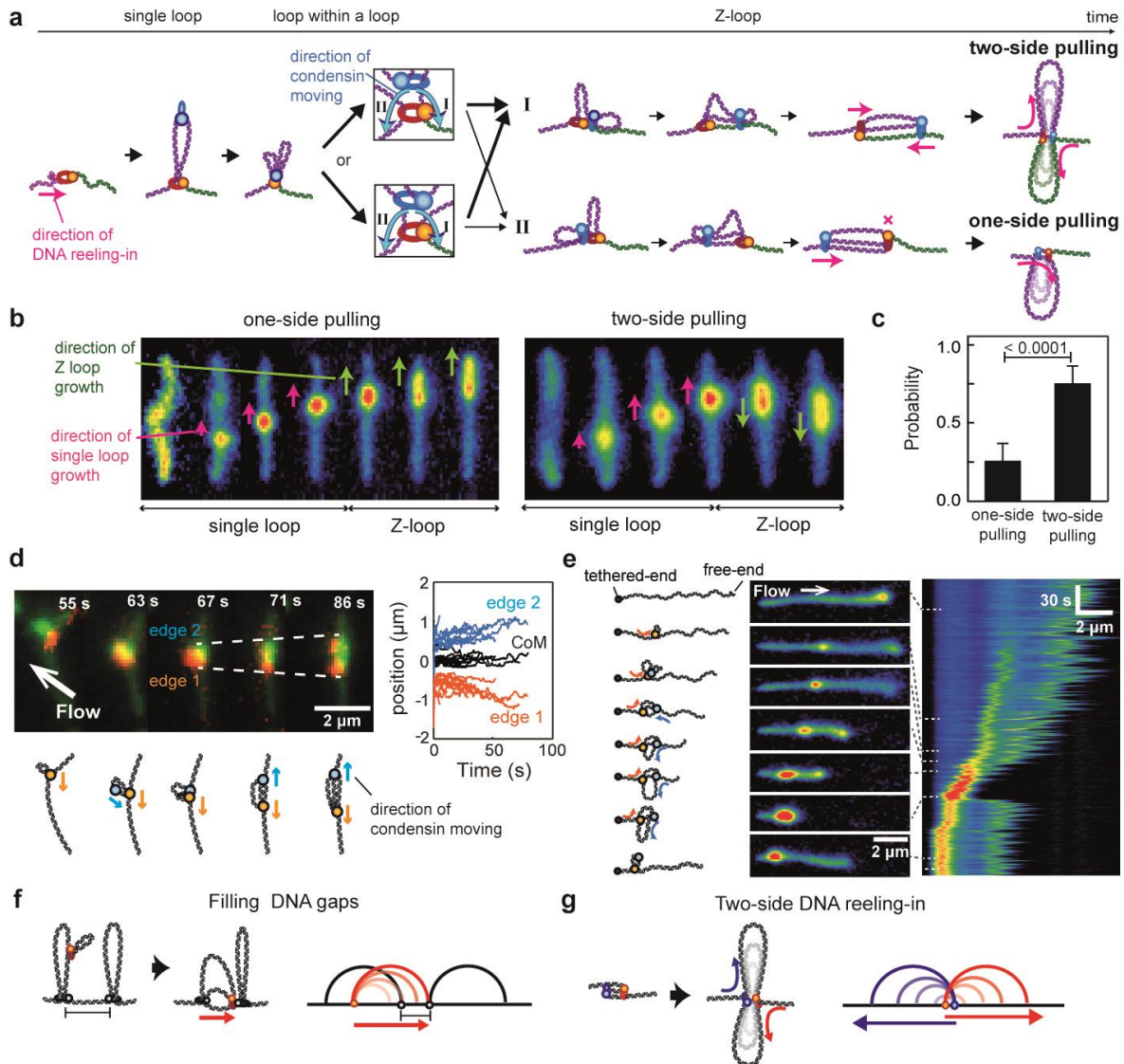


273

274 **Figure 2 | Condensins can traverse one another and form a Z-loop on DNA.**

275 **a**, Schematic of a Z-loop, which consists of three linearly stretched dsDNA molecules and two  
 276 condensins, one located at each edge of the loop. **b**, Images of DNA (left) and overlaid images of  
 277 DNA and condensin (right) revealing Z-loop by application of buffer flow. **c**, Probability of observing  
 278 different DNA conformations versus condensin concentration. Data show mean $\pm$ SD from 4

279 independent experiments per concentration ( $n_{tot}=476$  molecules). Lines are guides to the eye. **d**,  
280 Snapshots showing DNA intermediates in Z-loop formation from bare DNA (0 s), to a single loop  
281 (435 s), to an additional loop within the initial loop (~459 s), to a Z-loop (633 s), and to disruption into  
282 a single loop (726 s). Yellow arrows denote the moving DNA parts. **e**, Snapshots of condensin (top),  
283 and overlaid images of DNA and condensin (bottom) showing locations of two condensins during Z-  
284 loop formation. **f**, Snapshots of overlaid images of DNA and condensin tracing the locations of the  
285 second condensin during Z-loop formation after the first condensin is photobleached (149s). Yellow  
286 arrows in (e,f) denote the locations of condensins. **g**, Waiting time  $\Delta t_1$  and  $\Delta t_2$  (defined in schematic in  
287 c) versus the protein concentrations. Line represents fit for  $\Delta t_1$  (coefficient of determination,  $R^2=0.998$ ).  
288 Its slope for  $\Delta t_1$  was significantly different from zero (slope= $-0.28\pm 0.02$ ,  $P=0.03$ ) whereas that for  $\Delta t_2$   
289 did not significantly differ from zero ( $R^2=0.335$ , slope= $0.07\pm 0.09$ ,  $P=0.59$ ) (ANOVA test, significance  
290 set at  $P\leq 0.05$ ). Data show mean $\pm$ SD.  $n=12, 11, 13$  molecules for 1, 2, 3 nM (20 independent  
291 experiments). **h**, DNA-loop extrusion rate for single loops, loops within a loop, and Z-loops estimated  
292 for single- and double-tethered DNA (Methods). The box plots span from 25 to 75% percentile,  
293 showing median as center line, and max. and min. values as whiskers. All  $P$  values determined by  
294 two-sided  $t$ -test. **i**, DNA snapshots showing a Z-loop formed by merging of two separate loops. Two  
295 individual loops initiated independently of each other and subsequently converged. After the merger  
296 (1000 s), they transformed into a Z-loop, which was visualized by the application of buffer flow (1047  
297 s). Data in (b, d-f, i) represent 10, 20, 3, 10, 8 independent experiments, respectively. Schematic  
298 diagrams underneath the images in (b, d-f, i) provide visual guidance.  
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301

302

### Figure 3 | Possible impact of Z-loops on chromosomal compaction.

303

**a**, Model of DNA Z-loop formation by two condensins. Depending on the orientations of the two

304

condensins (zooms), the formed Z-loop can reel in DNA either from both sides of DNA (two-side

305

pulling) or from one side (one-side pulling). **b**, Series of snapshots showing two DNA molecules

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where the initial single loop and the subsequent Z-loop grow from the same side of DNA (left) or from

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opposite sides (right). Representative of 12 independent experiments. **c**, Probability that a Z-loop pulls

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from one side or from two sides. Data shows the mean  $\pm$  95% confidence interval.  $P$  value is

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determined using two-tailed Student's  $t$ -test,  $n=70$  molecules from 12 independent experiments. **d**,

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Snapshots (top left) and schematics (bottom left) of overlay of SxO-stained DNA and ATTO647N-

311

labeled condensin. For this molecule, binding of the second condensin occurred before the first

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condensin fully extruded the single loop, thus allowing for the first condensin to continue to reel in

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DNA during Z-loop extension. This results in a symmetric divergence of the two condensins.

314

Simultaneous change of positions of two Z-loop edges (blue and orange) and of the center of mass

315

(black) ( $n=11$  from 5 independent experiments; right). **e**, Schematics (left), snapshots (middle), and

316

kymographs (right) of loop formation on a single-tethered DNA. Initially a single loop is formed,

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whereupon a two-side pulling Z-loop is formed, which is visualized in the broadening, accompanied

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by a simultaneous decrease in DNA length outside of the loop in both directions. At some point in

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time, the Z-loop disrupted and terminated into a single loop because the DNA that was reeled on the

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right reached the free end. Representative of 3 independent experiments. **f**, **g**, Schematic diagrams

321

depicting possible implications of Z-loops for chromosomal compaction in real space (left) and 1D

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genomic space (right).

323

324 **Methods**

325

326 **Condensin holocomplex purification**

327

328 We used our previously published expression and purification protocols<sup>1</sup> to prepare the pentameric *S.*  
329 *cerevisiae* condensin complex.

330

331 **Fluorescent labeling of purified condensin complexes**

332

333 The purified condensin complexes were fluorescently labeled as described previously<sup>1</sup>. Briefly, a 10 %  
334 excess of ATTO647N-maleimide (ATTO-TEC) was coupled to Coenzyme A (Sigma) in  
335 deoxygenated 100 mM sodium phosphate buffer at pH 7.00 for one hour at room temperature. 10 %  
336 equivalent of tris(2-carboxyethyl)phosphine was included halfway through the reaction and coupling  
337 was terminated with an excess of dithiothreitol. The reaction mixture was used for enzymatic covalent  
338 coupling to ybbR acceptor peptide sequences within the kleisin subunit in condensin holocomplexes  
339 (Brn1[13-24 ybbR, 3xTEV141]-His<sub>12</sub>-HA<sub>3</sub>; C5066), using a 5-fold excess of fluorophore to protein  
340 and ~1 μM Sfp synthase (NEB) for 16 hours at 6 °C in 50 mM TRIS-HCl pH 7.5, 200 mM NaCl, 5%  
341 v/v glycerol, 1 mM DTT, 0.01% Tween-20, 0.2 mM PMSF, 1 mM EDTA. Labeled protein was  
342 separated from unreacted fluorophore and the Sfp synthase by size-exclusion chromatography on a  
343 superose 6 3.2/200 (GE Healthcare) preequilibrated in 50 mM TRIS-HCl pH 7.5, 200 mM NaCl, 5%  
344 v/v glycerol, 1 mM MgCl<sub>2</sub>, 1 mM DTT).

345

346 **Double-tethered DNA assay for single-molecule imaging**

347 Phage λ-DNA molecules were labelled with biotin at their both ends as described previously<sup>1</sup>. The  
348 biotinylated DNA molecules were introduced to the streptavidin-biotin-PEG coated glass surface of a  
349 flow cell at constant speed of 5 – 10 μL/min, resulting in attachment of DNA molecules with relative  
350 DNA extensions ranging from ~0.3 to ~0.6. The surface-attached DNA molecules were stained with  
351 500 nM Sytox Orange (Invitrogen) intercalation dye and imaged in condensin buffer (50 mM TRIS-  
352 HCl pH 7.5, 50 mM NaCl, 2.5 mM MgCl<sub>2</sub>, 1 mM DTT, 5% (w/v) D-dextrose, 2 mM Trolox, 40  
353 μg/mL glucose oxidase, 17 μg/mL catalase).

354

355 Real-time observation of multiple loop interactions by condensin was carried out by introducing  
356 condensin (1-10 nM) and ATP (5 mM) in the above specified condensin buffer. Although Z-loops  
357 were more frequently observed at higher concentrations (6-10 nM), most of the presented data were  
358 obtained in the concentration range of 2-4 nM. This was done to study single Z-loops and minimize  
359 measuring on DNA molecules that exhibited both a single loop and Z-loop simultaneously. For dual-  
360 color imaging of SxO-stained DNA and ATTO647N-labeled condensin, we also kept to this lower  
361 concentration range to minimize the background coming from both freely diffusing labelled condensin  
362 as well as from labelled condensins that transiently bound onto DNA without forming DNA loops.

363

364 Fluorescence imaging was achieved by using a home-built epi-fluorescence/TIRF microscopy. For  
365 imaging of SxO-stained DNA only, a 532-nm laser was used in epi-fluorescence mode. In the case of  
366 dual-color imaging, SxO-stained DNA and ATTO647N-labelled condensin were simultaneously  
367 imaged by alternating excitation of 532-nm and 640-nm lasers in Highly Inclined and Laminated  
368 Optical sheet (HILO) microscopy mode with a TIRF objective (Nikon). All images were acquired with  
369 an EMCCD camera (Ixon 897, Andor) with a frame rate of 10 Hz.

370

371 **Data analysis**

372

373 *Estimation of condensin density per DNA length in the in vitro experiments*

374 Condensin density per DNA length was estimated as follows. First, movie frames were mapped into  
375 intensity profiles along the tether length by subpixel interpolation mapping. This was done for both the  
376 DNA and condensin signal channel. Next, these intensities were mapped in position versus time

377 kymographs. Condensin was counted by simple peak detection on the condensin profiles associated  
378 with each time point of the condensin kymograph. To suppress noise, the profiles were smoothed  
379 and only peaks above a threshold were counted. This threshold was taken as two times the standard  
380 deviation of the background noise. Next, we obtained the density of condensin per DNA length by  
381 summing the total number of detected condensin molecules over all time points, and dividing this by  
382 the total observed DNA length. To avoid biasing by surface effects, we excluded DNA tether lengths  
383 and condensin counts that were within ~400 nanometres of the tether attachment points. In this way,  
384 we obtained an average condensin density per tether. Finally, we repeated this measurement for ten  
385 separate tethers to find an average plus error for the condensin density.

386  
387 For counting the number of molecules with two loops that were converging or kept a constant DNA  
388 gap in figure 1f, molecules exhibiting pronounced DNA slippage were excluded from the analysis.

#### 389 *Estimation of the DNA size within and outside of loops*

390 To estimate the size of the DNA loops and the distance between loops, fluorescence intensity  
391 kymographs as shown in e.g. Fig. 1d were built from the intensity profiles of DNA molecules per  
392 time point as explained in our previous paper<sup>1</sup>. From the kymographs for individual molecules thus  
393 obtained, a loop analysis (cf. Figs. 1h, Extended Data Fig. 1, 2 etc.) was carried out as follows. The  
394 start center position and start time of a loop was indicated through user input. Then, the position of the  
395 loop at each time point was found by center-of-mass tracking over a section of the DNA molecule.  
396 This procedure was repeated until a user-set end time for this loop was reached. The data was stored as  
397 a position-time trace per loop.

398  
399 For quantitation of the loop size, we define three regions per DNA molecule, namely 'Left', 'Middle',  
400 and 'Right', and collect the fluorescence intensities for the respective regions, viz., 'Left' as the  
401 intensity from the section left of the loop region, 'Middle' as the intensity of the DNA that is  
402 contained in the loop (Note that for Z-loops this includes the tether section below the loop region), and  
403 'Right' as the intensity from the section right of the loop region. These three intensities were then  
404 expressed as percentages of the total intensity count, adding up to 100%. Using this intensity  
405 information, the sizes of the DNA loops (in kbp) were obtained by multiplication of the percentages  
406 by 48.5 kbp, yielding the size of individual single loops (e.g. Fig. 1h, Extended Data 1b,c) or the size  
407 of DNA within Z-loops (e.g. Extended Data Fig. 9f). For the detailed analysis for the estimation of  
408 DNA length in between two loops, we refer the readers to Extended Data Fig. 1a.

409  
410 To obtain the observation frequencies in Fig. 2b, we performed 4 different experiments (i.e., in 4  
411 different flow cells) per concentration. Per experiment, we counted the fraction of molecules (out of 11  
412 to 40 molecules) that showed no loop/a single loop/two separate loops/a Z-loop for each frame, and  
413 divided that by the total number of molecules and by the number of frames. The error bars are the  
414 standard deviations from averaging the 4 different experiments.

#### 415 416 *DNA-loop-extrusion rate estimation for single and Z-loop expansion*

417 To estimate the DNA-loop-extrusion rates of single and Z-loops in the absence of flow, we first built  
418 the intensity kymographs and extracted the time traces of DNA size changes in the loop region during  
419 single/Z-loop formation (e.g. Extended Data Fig. 9f). For the extraction of the respective rates, a linear  
420 fit to the increase of DNA amount during the first 10 seconds of the single/Z-loop growth was used.

#### 421 422 *DNA-loop-extrusion rate estimation for a loop within a loop*

423 The rate of the DNA loop formation by the second condensin that docks within the DNA loop  
424 previously formed by a first condensin was estimated in the presence of buffer flow, as the change  
425 from a single loop to a nested loop can only be seen by flow-induced DNA stretching. For this, we  
426 built the intensity kymographs along the axis parallel to the extruded single loop (e.g. Extended Data  
427 9b). From these kymographs, the rate of loop within a loop formation was determined by the change in  
428 the physical length of the single loop and that of nested loop, divided by the time duration of the  
429 formation process of the second loop.

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432 *DNA-loop-extrusion rate estimation for single and Z-loop growth for single tethered DNA*  
433 The rate of single and Z-loop growth for single-tethered DNA was estimated from the change of the  
434 DNA end-to-end length divided by the time duration.  
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436  
437  
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